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SGPO 1

Androgen excess in an adolescent girl due to an ovarian tumor: diagnostic and therapeutic challenges

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Background: Virilization in pubertal girls raises suspicion for late onset congenital adrenal hyperplasia (CAH), androgen producing tumor of adrenals or ovaries or polycystic ovary syndrome. Signs of virilization are hirsutism, acne, clitoromegaly and amenorrhea. Adrenocortical tumors in children are rare (0.3–0.4 per million). Recent work revealed that androgen production occurs through several biochemical pathways, e.g. the classic and backdoor pathway, but the role of novel pathways in tumors is unknown.
Objective: To assess the role of backdoor androgen production in a rare ovarian, androgen producing tumor.
Case report, results and follow-up: We report on a 14-year-old girl with secondary amenorrhea, hirsutism and weight gain. On clinical examination hirsutism, normal blood pressure, Tanner B5 P6 were noted but no palpable abdominal mass. Laboratory work-up showed high androgen levels in serum and urine, AFP was normal. Ultrasound revealed a solid mass arising from the left ovary, MRI-scan confirmed an ovarian tumor. Laparoscopic left salpingo-oophorectomy was performed. Histopathological assessment showed a very rare steroid-cell tumor NOS ("not otherwise specified"). Specific immunohistochemical studies of steroid enzymes showed upregulated enzymes of the classic and backdoor pathway such as RoDH (retinol dehydrogenase), CYP17A1 (17α-hydroxylase) and AKR1C3 (aldo-ketoreductase family 1 C3). Postoperatively, androgen levels normalized and menstrual bleedings became regular. But 12 months later, severe acne and amenorrhea relapsed, and androgen levels were found elevated again. To preserve fertility and prevent ovarectomy, we tested whether tumoral hormone production is still responsive to regulation. Dexamethasone was found to suppress androgen levels significantly, while LH-RH did not inhibit hormonal activity. Because the tumor had upregulated enzymes involved in the classic and backdoor pathway, we started the patient on Androcur® (an inhibitor of the androgen receptor).
Conclusions: Our patient report shows that androgen production by the ovarian tumor occurs through the classic and the backdoor pathway. Drugs Abiraterone and Finasterid would inhibit CYP17 or 5α-reductase activities, respectively; both enzymes are part of classic and backdoor androgen production. Thus it is possible that those drugs that are in use for prostate cancer or CAH could be used to treat our patient's tumor. However, these drugs are not in routine use in pediatrics.

SGPO 2

Frequency of organ dysfunction and impact on mortality in children with blood culture-proven sepsis – results from The Swiss Pediatric Sepsis Study

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Background and aims: Recently, the definition of sepsis in adults has been refined as life-threatening organ dysfunction caused by a dysregulated host response to infection.
Objectives: We analysed the relationship of number of organ dysfunctions with case fatality rate in a prospectively collected dataset on sepsis in children.
Methods: Prospective observational cohort study of newborns and children <17 years with blood culture-proven sepsis admitted to ten paediatric hospitals in Switzerland between 9/2011 and 12/2015. Sepsis and organ dysfunctions were defined according to the 2005 pediatric consensus definition.
Results: Of 1204 blood culture-proven sepsis episodes, organ dysfunction was present in 474 (39%). In 590 (49%) episodes patients were admitted to the intensive care unit, and in 323 (55%) of those episodes patients required mechanical ventilatory support. In 90 of 1204 (7.5%) episodes the outcome was fatal in the first 30 days after sepsis onset. The odds ratio of death increased by 2.9 (95%CI 2.5–3.5, p <0.001) for every additional organ dysfunction; from a case fatality rate of 0.7% (95%CI 0.3–1.7) in 730 episodes with no organ dysfunction to 46% (95%CI 34.4–58.7) in 69 episodes with 4 or more organ dysfunctions.
Conclusion: Only a minority of children presenting with blood culture-proven sepsis as per 2005 pediatric consensus definition had an organ dysfunction. Presence and number of organ dysfunctions were strongly associated with mortality, and should be considered for future sepsis definitions to discriminate children with infection from children with life-threatening dysregulated host response to infection.

SGPO 3

Impact of introducing cerebrospinal fluid enterovirus polymerase chain reaction testing on duration of antimicrobial treatment and hospital stay in infants <90 days of age presenting with fever

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Background: Enteroviruses are the main cause of aseptic meningitis in infants presenting with febrile illness at the emergency room. Infants <90 days of age presenting with fever undergo a septical workup including lumbar puncture and receive empirical antimicrobial treatment not to miss serious bacterial infection. Introducing enteroviral polymerase chain reaction testing in cerebrospinal fluid (CSF EV-PCR) may impact duration of antimicrobial treatment and hospital stay of these infants, if the test result is readily available. We investigated this potential impact of introducing CSF EV-PCR in infants <90 days of age presenting with fever.
Methods: Infants <90 days of age presenting with fever at the University Children's Hospital Zurich between June 2015 and October 2016 and for whom CSF EV-PCR testing was performed were investigated by chart review. Infants with bacterial meningitis were excluded. Duration of empirical antimicrobial treatment and hospital stay were compared between children with positive and negative CSF EV-PCR results as well as to a corresponding control group of infants before the introduction of CSF EV-PCR.
Results: A total of 74 children were enrolled in the study, 47 in CSF EV-PCR group and 27 in the control group, respectively. The median age was 37 days in both groups (range 3 to 85 days). CSF EV-PCR tests were positive in 25 of 47 patients (53%). CSF EV-PCR positive patients showed a significant reduction in the mean duration of antimicrobial treatment compared to EV-PCR negative patients and to controls (23.1, 45.1 and 61.4 hours respectively, p <0.0001), as well as a significant reduction in the mean duration of hospital stay (3.4, 5.0 and 4.0 days respectively, p = 0.01). Introduction of CSF EV-PCR significantly reduced the mean duration of antibiotic treatment, not only if positive but irrespectively of test results, compared to control group before test introduction (33.4 vs 61.4 hours respectively p <0.0001), but had no impact on duration of hospital stay (4.1 vs 4.0 days, p = 0.83, respectively).
Conclusion: The use of CSF EV-PCR test in septical workup of infants <90 days of age presenting with fever significantly reduced antibiotic use not only in those with enteroviral meningitis but overall. These findings advocate for systematic use of CSF EV-PCR test in infants presenting with fever in the first 90 days of life in order to shorten unnecessary antibiotic use and possibly hospital stay.

SGPO 4

Temporal behavior of respiratory symptoms during infancy and associations with asthma at school age

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Background: A high number of respiratory symptoms during infancy increase the risk for later asthma development. However, if the temporal pattern of these symptoms may contain additional information on susceptibility for chronic disease is unknown. We developed an observer independent method to characterize this temporal pattern and prospectively tested their clinical utility.
Methods: In the BILD birth-cohort of healthy neonates, we assessed weekly respiratory symptom scores during infancy, resulting in a time series of 52 symptom scores. We then calculated transition states between two consecutive symptom scores and used those to construct a transition probability map. We quantified this map using a single entropy parameter for each infant. Based on entropy, we determined 4 temporal phenotypes and tested their association with asthma risk factors. Using logistic regression, we determined the association with asthma and atopy at school-age.
Findings: From 400 recruited neonates, 322 (81%) attended follow-up at 6 years and had complete data for ≥48 weeks of respiratory symptoms scores during infancy (16864 observations). In the high-risk phenotype, a one unit increase of entropy was associated with asthma (adjusted odds ratio; 95% CI) (OR 3.74; 1.08–12.95) and atopy (OR 3.45; 1.09–10.87). This phenotype was predominantly male (82%), and more infants were born to asthmatic mothers (23%) or to those who smoked during pregnancy (41%).
Interpretation: Temporal characteristics of respiratory symptoms in infancy might be a novel predictor for asthma at school age. We hypothesize that this temporal behavior may additionally reflect susceptibility and recovery pattern of the airways, rather than the number of environmental triggers alone.

SGPO 5

Lung function improvement and airways inflammation reduction in asthmatic children after a rehabilitation program at moderate altitude

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Background: Rehabilitational programs at moderate altitude (>1500 m–<2500 m) showed improvement of lung function and reduction of airways inflammation in asthmatic adults. Since concentration of pollen and house dust mites (HDM) is lower in such environment, allergen avoidance was postulated as the major effect of these improvements.
Objectives: To investigate whether asthmatic children benefit from a rehabilitation at moderate altitude, especially regarding different phenotypes.
Methods: 344 asthmatic children, who stayed in the rehabilitation clinic in Davos (Hochgebirgsklinik), were included in this study. Two spirometries or body plethysmographies, with at least 14 days between the first at baseline and the second at discharge, were performed. A sub population (n = 124) received two fractional exhaled nitric oxide measurements (FeNO). Associations with allergic sensitisation (skin prick testing and/or specific IgE), level of asthma control and level of inhalative cortison dose (according to Global Initiative for Asthma guidelines) were analysed.
Results: Pulmonary conditions improved significantly on average during the sojourn. Patients with uncontrolled asthma benefited most with an increase of FEV_1, MEF25 and MEF75 by 7.7, 9.9 respectively 12.7 percentage points (pp) ($p < 0.001$). FeNO decreased significantly by 36.9 parts per billion (ppb) in this group. FeNO also decreased significantly by 26.9 ppb for partly controlled and 11.8ppb for controlled asthma. In uncontrolled asthmatics, pulmonary improvement (FEV_1, MEF25, MEF75 and FeNO) was similar between patients with and without HDM sensitization. The pulmonary improvements of pollen sensitized patients were not dependent on the season of the sojourn. In patients with no change of the initial, inhalative cortison dose, FEV_1 increased by 4.9 pp ($p < 0.001$) and FeNO decreased by 21.9 ppb ($p < 0.001$). The increase of FEV_1 was slightly higher (6.6 pp, $p < 0.001$) for the group, in which the initial, inhalative cortison dose was raised by one GINA-level. The corresponding FeNO decrease was 29.7ppb ($p < 0.001$).
Conclusion: Inpatient rehabilitation in Davos improved pulmonary function in asthmatic children and adolescents independent from sensitization status to house dust mites or pollen. This result suggests the presence of further beneficial factors at moderate altitude other than allergen avoidance. Moreover, a positive effect is observed even for the group with no change in medication.

SGPO 6

Newborn screening for cystic fibrosis in Switzerland – evaluation after 6 years

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Background: Newborn screening (NBS) for cystic fibrosis (CF), based on immunoreactive trypsinogen (IRT)-DNA-IRT algorithm, was introduced in Switzerland in 2011. The program aims to detect all children with classic CF, but to avoid detecting children with "CF screen positive, inconclusive diagnosis" (CFSPID). Here we evaluate the diagnostic performance of the Swiss NBS for CF over the past 6 years.
Methods: We analysed data from the national CF screening database, including all children screened between January 2011 and November 2016. Children with positive screening results were referred to a CF-centre for further examination, including sweat test. We assessed the number of referred children and confirmed diagnoses. We calculated specificity, sensitivity, and positive and negative predictive values (PPV, NPV) of the screening procedure.
Results: Out of 512,396 births within 6 years, 533 children were screened positive and referred to a CF-centre. Of these, 145 (27.2%) were diagnosed with CF, 20 (3.8%) had CFSPID, 356 (66.8%) children were CF negative, and two have yet unknown diagnosis. Ten (1.9%) died of severe illnesses not related to CF (cardiomyopathy, heart failure, multiorgan failure etc.) and no further diagnostic testing was performed. However, nine of the 10 children had two elevated IRT and no CFTR mutation in the screening. In total, 7 children with negative screening result were later clinically diagnosed with CF (4.6% (7/152) false negatives). The sensitivity of the CF-NBS testing was thus 95.4% (145/152); the PPV was 27.2% (145/533), or 31.0% (165/533) when CFSPID cases were included. The specificity (511,856/512,244) and NPV (511,856/511,863) reached almost 100%.
Conclusions: After 6 years, the NBS for CF remains successful in detecting >95% of children with CF. In order to improve the PPV of the screening for CF, pancreatitis-associated protein (PAP) measurement, as a further technique or a more efficient safety-loop, is under evaluation.

SGPO 7

Phenotypes of atopic dermatitis depending on the timing of onset and progression in childhood

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Background: Atopic dermatitis is an inflammatory, pruritic skin disease that often occurs in early infancy with a chronic course. However, a specific description of subtypes of atopic dermatitis, depending on the timing of onset and progression of the disease in childhood, is lacking.
Objective: To identify different phenotypes of atopic dermatitis using a definition based on symptoms within the first 6 years of life and whether subtypes are more at risk for developing other allergic diseases.
Methods: 1038 children who participated in the Protection against Allergy-Study in Rural Environments (PASTURE) birth cohort were included in the current study. We used longitudinal latent class analysis (LCA) to identify different subtypes of atopic dermatitis in childhood based on the course of symptoms up to 6 years. Atopic dermatitis was defined as an itchy rash on typical locations from birth to 6 years.
Results: The LCA model with the best fit to PASTURE data separated 4 phenotypes of atopic dermatitis in childhood: 2 early phenotypes with onset within the first 2 years of life (early-transient: 9.2% and early-persistent: 6.5%), the late phenotype with onset after the 2nd year of life (4.8%), and the never/ infrequent phenotype (79.5%), defined as children with no atopic dermatitis. Parental history of allergies was a strong predictor for the early-persistent phenotype. Early phenotypes were strongly associated with food allergy and sensitization. The risk of developing asthma was significantly increased among the early-persistent phenotype. The late phenotype was only positively associated with allergic rhinitis.
Conclusion: Using LCA, 4 phenotypes of atopic dermatitis were identified depending on the onset and course of the disease. The prevalence of asthma and food allergy by 6 years of age was strongly increased among children with early phenotypes (within the first 2 years), especially with persistent symptoms. These findings are important for the development of strategies in allergy prevention.

SGPO 8

Stem cell transplantation for hemoglobinopathies in Switzerland

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Beta-thalassemia (Thal) and Sickle cell disease (SCD) are inherited diseases causing abnormal hemoglobin protein structure and impaired red cell survival. Conservative treatment options, e.g. red cell transfusions and iron chelation for Thal and hydroxycarbamide for SCD, mitigate the course of disease but ultimately fail to prevent debilitating conditions in affected patients. Life expectancy is limited to 3 to 5 decades in developed countries. Both disease- and treatment-related complications as well as impairments in activities of daily life are associated with high socio-economic costs. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) remains the only curative therapy. Here, we report on the transplant activity and outcome after allo-HSCT in pediatric Thal and SCD patients in Switzerland. Between 1991 and 2016, 21 patients with Thal and 8 with SCD (total n = 29) were transplanted at the University Childrens Hospital in Zurich. The median age at HSCT was 8.3 (3.0–19.9) and 8.6 (4.8–17.7) years, respectively. Conditioning before 2002 comprised oral Busulfan (14 mg/kg bw) and iv. Cyclophosphamide (200 mg/kg bw) (n = 10), from 2002–2005, i.v. Busulfan (dosed weight-based), Cyclophosphamide (200 mg/kg bw) and Thymoglobuline (rabbit ATG) (n = 7), from 2005–2009 targeted Busulfan (cum. AUC 60–80 mg/Lxh), Cyclophosphamide and Thymoglobuline (n = 3), from 2009–2016 targeted Busulfan, Fludarabine (180 mg/sqm) and Thymoglobuline or Alemtuzumab (n = 9). Donors were related siblings or relatives (MSD/ MRD, n = 28) and unrelated matched donors (MUD, n = 1). Stem cell source was bone marrow (n = 27), combined cord blood/bone marrow (n = 1) or peripheral blood stem cells (n = 1). Median follow up time was 8.1 (0.3–15.5) years. The overall/event free survival at the last follow-up was 93.1% (27/29 patients). Intractable acute Graft-versus-Host-Disease (GVHD) > grade 2 remained the main cause of transplant-related mortality (2 patients). No graft failures were encountered. All surviving patients remain transfusion-independent. The quality- of-life in survivors is excellent. Conclusion: MSD/MRD allo-HSCT demonstrates a high success rate and safety profile in this pediatric cohort of Swiss patients with Thal and SCD and implies timely consideration of this therapeutic option. Extending the stem cell donor pool to alternative donors, preventing GHVD and developing effective reduced-intensity conditioning regimens are mandatory and need future investigations.

SGPO 9

Epidemiology and treatment of Systemic-onset Juvenile Idiopathic Arthritis (SoJIA) in Switzerland through an international platform (JIRcohorte)

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Introduction: Systemic-onset juvenile idiopathic arthritis (SoJIA) is a potential life-threatening pediatric condition classified within the spectrum of juvenile idiopathic arthritis (JIA), but it is now believed to be an autoinflammatory disorder. SoJIA is characterized by remitting fevers, evanescent rash, generalized lymphadenopathy, hepatomegaly/ splenomegaly, and/or serositis. Non-responsiveness to standard therapy with corticosteroids and disease modifying antirheumatic drugs is not uncommon.
Objective: The aim of our study is to describe the epidemiology, clinical presentation and treatment of SoJIA patients in Switzerland.
Methods: This is a multicentric, descriptive, prospective and retrospective cohort study, through an international platform: JIRcohorte. Patients with SoJIA, diagnosed and followed in one of the nine participating centers in the JIR cohort project in Switzerland, are enrolled in the registry. The data are prospective since 2014 and retrospective between 2004 and 2014.
Results: 61 patients with SoJIA have been included; 38 were girls with a female: male ratio at 1.6:1. The median age at diagnosis was 5.6 years old and the median diagnostic delay was 51 days. Data for initial systemic manifestations were available in 39 patients: 97% presented with typical fever (38/39), 97% with rash (38/39), 33% with splenomegaly (13/39), 21% with hepatomegaly (8/39), 13% with adenopathy (5/39) and 13% with serositis (5/39). Among the 61 patients, 39 received a treatment with NSAID (64%), 26 systemic corticosteroids (43%), 5 intra-articular steroids (8%), 36 Methotrexate (59%), 8 Cyclosporin (13%), 2 IVIG (3%), 1 colchicine (1.6%). 44 out of 61 patients (72%) had a biologic treatment; the median time between the diagnosis and the introduction of the biologic agent was 161 days. Anakinra was administrated in 34% of patients (21/61), 18% received Canakinumab (11/61), 34% Tocilizumab (21/61), 31% Etanercept (19/61), 10% Infliximab (6/61) and 3% Abatacept (2/61).
Conclusion: We describe here the main epidemiologic-clinical characteristics and treatment of patients with SoJIA included in our international JIRcohorte platform since 2004. Biologic therapies, in particular anti-IL1 and anti-IL-6 agents, are widely used in the treatment of this disease.

SGPO 10

A demanding act of love: barriers and challenges in communication and decision-making for infants at the limits of viability

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Introduction: The birth of an extreme preterm infant often comes unexpectedly. Decisions, then, must often be made quickly and under circumstances that can be emotionally stressful for parents or morally burdensome for health care professionals. In the past decades, more emphasis has been placed on parental involvement in the decision-making, but a shared approach has remained understudied and difficult to implement in practice.
Methods: In order to explore parental attitudes and values in the end-of-life decision-making process, we conducted 13 qualitative semi-structured interviews with 20 parents. We recruited parents of extremely premature infants who were born alive and died in the delivery room or in the NICU at the University Hospital Zurich in the years 2013–2015.
Results: Parents described factors that resulted in either a smooth or friction-laden decisional process. Some elements favored good communication between parents and the health care team, such as transparent information, empathy and honesty. Elements such as lacking of transparent information and lacking continuity of care discouraged good communication. Parents experienced the end-of-life decision-making process in divergent ways. Although most parents did consider to be involved in decision-making, they were nevertheless satisfied with the process. In contrast, few parents were involved in the decision-making. They appreciated and valued the possibility to take part in the decision, but they did not perceive the decision as an act of autonomy. Rather they felt it was part of their parental responsibility and a demanding act of love.
Conclusion: The (non)involvement of parents in the end-of-life decision-making of their infant revealed that parental decision-making preferences are not homogenous, but highly case- and context-dependent. Health care professionals, who are closest to the parents, should aim to avoid either of these two opposites. Instead, they should enable and encourage parents' relationship with their child and create a space where parental preferences for involvement in end-of-life decisions can be assessed. Only then, it is considered a shared commitment.

SGPO 11

Catheter-associated bloodstream infection in very low birthweight infants after implementation of prevention bundles

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Background: Care of very low birth weight (VLBW) infants in pediatric intensive care units (PICU) substantially relies on venous access to provide medication or parenteral nutrition. Therefore, invasive central venous catheters (CVC) are essential for clinical management in VLBW infants. Besides, CVC are the major risk for catheter-associated blood stream infections (CABSI) which per se are related to significant morbidity and mortality. In consequence, programs to prevent CABSI are crucial to every PICU.
Methods: This is a prospective evaluation of central CVC management and care in VLBW infants in a 10-bed tertiary care PICU in Switzerland. During 2010 three prevention bundles (insertion, maintenance, "culture of safety") were implemented together with an automatized electronic outcome measurement of CABSI rates per 1000 catheter days. Interventions (e.g. CVC insertion) together with laboratory results were directly entered by the correspondent clinician or the laboratory technician to a central database. All VLBW infants age ≤90 days with placement of a CVC between January 2011 and December 2016 were included. CABSI was defined as presence of any pathogen in blood cultures of symptomatic VLBW infants more than two days after CVC insertion.
Results: During the 6-year period, 299 infants with a birth weight <1500 g were admitted and 428 CVC in 251 infants (84%) were placed resulting in a total of 2118 catheter days. CABSI per 1000 CVC days declined from 7 in 2011 to 3.8 in 2015 but did rise again in 2016 to 7.5.
Conclusions: Implementation of three prevention bundles resulted in a reduction of CABSI in VLBW infants but was not stable over time. Continued action is needed to further reduce CABSI incidence.

SGPO 12

Functional connectivity differences between term-born and very preterm born adolescents

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Background: Structural and functional magnetic resonance imaging (fMRI) studies showed aberrant trajectories of cerebral development and altered connectivity between various brain areas in children born very preterm. Using a network approach, alterations in neurocognitive networks are also seen in adults born very preterm.
Objective: To assess functional connectivity in the fronto-parietal cognitive control network (FPN) and in the default mode network (DMN) in adolescents born very preterm (VPA) compared to term-born adolescents (TBA).
Methods: Resting-state fMRI data was preprocessed using the CONN toolbox and involved the following steps: Realignment, functional and structural segmentation, normalization, outlier detection, and spatial smoothing. Between-group connectivity differences were assessed on the network level (FNC) comparing seeds using the DMN, saliency network (SN), dorsal attention network (DAN), sensorimotor network (SMN), visual network (VN), language network (LN), cerebellar network (CN), and the FPN ($p \leq 0.05$, FDR seed-level correction, two-sided). For the same networks, graph theory was applied to identify important network hubs ($p < 0.005$, uncorrected).
Results: 33 VPA (mean age: 12.9 ± 1.7 years, all born before 32 weeks of gestation) and 39 TBA (mean age: 13.0 ± 2.0 years) were analyzed. TBA showed higher connectivity between the left SMN (seed) and right VN, left SMN and right DAN and within the SMN relative to VPA. In contrast, VPA showed higher FNC between the bilateral SMN and two regions of the SN, and between the left SMN and two regions of the left FPN. Graph theory analysis revealed higher global efficiency and betweenness centrality for the preterm group in the left FPN (i.e. dorsolateral prefrontal cortex). In contrast, average path length was higher in this region in the term-born group.
Conclusion: Our results show altered functional connectivity and efficiency in VPA compared to TBA, especially within the FPN and the SMN. Higher global efficiency in the FPN could indicate that it is easier to reach one node from another node, making information transmission at the global scale more efficient in VPA. Higher betweenness centrality suggests that many information-processing pathways are passing through the left FPN and that this node thus could be of particular importance for cognitive functions in VPA. These differences may reflect long-term compensation mechanisms in individuals born very preterm.

SGPO 13

Central nervous system complications in rotavirus gastroenteritis: a systematic review of the literature

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Introduction: Rotavirus gastroenteritis can lead to complications involving the central nervous system ranging from convulsions with mild gastroenteritis (CwG) to severe encephalitis or cerebellitis. This study aimed to obtain a comprehensive overview on neurological complications of rotavirus infection, which have been described in case reports only.

Methods: Systematic review including articles published from 1984 to 2015. All cases with hyponatremia, hypoglycemia, pre-existing neurological disease and co-infections were excluded. Neurological complications were divided in 4 different groups: encephalitis, cerebellitis, encephalo-cerebellitis and CwG.
Results: Sixty-five reports described 158 cases: 99 CwG, 37 encephalitis, 17 encephalo-cerebellitis and 5 cerebellitis. More than 50% of cases were from Japan. The median age was 22 (IQR 14–29) months and children with CwG were significantly younger ($p < 0.01$) compared to the other groups. Status epilepticus, defined as a seizure lasting longer than 30 minutes, was observed in 5% of children with CwG and in 23% with encephalitis. The most frequently described finding on neuroimaging (CT or MRI) was a lesion of the splenium of the corpus callosum (35% in the encephalitis group, 41% in the encephalo-cerebellitis group and 12% in the CwG group). Four children died in the encephalitis group, whereas no death was described in the other groups. Among the surviving children, the encephalo-cerebellitis group showed a worse long-term outcome compared to the other groups ($p = 0.0004$). All cases of the CwG group recovered completely.
Conclusions: Rotavirus infection can rarely be associated with central nervous system complications, most frequently as benign convulsions, followed by encephalitis, encephalo-cerebellitis and isolated cerebellitis. The majority of the cases were described in East Asian Countries, suggesting a genetic predisposition. Complete recovery has been observed in all children suffering from CwG. However, important neurological sequelae are often described in children with encephalo-cerebellitis, encephalitis and cerebellitis.

SGPO 14

Behaviour change, depressed mood and catatonia: acute transient psychotic disorder or autoimmune encephalitis?

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Case report: 17 y.o girl presented abrupt change in behavior, deteriorating over 4 weeks: confusion, sleeplessness, mutism, depressed mood, feeding disturbance and dyskinetic movement of the orofacial region. Despite slight slowing EEG, first clinical evaluation led to diagnosis of acute transient psychotic disorder treated with risperidone. 48h later she presented general tonico-clonic seizure. 2nd EEG showed extreme delta brushes. Cerebral MRI revealed T2 hyperintensity in the left hippocampus and thalamus. CSF showed no pleicytosis, normal proteinorachy but oligoclonal bands were found in CSF. NDMAR antibody were negative in serum but positive in CSF (1/32). No ovarian teratoma was found. Initial treatment included 5 steroid bolus (1 g/d) and IV immunoglobulin, then rituximab. Because of dramatic outbreaks of auto mutilations, she was hospitalized in intensive care unit during several weeks for deep sedation. She recovered slowly but completely except for tiredness.
Discussion: Anti-N-methyl-D-aspartate (NMDA) receptor encephalitis is a severe form of autoimmune encephalitis associated with antibodies against NR1 and NR2 subunits of the NMDA receptor. Initially described in adult women with teratomas, it is now recognized among adult males and children, even without tumor. The stereotypical clinical course is non-specific flu-like prodrome followed by psychotic stage: bizarre behavior, paranoid thoughts, visual or auditory hallucinations and dyskinesia; some cases of pure psychiatric presentation exist. Brain MRI is either normal (50% of patients), or shows non-specific T2 hyperintensity in hippocampus, frontal and insular cortex. EEG is altered with background slowing. CSF abnormalities include lymphocytosis and oligoclonal bands. Diagnosis is confirmed by NMDA receptor antibodies in serum or cerebrospinal fluid. Improvement takes several months. Approximately 80% of patients have substantial or full recovery, with less than 5% mortality. First-step treatment includes steroid bolus and IVIg with eventual tumour resection; second line rituximab and/or cyclophosphamide. Yearly tumour check is advised.
Conclusion: It is important to consider anti-NMDA encephalitis in the differential diagnosis of acute psychiatric disorder. Some peculiar symptoms are in favor: auditory and visual hallucinations, dyskinesia, sleeplessness and feeding difficulties. Despite dramatic clinical presentation, full recovery can be expected with appropriate immunomodulatory treatment.

SGPO 15

Cardiovascular disease risk factors among male youths in Southern Switzerland: a transversal study

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Introduction: Cardiovascular diseases, first cause of death in Switzerland, are frequently attributable to risk factors already present in children and adolescents. The aim of this study was therefore to describe the prevalence of cardiovascular disease risk factors in 18- to 20-year-old males undergoing medical examination to assess fitness for recruitment into the army.
Methods: An exploratory transversal study, approved from the regional ethic commission, was conducted during the recruitment days. The analysis includes measurement of the anthropometric parameters, arterial brachial pressure, central arterial pressure and arterial stiffness (= pulse wave velocity in m/s). Moreover, a structured questionnaire addressing smoking behavior, sedentariness and familial cardiovascular risk factors, as well as blood analysis for determination of glycaemia, lipids and Vitamin D metabolism values was performed.
Results: In the period between 1/4/2014–31/12/2016, 1045 voluntary were included in our study. Following cardiovascular risk factors were present in this young male population: tobacco use (N = 449, 43%), body mass index >25.0 Kg/m^2 (N = 274, 26%); Abdominal circumference >94.0 cm (N = 117, 11%); Arterial pressure ≥140/90 mm Hg (N = 83, 8%); 25-OH-vitamina D3 rate ≤50 nmol/L (N = 201, 19%); total cholesterol ≥5.2 mmol/L (N = 54, 5%); uricaemia >500 µmol/L (N = 61, 6%); pulse wave velocity >10 m/s (N = 25, 2.5%).
Conclusion: The results of this study allow us to analyze the cardiovascular health of young males living in Southern Switzerland. These results clearly show that a high number of young male present at least one cardiovascular risk factor.
Funding: Swiss Society of Hypertension; Pierre Fabre Pharma Robapharm AG (unrestricted grant).

SGPO 16

Adaptation of the Graf hip ultrasound system for a newborn screening program of developmental dysplasia of the hip: The Swiss Mongolian Pediatric Project

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Background: Based on the study results published in 2013, the Mongolian Ministry of Health commissioned the Swiss Mongolian Pediatric Project (SMOPP) to implement a nationwide, ultrasound-based screening and preventive treatment program for developmental dysplasia of the hip (DDH). DDH is a major health issue in Mongolia because it may lead to lifelong pain and handicap if not or incorrectly treated. During the development of the program, which started in 2007, the researchers realized that the rather complicated Graf system of hip sonography was not only difficult to teach but to perform as screening method. As flexion-abduction devices were the only treatment necessary for those children with DDH, a simplification of its diagnostic criteria was also justified.
Methods: We adapted the system of 12 different hip types according to Graf, to only 4 types. Group A hips are healthy hips that need no further intervention and can be discharged. Group B hips are insufficiently maturated and need ultrasound-based follow-up to exclude progression to DDH; parents of these children were instructed to perform “hip-friendly” swaddling with abduction and flexion of both legs. Group C includes dysplastic hips that need immediate treatment with a flexion-abduction orthosis and, finally, the very rare Group D hips are dysplastic with trapped cartilage need, after an initial try with an orthosis, surgical management.
Results: The simplified classification system for DDH has been implemented in almost the whole of Mongolia. In the last 4 years only, more than 200.000 newborns were screened accordingly, whereof more than 3.500 children with DDH could be successfully treated within the first weeks of life. The number of treated children corresponds to an incidence of 17 per 1000 children with DDH, which is similar to our precedent study and internationally very low.
Conclusion: Early diagnosis and non-surgical treatment of DDH has superior outcomes and no sequelae.

SGPO 17

Introducing complementary medicine in the pediatric department of a Swiss teaching hospital: pilot phase outcomes of an integrative medicine program

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Introduction: Integrative medicine describes the combination of conventional and complementary approaches in a coordinated way. For the pilot phase of an integrative medicine program in the department of pediatrics, Fribourg Hospital HFR, we defined inpatient treatment algorithms for bronchiolitis, asthma and pneumonia, using medications and nursing techniques from anthroposophic medicine (AM). Parents could choose AM treatments as add-on to conventional care.
Material and methods: Patient charts were reviewed for diagnoses and treatments. Parents of AM users were asked at discharge to complete the Client Satisfaction Questionnaire (CSQ-8) and a questionnaire on experience with the AM treatment. Physicians and nurses were asked to complete an open-ended satisfaction questionnaire at 6 months into the pilot phase. Economic data for cost of staff training, medications and insurance reimbursements specific for AM were collected.
Results: During the 18-month pilot phase, a total of 351 children were hospitalized with bronchiolitis, asthma and pneumonia. Of these, 136 children (39%) received AM treatments and its use increased over time. 52 parents completed the questionnaire, 27 (54%) had never used complementary medicine for their child. Mean CSQ-8 score was 29.77 (95% CI 29.04 to 30.5), which is high in literature comparison. 96% of parents were mostly or very satisfied with AM; 96% considered AM as somewhat or very helpful for their child; 94% considered they learnt skills to care better for their child in the future; 87% thought they received sufficient information about AM. 6 physicians and 43 nurses filled the staff questionnaire. It revealed positive points such as enlarged care offer, a more listening and close contact with the child, more relaxed children and a greater role for parents in the care; weak points included insufficient knowledge and practice of AM methods and additional nursing time needed. Cost for staff training and medications was nearly compensated by AM related insurance reimbursements; no additional staff positions were created.
Conclusions: The introduction of complementary, anthroposophic treatments for respiratory disorders as part of an integrative medicine concept in a Swiss pediatric inpatient department was well accepted by patients and staff and led to high parent satisfaction. Cost was compensated by additional insurance reimbursements.

SGPO 18

Abusive Head Trauma: unique or multiple event?

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Objective: The study raises the question of the frequency of multiple Abusive Head Trauma (AHT) and the number of initial events not recognized by paediatricians.
Method: Zero to 2 year patients with medically proven AHT were recruited at the University Children's Hospital of Lausanne between 2002 and 2015. Two independent neuradiologists review the characteristics of cerebral haemorrhages to determine the occurrence of multiple events. Recent history of these patients was studied to look for the frequency of previous paediatricians' visits and initial diagnosis.
Results: During the study period, 20 patients met admission criteria of AHT. Twelve (60%) presented with imagery evidence of multiple events. 9 of these 12 (82%) underwent a previous medical visit within 3 months with non-specific symptoms leading to a wrong diagnosis before the final diagnosis of AHT.
Discussion: Frequency of multiple shaken events is much higher than previously assumed. Clinical manifestation of lesions met in AHT is variable, mimicking in most cases a banal paediatric illness. The diagnosis is often missed by paediatricians at the initial consultation.
Conclusions: Paediatricians must be critical when non-specific symptoms compatible with an intracranial pathology occurs. They must perform without delay cerebral imaging and fundus examination before to exclude non-accidental cerebral bleeding in the age group of AHT. Missing the diagnosis of AHT can lead to repeated episodes of shaking with subsequent permanent neurologic damage or death.

SGPO 19

"Feed forward" and interprofessional feedback to support learning of pediatric emergency medicine residents: results of a focus group study

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Background and objectives: Residents sometimes feel that they are not well prepared for handling of a critical ill pediatric patient and rarely they get structured feedback, and if so usually only by the attending physician. The goal of this study was therefore to investigate whether and how the use of "feed forward" (discussion of the patient before arrival) and interprofessional feedback (after seeing the patient) support learning as perceived by residents, attending physicians and nurses handling critical ill patients at a pediatric emergency unit.
Methods: The study was conducted from April till June 2016 at the Pediatric Emergency Medicine Department, University Children's Hospital, Inselspital Bern. At the beginning of the study period the "feed forward" checklist and interprofessional mini-CEX as feedback instrument were introduced to physicians and nurses, especially focusing on how to support learning of residents. To evaluate people's perspectives as well as the underlying considerations we conducted focus groups interviews with all three stakeholders, two groups with residents, one with attending physicians and one with nurses (each group with 4–5 participants (N = 18)). The interviews were recorded, transcribed and analyzed qualitatively.
Results: The analysis resulted in five themes: (i) identification of knowledge gaps/teaching moments before and after a clinical encounter supports learning interprofessionally, (ii) teamwork is promoted through inputs from all team members which leads to a common plan, clear role allocation, (iii) improvement of patient care and patient safety along with increased competency of residents; (iv) feed forward is perceived as more feasible than giving interprofessional feedback, which partly is due to time, space and feedback culture and (v) overall acceptance is high since focus is more on learning than on accomplishment. Also ideas for further improvement of the project were generated.
Conclusions: Especially "feed forward" not only has a positive impact on learning but also on teamwork and patient care. Although as supportive perceived by all team members interprofessional feedback has some challenging aspects.

SGPO 20

Elevated blood pressure in children referred to a pediatric cardiology clinic: frequency and management

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Introduction: Elevated blood pressure (BP), a major risk factor for cardiovascular diseases, might begin in childhood and tracks overtime. However, frequency and management of elevated BP is not well described among pediatrics outpatients.
Objectives: 1) To establish the frequency of elevated BP in children referred to a cardiology clinic; 2) To determine the association with the diagnosis of an heart condition and the proportion of cases reported in the medical report and for which the cardiologist made a management proposal.
Method: We performed a retrospective study of BP measurements of all outpatients having had an echocardiographic exam between 2005 to 2014 at the Cardiology Unit of the Lausanne university hospital (CHUV). BP values, demographic and anthropometric data from children 1 to 18 years old seen at the outpatient clinic were extracted. BP values were expressed in percentiles according to international references. Elevated BP was defined as a systolic or diastolic BP[3] 95th percentile. Medical reports of an approximately 10% sample of children with elevated BP were reviewed to assess the diagnosis of a heart condition, if elevated BP was reported and if any management was proposed.
Results: Among 10'779 outpatient visits (from 4'829 children; 57% of boys, mean age: 8.8 years, SD: 4.64), an elevated BP was found in 1799 (16.7%). In the sample of 222 children with elevated BP, 163

(73%) had a cardiac condition. An elevated BP was reported in the medical report in 15.3% of all cases (9.8% of cases with and 30.0% without a cardiac condition, respectively). When an elevated BP was reported, a management was proposed in 82.4% of cases.
Conclusion: The frequency of elevated BP at a single visit in children referred to the cardiology clinic at the CHUV is close to the proportion found in the general population. Reporting of elevated BP in medical reports is relatively low, and three times lower in children with a cardiac condition. In case of elevated BP, cardiologists often make management recommendation. However, the clinical signification and the appropriate management of elevated BP at a single visit remain to be established.

SGPO 21

FODMAP in IBS – more than acronyms?

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Introduction: Functional abdominal pain disorders are highly prevalent in children and adults. Irritable bowel syndrome (IBS) is a subgroup among these functional gastrointestinal disorders defined according to the Rome IV classification by abdominal pain or discomfort associated with changes in form or frequency of stool and/or pain related to defecation. The treatment focuses mainly on drugs, behavioural and more and more also nutritional approaches. Low FODMAP-diet (fermentable oligosaccharides, disaccharides, monosaccharides and polyols) as therapeutic approach aims to exclude poorly fermentable carbohydrates as a trigger of the visceral hypersensitivity and osmotic load in the gut. Studies in adult IBS patients have shown its efficacy in relieving symptoms in up to 70% in short term. However, there are only few studies on FODMAP-diet in children so far. In our case study we aim to describe the results of a low FODMAP-diet in children diagnosed with IBS at our hospital.
Methods: This is a retrospective case study performed at the pediatric hospital in St. Gallen, Switzerland. Children and adolescents diagnosed with IBS who had an intervention with a low FODMAP-diet were included. After counselling with a dietician, a low FODMAP-diet was established for four to six weeks followed by a stepwise reintroduction of different carbohydrate groups to the diet. The clinical response and final food patterns were obtained by chart reviews.
Results: Six patients from 10 to 19 years were included (5 female). Four patients were diagnosed with a diarrhea predominant IBS, one IBS with predominant constipation and one mixed-type IBS. During low FODMAP-diet three patients had an immediate reduction of symptoms, one patient showed a partial response, one girl achieved no symptom relief by the diet and one patient was still in the testing phase. After reintroducing carbohydrates in the daily food plan, three patients stayed asymptomatic and tolerated the diet well, one with no food restrictions, one with lactose free diet and one with wheat, lactose and low fructose diet. The average follow up time was 7.3 months.
Conclusion: The low FODMAP-diet is a valuable therapeutic approach for (older) children with IBS. In our case study three of five patients stayed asymptomatic after initial treatment. However, it is still unclear how to select patients qualifying for this treatment and what impact diet changes will have in a longer perspective.

SGPO 22

Interprofessional confidence, patient safety culture and CPR preparedness among pediatric health care providers at a primary care hospital

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Introduction: The safety culture practiced by health care professionals (HCP) is influenced by a variety of factors including safety climate, interprofessional morale, teamwork, supervision, peer support and medical education. We seek to assess safety skill and attitude, specifically CPR preparedness, interprofessional confidence and failure management culture among nurses and physicians in pediatrics in Switzerland.
Methods: During the last quarter of 2016 we conducted a cross-sectional anonymous survey to measure confidence and safety attitudes among clinically active staff of all 3 pediatric departments at Bern's University Hospital.
Results: We report a 55% response rate stemming from 1007 distributed and 550 returned surveys. Roughly 1/3 of respondents were physicians and about 2/3 were nurses. Half of those polled report encountering life threatening situations at least once a month. Three quarters believe they recognize clinical deterioration early in a child and two thirds believe they are in theory well prepared to deal with resuscitation. Less than half feel they possess the practical skills required for resuscitation. Nurses rank their emergency preparedness significantly higher ($p = 0.008$) than physicians and close to two thirds of residents express concern regarding their resuscitation preparedness. Patient safety education is deemed sufficient by 48% of respondents with nurses rendering a significantly higher approval rating than physicians ($p = 0.001$) and junior staff physicians disapproving at the highest rate (75%). Seventy-seven percent view nurse-physician collaboration as collegial rather than hierarchical but nursing's assessment was significantly less optimistic ($p = 0.001$) compared to physicians' assessment. When dealing with life-threatening situations, nurses are also significantly more likely to voice uncertainty ($p = 0.001$).
Discussion and Conclusion: Despite encountering life threatening situations relatively often, a majority of physicians do not feel sufficiently prepared to deal with them. Patient safety education appears to fall short of HCP staff expectations. Interprofessional and intraprofessional collaboration enjoys broad support, but differences in assessment are paralleled by the hierarchical slopes of the respective professions. Practical education surrounding interprofessional confidence, patient failure management and CPR preparedness warrants improvement in the eyes of both physicians and nurses in pediatrics.

SGPO 23

Childcare correlates of physical activity, sedentary behavior and adiposity in preschool children (SPLASHY)

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Purpose: No previous study has investigated the impact of the childcare (CC) environment on overall objectively measured physical activity (PA), sedentary behavior (SB) and adiposity. The aim of the study was to identify CC correlates of PA, SB and adiposity in a large sample of 2–6-year-old preschoolers.
Methods: 84 CC participated in the Swiss Preschoolers' Health study (SPLASHY). CC environment was evaluated through a modified nutrition and physical activity self-assessment for child care questionnaire. Based on the Ecological model of health behaviour (Sallis et al.), 5 domains were used for the selection and categorisation of 33 variables in addition to age and sex: demographic/biological, psychological/cognitive/emotional, behavioural, socio-cultural, and physical environment. PA and SB were measured using accelerometers which were worn at least 10 h/day over a week. Analyses were performed using total PA (TPA), moderate-and-vigorous PA (MVPA), sedentary behavior (SB), skinfold thickness (SF), and BMI as the main outcomes.
Results: 476 preschool children (mean age 3.9 ± 0.7 yrs; 47% girls, 23% overweight & obese) participated in the study. TPA was 621.5 ± 153.6 counts per minutes, MVPA was 45.5 ± 23.13 minutes per day, and SB was 365.68 ± 56.19 minutes per day. Using 50 different imputed datasets and multiple regression analyses, we identified the following correlates for TPA and MVPA during CC (all $p < 0.047$): age, sex, child's temperament in CC, written PA convention, general, not PA-specific, staff support (inverse), and mobile equipment. Significant correlates for SB were written PA convention (inverse), sociocultural region and child's temperament in CC. For overall PA, none of the CC correlates remained significant. Correlates for SF were: sex, and general staff support (inverse) and for BMI only age.
Conclusion: Some CC correlates such as child's temperament and behavior in CC, general staff support, having a written PA convention and presence of mobile equipment were related to PA during CC, and less so to overall PA. CC social support might be protective against body fat. In Switzerland, individual and sociocultural factors seem to be stronger drivers of children's PA than CC correlates.

SGPO 24

Methods to prevent recurrent cystitis: Where's the evidence?

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Introduction: A considerable number of children suffer from recurrent lower urinary tract infections. Most of them are otherwise healthy girls, without any anomaly of the urogenital tract. Unfortunately, antibiotic treatment of the acute infection normally does not lead to persistent healing, but just to a short break until the next cystitis with the same or another bacterium some weeks later. As a consequence, these children will get repeated antibiotic treatment, including the known risks of adverse effects and drug resistance development. Therefore, methods to prevent cystitis could be an attractive alternative.
Methods: From Cochrane and Pubmed database, we collected the studies investigating the effectiveness of methods to prevent recurrent cystitis. We completed these results with a search on the internet related to some methods without any studies on the mentioned platforms.
Results: In literature, we found approximately two dozen methods to prevent recurrent cystitis, among them treatments of western and alternative medicine, drugs and non drug-related interventions. They should reduce the bacterial colonization of the foreskin, complicate the ascension and adhesion of bacteria in the urinary tract, support or stimulate the kidneys, minimize residual urine or operate by antibacterial treatments and immunological approaches. Overall, the only well investigated method to prevent recurrent cystitis with good evidence is the administration of low dose antibiotics. For most of the other searched methods, we found studies of lower quality, which suggest a certain effectivity.
Limitations: The main limitation is due to the absence or low quality of the found studies. For most of the investigated treatment methods, there are just a few studies with small patient numbers and short follow-up periods, observing only adults or women. Other preventive methods are not yet being investigated at all.
Conclusion: There is a limited evidence about the effectiveness of some methods to prevent recurrent lower urinary tract infections. However, their application could be helpful in patients suffering from recurrent cystitis. The choice of the adequate method among the therapies of comparable evidence level relies on a careful evaluation of the application form, side effects and costs.

FREE COMMUNICATIONS SWISSPEDNET

SPN 1

The relation of stress reactivity, behavioural problems and temperament in preschool children

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Stress reactivity is influenced by children's temperament, especially by high negative emotionality. Previous research has shown that children with high emotionality show a stronger reactivity in response to stressors. In contrast, findings on the relationship of stress reactivity and behavioural problems are mixed, probably due to different stress assessments. As both temperament and behavioural problems are both independently related to stress reactivity and interplay with each other, both factors need to be considered in the analysis. The aim of this study was to investigate stress reactivity during an age-adapted stress task and its relation with behavioural problems and the child's temperament in preschool aged children. A total of 476 2–6-year-old children participated in the Swiss cohort study SPLASHY. Saliva samples were taken before, during and after the task and salivary cortisol and salivary alpha-amylase (SAA) were analysed. Besides this, parents were asked to complete a set of online questionnaires to assess the child's temperament and current behavioural problems of the child. Cortisol patterns displayed three different groups of stress reactivity including a first pattern with an expected peak after the stress task and a decrease of cortisol levels during the recovery period, a second group with a blunted response and a third group with delayed cortisol reactivity. Comparison of all three groups showed no differences in sAA, temperament or behavioral problems but a significant age difference between the groups. Within the group with blunted stress response age and peer problems predicted overall cortisol release during the stress task. Conduct problems significantly predicted overall cortisol release in the delayed reactivity group. In contrast to previous research, children's overall cortisol release during the age-adapted stress task showed different response patterns. The groups of cortisol patterns differed in age only, however cortisol levels within the blunted and delayed cortisol group were both associated with behavioural problems, but not with temperament or sAA. Cortisol reactivity patterns seem to differ within younger and older preschool children, and unusual stress patterns were related to behavioural problem. The long-term impact of delayed or blunted stress patterns needs to be investigated within the longitudinal design to improve the understanding of the relation between stress reactivity and persistent behavioural problems.

SPN 2

Regional socio-cultural differences as important correlate of physical activity and sedentary behaviour in Swiss pre-schoolers

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Question: Regional differences in physical activity (PA) in school-aged children and adults even within one country with the same political and health care system were observed and could not be explained by socio-demographic or individual variables. We analysed whether such differences were already present in pre-schoolers.
Methods: Swiss children from 84 childcare centres in 5 cantons (Aargau, Bern, Fribourg, Vaud, Zurich) comprising about 50% of the country's population participated. PA was quantified by accelerometers (ActiGraph, wGT3X-BT) and potential correlates were assessed with measurements at the childcare centre or questionnaires. Mixed regression models were used to test associations between potential correlates of total PA (TPA), moderate-to-vigorous PA (MVPA), light PA (LPA) or sedentary behaviour (SB) with a special focus on regional differences.
Results: 394 of 476 children (83%) provided valid PA data (at least 2 week- and 1 weekend-day with 10h recording; mean age 3.9 ± 0.7 years, 54% boys) with 26% and 74% living in the French- and German-

speaking parts, respectively. Days consisted of (mean ± SD) 1.5 ± 0.5h MVPA, 5.0 ± 0.6h LPA, and 6.3 ± 0.8h SB with an average of 624 ± 150 counts/min TPA. TPA and MVPA (but not SB or LPA) increased with age, were higher in boys and children with better motor skills. Despite controlling for individual characteristics, familial factors and childcare exposure, children from the French-speaking part of Switzerland showed 13% less TPA, 14% less MVPA, 6% less LPA and 8% more SB than German-speaking children.
Conclusion: Beside motor skills and non-modifiable individual factors, the regional socio-cultural difference was the most important correlate of PA and SB. Therefore, regionally adapted public health strategies may be needed.

Reference

Leeger-Aschmann CS, Schmutz EA, Radtke T, Kakebeeke TH, Zysset AE, Messerli-Bürgy N, Stülb K, Arhab A, Meyer AH, Munsch S, Jenni OG, Puder JJ & Kriemler S. Regional sociocultural differences as important correlate of physical activity and sedentary behaviour in Swiss preschool children. Swiss Med Wkly. 2016;146:w14377.

SPN 3

Relationship between temperature variability and brain injury on magnetic resonance imaging in cooled newborn infants after perinatal asphyxia

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Background: Therapeutic hypothermia (TH) is standard treatment for hypoxic-ischemic encephalopathy (HIE) in term newborn infants, reducing mortality and neurodevelopmental disability at 18–24 months of age. This effect seems to persist into childhood. However little is known about the effect of temperature management during TH on the severity of brain injury.
Methods: Prospectively collected register data from the National Asphyxia and Cooling Register of Switzerland were analysed. An injury severity score for analysing the cranial magnetic resonance imaging (cMRI) was used. During established TH, temperature variability within the target range, temperatures measured outside the target range and their correlation on cMRI findings were analysed.
Results: Fifty-five newborn infants were cooled for 72 hours with a target temperature range of 33–34 °C. The majority of newborn infants were classified as being Sarnat stage 2 (n = 33, 60%), some Sarnat stage 1 (n = 8, 14%) and Sarnat stage 3 (n = 14, 26%). Temperatures in the target range were achieved 80% (95%CI 75–85) of the time but were above target temperature 10% (95% CI 6–15, range 34.1–37.2 °C) and below 10% (95% CI 7–13, range 32.9–30.3°C) of the time. Individual temperature variability (OR 40.17 [95% CI 1.37–1037.67]) and percentage of temperatures within temperature target range (OR 0.95 [95% CI 0.90–0.98]) were associated with the severity of brain injury seen on cMRI after adjustment for Sarnat stage during admission. The analysis revealed no significant correlation between the MRI findings and postnatal age at start of cooling (OR 0.97 [95% CI 0.70–1.34]), percentage of temperature measurements above (OR 1.08 [95% CI 0.96–1.21]) or below (OR 0.99 [95% CI 0.92–1.07]) the target temperature range.
Conclusion: In our national cohort of cooled newborn infants with HIE, temperature variability within the target temperature range and the amount of time spent within the target temperature range are associated with the severity of brain injury as assessed on cMRI even after adjustment for the degree of encephalopathy. Over- and undercooling to a minimal extent did not influence the severity of brain injury. Hence, clinicians should pay attention not only at reaching target temperature but also at avoiding variability of temperature during TH. Whether less temperature variability reduces poor neurodevelopmental outcome in newborn infants with HIE has to be seen.

SPN 4

Neonatal sepsis in Switzerland: a prospective nationwide study

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Background: Neonatal infection is a major cause of morbidity and mortality. Recent studies have reported an incidence of early-onset neonatal sepsis (EOS) of 0.5–0.9 per 1000 livebirths. Late-onset sepsis (LOS) affects mainly preterm newborns with an incidence of 15–25% in infants born <32 weeks.
Objective: To evaluate the epidemiology of blood culture-proven sepsis in newborns in Switzerland.
Methods: Newborn infants admitted to the ten Swiss neonatal intensive care units and presenting with blood culture-proven sepsis between 9.2011 and 12.2015 were included. EOS was defined as infection occurring <72h of life. LOS was defined as infection presenting ≥72h of life. In infants with LOS, those presenting with signs of infection before or ≤48h after admission were classified as having community-acquired (CA) LOS, while infants presenting with signs of infection >48h after admission were classified as having hospital-acquired (HA) LOS.
Results: We identified 444 episodes of blood culture-proven sepsis in 430 infants; 87 (20%) were EOS and 357 (80%) were LOS. Among episodes of LOS, 278 (62%) were HA-LOS and 79 (18%) were CA-LOS. The incidence of EOS, HA-LOS and CA-LOS was 0.27 (95% CI 0.22–0.33), 0.82 (0.72–0.92) and 0.26 (0.21–0.32) per 1000 livebirths. In infants born <32 weeks, the incidence of EOS, HA-LOS and CA-LOS was 1.0% (95% CI 0.7–1.4), 6.1% (5.3–7.0) and 0.1% (0.03–0.3). Median gestational age at birth was 34 (IQR 29–39), 27 (26–32) and 40 (39–41) weeks for EOS, HA-LOS and CA-LOS. Mortality was 18% (16/87), 12% (33/278) and 0% (0/79) in EOS, HA-LOS and CA-LOS. The proportion of infants that presented with septic shock was 30% (26/87), 19% (53/278) and 3% (2/79) in EOS, HA-LOS and CA-LOS. The most frequently isolated pathogens were Group B Streptococcus (38%, 33/87) and Escherichia coli (23%, 20/87) in EOS, Coagulase-negative Staphylococci (40%, 111/278), Staphylococcus aureus (16%, 44/278) and Escherichia coli (15%, 43/278) in HA-LOS, and Escherichia coli (42%, 33/79) and Group B Streptococcus (39%, 31/79) in CA-LOS.
Conclusions: This national study shows a lower incidence of neonatal sepsis in Switzerland compared to recently published studies from other industrialized countries. However, mortality and morbidity of EOS and HA-LOS remains considerable. Among neonatal sepsis, we describe three entities with distinct clinical presentation, pathogens and outcomes.

SPN 5

Nebulised hypertonic saline therapy compared to supportive care in moderate to severe bronchiolitis: a randomized controlled trial

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Importance: Bronchiolitis is the most common respiratory tract infection in the first two years of life. Nebulized hypertonic saline has shown potential benefit in reducing length of hospital stay. Most studies used normal saline nebulization as control group which is not a true placebo.
Objective: To investigate the impact of hypertonic saline nebulizations in the treatment of moderate to severe bronchiolitis compared to standard supportive care.

Design: We conducted a randomized clinical trial from April 1, 2013 to March 31, 2016.
Setting: Two Swiss children hospitals
Participants: We recruited patients aged 6 weeks to 24 months with a primary diagnosis of viral bronchiolitis with moderate to severe score at Wang clinical severity score. We excluded children with mild bronchiolitis, previous episodes of wheezing, cardiac disease, chronic respiratory disease, immunodeficiency, prematurity (gestational age <34 weeks), critical illness requiring immediate Intensive Care Unit (ICU) admission, immunoprophylaxis therapy, corticotherapy in the preceding 2 weeks or bronchodilators inhalations within 24 hours before presentation.
Interventions: Patients were randomized to receive standard supportive treatment with inhalations of 4 ml of 3% sodium chloride (hypertonic saline [HS Group]) every 6 hours or standard supportive care (SC Group) with no inhalations.
Main outcomes and measures: Lenght of hospital stay, length of oxygen therapy, transfers to pediatric intensive care unit (ICU), readmission in the next 7 days following discharge, adverse events.
Results: A total of 121 children were randomised. No statically significant differences were found between treatment group (age, Wang score, atopic history, smoking exposure). Children in the HS group had a non significant length of stay 47 hours (95%IC 39–56) compared with SC group 50 hours (95%IC 39–61). There was no difference also for the duration of oxygen therapy (29 hours versus 31). No transfer to ICU occurred in HS group versus 3/60 in SC group (p = 0.1). No difference for the readmission or for adverse events between group.
Conclusion and relevance: Hypertonic saline nebulizations given to children with moderate to severe bronchiolitis do not decrease length of hospital stay compared to standard supportive care. However hypertonic saline nebulizations could decrease ICU transfer need especially in the most critically ill patients.

SPN 6

Vaccination coverage in children with rheumatic autoimmune diseases with and without immunosuppressive therapy in Switzerland

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Background: Pediatric patients with rheumatic diseases (PedRD) are more susceptible to invasive infectious diseases, due to their underlying disease and immunosuppressive therapy (IT). Correct immunization reduces risk of vaccine preventable infections and its associated morbidity and mortality. Information about vaccine coverage rates in PedRD patients in Switzerland is scarce.
Objectives: Assessment of vaccine coverage in PedRD patients with or without IT during disease course in Switzerland.
Methods: Multicenter retrospective prevalence study based on the Juvenile Inflammatory Rheumatism (JIR) cohort, an international registry for rheumatic diseases. PedRD patients treated in Swiss pediatric rheumatology centers in Basel, Geneva, Lausanne, Lucerne and Zurich were included in the study. Vaccine coverage was assessed at the date of diagnosis and as point prevalence on 12 April 2016 for routine and for specifically indicated vaccinations according to the Swiss Federal Office of Public Health (FOPH).
Results: From initially 620 eligible patients, 242 with updated vaccination cards were included. Median age was 6.6 years (IQR 3.0–10.4) at diagnosis; 11.0 years (IQR 7.0–14.3) at follow up. 138 (= 57%) PedRD patients received IT (conventional disease-modifying antirheumatic drugs (DMARDS) [n = 52], biological DMARDs [n = 26], combined therapy with conventional and biological DMARDS [n = 59], systemic steroids [n = 1]). At diagnosis, 182 (= 75%) patients were completely immunized for routine vaccinations. Coverage rates for routine vaccination decreased by 8% during the observation period. Compared with age adapted healthy Swiss population vaccine coverage was 14% lower in PedRD patients at reference date. Varicella-zoster virus (VZV) history was documented in 138 patients. 36 patients fulfilled the indication for VZV vaccination; of those, 20 had a complete VZV vaccination. Moreover, specially indicated vaccine coverage was 55% for pneumococcus, 48% for hepatitis B, 33% for human papilloma virus and 30% for influenza vaccination. Only 22% of PedRD patients showed complete coverage for routine and specially indicated vaccinations.
Conclusion: Vaccination coverage in PedRD patients was lower compared to age adapted Swiss population. Documentation of vaccination status and VZV history was limited. Systematic evaluation by the rheumatologist and the primary health care provider is essential to optimize protection from vaccine-preventable infections in this vulnerable population.

SPN 7

The Swiss Paediatric Airway Cohort – embedding research in clinical routine

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Background: Respiratory symptoms are highly prevalent in children and lead to numerous outpatient consultations in Switzerland. Yet etiology, treatment, and disease course are largely unknown. Up to date, most research was conducted in population-based birth cohorts, which include mainly healthy children, and mildly affected ones. A large national cohort of patients with variable respiratory disease phenotypes may overcome current knowledge gaps. Currently we are setting up the Swiss Paediatric Airway Cohort (SPAC) as a national research platform. Here we describe the objectives and study design.
Methods: The SPAC is a prospective observational cohort study of children aged 0–16 years, who are referred to a paediatric outpatient clinic for evaluation of wheeze, recurrent cough, or exercise-induced and sleep-related respiratory problems. Patients with specific diagnoses like cystic fibrosis, primary ciliary dyskinesia or congenital malformations are excluded. Investigations and treatment are done as per national consensus and in-house guidelines. Data are recorded in a REDCap database. At baseline we collect detailed data from the outpatient visit, including a) a standardized history (from a parental questionnaire) on symptoms, previous treatments, health behaviors, socioeconomic and environmental factors; b) clinical test results (such as lung function and allergy tests, or imaging) and; c) diagnosis and prescribed treatments. Information on disease course will be obtained from questionnaires sent to the families in yearly intervals.
Preliminary results: The study starts in March 2017 and we expect a response rate of at least 80%, as the study is embedded in the clinical routine and does not induce a burden on the patients, apart from the questionnaires. Bern, Zurich, Lucerne, St. Gallen and Basel will participate already in 2017; other clinics have expressed interest to join later. We plan to collect data from at least 3000 patients, to have representative numbers also for rarer entities.
Conclusion: SPAC will be the first national cohort study of children with common respiratory symptoms in Switzerland, and will provide the basis for better understanding of disease and treatment burden and respiratory disease outcomes in Switzerland.

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SPN 8

Excess body weight in children referred to a pediatric cardiology clinic: frequency and management

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Introduction: Excess body weight (BW) in children is a major risk factor for further cardiovascular and metabolic diseases. However, frequency and management of EBW among children attending a pediatric cardiology clinic is not well described.
Objectives: 1) To establish the frequency of excess BW in children referred to a cardiology clinic; 2) To determine the proportion of cases reported in the medical report and for which the cardiologist made a management proposal.

Methods: We performed a retrospective study on excess BW in all outpatients having had an echocardiographic exam between 2005 to 2014 at the Cardiology Unit of the Lausanne university hospital (CHUV). Demographic and anthropometric data from children 1 to 18 years old seen at the outpatient clinic were extracted. BMI was calculated and expressed as a z-score. Excess BW was defined as a BMI z-score >1.0. Medical reports of all severely obese (BMI z-score >3.0, n = 152) and a representative sample of obese (BMI z-score >2.0, n = 165) children were reviewed to assess the diagnosis of a heart condition, if elevated BP was reported and if any management was proposed.
Results: Among 14'040 visits, the frequency of excess BW was 15.8%. The proportion of excess BW increased from 13.6% in 2005 to 17.3% in 2014. Excess BW was mentioned in 45.8% and 37.3% of medical reports of children with obesity and severe obesity, respectively. When excess BW was reported, a management was proposed in 8.3% and 7.6% for obesity and severe obesity respectively.
Conclusion: The frequency of excess BW in children referred to the cardiology clinic at the CHUV had an upward trend between 2005 and 2014, similar to that of the general population. The proportion of excess BW reporting in medical reports is relatively low, and pediatric cardiologist rarely proposed a management. Implementation of automated BMI z-score calculation and standardized protocol for excess BW management in children attending an outpatient clinic might be helpful for further preventing cardiovascular and metabolic diseases.

SPN 9

Clinical signs of hypoxemia in children with acute respiratory distress in developing/non-developing countries

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Objective: The aim of this study was to assess the accuracy of clinical signs and combinations of signs predicting hypoxemia in children from 2 months to 5 years of age with acute respiratory distress in primary healthcare.
Methods: We conducted an observational study in 4 emergency units, 2 in Switzerland and 2 in Senegal. Patients from 2 months to 5 years of age with acute respiratory distress were eligible to be included. We compared clinical signs to the level of transcutaneous blood saturation (SaO_2).
Results: A total of 111 children were recruited. The prevalence of hypoxemia was 13%. We found that cyanosis was the only sign with high specificity, moderate sensitivity and a positive likelihood ratio >5. We analyzed 12 models of combined symptoms and the WHO model when oxygen supply is ample had the highest diagnostic performance with a sensitivity of 0.93, a specificity of 0.60 and a LR– of 0.12.
Conclusions: The diagnosis of hypoxemia shouldn't be based only on a clinical sign or a combinations of sign. Cyanosis had a good diagnosis performance but only if the prevalence of hypoxemia was high and couldn't be used to eliminate hypoxemia. The current WHO model for ample oxygen supply was the best clinical diagnosis but a lot of non-hypoxemic children were treated unnecessary.

SPN 10

Development of a population pharmacokinetic model describing the pharmacokinetics of vancomycin in infants, children and adolescents, comparing creatinine and cystatine C as potential markers of renal function in childhood, as well as evaluation of its performance

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Background: Vancomycin is a renally-excreted antimicrobial with high interindividual variability. Sufficient drug levels are pivotal for therapeutic efficacy and to avoid resistances selection.
Methods: A retrospective population pharmacokinetic study based on routine therapeutic drug monitoring data was performed. 189 vancomycin concentrations (immunoenzymatic method) from 53 patients were analyzed with the nonlinear mixed-effects modelling (NONMEM®) software using the first-order conditional estimation method with interaction. Choice of base and error models were followed by covariate screening, stepwise forward selection and backward elimination. One model based on creatinine (enzymatic method) and one based on cystatine C (latex-potentiated immunoturbidimetric method) were selected. Both models were internally validated using normalized prediction distribution errors, visual predictive checks (VPCs) and the bootstrap procedure.
Results: 189 vancomycin concentrations from 53 patients (32 females, 21 males), median 8.6 (IQR 3.5–14.8) years, were analyzed. A 1-compartment model with 1st order elimination was selected. Five covariates on volume of distribution (Vd) and 14 on clearance (Cl) passed the screening. Both models inglobed weight as a covariate on Vd and Cl. The creatinine-based model included the simplified Schwartz GFR equation, the cystatine-based the Filler GFR equation, as additional covariate on Cl. Both models showed an interindividual variability on Vd of 35.4% and on CL of 26.4%, residual variability was 34.2%. Bootstraps showed stable estimates for both models. The VPCs of the creatinine-model showed 5.82%, that of the cystatine C-model 6.88% of observed concentrations outside the 95%-confidence interval of predictions.
Conclusions: Creatinine and cystatine C based pharmacokinetic models of vancomycin have good and equivalent prediction performance in the studied population. Because of availability and lower costs, the creatinine-based model might be preferable in clinical practice.

SPN 11

Medical students knowledge of and beliefs about LGBT and their health care needs: what's the impact of a class on LGBT adolescent health?

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Objectives: Lesbian, gay, bisexual, and transgender (LGBT) adolescents have specific needs in health care and are susceptible to health care disparities. It has been shown that lack of specific knowledge and prejudice on the part of medical professionals towards LGBT people have a negative effect in terms of access to care and health. The aims of this study are to explore the knowledge and beliefs of medical students towards LGBT people and to assess the potential impact of a class on LGBT adolescent health.
Methods: Fourth-year medical students at the medical school of the University of Lausanne attended a compulsory one-hour course about sexual orientation and gender identity development in adolescence, including specific health issues for LGBT adolescents, given by an experienced paediatrician trained in adolescent medicine. We developed a questionnaire with an assessment scale (5-point Likert scale) to elicit students' knowledge of, and beliefs about LGBT health issues. Students were invited to complete online anonymous questionnaires by e-mail one week before the course and one month after the course.
Results: Out of a total of 157 students, 105 (66.9%) responded to the pre-intervention questionnaire and 96 (61.1%) to the post-intervention questionnaire. Eighty-six (54.7%) students responded to both questionnaires. Among these 86 students, 64 (74%) attended the course. A significant proportion (14.4% of all respondents) identified as LGBT or questioning. Preliminary results suggest that most of medical students already possessed non-rejecting beliefs about LGBT and certain knowledge about LGBT health issues. Those who attended the course demonstrated a significant increase in knowledge about LGBT health issues one month after the course.
Conclusion: A single one-hour course about sexual orientation and LGBT health issues increases knowledge among medical students. All medical students and professionals should receive such training to increase their knowledge about LGBT patients to provide less stigmatizing care to this population.

SGPP 1

Concept for quality control of ultrasound-based screening and treatment of hip dysplasia in Mongolia: The Swiss Mongolian Pediatric Project

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Background: Developmental dysplasia of the hip (DDH) is a major health problem which can lead to lifelong treatment or handicapped individuals if diagnosis is missed in early weeks of life. DDH has implications not only for the baby itself and its entire family but is of enormous consequence for public health. The Swiss Mongolian pediatric project brought the knowledge and the hardware (US-machines, cradles, treatment devices) to Mongolia, to detect and treat neonatal DDH and realized from a early state on that the quality of the ultrasound examinations cannot be guaranteed with these measures alone. Quality checks and possibilities for feedback are important in daily practice to reduce wrong diagnoses or overtreatment. This is especially true for developing countries where resources and knowledge is sparse.
Methods: We therefore decided to implement a web-based quality control tool which offers the possibility to control and supervise Ultrasound pictures of all hip examinations of screeners by locally trained experts and Swiss supervisors.
Results: In order to implement the quality system, screeners can enter specifically programmed hotkeys for side of hip (left, right) and diagnosis (according to adapted Graf) and regular information (name, age, gender, and social security number) on their ultrasound machines in Mongolia. The resulting DICOM files are exported daily by the screener to a USB flash drive and uploaded to HipScreen. HipScreen is our web portal which is developed and regularly updated from Switzerland and offered to the Mongolian partners free of charge. HipScreen automatically extracts essential information from the files when uploaded by the screeners. Experts have access to those examinations including all pictures. They can either close the case (healthy hips, good quality of pictures) or give comments and return the examination to the screener; this procedure allows the screener to learn from mistakes. The experts decide on therapy and are allowed to send cases to the supervisors for further advice.
Conclusion: A 3-level control and teaching tool allows standardized diagnosis and treatment of DDH. The Mongolian database is probably becoming the biggest cohort of screened newborns ever. The tool will serve as a model for a similar DDH project in Switzerland and is open for interested pediatricians in other fields.

SGPP 2

Birth weight is an important information for cardiovascular disease risk stratification: have we forgotten it?

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Introduction: Low birth weight is a recognized risk factor for cardiovascular diseases. The Barker's hypothesis postulates the programming of metabolic and cardiovascular functions during fetal life (fetal programming), which may predispose to health or disease in later life. Consequently, it has been recognized that birth weight is an important information for cardiovascular disease risk stratification. The aim of the present study was to assess the knowledge of the personal birth weight among adults.
Methods: The employees of the EOC were invited to fill an internet-based questionnaire about knowledge of their own birth weight, birth length, duration of gestation, and cardiovascular risk factors or diseases.
Results: A total of 1369 valid answers were analyzed in detail. Mean age of the participants was 40.0 ± 11.0 years, 77% were female. Mean BMI was 23.1 ± 4.0, 26% were overweight or obese, 13% presented at least one cardiovascular risk factor or disease. Birth weight was known in 85% of the participants, the majority (70%) know these data by heart. Age above 40 years was significantly associated with a worse knowledge of birth weight when compared to younger employees (82.7% vs. 87.6%, p = 0.01). A trend towards an association between low birth weight and higher BMI or cardiovascular diseases was found in this cohort.
Conclusions: The vast majority of the participants know their personal birth weight. Hence, it seems to be reasonable to always ask to the adult patient this important information in order to comprehensively assess his risk for cardiovascular diseases.

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SGPP 3

Recurrent superficial insect bite lymphangitis

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Introduction: Superficial lymphangitis of the anterior chest wall is an unusual finding and may be a diagnostic challenge. We present a patient with this condition who had recurrent episodes.
Case presentation: An 8-year-old boy presented with recurrent erythematous streaks on the anterior chest. The first episode occurred in spring with a linear eruption on the left side after an insect bite at night. The lesion extended from the area of the bite toward the left axillary fold. He was in excellent general condition without fever or lymph node enlargement. He took no medications and his medical history was unremarkable. The eruption resolved within hours. The second episode occurred in summer with identical self-limiting linear eruptions after several insect bites on the right side.
Conclusion: Superficial lymphangitis after insect bite is an underrecognized entity mimicking acute bacterial lymphangitis. It is characterized by the absence of fever and lymph node enlargement and a rapid spontaneous regression. Pathogenesis likely involves an allergic immune reaction to insect toxins. The diagnosis should be considered particularly when recurrent or multifocal lymphangitis occurs after insect bites. Antibiotics should be avoided in such cases.

SGPP 4

Functional and morphological outcome analysis of pediatric cataract surgery

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Introduction: Long-term outcome of pediatric cataract surgery is dependent on multiple factors such as age at cataract presentation, associated ocular anomalies, or aphakic glaucoma. Analysis of a large patient cohort will provide knowledge for critical management analysis but also for patient and parental support.
Methods: Retrospective chart review of consecutive cases, which underwent cataract surgery until 10 years of age, during a 10-year period from 2004 to 2014 at the University Hospital Zurich, Switzerland. Analysis included functional and morphological data at initial presentation and last follow up as well as management of glaucoma. The study was approved by the local Ethics committee.
Results: 63 children (28 female, 94 affected eyes) with bilateral (68/94) or unilateral (26/94) cataracts were identified. Diagnosis of cataract were: congenital (82/94), juvenile (12/94), acquired (8/94, 3 of these post retinopathy of the premature treatment, 2 due to juvenile rheumatic arthritis, 3 due to trauma). Surgery was performed at a median age of 22 months: 49 of 94 eyes received an intraocular lens (median age 50.7 months at surgery) and 45 of 94 eyes were left aphakic (median age 1.5 months at surgery). At the last follow up visit (22 days – 8.6 years, median 31 months) visual acuity was =/>0.4 decimal Snellen equivalent in 23 patients (34/94) and =/<0.2 in 10 patients (12/94). Aphakic glaucoma was diagnosed in 12 of 45 eyes (9/28 patients) at a median of 6.8 months after surgery. Microcornea (5/12), anterior segment dysgenesis (1/12), aniridia (1/12), or Lowe Syndrome (1/12) were associated with glaucoma development.
Conclusion: Congenital cataracts are the main cause of surgical intervention in our pediatric cataract group. In agreement with previously published data, aphakic glaucoma is one of the main postoperative complications and is often associated with abnormal ocular development.

SGPP 5

Variations of Currarino triad

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Backround: Currarino triad is a rare congenital disease with a prevalence of 1–9/100.000 births. Although it can occur spontaneously an autosomal-dominant transmission is described. The disease consists of an anorectal malformation (anal stenosis or atresia), a sacral deformity and a presacral mass with tethering of the spinal cord in approximately 50%. The assumed cause is seen in a defective separation of entodermal and neuroectodermal layers that are intended to form the notochord. The presacral mass consists of ether a germ cell tumor that basically can be resected or a ventral spinal dysraphy that can't in most cases.
Results: We present five patients that show the complete pattern of Currarino triad. In all patients the disease was detected in infancy due to the anorectal malformation. 3 out of 5 presacral masses are ventral meningoceles which could not be completely removed. A neurogenic bladder dysfunction also exists in 3 out of 5 patients. Neurological disorders of the lower extremity are not present in any patient. Constipation is the most important symptom. All patients require an individual bowel management due to severe constipation.
Conclusion: Patients with Currarino triad mostly suffer from constipation on the long run. Although postulated in literature a complete resection of the presacral mass is not feasible in many cases. A neurogenic bladder must not be overseen.

SGPP 6

Factor XIII deficiency and Noonan Syndrome: a rarely described association

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Background: Noonan syndrome (NS) and related disorders, collectively called the RASopathies, are autosomal dominant disorders caused by mutations in several genes, which encode proteins belonging to the signal transduction pathway (RAS-MAPK). NS is known to be associated with different coagulopathies including thrombocytopenia, platelet function disorders and coagulation factor (F) deficiencies, particularly FXI, FXII, and FVIII. Haemostatic investigations are recommended in all patients newly diagnosed with NS. An increased prevalence of coagulopathies is described in NS caused by a mutation in the PTPN11 gene. Only one case of mild FXIII deficiency (FXIII 59%) in a 18-year-old girl with NS was reported so far.
Case: A 6-year-old girl presented with fever and acute abdominal pain. On admission, she underwent diagnostic laparoscopy with resection of a normal appendix and Meckel's diverticulum. Few hours after surgery, severe bleeding from the trocar wounds was observed. Her medical history so far was uneventful. There was no personal or family history suggestive of bleeding disorders. Laboratory tests revealed a very low FXIII of 4% with no other haemostatic abnormalities. Daily FXIII replacement of 30 IU/kg during 5 days was required to overcome rapid FXIII consumption with trough levels below 5%, and to resolve the bleeding. Further investigations showed normalization of FXIII activity by mixed plasma test, no mutations on FXIII gene and normal FXIII activity in both parents. These results ruled out the presence of an inhibitor and congenital FXIII deficiency, respectively. A more accurate clinical examination of the girl revealed facial features suggestive for NS, which was confirmed by genetic testing showing a c.188A>G mutation on the PTPN11 gene. On follow-up, FXIII activity normalized within 2 months and no further bleeding occurred.
Conclusions: We report the first case of a severe FXIII deficiency in a patient with NS. In this patient, NS was diagnosed only after a first bleeding complication occurred. In addition, FXIII deficiency was transient and recovered spontaneously 2 months after the bleeding episode. Our case suggests that a negative bleeding history or normal haemostatic results do not exclude the occurrence of bleeding complications in risk situations in patients with NS. Thus, timely postoperative monitoring of haemostasis is required to prevent bleeding complications in these patients.

SGPP 7

Comparison of parental report and objectively measured fundamental motor skills in preschool children: data from SPLASHY

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Background: Competence in fundamental motor skills is assumed to be linked with health related outcomes (e.g., physical activity, weight status). Therefore, the evaluation of motor skills in early childhood and the identification of abnormal motor development is crucial in pediatric practice (e.g., during well-child visits). We constructed a 6-item parental questionnaire of fundamental motor skills (e.g., riding a bicycle, throwing, balancing) based on frequently asked questions in pediatric practice. The aim was to compare the answers from the parental questionnaire with a standardized motor test.
Methods: The data were collected within the frame of the Swiss Preschooler's Health Study (SPLASHY). 449 children typically developing children between 2 and 6 years of age (213 female, M age = 3.9 years, SD = 0.7, range 2.5–6.6 years) we examined. Motor skills were examined using the Zurich Neuromotor Assessment 3-5 (ZNA3-5) and parents filled out an online questionnaire on swimming, climbing stairs, hopping, riding, balance, throwing, answering categories (scale 0–2).
Results: We found small sex differences in the questionnaire and in the ZNA3-5: Boys scored higher on the questionnaire items riding (effect size r = .140, $p < .01$), and throwing (effect size r = .162, $p < .01$), while girls scored higher in the ZNA3-5 component static balance (effect size r = .143, $p < .01$). Partial correlations, controlled for age and sex, revealed that out of all six questionnaire items the four items climbing stairs, jumping, riding and balance correlated weakly with the gross motor component of the ZNA3-5 (r = .125 – .247, $p < .05$). Furthermore, we tested whether children with delayed motor development according the parental report (<5th percentile, score <5, n = 22) differed from children with normal motor development (score ≥5) in their gross motor skills. In fact, we found that children with delayed motor development scored significantly different in gross motor skills (effect size r = .142, $p < .01$), although the magnitude of the correlation was small. We conclude that the described effects are rather small, and thus, parental report may not allow a reliable identification of children with delayed motor development.

SGPP 8

Correlates of preschool children's objectively measured physical activity and sedentary behavior: a cross-sectional analysis of the SPLASHY study

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Background: Identifying ways to promote physical activity and decrease sedentary time during childhood is a key public health issue. Research on the putative influences on preschool children's physical activity (PA) and sedentary behavior (SB) is limited and has yielded inconsistent results. Our aim was to identify correlates of PA and SB in preschool children.
Methods: Cross-sectional data were drawn from the Swiss Preschoolers' Health Study (SPLASHY), a Swiss population-based cohort study. Of 476 two to six year old children, 394 (54% boys) had valid PA data assessed by accelerometry. Information on exposure data was directly measured or extracted from parental questionnaires. Multilevel linear regression modeling was used to separately assess associations between 35 potential correlates and total PA (TPA), moderate-to-vigorous PA (MVPA) and SB.
Results: In total, 12 correlates from different domains were identified. TPA and MVPA were greater in boys than girls, increased with age and were positively associated with gross motor skills. Children from single parent families had a higher level of TPA and spent less time sedentary than those living with two parents. Time spent outdoors was positively associated with TPA and negatively with SB. The child's activity temperament was related all three outcomes, whereas parental sports club membership, living area per person and neighborhood safety were associated with SB only. Fixed and random factors in the final models accounted for 28%, 32% and 22% of the total variance in TPA, MVPA and SB, respectively. Variance decomposition revealed that age, sex and activity temperament were the most influential correlates of both, TPA and MVPA, whereas the child's activity temperament, time outdoors and neighborhood safety were identified as the most important correlates of SB.
Conclusions: A multidimensional set of correlates of young children's activity behavior has been identified. Personal factors had the greatest influence on PA, whereas environmental-level factors had the greatest influence on SB. Moreover, we identified a number of previously unreported, potentially modifiable correlates of young children's PA and SB. These factors could serve to define target groups or become valuable targets for change in future interventions.

SGPP 9

The role of physiotherapy in prevention of positional plagiocephaly. A systematic review

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Introduction: Plagiocephaly is a general term for misshapen head. It is characterized by flattening of one side of the back of the head and ipsilateral forehead prominence, contralateral flattening of the forehead and ear shift. A variety of causes and risk factors are described and discussed. There has been little research of the consequences.
Objective: To find studies relating to the prevention of positional plagiocephaly and any relevant role that pediatric physiotherapy might play.
Method: Systematic literature searches were carried out by two independent experts in five databases without any restriction on time period.
Results: The searches yielded 290 studies four of which matched the inclusion criteria. Interventions in these studies were based on the recommendations of the American Academy of Pediatrics for the prevention of plagiocephaly. None of the studies explicitly researched pediatric physiotherapeutic management.
Discussion: The four studies under review were so heterogeneous as to be almost incomparable, whereby their results were essentially similar. The results were also confirmed by other authors.
Conclusions: The prevention of asymmetrical head shape in infants is possible. It requires early examination of all neonates to assess mobility of the cervical spine and identify any abnormalities of head shape. The parents must be given detailed instructions about organizing the child's environment, positioning and handling of the infant as this is the most important aspect in the prevention of positional plagiocephaly. Pediatric physiotherapists have an important contribution to make through interdisciplinary team participation and parental support.
Key words: positional plagiocephaly, prevention, physiotherapy

SGPP 10

Behavioural problems, eating disorder and short starure: is there a genetic disorder?

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Background: The 16p11.2 BP4–BP5 duplication, is the copy number variant most frequently associated with autism spectrum disorder (ASD), schizophrenia, and comorbidities such as decreased body mass index (BMI).
Objective: Identify possible cases of duplication 16p11.2 in the family of a patient with duplication 16p11.2 BP4–BP5.
Methods: We created a family tree with at least three preceding generations and analyzed each individual with a predefined protocol that included problems frequently linked to 16p11.2 duplication.
Case: 12 yo patient, hospitalised for an alcoholic intoxication. He had a difficult behavior since he was a baby, with difficulties to manage frustration, behavioral problems and a deficit of comprehension, treated with Methylphenidate (Ritaline®) et Atomoxetine (Strattera®). He furthermore had a developmental delay of language (dyslexia), sleeping troubles, diurnal enuresis and eating disorder (picky eater). During his hospitalization, a small stature was noticed, as compared with height for age percentiles and with final height calculated to their mid-parental target height 180 ± 8.5 cm.
Results: We found that the mother was a carrier of the gen and one brother have the same 16p11.2 BP4–BP5 duplication. Then, we suspect that 8 members of the mothers branch family could be affected by the same duplication.
Conclusions: Once the diagnosis was established, our patient obtained the disability insurance and got the financial support for special school, psychologic and educational follow-up. The mother was relieved because she understood why her son's behavior had always been so problematic, and there was less social exclusion at school However, parents got divorced. As pediatricians, we should be more aware of the more striking features linked to this genetic disease and other similar genetic problems (speech and motor impairments, growth abnormalities, tremor, sacral dimples when evaluating individuals with developmental delay, intellectual disability, ASD and/or language disorders) in order to provide earlier and adequate therapies and support to affected children and families.

SGPP 11

What practitioners need to know about thalassemia major: a case report full of comorbidities

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Background: Patients with thalassemia major suffer from sever chronic anemia and require regular blood transfusions, causing iron overload. The present case is representative for the essential comorbidities of thalassemia major.
Case report: A 15-year-old adolescent from Syria was admitted to our hospital with beta-thalassemia major. He had received regularly blood transfusions. After splenectomy, he was still in need for transfusions every 3–4 weeks. He presented in an altered general state, with a serum ferritin level of 8015 µg/l (reference range 7–140 µg/l) and a history of tachycardia, treated with antiarrhythmic agents. The ECG showed intermittent first degree AV-block. A T2* MRI confirmed myocardial iron overload, whereas echocardiography displayed a normal function. Abdominal imaging showed liver fibrosis and portal hypertension most probably due to the combination of iron overload and transfusion transmitted chronic Hepatitis C. Gastroscopy displayed esophageal varices. The boy had a short stature because of growth hormone deficiency and delayed puberty due to hypogonadotropic hypogonadism. MRI of the head confirmed iron overload of the adenohypophysis. Thyroid and cortisol axes presented normally, whereas pancreatic iron overload caused insulin dependent Diabetes mellitus. Combined therapy with two iron chelators (Deferiprone and Deferasirox) reduced ferritin levels to <1500 µg/l. After one year of treatment T2* MRI demonstrates no improvement of iron overload in liver and heard. Cardiac arrhythmias disappeared and antiarrhythmic agents could be stopped. Hepatitis C will be treated with Grazoprevir/Elbasvir to stop progression of liver fibrosis. Regular screening for hepatocellular carcinoma is required. To compensate the hormonal deficiencies, therapy with growth and sexual hormones is started. Despite reduction of the iron overload, diabetes mellitus did not improve. Regular blood transfusions once a month are still required and the patient will depend on interdisciplinary therapy and management for the rest of his life.

Conclusion: Where available, the contemporary iron chelation reduced comorbidities of thalassemia major dramatically. With the increase of children off refugees from countries with endemic occurrence of thalassemia in Western Europe, we will be confronted more frequently with complex clinical pictures. Swiss physicians should be aware of the medical impact of severe iron overload to ensure optimal treatment of these children.

SGPP 12

Severe multiforme mucosal reaction

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Introduction: Erythema multiforme (EM) is an immune mediated disease, typically characterized by acrally located target-lesions on the skin. It can be triggered by many factors, most commonly infections (mainly HSV and Mycoplasma pneumoniae) and rarely drugs. Mucous membranes are often involved. In rare cases, EM can present with mucous membrane lesions alone.
Case report: We report the case of a 9-year-old boy who presented to our emergency department with severe enoral mucositis, conjunctivitis and urethritis of five days duration, preceded by a febrile illness and cough. While his lips, tongue and oral mucous membranes showed severe erythema and ulcerations, his skin was spared. A clinical diagnosis of a multiforme mucosal reaction was made. Due to the subacute presentation, we decided against giving him systemic steroids. He was in severe pain and needed intravenous opioids and parenteral nutrition for seven days. Under intensive local treatment, the oral lesions healed within two weeks, whereas conjunctival scarring in his right eye persisted. A PCR for Mycoplasma pneumonia was negative. His HSV IgG was positive, but IgM was negative. He showed a positive provocation test after oral administration of ibuprofen: he developed oral ulcerations. Intolerance to ibuprofen is thus presumed to be the trigger for his disease.
Discussion: Erythema multiforme is an immune mediated disease of skin and mucous membranes. Acral target lesions are the hallmark of EM. Oral, ocular and/or genital mucosa is often involved, and lesions manifest as painful erythema, erosions and ulcerations. Infections are by far the most common trigger of EM, particularly HSV. Drugs induce EM in less then 10 percent of cases. Steven-Johnson syndrome is an often drug-mediated mucocutaneous reaction and a distinct entity from EM, characterized by painful, ill-defined erythematous macules and blisters, mainly affecting the trunk and face, and severe mucosal involvement. There are cases of multiforme mucosal reactions described as 'atypical Steven-Johnson syndrome' or 'EM without skin involvement' in literature. In pediatric populations, the vast majority is caused by Mycoplasma pneumoniae. The rare cases not due to M. pneumoniae may occur in association with a specific drug.
Conclusion: Multiforme mucosal reactions are in most cases triggered by infections, typically Mycoplasma pneumonia. Drugs are a rare cause, yet careful allergologic testing is crucial to identify preventable triggers.

SGPP 13

Pediatric Dermatology in a non-university hospital in Southern Switzerland: an overview

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Introduction: Dermatological problems in the pediatric practice are very frequent. In the absence of a specific Pediatric Dermatology advice in our region, we've decided to create a joined consultation of Pediatrics and Dermatology. The aim of this analysis was to get an overview of this new opportunity, and briefly illustrate the most frequent pediatric skin diseases encountered in Southern Switzerland.
Methods: Retrospective data collection from September 2015 (begin of the joined pediatric-dermatological consultation) to December 2016. Age, sex, dermatological diagnosis, follow-up and referring physician were analyzed in detail.
Results: During the first 16 months, the consultation was performed 33 times (twice/month). Ninety-eight patients were examined. The median age was 3.7 (IQR 0.8–8.2) years, 50% were males. The median number of consultation per patient was 1 (1 to 6). General pediatricians were the most frequent referring physicians (80%), followed by pediatrics subspecialists, dermatologists and parents. The most frequent diagnoses were: inflammatory diseases (like atopic dermatitis) followed by infantile hemangiomas, benign cutaneous tumors, viral infections (like molluscum contagiosum and warts), angiomas or other vascular malformations, congenital hemangiomas, genodermatoses, hair problems, bacterial infections, nails problems, other vascular tumors and parasitic diseases. 90% of the infantile hemangiomas, receiving a specific β-blocker therapy (n = 12), has shown a good response.
Conclusion: A specific Pediatric Dermatology consultation seems to be required also in peripheral regions of the Switzerland. General pediatricians are grateful about the possibility to collaborate with experts in Pediatric Dermatology, particularly in the case of frequent diseases like atopic dermatitis or infantile hemangiomas, which usually require complex management and follow-up.

SGPP 14

Iron supplementation in pediatrics: primum nil nocere

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Introduction: Lack of iron remains one of the most common nutrients deficiencies. Iron deficiency anemia and iron deficiency without anemia in infants and young toddlers can have longlasting detrimental effects on neurodevelopment. In older children, iron deficiency without anemia may be associated with lower cognitive test scores and improved physical performance. However these concerns doesn't justify unsighted iron supplementation regarding their considerable side effects.
Case report: We present a 6-year-old boy who was treated by intravenous iron supplementation for the diagnosis of iron deficiency. The infusion was described as painful and a permanent skin pigmentation remained on the whole forearm leading to teasing at school and consecutive psycholgical impairement. The patient was presented to a dermatologist and was recommanded to start a laser therapy, which was declined by the boy's parents because of the uncovered cost of more than 1000 CHF.
Discussion: In the presented case, survey of history and laboratory analysis showed no clear indication for intravenous iron supplementation therapy. View the fact, that in the pediatric daily routine untargeted iron supplemenation and consecutive morbidity is common, we present a review of literature discussing the indications for iron supplementation in iron deficency with and without anemia for different age groups.
Conclusion: Screening for iron deficiency should be part of the pediatric routine. The cornerstone is a careful anamnesis including the dietary habitudes. Labaratory analysis are needed to confirm clinical suspicion of iron deficiency and anemia but should not be used as a broad screening. Dietary advice and introduction of red meat and vegetables with higher iron concentration is the first step of the therapy followed by oral iron supplementation if needed. Intravenous iron administration remains an exceptional procedure.

SGPP 15

When Easter bunny comes at Christmas

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Introduction: Suspected foreign body ingestion and aspiration is a common cause for presentation in the emergency department. We present an interesting case which shows the challenge whether an invasive procedure like gastroscopy or bronchoscopy is justified when clinical symptoms disappear.
Case presentation: We present a case of a nearly three year old girl who accidently ingested a small plastic bunny. She started to cry, choked once and coughed productively and told the parents about the bunny. She never had dispnoe or stridor. Later on she told that the bunny would sit in her lower neck, without problems swalloing fluids. Three days earlier she had flu-like symptoms with cough, rhinitis and fever and since then a productive cough and increased salivation. In the emergency department she presented without any respiratory distress, normal oxygen saturation, no wheezing, no stridor. There was no foreign body apparent intraorally or in the throat. The x-ray of the chest showed a reduction of transparency paratracheal, bigger than the trachea and normal ventilated lungs. But now the foreign body sensation disappeared and the parents wanted/insisted to go home. To convince the family a barium swallow was performed which showed very obviously the sitting bunny in the oesophagus. Via gastroscopy in sedation the animal was removed without any further complications.

Discussion: Suspected ingestion and aspirations of foreign bodies is common. If findings and anamnesis is not distinct, it is challenging to decide whether an invasive procedure like gastroscopy or bronchoscopy is mandatory. Especially if a respiratory infection is ongoing and causes confounding symptoms the decision is difficult. Because of the risk of fatal suffocation through moving foreign bodies in the trachea and upper oesophagus, they have to be removed without delay. Not accepting fatal consequences we have to be ready to perform unnessecessary procedures in unclear cases.
Take home message: All suspicions of a foreign body ingestion or aspiration need careful examinations and interventions should be performed liberally if a sticking foreign body cannot be ruled out.

SGPP 16

Manifestation of glutaric aciduria in an infant with false negative newborn screening

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Background: Glutaric aciduria type I (GA-1) is a rare autosomal-recessive disorder of the degradation of the amino acids lysine and tryptophan caused by mutations of the GCDH gene encoding glutaryl-CoA-dehydrogenase. Affected patients typically present with progressive macrocephaly and an acute encephalopathic crisis often caused by a catabolic state e.g. a febrile viral illness. Accumulation of toxic metabolites may lead to irreversible damage of the basal ganglia and severe permanent dystonic cerebral palsy. Newborn screening (NBS) for this condition is done in Switzerland since November 2014 based on elevated levels of glutarylcarnitine (C5DC) in dried blood spots (DBS).
Case: In July 2016, a so far healthy 9 month old boy with normal head circumference presented with a Rota-positive gastroenteritis. He developed an acute encephalopathy with lethargy, muscular hypotonia, paroxysmal eye movements and dystonic posturing resembling epileptic seizures. Basic chemistry values were unremarkable. Cranial MRI revealed symmetrical increased signal intensity in T2-sequences and diffusion restriction of basal ganglia suspicious of GA-1. The boy was treated with high caloric intake and carnitine substitution. Organic acid profile in urine was consistent with GA-1 with however only mild elevations of characteristic metabolites. Plasma acylcarnitines showed slightly elevated C5DC. Re-evaluation of acylcarnitines in the NBS confirmed a C5DC concentration below the cut-off. Several consecutive controls showed again normal C5DC concentrations in DBS and only one a borderline C5DC concentration. The diagnosis of GA-1 was confirmed genetically by demonstrating compound heterozygosity for 2 GCDH-gene mutations. At last follow-up with 14 months, the boy showed a dystonic movement disorder and a severe global developmental delay.
Conclusion: In case of clinical suspicion of an inborn error of metabolism a thorough workup has to be performed, even if the respective defect (here GA-1) is part of the NBS scheme and screening results had been negative. Screening tests can neither be 100% specific nor 100% sensitive. Therefore, false negative results always have to be taken into account, as well as false positives. Cases with an only mild biochemical phenotype can easily be missed by NBS as this example shows.

SGPP 17

Glycogen storage disease Ib and hereditary spherocytosis: the diagnostic challenge of concomittant rare diseases

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Glycogen storage diseases type I (GSD-I) are rare inborn errors of metabolism causing severe fasting hypoglycemia and increased glycogen storage in liver and kidney. Two different GSD-I forms exist: the more common GSD-Ia affects only liver and kidney and is caused by G6PC mutations (OMIM #232200); the extremely rare GSD-Ib is associated with neutropenia, recurrent infections and chronic inflammatory bowel disease. It is caused by mutations in SLC37A4 (OMIM #232220). Hereditary spherocytosis (HS) is a genetically heterogeneous non-immune mediated hemolytic disorder in which microspherocytosis, abnormal osmotic fragility and hemolytic anemia can be present at various levels of severity. Mutations in ANK1 are responsible for approximately half of all HS cases.
Case report: We report the case of a 6-year-old boy of Syrian-descent diagnosed with GSD-I at age 8 months by liver biopsy and treated with allopurinol, fenofibrate and frequent cornstarch feedings. Upon arrival in Switzerland he presented with dehydration, electrolytic imbalances, recurrent fasting hypoglycemias, severe lactic acidosis, growth failure, developmental delay, hypertriglyceridemia, hyperuricemia, hepatosplenomegaly, anemia, mild neutropenia and proteinuria. He was hospitalized for IV rehydration. Continuous nocturnal tube feeding was introduced, and his daytime diet was adjusted to his needs. To define the diagnosis of GSD-I more precisely, we performed genetic testing by panel approach (TruSight) which showed a homozygous mutation in SLC37A4 confirming the diagnosis of GSD-Ib. Due to the presence of a normochromic normocytic regenerative anemia without signs of iron deficiency and splenomegaly (not typical for GSD-I), the peripheral blood smear was reviewed revealing features of HS. An osmotic fragility test confirmed the diagnosis of HS. A re-analysis of the TruSight data revealed a yet undescribed heterozygous mutation in the spectrin-binding domain of ANK2. Since mutations in ANK2 are known to cause long QT syndrome 4 (OMIM #600919), an ECG was performed and showed a normal QTc interval.
Discussion: This clinical situation of concomitant rare disorders underlines the need for further diagnostic investigations when not all symptoms of a patient correlate with the expected phenotype of an existing diagnosis. The pathogenic nature of the ANK2 mutation needs to be further evaluated, and illustrates the challenges and limitations of molecular diagnosis by next generation sequencing tools.

SGPP 18

Study, read, learn, be a bit lucky and save money: think of Parechovirus!

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Since development of a specific real time (rt) PCR, Human Parechovirus (HPeV) has become a quickly and easily detected pathogen. Retrospective studies analyzing stored cerebrospinal fluid (CSF) samples conclude that HPeV might be a common cause of sepsis-like central nervous system infections (CNS) in newborns. We present 3 cases of neonatal CNS HPeV. Common symptoms were fever, irritability, sepsis-like syndrome, abdominal discomfort and hypotonia. Two of three cases presented with apneas. Blood cultures, complete blood count and CSF analysis showed no sign of bacterial infection despite high clinical suspicion. No co-infection was found. In two patients, MRI was performed and showed HPeV typical CNS white matter lesions. 2/3 patients were normal at 4 and 6-months clinical follow-up. One patient developed hemiparesia of unclear origin. In all three cases CSF HPeV RT-PCR led to diagnosis. In one case, it was an incidental finding thank to a CSF broad RT-PCR analysis (the detection of this virus is part of a PCR multiplex panel for meningitis/encephalitis cases since May 2016). This curious discovery prompted us to suspect a CNS HPeV infection in another case a few days later. In contrast, without the same initial lucky hazard, the third case remained mysterious for enough time to lead to multiple investigations, such as large metabolic, genetic, and infectious screening, until CNS MRI finally prompted towards parechovirus infection.
Conclusion: We already knew that our medical knowledge can be fed by both scientific culture and clinical experience. HPeV RT-PCR illustrates well how serendipity can also help us to improve our differential diagnosis and increase our efficiency. Thank to our learning curves, we could save money, energy, pain, worries and time. Parechovirus is not rare and no more unknown; do not forget it!

SGPP 19

Congenital hyperinsulinism in a neonate due to a rare homozygous autosomal recessive ABCC8 mutation: a case report

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Introduction: Congenital hyperinsulinism (CHI) is a rare glucose metabolism disorder characterized by an unregulated oversecretion of insulin leading to persistent hypoglycemia. Most cases of CHI are caused by autosomal recessive or dominant mutations in ABCC8 and KCNJ11 genes. Diazoxide is the first line therapy for CHI but it has been reported to be ineffective in recessive CHI.
Case report: A preterm macrosomic male infant was born at 33 1/7 weeks of gestational age to 1st degree consanguineous parents. At 2 hours of life, he showed severe hypoglycemia with undetectable blood glucose level (<0.1 mmol/l). Serum insulin level was elevated (440 mU/l), beta-hydroxybutyrate (<10 mcmol/l) and free fatty acids (<0.1 mmol/l) were suppressed. In the following hours, 18 mg/kg/min of intravenous glucose were necessary to achieve normoglycemia. Molecular genetic analysis for CHI showed a homozygous ABCC8 missense mutation (p.Val601Ile, c1801G>A), inherited from heterozygous unaffected parents and consistent with the diagnosis of autosomal recessive CHI. F-DOPA PET-CT showed a diffuse CHI. Oral diazoxide was initiated but had to be stopped at day 3 due to intestinal malrotation with volvulus which needed a resection of 76 cm of the small intestine. The patient did not respond to continuous s.c. octreotide. Subtotal pancreatectomy was considered due to the persistant hypoglycaemic episodes. The genetic results prompted a second diazoxide attempt and the patient could be successfully weaned off the glucose infusion with 10 mg/kg/d of diazoxide.
Discussion: Contrary to dominant ABCC8/KCNJ11 mutations, recessive ABCC8/KCNJ11 mutations are reported to be unresponsive to diazoxide. In our patient, the rapid genetic confirmation of a rare mutation associated to diazoxide responsiveness (in one case report) avoided subtotal pancreatectomy.
Conclusion: To our best knowledge, the association of CHI with an intestinal malrotation and volvulus has not been described in the literature yet. Rapid genetic testing in the presence of CHI may guide therapeutic strategies. In our patient, subtotal pancreatectomy could be avoided.

SGPP 20

A severe case of mucositis

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Background: Fuchs Syndrome (FS) is described as a Steven-Johnson Syndrome without skin lesions with isolated involvement of mucous membranes. This condition is often triggered by infections, with Herpes simplex Virus (HSV) and Mycoplasma pneumoniae (MP) being the most common. An important differential diagnosis is Behçet Disease (BD), a systemic inflammatory condition sharing the clinical features of both autoinflammatory disease and vasculitis.
Case description: We report the case of a 14-year-old boy who presented to our emergency department with fever, cough, blepharoconjuntivitis and severe oral and genital mucositis. A careful medical and drug history revealed two previous episodes of oral mucositis in the past two years, and no drug intake before the onset of symptoms. On admission high inflammatory markers and signs of pneumonia on chest X-ray were found. An abdominal US showed a moderate hepato-splenomegaly. The microbiological investigation detected a positive nasopharyngeal swab PCR for Chlamydophila pneumoniae (CP), whereas HSV and MP were negative. Given the clinical presentation, supported by laboratory, radiologic and microbiological examinations, the diagnosis of CP associated FS was made. The patient needed oxygen supplementation for five days and recovered promptly after the introduction of antibiotic treatment and steroids.
Discussion: The clinical presentation with atypical pneumonia due to CP and mucositis led us to the diagnosis of a CP associated FS. Although the most common triggers for FS are HSV and MP, a negative result for these pathogens does not exclude an infectious cause of the mucositits and the search for CP may be helpful for the diagnosis and treatment. As mentioned above, an important differential diagnosis is BD which has been discussed. Our patient did not fulfill the diagnostic criteria for BD, but it is known that other clinical manifestations as uveitis or retinitis may develop several years after onset of the first symptoms.

SGPP 21

From frontal teaching to emotional understanding – a modern concept in childhood obesity therapy

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Introduction: In Switzerland, the prevalence of overweight and obesity in children has stabilized over the last ten years, but 20% of children remain overweight and urgent efforts are needed to control the epidemic. So far, existing interventions prove limited effectiveness and sustainability. From several new studies we know that a good quality of life and mental health are predictive for subsequent weight loss.
Intervention and results: Our multiprofessional group program includes lessons in nutrition, physical activity, medical information and behavioral coaching. Most important components of the program consist in initial home visit and a one-week camp during the summer holidays to strengthen the social cooperation. Own results from the 2016 group program showed an increase of BMI-SDS (+0.062) during the intervention. Additionally, about 50% of obese children show signs of psychological/psychiatric disorders. The evaluation of the KIDSTEP questionnaire revealed 40% anxiety and sleeping disorders. Drop-out rate was about 30%. Only during those lessons with specially trained artists performing a concept to transform learning processes into positive experiences with humor and pleasure, we observed higher levels of attention and motivation.
Conclusions: It becomes obvious that the educational character in teaching the patients is not sufficient to increase adherence, motivation and success and positive experience and a new design of multiprofessional group programs is planned. The lessons in nutrition, physical exercise and medical background formerly teached face to face should be replaced. The fun and emotional experiences conveyed by the humorous artists may motivate patients for more sustainable learning processes into a healthier lifestyle. In addition, the clowns could help the family to open up hidden psychological problems. The increase of psychological / psychiatric comorbitities in the families requires a fundamentally higher weight of systemic family therapy as a fixed component of the program to improve quality of life, physical activity and eating habits sustainably.

SGPP 22

Prepubertal girl with vaginal bleeding

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Case report: A 4 y.o. african girl was hospitalized for vaginal bleeding that had started few hours before. Her medical and surgery histories were uneventful. She denied pain, fever, lethargy, abdominal distension or dysuria. She had no history suggestive of sexual abuse, trauma or foreign body to the genital area. The patient was kept by her big siblings during the day without a remarkable event. Her vital signs and physical examination were normal. Tanner: P1S1A1. Examination of the genitalia revealed vaginal blood clot without any local injury. Complete blood count and clotting factors were normal. Further examination wasn't possible at bedside. A cystoscopy and vaginoscopy were done under a general anesthesia. A tumor was found in the urethral inferior lip. No gynecologic disease was observed. Biopsy revealed an overlay with inflammatory and fibrin tissue, and no malignancy. An urethral caruncle was diagnosed. 1 week later urethral inflammation was still present; corticoid cream was prescribed for 2 weeks allowing disappearance of residual mass. Follow up was free of recurrence.
Discussion: Common causes of vaginal bleeding in pre-puberty girls are neonatal withdrawal bleeding, trauma, foreign bodies, infections (vulvo-vaginitis / peri-urethral gland abscess), hematuria, urethral prolapse, precocious puberty, tumor and sexual abuse. An exhaustive anamnesis and physical examination, usually under general anesthesia, are crucial for the diagnosis. If a tumor is found, biopsy is required to exclude malignant tumors. Urethral caruncles are usually confined to women urethra and are rarely found in pre-puberty girls. They are present in the urethral meatus and bleeding is the most

common symptom, but persistent pain or urination difficulties are also described. Microscopic findings are interstitial oedema with inflammatory cell infiltration and hyperplasia of the connective tissue. Two therapy approaches are possible: steroid ointment (because of chronic inflammation) and/or surgical resection. A potential problem is meatal stenosis. No relapse is described in the literature.
Conclusions: Urethral caruncles are one of the rare causes of vaginal bleeding in children. Topical steroids are recommended for a few weeks. If this treatment fails or suspicion of malignancy exists biopsy ± excision is required. Surgery side effect is meatal stenosis and steroid ointment may be required if inflammation (or incomplete resection) persists after surgery.

SGPP 23

Not as quick as expected – observation and treatment of an intentional Marcoumar overdose

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An intentional overdose with Marcoumar (phenprocoumon) or other coumarin derivates is rare and due to possible bleeding complications a potential life-threatening condition. Therefore, few guidelines exist how to observe and treat patients with an ingestion of high dosages of phenprocoumon. Most publications describe patients, which are under preexisting oral anticoagulation to prevent thromboembolism or intoxications with superwarfarins (rodenticides). Only few cases describe adolescents with acute warfarin / phenprocoumon overdoses. We present the case of a 14-year-old adolescent girl who ingested 390 mg of Marcoumar in suicidal intention. At initial presentation two hours post ingestion she had an international normalized ratio (INR) of 1.0 and a Quick value of 98%. After initial preventive therapy with oral and intravenous vitamin K she was admitted to our hospital for further treatment. Regularly coagulation tests were performed and when the Quick fell below 65% 10 mg of intravenous Vitamin K was administered. The INR/Quick in our case showed a rebound tendency during an overall period of 26 days. There were no severe bleeding complications and the patients had no other symptoms during the treatment period. Due to a complex social situation, the patient was treated in the hospital setting during the complete period until stable coagulation values were reached. Once the INR/Quick stayed at a level above 65% the patient was discharged for further psychiatric treatment. Our case highlights the risk of a rebounding effect on the coagulation system for almost one month after an acute phenprocoumon overdose and the need for regular treatment with vitamin K. Most past studies were performed with warfarin and described an effect on coagulation between 12 hours to two weeks. In comparison to warfarin, phenprocoumon has a longer half-life between about 80 and 270 hours. Single large doses of vitamin K do not show a benefit compared to small doses because the half-life of phenprocoumon is longer than the elimination time of vitamin K. Our patient took tablets that belonged to a relative. In Switzerland package sizes of 100 tablets (total of 300 mg) per package are available. In children and adolescents 300 mg of Marcoumar can cause fatal bleeding. Limitating the amounts of tablets available at home can be helpful in preventing suicide attempts or accidental ingestions especially in children and adolescents.

SGPP 24

Ingestion of a button battery in a one-year's child

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Introduction: These last years the number of major outcomes after the ingestion of button battery (BB) has increased. We report a case of severe complication.
Case report: A one-year's child consults after a loss of consciousness following a crying episode. The laboratory shows a normocytic normochromic anemia without history of bleeding. Few hours later she presents a new discomfort and fresh blood in Stool. By suspecting a gastrointestinal bleeding she's transferring to a university hospital. The esophagogastroduodenoscopy (EGD) shows an esophageal injury and a BB in the stomach. The battery is removed and a treatment with proton pump inhibitor and antibiotic is started. After one week she presents a new intestinal bleeding. No source of active bleeding is seen during the EGD. Two weeks after the initial presentation she presents a hemorrhagic shock. This time during the EGD an aortoesophageal fistula (AEF) is suspected and confirmed with a MRI and a CT. She undergoes a successful resection of the fistula.
Discussion: A BB, lodged in the gastrointestinal tract, liberates electron at her negative pole and generate hydroxide. The production of hydroxide causes an alkaline caustic injury leading to tissue liquefaction and necrosis. Lesion can already happen after two hours. 20 mm Lithium cells are most often associated with severe outcome. Parents who observe their children swallowing a BB should be advise to go promptly to the emergency department. An X-ray will localize the BB. If the BB is located in the esophagus emergent endoscopic removal is necessary. Recommendations diverge when the BB is in the stomach. The NASPGHAN recommend an endoscopic assessment of the esophagus and the removing of the BB by children under 5 years and BB ≥20 mm. In contrast Litovitz & co propose an observation with X-ray in 4 day when the child is asymptomatic. Depending of the localization of the BB, different kind of complications can appear: AEF, esophagus stenosis, tracheoesophageal fistula, mediatinitis and spondylodiscitis. Sever complications like AEF can appear until 3 weeks after the removal of the battery. MRI seems to be the best exam to evaluate the extension of lesion. Esophagram helps to diagnose stricture or perforation of the esophagus.
Conclusion: Life threatening complication can follow BB ingestion. It is important to know them in order to avoid sever outcome. Parents should also be aware of the danger associated with ingesting BB.

SGPP 25

Fever after travel in children is not always an infection!

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Introduction: We present the case of a 13-year-old Asian girl returning from a two-month trip in Thailand. The patient presented to the emergency room on two occasions. On the first visit, the patient presented with a facial rash and fatigue. Two weeks later, the patient returned to the emergency room with headaches, vomiting, persistent facial rash and fatigue.
Case Presentation: On physical exam, the patient was found to have a confluent facial rash, paleness, poly-articular arthritis (knee, cervical and lumbar spine), seven days of fever, periorbital edema, and hepatosplenomegaly. The vital signs were normal. The differential diagnosis for the patient at that time was focused on the possibility of tropical illness. The initial work-up revealed a macrocytic anemia (49 g/l), a positive direct Coombs test, low haptoglobin, elevated ferritin, hypertryglyceridemia, and hypoalbuminemia. The basic metabolic panel did not demonstrate any anomalies and inflammatory markers were within normal limits. Urinalysis demonstrated nephrotic range proteinuria. The patient was admitted to the hospital for transfusion and further investigation. During the hospitalisation, tests for tropical and infectious illnesses were negative, and the patient's anemia improved after transfusion and treatment with corticosteroids. The patient also developed oral ulcers. The investigation was pursued to exclude a macrophage activation syndrome and a systemic lupus erythematosus (SLE). The diagnosis of SLE was retained when lab results demonstrated a low complement level, a positive anti-nuclear (ANA) and anti-dsDNA antibodies. The patient has been followed-up by the rheumatology team and has improved (disappearance of the rash, normalisation of anemia, and decreased arthritis) since being treated with Cellcept.
Conclusion: Lupus, particularly in children, has an array of different clinical presentations. It is usually more severe compared to adults, and morbidity-mortality depends on the affected organs. This severity implies an early diagnosis, treatment, and multi-disciplinary managment in order to prevent long-term complications.

SGPP 26

"Here's looking at you, kid." – A Case report of an unusual ophthalmic disease with iatrogenic Cushing's syndrome due to dexamethasone containing eye drops

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Introduction: A primary glaucoma is a rare condition with a reported prevalence of 2.85 cases per 100 000 births. The diagnosis and the management is highly complex and besides the surgical interventions the medical treatment includes the potential of exceptional side effects.
Case: A 3 months old term male baby with normal intrauterin growth was seen in our office with recurrent conjunctival injections and tearing. A bilateral mydriasis pronounced in the right eye and a poor red reflex ipsilateral led to a referral to a specialized center. A primary glaucoma in both eyes were suspected and consecutively diagnosed. The patient underwent a deep sclerectomy and a trabeculotomy on both eyes at the age of 5 months at the Department of Ophthalmology, University Hospital, Zurich. Postoperatively, a topical treatment with dexamethason-containing eye drops (0.1% solution) was established to prevent intraocular inflammation and scarring. The dexamethason dose was tempered after the first 2 weeks. $1^1/_2$ months after starting the glucocorticoid treatment the child presented with a round, moon-shaped face and growth failure. As a potential complication due to the locally administered eye drops the patient developed an iatrogenic Cushing's syndrome. The diagnosis was confirmed at a specialist center (Dept. of Endocrinology, Children's Hospital, Zurich) with an euglycemic metabolic status, suppressed cortisol and ACTH levels. On the right eye the child underwent a second operation, a trabeculectomy at the age of 7 months. In regard to this operation the dosage of the dexamethason-containing eye drops was temporarally increased to the primary dose in the right eye, while the dosage was further gradually reduced in the left eye. The topical steroid treatment was finally stopped around 5 1/2 months after its initiation. During the subsequent follow-up the patient's cushingoid face disappeared and he showed the beginnings of catch-up growth. Nevertheless at the age of 13 months the bone age (right hand and wrist x-ray) was delayed by approximately 6 months compared to the chronological age.
Conclusion: An iatrogenic Cushing's syndrome is a very rare complication in the management of postoperative medical treatment of a primary glaucoma. Therefore, medical professionals using topical steroids should be aware of this side effect and a close patient's follow-up in a multidisciplinary team is essential.

SGPP 27

Mild hypothyroidism in an infant – due to prematurity or consumption in benign neonatal hemangiomatosis and how to treat?

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Introduction: Infantile hemangiomas (IHs) are the most common benign tumors of infancy and more frequent in premature infants. Benign neonatal hemangiomatosis (BNH) is a rare disorder, in which multiple cutaneous hemangiomas appear at birth or shortly after, without visceral complications. In contrast to BNH, diffuse neonatal hemangiomatosis (DNH) involves life threatening visceral hemangiomas and represents the most frequent type of neonatal hemangiomatosis. In DNH, a high level of expression of type 3 iodothyronine deiodinase in hemangiomas may convert thyroxine (T4) into inactive reverse triiodothyronine (rT3) and cause severe consumptive hypothyroidism resistant to T4 therapy [1]. Hypothyroidism in association with BNH has been reported in only two cases [2, 3].
Case Report: We present a 6 weeks old preterm male infant (GA 26 weeks) with more than 80 cutaneous hemangiomas of various size and morphology scattered over the whole body. While their number is steadily increasing since birth, normal abdominal, intracranial and thyroid ultrasonography excluded a visceral or hepatic involvement. Due to low free T4 and free T3 (8.0 and 4.5 pmol/L) levels and increased TSH (6.5 mu/L), a T4 replacement therapy (7.5 µg/kg BW) was initiated after confirmation of mild hypothyroidism. One week later, thyroid function was normalized.
Conclusion: Thyroid dysfunctions should be considered in infants with benign neonatal hemangiomatosis, especially during the growth phase of the disease, because hypothyroidism at this age impairs brain maturation and leads to permanent cognitive deficits. Nevertheless, in face of the high prevalence of hemagiomatosis in infants, there is no evidence, whether thyroid tests are necessary in all children concerned. Therefore, further research is needed, all the more as BNH shows a spontaneous regression of the lesions within the first two years of life and usually needs no treatment. In case of complications like hypothyroidism, besides T4 replacement, a therapy with propranolol may be recommended [4].

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SGPP 28

Unexplained dystonia: think Ataxia-Telangiectasia

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Ataxia-Telangiectasia (AT) is a rare autosomal recessive disease, due to pathogenic variants in ATM gene, with a multisystem phenotypical expression. It represents the second most common form of inherited ataxia. Despite its name, ataxia can be at onset very subtle and conjunctival telangiectasia may appear much later. We present here two cases where dystonia as presenting feature led to erroneous initial diagnosis. The first one is a two-year-old male, referred to neurology for delayed gross motor milestones. Isolated dystonic postures were observed suggesting a primary dystonic disorder. Subtle gait ataxia was noticed later. Initial large radiological and metabolic work-up, including CSF neurotransmitters, were all normal. The unexpected finding of extremely low level of serum IgG led to consider AT as a potential diagnosis. This was rapidly confirmed by elevated alpha-fetoprotein (AFP) level, and finally by molecular genetics, which revealed a compound heterozygous state for two pathogenic variants in ATM. Further investigations confirmed a combined severe immunodeficiency. He was rapidly started on monthly intravenous immunoglobulin substitution. The patient has so far not developed any severe infections and the neurological symptoms are stable. The second patient is a 24-year-old male with a long history of presumed non-progressive extrapyramidal symptoms since early childhood. During adolescence, the combination of mild dystonia and axial myoclonus of the upper trunk suggested possible primary dystonia. Mild resting tremor at the age of 22 prompted to repeat a brain MRI, which showed significant cerebellar atrophy, retrospectively already present to a lesser degree 10 years earlier. Exome sequencing with targeted bioinformatics analysis showed a compound heterozygous state for two likely pathogenic variants in ATM. Further investigations showed elevated AFP, low levels of immunoglobulin and signs for an axonal polyneuropathy consistent with the diagnosis of AT. Interestingly, the patient has still no telangiectasia and a very mild ataxia at tandem gait. From these two examples, it should be kept in mind that AT is a misleading name as the disorder does not always present with ataxia or telangiectasia. Prominent dystonia is not unusual and should not disregard the possibility of AT. Reaching the correct diagnosis is crucial given the risk of immunodeficiency, the radiosensitivity and the risk of malignity associated with this disorder.

SGPP 29

Acute abdominal pain in an adolescent girl with Mayer-Rokitansky-Kuster-Hauser-Syndrome

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Introduction: Acute abdominal pain is one of the most common symptoms leading to emergency consultation. Acute surgical condition must be ruled out. In presence of congenital syndromes the evaluation of this common condition becomes more challenging.
Case report: A $15^7/_{12}$-year-old girl with previously known Mayer-Rokitansky-Kuster-Hauser-syndrome was admitted for acute abdominal pain in the right lower quadrant. The clinical examination, laboratory results (CRP 1.3 mg/l, Lc 12.3 G/l) and sonographic examination weren't conclusive. Abdominal MRI suggested an infectious process in the right lower abdominal region. An explorative laparoscopy was perfomed and

showed a ruptured follicule in her right ectopic located ovary and some blood collection in the douglas pouch. The appendix and colon showed no signs of infection.
Discussion: In adolescent girls, acute abdominal pain in the right lower quadrant includes the differential diagnosis of an ovulation in addition to the well known appendicitis, constipation, gastrointestinal infectious disease, cystitis, psychosomatic conditions ndmany more. In this case the diagnosis was complicated by the fact, that the intraabdominal anatomy was different because of the Mayer-Rokitansky-Kuster-Hauser-syndrome, a rare congenital condition which affects the reproductive system causing the uterus and vagina being underdeveloped or absent.
Conclusion: Acute abdominal pain should always be carefully investigated. In combination with Mayer-Rokitansky-Kuster-Hauser-syndrome with aberrant anatomical situation, the management should be performed by a multidisciplinary team consisting of pediatricians, gynecologists, surgeons and radiologists.

SGPP 30

Periorbital emphysema in pediatric age: nose blowing leading to a blown orbit

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Introduction: Periorbital emphysema is a condition that occurs following forceful injection of air into the orbital soft tissue spaces. In many cases there is a history of trauma and fracture of an orbital bone.
Case report: A 10-year-old girl presented to our emergency room several hours after been hit in her right eye by her sister. Following the impact the patient said that her eye was lightly sore, but she did not experience blurry or double vision. Later the patient blew her nose felt like the skin around his eye "puffed out" Following this swelling, there was no deterioration or change in his vision and no other symptoms. There was mild swelling of the right upper eyelid, but no crepitus or palpable bony step-off around the orbital rim. There was no proptosis and the remainder of his optical exam was normal including extraocular motion and pupillary reflex. In light of the mechanism of injury and the abrupt onset of orbital swelling after the patient blew her nose, there was a high suspicion for an orbital fracture with concomitant periorbital emphysema. Computed tomography (CT) of the facial bones showing a fractures of the floor of the orbit and a periorbital subcuteaneus Emphysema. Surgical repair and decompression were not performed, and several weeks later the patient was doing well without sequela. We treated her with a course of oral antibiotics, and instructions to use nasal decongestants and avoid blowing his nose.
Discussion: The presence of periorbital emphysema in the absence of an apparent orbital wall fracture suggests an occult fracture of the orbit In such cases, a careful history is helpful in making the correct diagnosis. Fractures of the floor of the orbit, sometimes known as "blowout fractures", typically occur when a small round object strikes the eye. A significant consequence of fractures of the orbital floor is entrapment of the inferior rectus muscle and-or orbital fat. Surgical repair of orbital fractures within two weeks is indicated in patients with diplopia and CT evidence of entrapped muscle or periorbital tissue, large fractures (>50% of the wall), and enopthalmos that does not resolve. Conservative management is generally reserved for patients with minimal diplopia, preserved ocular motility and the absence of marked enopthalmos.

SGPP 31

Mitral valve endocarditis in the course of invasive Haemophilus influenzae type b infection reveals a primary combined immunodeficiency syndrome

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Introduction: Out of 7 different serotypes, the encapsulated H. influenzae type b (Hib) is the most pathogenic type. It is protected from phagocytosis by a polysaccharide capsule and with adherence factors it sticks to the respiratory epithelium. Invasive Hib infections result in meningitis in over 50%. Other common clinical manifestations include acute epiglottitis, pneumonia, sepsis, osteomyelitis or endocarditis. An effective conjugate vaccination was introduced in Switzerland in 1990. Primary immunodeficiency diseases include more than 300 genetic disorders. Common manifestations include an increased susceptibility to recurrent, severe or persistent infections or signs of immundysregulation such as autoimmune cytopenias. Treatment options include immunoglobuline substitution, immunosuppression, stem cell transplantation or gene therapy.
Case report: A 13-year-old, unvaccinated girl with dysmorphic features of unknown aetiology including minor mental retardation presented in our emergency department with signs of epiglottitis. After blood cultures and a first dose of i.v. antibiotics she was transferred to a child intensive care unit for further investigations/treatment. With positive blood cultures for Hib she was treated for 14 days with Amoxicillin. 5 days after the treatment, she was admitted with pneumonia and again blood cultures proved growth of an amoxicillin-sensitive Hib. Antibiotic treatment was initiated. Searching for the source of the recurrent infection, a whole body MRI was performed and showed right tibial epiphysis osteomyelitis. Further searching revealed an endocarditis of the mitral valve, which presumably had spread the Hib infection. The girl was treated for 6 weeks with weekly echocardiographic controls. Due to the severity of the infection, a history of frequent infections in the past and recurring thrombocytopenia in a girl with dysmorphic features, we suspected a primary immunodeficiency disease. Serum immunoglobulin levels were low. There was only a weak vaccination response to Tetanus. After several assessments, a combined immunodeficiency syndrome is suspected, further immunological examinations are still pending to date. Immunoglobuline substitution was initiated and well tolerated.
Conclusion: In patients with recurrent infections with the same bacteria, it is crucial to find the spreading lesion and screen for immunodeficiency. Hib disease must be considered in the differential diagnosis of unimmunized /undervaccinated children.

SGPP 32

Stroke-like phenomena in paediatric lyme neuroborreliosis

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Background: Neuroborreliosis is a disease caused by an infection with the spirochete Borrelia burgdorferi after a tick bite. In children, neuroborreliosis is usually associated with a peripheral facial nerve palsy and lymphocytic meningitis and only rarely with hemiplegia.
Patient description: We present a 3-year-old boy with a left hemiplegia without facial asymmetry. He had neither fever nor headache. A tick bite behind the ear was recorded one month before the onset of the symptoms. Initially, because a stroke was suspected, he was transferred to the university hospital of Basel. The MRI revealed a flux restriction in the anterior and middle cerebral arteries, but no thrombus was described. The Lumbar puncture was notable for lymphocytic pleocytosis. Serologic testing demonstrated positive Lyme antibody (IgG and IgM). Cerebrospinal fluid was also positive for anti-Lyme immunoglobulin M. The patient was treated for a Neuroborreliosis with parenteral ceftriaxone for 14 days with complete resolution of his symptoms.
Discussion: Children affected by Lyme neuroborreliosis typically show cranial nerve plasy or aseptic meningitis. But in rare cases, they have an unusual presentation similar to an ischemic stroke which can be due to vasculitis or subarachnoidal/intracranial hemorrhaging. The criteria for diagnosis of Lyme neuroborreliosis is the presence of neurological symptoms, CSF pleocytosis and intrathecal synthesis of B. burgdorferi antibodies [1–3]. MRI is the reference tool to diagnose early signs of ischemic lesions [2]. A CSF analysis is the Gold-standard if neuroborreliosis is suspected. A treatment with intravenous Ceftriaxone for 14 days allows a complete regression of the neurological deficits.
Conclusion: Neuroborreliosis with hemiplegia is a rare presentation of pediatric Lyme disease but should be considered as a possible differential diagnosis when assessing a patient with hemiplegia in an endemic area.

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SGPP 33

HSV type 2 conjuctival infection with bacterial coinfection

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Neonatal conjunctivitis is a relatively common perinatal infection. The causes are chemical agents (silver nitrate gonococcal prophylaxis), viral (herpes simplex virus), Chlamydia trachomatis, typical bacteria, Escherichia coli, gram-negative enteric bacilli and Neisseria gonorrhoeae. We present the case of neonatal conjuctivitis in a child born with natural childbirth at 40 weeks gestational age. Mother's medical history was positive for a previously treated Chlamydia trachomatis infection, and negative for HSV infection. Serologic screening during pregnancy did not reveal other risk factors. On the 4 day postpartum, the newborn presented with bilateral conjunctival erythema and purulent secretions with no fever. On the 8 day postpartum and based on clinical diagnosis of bilateral bacterial conjunctivitis, the newborn was treated empirically with Polymyxini B sulfas and Neomycinum drops. At 10 days postpartum, a conjunctival swab was obtained due to persistence of symptoms and treatment was switched to Ofloxacine drops, pending results. Culture results were positive for Chlamydia trachomatis and HSV-2 infection,and treatment was modified appropriately. Conjunctivitis with HSV type 2 is rare (HSV <1%) and may present as disseminated form with CNS involvement in 25% of cases and SEM disease (skin, eyes, mouth) in 45% of cases. Mean age at diagnosis is at 10 days postpartum. Vertical transmission rate during childbirth is 50–60% in the presence of primary maternal infection and less than 3% with recurrent maternal infection. In the case reported here, laboratory findings highlighted a bacterial (Chlamydia trachomatis) and viral (HSV-2) infection.It was treated with oral Azithromycin 20 mg/kg/day,oral Acyclovir 40 mg/kg/day,Trifluridine ophthalmic 1 drop/2h. The newborn recovered successfully with no ophthalmologic sequale up to most recent follow up. The reported case presents a rare case of conjunctival coinfection of Chlamydia trachomatis and HSV. Moreover, the patient presented with a HSV type 2 infection which occurs in less than 1% of all cases. The patient was treated at the referral university hospital with oral therapy and not with intravenous treatment, which is the treatment recommended in the literature. HSV type 2 conjuctival infection is rare and may present either as isolated viral infection or with bacterial coinfection. Involvement of the CNS must be excluded. The outcome of isolated conjunctivitis with no systemic involvement is good following appropriate antiviral therapy.

SGPP 34

Extremity pain in a child with sickle cell disease

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Introduction: Sickle cell disease (SCD) is a serious haemoglobinopathy showing increasing incidence in developed countries through migration. In our case report we highlight the difficulty distinguishing between a vaso-occlusive crisis (VOC) and an acute haematogenous osteomyelitis (OM) in a child with limb pain. Overall the risk for OM is increased in children with SCD.
Case report: A 7-year-old girl with known homozygous SCD and a history of several VOC in the past presented to our ER with a painful left thigh for one day with no response to oral Paracetamol and Ibuprofen. She was on daily treatment with Hydroxycarbamid (20 mg/kg/d), Amoxicillin (20 mg/kg/d, prophylaxis) and folic acid. On examination she was subfebrile (37.4 °C) and had a tender left thigh without limb swelling. CRP and white blood count were 10 mg/L and 9.1 g/L respectively. She was admitted to intensify analgesia and for rehydration. Working diagnosis: VOC. After 3 days CRP increased to >100 mg/L (max. 145 mg/L on day 6) and the pain persisted. The child was afebrile. Work-up for OM was done. Blood cultures were taken on day 3 and 4 and a MRI was performed on day 5. The latter couldn't exclude an OM in an area of possible bone infarction in the upper third of the femur. The orthopaedic team performed a biopsy and sent material for culture and histology. Empirical treatment with Clindamycin and Ceftriaxon was started on day 5. The symptoms improved. Blood cultures were negative. Histology and culture of the biopsy were not suggestive for OM.
Conclusion: In retrospect the high CRP and inconclusive results from the MRI influenced the management towards OM. CRP, however, is an inflammatory marker which may also be increased in VOC. As the case progressed and negative culture results were available, OM was unlikely. Several previous studies highlight the difficulty of differentiating OM from VOC in children with SCD. Predictive factors suggesting OM rather than VOC are: increasing number of days of fever and pain before admission and a swollen limb at the time of on presentation. These factors can be found after a good history and physical examination.

SGPP 35

Paediatric musculoskeletal infections by Panton-Valentin leucocidin

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Musculoskeletal infections by Staphylococcus aureus carrying the Panton-Valentin leucocidin (PVL) constitute a rare, but highly critical event. They stand out by a rapid course of distinct inflammation, degradation under conservative therapy and a high rate of recurrence. Early surgical intervention and aggressive, prolonged intravenous antibiotic treatment are required to prevent short-term complications like toxic shock as well as long-term effects like impaired growth. This study resumes nine paediatric patients with PVL-positive musculoskeletal infections which were treated at the University Hospitals of Geneva and Lausanne from 2010 to 2016. Seven of the nine patients were male (mean age 9.8 years, SD 4.7). While one patient demonstrated a localised soft tissue infection of the finger with good response to treatment, the other patients stood out by a short history of complaint, high fever (mean 39.1 °C, SD 1.3), slightly increased level of leucocytes (mean 14.7 G/l, SD 5.8) and remarkably high CRP levels (mean 147.9 mg/l, SD 72.6). There were six osteoarticular infections (shoulder, hip, knee, ankle) in adolescents (mean 12 years, SD 1.9) with even more notable CRP levels (mean 171 mg/l, SD 43.1). They required repeated surgical interventions and prolonged antibiotic treatment (up to 2 months intravenously) resulting in an extended duration of hospitalisation (mean 36.5 days, SD 16.1). Complications included local recurrence, growth disturbance and psychological deterioration, resulting in suicidality. The remaining two 2-year-old patients presented subcutaneous abscesses with a quicker response to treatment, but were likewise complicated by local recurrence. Indeed, the number of nine cases illustrates the low incidence of these infections in the general paediatric population. Yet, regarding the duration of hospitalisation, the recurrence rate and the importance of complications, it becomes evident that a targeted approach is essential to assure a good outcome. Fundamental knowledge of this particular type of infection in paediatricians is therefore essential to prevent avoidable risks.

SGPP 36

Tick-borne encephalitis despite immunisation

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Introduction: Tick-borne encephalitis (TBE) is endemic in Switzerland. Significant neurological sequelae including death are possible in adolescents and particular in adults. Children generally have a much better outcome. Active Immunization against TBE is recommended by EKIF/BAG for children living in endemic areas from the age of six years. A booster dose is recommended every ten years in Switzerland. Other European countries recommend a booster already after 3–5 years. We describe a rare case of an adolescent boy who presented with TBE despite vaccination.
Case report: A 14-year-old boy was referred to the paediatric emergency department presenting with fever, severe headache, vomiting, photophobia, dizziness and diplopia. He remembered a tick bite 2 weeks prior, since he was out in the forest area frequently. He had been fully vaccinated 8 years ago against TBE according to the usual schedule. He presented with meningism and no further neurological sings. The cerebrospinal fluid (CSF) showed a pleocytosis (210 cells/ul; 90% mononuclear), further parameters unremarkable. Empirical treatment was initiated with ceftriaxone and acyclovir. Intrathecal TBE-specific antibody response was positive as well as TBE specific IgM and IgG serology. Cerebral MRI showed focal oedema of the right thalamus. Further treatment was symptomatic. The patient was discharged after 8 days with a mild gait ataxia and terminal nuchal rigidity. Follow up serology after four weeks showed a quantitative increase of TBE specific IgG and IgM. Clinically he was nearly back to his usual self. He plans to become a forest ranger.

Conclusion: Despite an excellent effectiveness and safety profile of TBE vaccines, break-through wild type infection can occur. As this number is extremely low in Switzerland a booster dose is recommended every 10 years. We postulate that certain individuals who have a high exposure to tics (for eg. forest rangers) and living in endemic areas may benefit from a shorter booster interval (as recommended in Austria and Germany).

SGPP 37

Bacterial superinfection of a tick bite? Take a closer look

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Introduction: Tics are able to transmit infectious diseases. In Switzerland the most well known diseases are tick bite encephalitis, which is endemic in certain regions, and Lyme borreliosis (LB). The less frequent but important Tularemia, caused by the Gram negative, highly contagious, intracellular bacterium Francisella tularensis, may also be transmitted by ticks and can present with different clinical syndromes – the most common being ulceroglandular disease.
Case report: A 13-year-old female scout was referred to our emergency department for a pruritic maculopapular generalised rash. She had been taking Amox.-Clav. since 8 days for a presumed bacterial superinfection with cellulitis behind her ear after a tick bite. On examination she was afebrile and in good general health. She remembered removing a tick around 2 weeks ago. Initially the area was swollen and red, some pus and crusted debris could be removed. During the course the area around the bite became inflamed with a red-blueish appearance. She was admitted and treated with antihistamines and clindamycin for a working diagnosis of cellulitis and beta lactam associated non-immediate allergy. On further examination by the paediatric infectious diseases specialist an ulcerative lesion at the former tick bite area and retroauricular lymphadenitis was identified. She denied any fever episodes, erythema migrans or contact with cats. Francisella tularensis serology was positive for IgM. Oral treatment with Ciprofloxacin was started. Follow-up 2 weeks later showed remission of the ulcer and retroauricular lymphadenitis. Repeat serology demonstrated F. tularensis seroconversion. The diagnosis of ulceroglandular tularemia was confirmed.
Conclusion: Tularemia, perhaps better known to be transmitted by direct contact or consumption of infected meat or skin of rabbits, may also be transmitted by the bite of ticks, flies or mosquitoes. In Europe the most common clinical syndrome is ulceroglandular or glandular disease. Gastrointestinal, respiratory or typhoid manifestations are far less common. Tularemia should be part of a differential diagnosis in a patient presenting with cutaneous ulceration and/or glandular disease particularly after an arthropod bite or contact with contaminated food, water or rodents.

SGPP 38

A seizure can open another door – at the right time

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Background: For the work up of a non-febrile generalised seizure in a child usually EEG and often cranial MRI are performed. Imaging can reveal an unexpected pathology that may not have been associated with the primary clinical presentation. These unexpected findings however, may have serious consequences.
Case Presentation Summary: A 3-year-old girl was referred to our neurology department for evaluation of 2 recent non-febrile generalised seizures. EEG detected a focal epileptogenic area with sharp-slow wave complexes suggesting structural epilepsy. Cranial MRI showed a focal subcortical heterotopia. On second look we noticed bilateral cystic lesions of the parotid glands. The child was referred to our paediatric infectious disease specialist. An HIV screen was positive. From personal history she had been well and had a normal development, no recurrent or severe infections, only mild flares of atopic eczema and self-limited papular skin lesions. On examination, parotid glands appeared normal. Her parents had adopted her at the age of 14 months from Kenia. Screening in Kenia allegedly had been "normal" and had not been repeated after arrival in Switzerland. Further HIV work-up showed a viral load of 53'000 copies/ml and a CD4 count of 730/µl (29%). No clinical, laboratory or radiological signs of opportunistic diseases were evident. Treatment with ABC, 3TC, LPV/r was started.
Learning Points/Discussion: When imaging is done, all organs should be judged and, if abnormal, evaluated. In children bilateral parotid enlargement as well as cystic lesions found by imaging should always prompt to search for HIV. Retrospectively she had no warning signs for this chronic viral infection. Every adopted child should have repeated screening, preferably by a paediatric infectious disease specialist lead adoption/immigration clinic.

SGPP 39

RSV associated acute encephalitis with basal ganglia involvement: a pediatric case report

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Background: Respiratory syncytial virus (RSV) causes every year millions of respiratory infections worldwide. Common complications are pneumonia and middle ear infection. Neurological complications such as seizures, encephalopathy and encephalitis are very rare, but have been reported especially in children younger than two years of age.
Case description: We describe the case of a previously healthy 9-year-old boy, who presented to our emergency department with a 2-days history of fever, cough and vomiting. After 3 days of acute flu-like symptoms, the boy became afebrile, but developed an acute loss of consciousness, slurred speech, lethargy, weakness and photophobia. No neck stiffness. Peripheral blood count didn't show leukocytosis and CRP was negative. Cerebral spinal fluid (CSF) analysis showed mild pleocytosis with no protein and glucose abnormalities. Neurotropic viruses, including HSV, were tested negative in CSF. Electroencephalography (EEG) showed continuous generalized slow activity without epileptiform discharges. Brain magnetic resonance imaging (MRI) showed basal ganglia involvement. By missing clinical, radiological and EEG suspicion for an HSV associated encephalitis, no antiviral treatment was initiated. View the associated respiratory symptoms, an analysis for M. pneumoniae and respiratory virus in nasopharyngeal swab was performed, showing a positive result only for RSV. CSF was also tested with RT-PCR for RSV and resulted negative. Nevertheless, the clinical symptoms are highly suggestive of a RSV associated acute encephalitis. The patient showed a significant improvement in clinical and neurological symptoms within 7 days, with normal EEG findings. The patient was discharged with a close clinical and neuroradiological follow-up.
Discussion: The clinical presentation with respiratory symptoms followed by acute neurologic symptoms and detection of RSV as viral pathogen, led us to the diagnosis of RSV associated acute encephalitis. The negative RT-PCR on CSF doesn't exclude our diagnosis, since in the literature only up to 50% of patients with RSV encefalitis had a positive PCR in CSF. The cerebral MRI images showed basal ganglia involvement, which have also been described in the literature. Fortunately the recovery is quick and no specific treatment is required. Little is known about the long-term neurodevelopmental outcomes of children developing RSV associated acute encephalitis, so a prolonged period of neurologic follow up can be recommended.

SGPP 40

Intraorbital abscess caused by complicated sinusitis

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Case report: A 3 y.o. child presented with an important peri-orbital edema. He had fever for a week (39 °C), otalgia, rhinorrhea, cough and weight loss. Six days earlier, he started amoxicillin for bilateral acute otitis media. Three days after ambulatory ceftriaxone was initiated, he became afebrile and periorbital edema reduced, but left-sided morning-predominant hemicrania appeared without other neurological signs. Eye movements were normal, no trouble of vision nor diplopia. A CT-scan showed a collection (8x3 mm) in the left orbital cavity in favor of a subperiosteal intra-orbital abscess. There was focal erosion of the papyraceous lamina, filling of the left ethmoidal cells, maxillary and sphenoidal sinuses bilaterally. The patient underwent endoscopic drainage of the abscess associated with anterior ethmoidectomy and middle meatotomy in the left side. Corticotherapy was given for two days. Intravenous co-amoxicillin was administered for 5 days, and relayed orally for 10 days. Evolution was favorable. He was asymptomatic at discharge except for nasal obstruction treated by xylometazoline spray.

Discussion: Intra-orbital complication of acute sinusitis is a known but rare condition, more frequent in children. It is usually secondary to sinusitis, but can also be linked to dental infections, trauma or foreign bodies. Symptoms include eyelid edema, erythema, chemosis, proptosis, blurred vision, diplopia and fever. Orbital abscess is a serious complication with possible loss of vision, thrombosis of the cavernous sinus or intracranial extension. Suggestive symptoms are ocular pain, headache, mydriasis, loss of visual acuity and oculomotricity. Incriminated germs are those found in sinusitis: Haemophilus influenzae, Streptococcus and Staphylococcus species. The CT-scan allows to differentiate orbital cellulitis, subperiosteal and orbital abscess. Chandler's classification allows grading the severity of periorbital infection. When intracranial complications are suspected, MRI is indicated.
Conclusion: Acute orbital infection is an emergency and requires prompt diagnostic and adequate management. Intravenous antibiotics are necessary, as well as surgical drainage of the abscess and the affected sinus. In case of headache, a CT scan is mandatory to rule out any intracranial complications and allows a better evaluation of the extension of the infection inside the orbital cavity.

SGPP 41

Something is moving into my eye: Loiasis

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Case Report: A 14 y.o. Swiss girl came during the night to pediatric emergency with foreign body sensation, eye itching, visual disturbances and feeling something moving inside of her left eye. A first consultation, in the afternoon, at a medical center concluded at a non-specific eye infection and topical antibiotic with steroids were prescribed. Our physical examination discovered a white worm moving behind the conjunctiva. Medical history revealed a 3 weeks stay during holidays in Cameroon (her dad's country of origin), 7 years ago. Clinically, diagnosis of Loa loa infection (loiasis) was made. The eosinophilic count was normal. Serology and specific search of microfilariae (blood smear) were negative. Patient was referred to an ophthalmologist who saw no worm to extract, and to tropical medicine clinic. She was treated for 3 weeks with Diethylcarbamazine (DEC). The worm spontaneously disappeared from the eye two days after the first consultation and was never seen again.
Discussion: Loiasis, also known as African eye worm, is caused by the nematode filaria Loa loa. The affection is transmitted by the day bite of a Chrysops fly wich lives in rainforest of west and central Africa. More than 10 millions of people are estimated to be infected. In some regions, prevalence of infection exceeds 40%. The disease is rare in travelers. Adult worm lives in subcutaneous tissue and produces microfilaria, which reach the blood stream. Adult worms can survive more than 20 years into the body. Most patients are asymptomatic. Cardinal clinical manifestations are due to adult worm migration: Calabar swelling (transient localized subcutaneous swellings), eye itching, visual disturbances. Visualizing organisms (migrating adult worm in the subcutaneous tissue or conjunctiva) or detecting microfilariae in a blood smear establish the diagnosis. Eosinophilia is often associated. First line treatment is DEC. Rouling out onchocerciasis infection and quantification of blood microfilaria is recommended before treatment initiation as serious side effect like encephalitis and coma have been reported in patients with coinfection or high microfilariae count. In those cases, pre-treatment with albendazole or ivermectin has to be considered. Relapses are frequent.
Conclusion: Loiasis is an unusual ocular disease, which is rare outside Africa, but can occur years after exposure. Loa loa infection should be considered in atypical visual symptoms long after stay in Africa.

SGPP 42

Lumbar pain not always urinary tract infection: a case of sacro-iliitis

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Case report: A previously healthy 13 y.o. girl presented with unilateral low back pain with fever. Symptoms began 3 days earlier, with pain located in the right flank, worsened by movement and orthostatism. Pain was associated with fatigue and headache one week ago. Because fever became associated with chills and vomiting, she came to hospital. Laboratory findings were unremarkable and urinalysis showed leucocytes and blood. Pyelonephritis was suspected and ambulatory oral antibiotic (cephalosporine) initiated pending results of urine culture. Ultrasonography of urinary tract and ovaries was normal. The day of admission, temperature was 40 °C and back pain persistant. Hematological parameters were: WBC 12.8 G/L (immature neutrophils 10%), CRP: 190 mg/L. The CT scan was not conclusive, but MRI revealed a sacro-iliitis. Parenteral antibiotic (amox.-clavulanate) was given. Blood cultures grew Staphyloccocus aureus, and therapy was changed to flucloxacine. Analgesia was achieved with paracetamol, ibuprofen, and morphine. Additional work-up with cardiac ultrasonography excluded endocarditis and immunological tests were normal (ANA <160 1/DIL, HLAB27: negative). After 15 days of iv therapy, antibiotics (sulfaméthoxazole + triméthoprime) were given orally for 4 more weeks.
Discussion: Pyogenic sacroiliitis is particularly rare in paediatrics (~1–2% of osteoarticular infections). Staphyloccocus aureus is the most frequent pathogen. Clinical presentation of septic sacro-iliitis is variable and may include nonspecific signs such as febrile back pain and fatigue. This makes diagnosis challenging and explains the delay in diagnosis. Laboratory tests may reveal inflammatory syndrome. MRI is the most reliable imaging modality for the diagnosis.
Conclusion: Even though clinical septic sacro-iliitis is uncommon in infants and children, it should be suspected in children with an acute onset of low back pain and fever. MRI is the radiologic exam to perform for diagnosis.

SGPP 43

Anomalous origin of the Left Coronary Artery from the Pulmonary Artery (ALCAPA) – rare differential diagnosis of failure to thrive in children

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Introduction: Failure to thrive and malnutrition is a common problem in infants and children. Heart failure and congenital heart disease are rarely found, but is an important differential diagnosis. Anomalous left coronary artery from the pulmonary artery (ALCAPA syndrome or Bland-White-Garland syndrome), is a rare congenital cardiac disease that affects 1 in 300'000 live births and can cause myocardial infarction, heart failure and even sudden cardiac death in children. Tachypnea, dyspnea and failure to thrive can be the first symptoms in neonates and infants.
Case report: A 4-month-old female girl was admitted to the paediatric ward with failure to thrive. The mother reported inappropriate weight loss over the last 3 weeks, decreased activity and intermittent vomiting. The clinical examination revealed discrete tachypnea (40–50/min) and no other pathological findings. Results of laboratory tests, including complete blood count, CRP, electrolytes and tests of liver- and kidney- function, as well as urine analysis, were normal. The chest x-ray demonstrated a distinct cardiomegaly with signs of a pulmonary edema. Echocardiography showed a dysfunctional and dilated left ventricle (LV) with low ejection fraction, a severe mitral regurgitation and an anomalous left coronary artery arising from the pulmonary artery. The girl underwent surgical correction in Zurich, (reimplantation of the LCA to the aortic root, mitral valve reconstruction, Patch plastic for the pulmonary artery). Due to already severe impaired LV function and very high catecholamine support after weaning from cardiopulmonary bypass, a left ventricular assist device (LVAD) was implanted as bridge to myocardial recovery. Within the following days echocardiographic signs of recovery were seen and the LVAD was successfully explanted after 6 days on support. The infant was discharged after 25 days with medical heart failure therapy including beta-blocker, ACE inhibitor and Spironolactone. The ventricular function continued to recover and is 1 year after correction almost normal.
Conclusion: Even if failure to thrive and breast feeding problems is often not due to an underlying organic reason, in rare cases they can be the first symptoms of a life-threatening congenital heart disease such as an ALCAPA syndrome. Early diagnosis and correction has a strong impact on heart function and outcome. Short term support using LVADs are increasingly used if LV function is severely impaired.

SGPP 44

A rare manifestation of Lyme disease

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Complete heart block is a total conduction block through the atrioventricular (AV) node resulting in AV dissociation with bradycardia. It is uncommon in childhood, so a high degree of suspicion is required, especially when occurring in healthy children. Symptoms include syncope, dizziness, diminished exercise tolerance and severe fatigue. Differential diagnosis of third-degree AV block in children should include Lyme carditis, myocarditis, inflammatory disease, e.g. systemic lupus erythematodes, and congenital heart block as a diagnosis of exclusion. Here we show the case of an adolescent presenting with third-degree AV block due to Lyme carditis. A 14-year-old boy presented with fatigue and episodes of blurred vision going on for three days, as well as dyspnea on exertion since the previous day. He denied fever and any other infectious symptoms. Physical examination revealed no abnormalities despite regular bradycardia with a heart rate of 40 bpm. Because of this finding, an ECG was performed showing third-degree AV block. Laboratory tests revealed slight elevation of troponine T (18.6 ng/l) and significant elevation of NT pro-BNP (1658 pg/ml). Chest radiograph as well as echocardiography showed no abnormalities. The patient's history was reassessed and revealed a tick bite one year before and the participation in a scout camp one month before the onset of symptoms. The hypothesis for the cause of the complete heart block was therefore Lyme carditis and treatment with IV Ceftriaxone was begun. Positive serology for Borrelia burgdorferi confirmed the diagnosis. The clinical course was favorable with rapid response to treatment. Repeated ECGs showed second-degree AV block type Mobitz II after two days, and first-degree AV block after three days of antibiotic therapy. Ceftriaxone was then switched to oral Doxycycline for a total duration of 21 days. ECG and Holter ECG at the end of the therapy were normal. This case illustrates that complete heart block can be a rare cause of fatigue and can be the only manifestation of Lyme disease. Performing an ECG is therefore important in patients with symptomatic bradycardia. In case of third-degree AV block, high suspicion should be raised for Lyme disease as this is endemic in Switzerland and Lyme carditis is rapidly reversible with antibiotic treatment. We suggest beginning treatment immediately if the patient's history is compatible with Lyme disease, even before confirming the diagnosis with Borrelia serology.

SGPP 45

How a drunkenness can positively change life

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Introduction: Early diagnosis of coarctation of the aorta in children is historically difficult. Though prenatal diagnosis has made progress in the early detection of heart diseases, coarctation of the aorta remains difficult to be diagnosed in infancy. Whereas late diagnosis is associated with high morbidity and mortality. We present the case of an adolescent brought in by ambulance to our pediatric emergency because of reduced level of consciousness. Incidental finding on brain scan led to the diagnosis of coarctation of the aorta, which could be treated quickly and without complications by endovascular intervention, preventing the long-term sequelae of a missed diagnosis.
Clinical case: A 14-year-old boy presented to the emergency department with suspected acute alcohol intoxication. Because of the reduced general condition, the unconsciousness (Glasgow Coma Scale 7) and the lack of information about the happenings, a CT scan of the brain was performed to rule out cerebral bleeding or consequences of a trauma. As incidental finding the radiologist reported signs indicative for a coarctation of the aorta as collateralisation of the intercostal arteries, prominent mammarian artery and brachiocephalic trunk. Compatible with this, there was a history of chronic leg cramps on exertion. Cardiovascular examination revealed a 2/6 systolic murmur and a blood pressure difference of 20 mm Hg between the upper and lower extremities. Chest X-ray revealed rib-notching of the lower surface of the third left rib. Subsequent echocardiography and MRI confirmed the diagnosis: a high-grade coarctation of the aorta, a moderate hypoplasia of the aortic arch with extensive collaterals. The patient underwent an uncomplicated endovascular aortic stent graft and is doing well.
Conclusion: Unexpected findings on imaging studies may reveal more or less relevant pathologies. In our case, the CCT performed in a comatose drunk adolescent allowed to discover an hitherto undiagnosed and serious cardiovascular disease.

SGPP 46

When enough is not enough: does the dosing of adenosine have to be adapted to the intraosseous route?

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Introduction: Supraventricular tachycardia (SVT) is the most common tachyarrhythmia in the pediatric population, requiring immediate treatment. Adenosine is the drug of choice and should be administered through intravenous (IV) access. Intraosseous (IO) access is a rapid and safe alternative for unsuccessful IV access, but the efficacy of this route for the administration of adenosine is poorly established. We present a case where IO adenosine infusion was successful.
Case Report: A full-term newborn from a drug addict mother was admitted to our neonatal care unit for withdrawal syndrome. He presented a first episode of SVT, which converted to normal sinus rhythm when exposed to sudden bright light. A few days later, the SVT recurred with a heart rate of 300 bpm, which was hemodynamically well tolerated. Vagal maneuvers were unsuccessful. After multiple attempts of IV line placement, an IO access was established and a first bolus of adenosine, 0.1 mg/kg, was administered with no effect. Subsequent doses of adenosine with increasing dosage were given. It was only at a dosage of 0.4 mg/kg of adenosine that conversion to sinus rhythm was observed, 15 seconds after injection. After this episode, the patient was started on propranolol, on recommendation of the pediatric cardiologist.
Discussion: When vagal maneuvers are unsuccessful in converting SVT, and the patient is hemodynamically stable, adenosine is indicated. Rapidly metabolized by cell enzymes, adenosine has a half-life of 10–15 seconds and an effect is expected in 20 seconds. Recommendation is the administration in a proximal vein as a rapid bolus followed by a saline flush. Few cases report IO administration and only one describes efficacy with 0.1 mg/kg/dose. The administration of 0.4 mg/kg as in our case, is higher than usually recommended, but remains still in the safe range. We postulate that the injection in a relatively distant site and in a highly cellular environment (bone marrow) explains the need for a higher than usual dosage.
Conclusion: SVT is an emergency requiring immediate treatment. When IV access in unavailable and the patient is hemodynamically stable, IO route may be an alternative way to administrate adenosine. The optimal dosage for this route of administration has yet to be established. Further research is needed to establish the optimal dosage of adenosine for IO administration. Hemodynamically unstable patients however should undergo electrical cardioversion without delay.

SGPP 47

Hereditary hemorrhagic telangiectasia – evolving symptoms in a familial rare disease

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Introduction: Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant disorder with a prevalence of 1:5000–1:8000. Clinical manifestations are mostly absent at birth, but usually evolve with increasing age. First symptom during childhood often is epistaxis, followed by symptoms of pulmonary arteriovenous malformations (PAVMs) in 70% of all patients by the age of 16.
Case report: We report a case of a father with proven HHT and his three children (a boy of 16 (A), a girl of 13 (B), and girl of 11 (C) years). The father became symptomatic with an ischemic stroke most likely due to paradoxical thrombus embolism via PAVMs at the age of 46. Further clinical signs as repetitive epistaxis and mucocutaneous telangiectasia led to the diagnosis of HHT. Similar symptoms in a paternal uncle prompted the parents to present their three asymptomatic children to evaluate their risk for HHT.

Echocardiography, oxygen saturation level (also on exertion) and abdominal ultrasound showed normal findings in all children. A thoracic CT scan revealed in A a PAVM of 2 cm in the posterior left upper lobe and in B a PAVM in 0.8 cm in the superior left lower lobe. As diagnosis of PAVM was made by CT, we abstained from doing an echocardiographic bubble study. Preventive transcatheter coil embolisation of the PAVM in A was performed. Since PAVM in B still was tiny with no desaturation at exertion, no intervention was done at that time. At follow up 4 years later, A and B still were clinically asymptomatic with normal saturations, but both had a pathological bubble test suspicious of a new PAVM. C showed epistaxis as the only clinical finding of HHT. Genetic testing in A confirmed the clinical diagnosis of HHT with a typical mutation in the Endoglin gene. Genetic testing of child B and C is pending.
Conclusion: Diagnostic criteria for HHT include epistaxis, mucocutaneous telangiectasia, visceral involvement (PAVMs, cerebral, hepatic or gastrointestinal arteriovenous malformations) and/or a first degree relative with HHT. According to our case, symptoms can evolve over time. The bubble test is a convenient non-invasive method to detect arteriovenous malformations, though it remains difficult to define relevant findings which possibly will need coil closure or surgical resection. Importance of genetic testing within a family with affected members is to identify unaffected members to avoid unnecessary investigations.

SGPP 48

Unexpected diagnosis of an ALCAPA-Syndrome in an 8-day-old newborn presenting an ALTE at the emergency unit

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Introduction: We report on a newborn with a new diagnosis of an ALCAPA-Syndrome who was admitted to our emergency department after an apparent life threatening event (ALTE). The anomalous origin of the left coronary artery from the right pulmonary artery (ALCAPA) is a rare congenital malformation with an incidence of 0.25% to 0.5% of all congenital cardiac malformations.
Case Report: An 8-day-old newborn was admitted to our emergency unit after he had been bottle-fed for the first time. During drinking he manifested perioral cyanosis, a pale-greyish integument and bradypnea. This episode lasted for 2–3 minutes. During examination the patient was in an inconspicuous condition with no signs of heart-failure. Because of a marked right axis deviation and signs of left ventricular hypertrophy in the ECG an echography was performed. To our surprise the test revealed an ALCAPA syndrome with a distinctive retrograde flow and dilatation of the anterior interventricular branch of left coronary artery. A surgical correction was successfully performed and regular controls showed a favorable development of the patient.
Discussion: Typically, children suffering from ALCAPA syndrome present signs of severe heart failure like dyspnea, pallor, failure to thrive or profuse sweating in the first weeks or months of life. If left untreated up to 90% of the patients die within the first year. Our patient did not show any signs of heart failure during rest. The marked right axis deviation in the ECG rose suspicion of right ventricular hypertrophy, so echocardiography was performed. Surprisingly, we detected an ALCAPA syndrome that is typically associated with marked Q-Waves and ST-Changes as signs of myocardial ischemia, which the patient's ECG did not show. Right axis deviation is not a sign of ALCAPA syndrome. ALTE is a common cause for children admitted to the emergency unit. Incidence is reported to be 0.6 to 2.46 per 1000 live births. In 35% to 50% of ALTE no conclusive cause can be determined. The remaining 65–50% can be attributed to gastrointestinal (50%), neurological (30%), respiratory (20%), cardiovascular (5%) and other causes.
Conclusion: This case report shows a potentially life-threatening cause of an ALTE and highlights the necessity of thorough anamnestic evaluation and diagnostics to identify rare causes and guarantee an appropriate treatment. In our patient, the quick diagnosis ensured a good outcome and prevented subsequent damage such as severe heart failure.

SGPP 49

Rectal bleeding in neonates due to Campylobacter enteritis: 2 case reports and review of the literature

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In newborns, bacterial enteritis is not a common cause for hematochezia. Especially in an otherwise asymptomatic patient, blood in the stool is often interpreted as CMP intolerance leading to unnecessary prolonged dietary changes. We present two cases of hematochezia due to campylobacter enteritis in the first month of age and because Campylobacter enteritis in neonates is rare, we reviewed the literature to collect additional data and propose an exhaustive analysis of all published cases to date. This literature review confirms the self-limited nature of campylobacter infection in newborns. In otherwise well appearing infant empirical symptomatic treatment is reasonable. Hematochezia can be the only symptom of bacterial enteritis and children often undergo dietary changes to exclude CMP without further testing leading to prolonged and sometimes unnecessary CMP exclusion from the diet. Therefore, we recommend stool cultures in neonatal hematochezia to rule out infectious causes and shorter CMP free diet.

SGPP 50

Trimethylaminuria – the fish odor syndrome: two cases and review of the literature

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Introduction: Trimethylaminuria (TMAU) is an autosomal recessive metabolic disorder, caused by a deficit in the enzymatic activity of FMO3 (Flavin-containing monooxygenase 3). Accumulation and consequentially increased excretion of trimethylamine (volatile substance with typical fish odor) are consequences of the impairment of enzymatic activity. A fish like smell, is the only clinical feature as there are no dysmorphic features or other associated symptoms. TMAU is therefore also known as "fish odor syndrome" (FOS). We report two pediatric patients (aged 5 and 10 years, respectively) with TMAU.
Cases report: The first case is a 10-year-old-boy, who from his birth emanated a strong smell of fish associated with febrile episodes, intake of certain foods or physical activities. The medical history and clinical picture as well as laboratory tests suggest a Trimethylaminuria of genetic origin. The second case is a 5-year-old child, who presented within a few months of birth with a smell of fish. This occurred in particular after the ingestion of fish, or more commonly upon the intake of foods rich in choline and lecithin. The medical history and laboratory tests suggested a Trimethylaminuria from substrates overload.
Discussion: Both patients show an unpleasant body odor as their unique clinical characteristic, without any other dysmorphic features, symptoms or signs. Faced with this clinical situation it is imperative to consider rare metabolic diseases as possible differential diagnosis. Additionally, acquiring the correct diagnosis may provide somatic relief for the patient and parents from a psychosocial perspective. Integrating the information related to our two cases with the literature's guidelines we were able to build a diagnostic algorithm for this disease; from medical history up until the molecularly diagnostic tests.

SGPP 51

Improvement of gross motor skills in a GSD-IV patient after liver transplantation

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Glycogen storage disease type IV (GSD-IV) is an autosomal recessive disorder characterized by the accumulation of amylopectin-like structures in liver, brain, lymph nodes and heart, caused by mutations in the GBE1 gene (OMIM: 607839). While the clinical course of this

ultrarare disease (incidence 1:800'000) is heterogenous, this case report describes a male patient presenting with the classic hepatic form. At the age of 3 months, he was hospitalized with failure to thrive, hepatosplenomegaly and generalized muscular hypotonia. Initial metabolic and neurological laboratory workup revealed normal results, however biochemical signs of liver cirrhosis were noted. Repeated neurological exam showed delayed gross motor skills with stagnation at the age of 8 months and a persistent generalized hypotonia. Brain MRI was normal as was cardiac exam. Elevated biotinidase activity and abnormal bone marrow aspiration raised the suspicion of glycogen storage disease, and subsequently biopsies of liver tissue showed typical histological findings of GSD-IV. Final enzymatic and genetic confirmation were achieved at the age of 18 months. A multidisciplinary team recommended liver transplantation (LT), which is currently the only treatment option, even though outcome after LT in GSD-IV is controversial. LT was performed at the age of 21 months with 13 months follow up. Contrary to reported cases of cardiac death owing to amylopectin accumulation in the myocardium, echocardiography and ECG investigation showed no cardiac complications in spite of drug-induced hypertension. From a liver stand point, post operative course was remarkable for early acute rejection now resolved. Thorough post LT neurological assessment showed: 1) rapid progress in development of gross motor skills, achieving a Griffith sub score of 18.5 months at the chronological age of 30 months; and 2) normal development in all other areas of neurological development according to the patients' chronological age. While the long-term outcome of our patient must be monitored closely, short-term results are promising. To the best of our knowledge this is the only child in Switzerland with GSD-IV. It illustrates the diagnostic odyssey due to lack of specific non-invasive biomarkers and the feasibility and satisfactory outcomes of LT in this rare indication. GSD-IV should be considered when distinct clinical symptoms coincide with elevated biotinidase activity, and timely referral for LT may improve neurological outcomes.

SGPP 52

Assessment of skinfold thickness equations in estimating body composition in children with inflammatory bowel disease

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Aim: to assess the agreement of commonly used skinfold thickness equations to estimate percentage of Fat Mass (FM%) in pediatric patients with IBD, in comparison with dual energy X ray absorptiometry (DEXA).
Methods: twenty-one pediatric IBD patients were included: 11 females and 10 males; mean age for the entire group: 14.3 years, range 12–16 years, 16 with Crohn's disease, 5 with ulcerative colitis. The FM% was calculated using 6 established pediatric skinfold thickness equations and compared to the results obtained by DEXA. The statistical analysis was performed using Spearman's correlation, Lin's concordance correlation coefficient and corresponding 95% confidence interval, Bland-Altman's limits of agreement method, and the Bradley-Blackwood test.
Results: Correlation between skinfold and DEXA values ranged between 0.85 (Deurenberg) and 0.92 (Durnin & Rahaman and Johnston), all p <0.001. Lin's concordance correlation coefficients and (95% confidence interval) ranged between 0.702 (0.512–0.891) for the Deurenberg equation and 0.876 (0.779–0.972) for the Brook equation. Average differences between skinfold and DEXA values ranged between –3.6% (Deurenberg) and 2.5% (Weststrate). Bland- Altman limits of agreement were wide, spanning over 10%. Finally, the Bradley-Blackwood test of equality of means and variances was significant in all but the Durnin & Rahaman equation.
Conclusion: In adolescents with IBD, fat mass calculated from six skinfold thickness equations showed good correlation but poor agreement with reference values from DEXA. Assessment of body composition using skinfold thickness equations cannot be recommended in pediatric patients with IBD.

SGPP 53

Microgastria – a rare cause of recurrent respiratory tract infections, vomiting and failure to thrive in infancy

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Introduction: Congenital microgastria is a very rare malformation caused by an interruption of the forearm development in the embryonic period. Characteristics of this anomaly are a hypoplastic tubular stomach with abnormal function and megaesophagus. Due to gastric hypoplasia, the ability to store food is diminished. Moreover, the absence of a gastric fundus lacks any antireflux mechanism. This can lead to recurrent vomiting, failure to thrive, aspiration pneumonia and esophageal ulceration. Microgastria has been usually associated with a variety of additional malformations like malrotation, esophageal or duodenal atresia, asplenia or renal, cardiac and skeletal anomalies.
Case report: A 7-month-old boy was referred to our gastroenterological service due to failure to thrive. Till the age of 3 months he was fully breastfed. Due to a lack of weight gain formula milk was added first and complementary food was introduced at the age of 5 months. From the beginning he showed very little interest in food and ate only very small portions. Clinical work-up did not reveal malabsorption, or any organ dysfunction, therefore hypocaloric nutrition was most likely. In addition he has been suffering from recurrent respiratory tract infections since the age of 3 months and was treated once with antibiotics for pneumonia. Because of persistent cough and recurrent vomiting, an upper gastrointestinal contrast study was performed which revealed microgastria and intestinal nonrotation. Except for a pelvic ectopy of both kidneys no associated malformations were detected. As feeding problem persisted, a laparoscopic hiatoplasty and fundopexy were performed at the age of 2.5 years. Postoperative course was uneventful and our patient was able to tolerate oral feeding without symptoms of gastroesophageal reflux. At follow-up 7 months after surgical intervention, he presented with normal weight gain and no further respiratory infections occurred.
Conclusion: In children with failure to thrive, signs of gastroesophageal reflux and recurrent respiratory infections, radiological work-up of the GI-tract is essential. In the case of microgastria, surgical intervention can lead to complete resolution of symptoms.

SGPP 54

Intense epigastralgia in an adolescent boy revealing complicated acute cholelithiasis

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Case report: A 15 y.o. boy was evaluated for severe abdominal pain located in the upper abdomen, evolving for a week. Pain was associated with vomiting and secondarily with fever. Body weight (68 kg) was normal, there was no family history of gallstones, no toxic ingestion. Patient admitted, however, frequent alcohol drinking in the past weeks. Omeprazol treatment initiated few days earlier brought no relief. As symptoms worsened, he consulted our ED. Physical examination revealed altered general status, hyperalgic and agitated patient with tender epigastrium on deep palpation. Murphy sign was negative. Biochemical investigations showed raised pancreatic enzymes (Lipase 5830 U/L [N <60]; Amylase 1610 U/L [N <110]) and liver function (LF) tests (ALAT 4647 U/L, ASAT 441 U/L, GGT 274 U/L, bilirubin 148 µmol/L). Abdominal sonography showed cholelithiasis. Cholangio-MRI (MRCP) showed biliary pancreatitis with non dilated intra or extra hepatic ducts. Endosonography don't show any stone in the common bile duct (CBD). He was fasted, received IV drip and antalgia. Prognosis was complicated by the occurrence of acute respiratory insufficiency with bilateral pleural effusion requiring intubation, and endocrine pancreatic insufficiency with diabetes. Etiological screening remained unremarkable. Genetic study of the ABCB4 gene was not performed. Management consisted of insuline therapy, and elective cholescystectmy 2 months post acute phase.
Discussion: Cholelithiasis is rare in children (prevalence ~2%). Most cases are asymptomatic, but some may present with typical bilary colics or non specific abdominal symptoms. Complications include pancreatitis, cholecystitis or cholangitis. Abdominal sonography is first line diagnostic tool. MRCP is useful to rule out anomalies of biliary tree. Pancreatic and LF tests may be altered in some cases. Differential diagnosis include hemolytic, metabolic, and hormonal

disorders, cystic fibrosis, ileal disease, congenital biliary or hepatic disease, drugs, prolonged parenteral nutrition, aminoacidopathy, hypercholesterolemia, obesity. Cases of ABCB4 gene mutations have also been reported. Therapeutic options are cholecystectomy or ERCP (Endoscopic Retrograde Cholangiopancreatography).
Conclusion: Cholelithisiasis must be considered in children with upper abdominal pain. High level of suspicion and sonography often lead to diagnosis. Close monitoring of diagnosed cases is mandatory as serious complications may occur.

SGPP 55

Technical difficulties in management of neonatal diabetes mellitus in the preterm infant

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To date little data has been published on treatment of neonatal diabetes mellitus (NDM) in premature infants, whether transient neonatal diabetes mellitus (TNDM) or monogenic permanent neonatal diabetes mellitus (PNDM). Initial treatment usually consists of continuous insulin application. If a genetic mutation is detected in genes responsible for potassium channels (e.g. KCNJ11 and ABCC8) treatment may be switched to oral sulfonylurea, overlapping with continuous insulin application. Particularly the technical management of NDM in the preterm infant is heavily underrepresented in current literature. We present the case of a female preterm infant born at 36 3/7 weeks gestational age with severe symmetrical IUGR and a birth weight of 1600 g (P <1). On day 8 she developed clinical late onset sepsis and was treated with amoxicillin/clavulanate and gentamycin, blood cultures came back positive for staph. epidermidis. Initial presentation of hyperglycemia was assumed to be caused by sepsis, therefore insulin was administered intravenously. Subsequently we observed persistent hyperglycemia, leading us to suspect NDM. Insulin infusion was therefore started again before switching to continuous subcutaneous insulin infusion (CSII) on day 20. Basal rate was adjusted for blood glucose levels, meal boluses were not administered. Genetic testing for mutations in KCNJ11, ABCC8 and INS genes was carried out at Exeter Molecular Genetics Laboratory, no mutation was found. Insulin requirement was continuously reduced and CSII was discontinued on day of life 34. When switching from i.v. insulin to CSII we encountered various technical difficulties in administering an adequate dose of Novorapid® preventing hypoglycemia. Firstly, Insulin U100 had to be diluted to U10 in order to maintain appropriate flow rate in the catheter. Secondly, all subcutaneous devices readily available are decidedly large when used on an infant weighing under 2000 g. Thirdly, localization of sufficient subcutaneous fatty tissue in a severely IUGR infant posed a major complication, inducing stress for the infant and its parents. Last but not least flow rate on the Medtronic Minimed 640G pump could not be switched to U10 concentration, the displayed dose had to be calculated separately and dilution and stability of the insulin was critical.
Conclusions: When treating severely IUGR and/or premature infants with CSII modifications should be made according to patient size and weight.

SGPP 56

Causes of neonatal death in a Swiss perinatal centre

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Background: Understanding the causes of neonatal death in a perinatal centre is an important step in guiding clinical efforts and in setting a focus in prevention.
Methods: This is a retrospective study performed by analysis of the patients' medical history. All liveborn newborns from 24 0/7 weeks of gestation born at the University Hospital Zurich between July 2009 and June 2014 and who died in the first three months of life were included. We analysed the causes of death, particularly related to the timing of death and the gestational age. All data were treated anonymously.
Results: Within the five years period, a total of 179 newborns were born alive and died during the first three months of life. 92 (51.4%) of these infants were born extremely preterm with a gestational age between 24 0/7 and 27 6/7 weeks. The most common cause of death was found to be congenital malformations (52; 29.1%). Further deaths were attributed to respiratory distress (34; 19.0%), neurological pathologies (29; 16.2%), immaturity (28; 15.6%) and infections (21; 11.7%). Overall 52 newborns (29.1%) died within the first two hours after birth. In 43 newborns (24.0%), palliative care was decided antenatally, together with the parents, mainly because of extreme immaturity or severe congenital malformations.
Conclusions: We found the most common cause of death to be malformations, where medical efforts have few influence on. Focus should be set on the further causes of death like infections or management of respiratory distress, as well as on the prevention of extreme prematurity.

SGPP 58

Fetal akinesia as a diagnostic challenge

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Introduction: We report our management of a newborn with fetal akinesia, severe hypotonia, muscle weakness and congenital chylothorax.
Case Description: The infant was born to healthy parents by cesarean section at 30 weeks of gestation. There was no consanguinity. Family history was unremarkable besides of a sister, who died due to asphyxia and had a mutation of Exon5CFHR4, possibly responsible for various disease but also known to have no relevance. The older brother is healthy. Pregnancy was complicated with decreased fetal movements, polyhydramnion, pleural effusion, edema and the same heterozygote mutation (Exon5CFHR4). Apgar score were 4-3-4 at 1, 5, and 10 minutes respectively. At birth, the baby had muscular hypotonia and showed no respiratory effort, so intubation was required. Weight, crown-heel-length and head circumference were normal. The first clinical examination showed missing vigilance, absence of active movements, absent reflexes, arthrogryposis, and dysmorphic features with expressionless triangular low-set ears, an inverted v-shaped upper lip and bilateral cryptorchidism. The infant remained intubated and ventilated. Extubation was failed trice. Further course was complicated by a chylothorax managed by chest tube drainage and fat free nutrition. At present the infant is 3 months old and still hospitalized. Regarding neuromuscular diseases, we excluded central causes of muscular hypotonia by brain MRI showing no signs of hypoxic ischemic encephalopathy and whole-exome sequencing showing no signs for chromosomal disorders, except the mentioned mutation. Concerning peripheral causes, genetic and laboratory investigations did not verify anterior horn cell disease, neuromuscular junction disease [transient/congenital neonatal myasthenia], or metabolic disease. Normal electromyography and nerve conduction velocity allowed us to rule out congenital muscular dystrophies, metabolic myopathies and congenital myopathies. Thus, the etiology of the symptoms presented is still unclear.
Conclusion: Fetal akinesia syndrome may be the leading symptom for abnormalities of the central nervous system, neuromuscular diseases or genetic disorders. Identifying the specific etiology is challenging, but can be crucial for both treatment and prognosis. In approximately half of affected individuals a diagnosis cannot be found in the newborn period.

SGPP 59

"Chaotic arrhythmia" during successful resuscitation after ingestion of yew (Taxus baccata) needles

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Objective: To describe the management of a case of life-threatening yew (Taxus baccata) intoxication.
Background: The needles of the yew tree contain highly cardiotoxic taxines. Intoxication with taxines, typically as part of suicide attempts, may lead to potentially lethal arrhythmias which often require prolonged cardiopulmonary resuscitation and other supportive measures. No specific therapy has been described. In some cases extracorporeal life support (ECLS) has been used.
Case report: After an attempted suicide with yew needles and out-of-hospital cardiac arrest a female adolescent was resuscitated for 6 hours according to Advanced Cardiovascular Life Support (ACLS) guidelines. Complex ventricular tachycardias were treated by repeated DC shocks and broad complex bradycardia managed with transvenous

cardiac pacing. Antiarrhythmic drugs (amiodarone, lidocaine), magnesiumsulfate and supportive measures (intravenous lipids, sodium bicarbonate) were provided. The arrhythmias finally resolved and the patient did not show any significant neurological or cardiac short-term sequelae after 24 h.
Results: We describe the successful management of a case of severe taxine intoxication by prolonged conventional advanced cardiac life support lasting over 6 hours.
Conclusions: In life-threatening yew intoxication prolonged cardiopulmonary resuscitation is absolutely essential due the long duration of the cardiotoxic action of taxines and can lead to an outcome without cardiac or neurological sequelae.

SGPP 60

Neonatal tooth – clinical presentation and management

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The presence of a tooth in a newborn or in the first month of life is a rare phenomenon. A neonatal teeth is a tooth who appears in the first month of life while a natal tooth is present at birth. Neonatal teeth are three times less common that natal teeth. In our case report we will present a newborn who had an alteration of the mucous membrane at the upper dental ridge. It was present directly after birth. We will show the clinical course, our management with the differential diagnosis and give an overview of neonatal teeth in the literature.

SGPP 61

Unexpected genetic findings in a female newborn with severe IUGR and epidermiolysis bullosa (EB)

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Introduction: Intrauterine growth reduction (IUGR) is a common reason for premature birth. In most of the cases prenatal investigations such as TORCH serology, sonomorphologic screenings and genetic evaluations are performed, respectively. Sometimes no further pathologic findings can be detected while the newborn's development and growth is unsuspicious. Besides, there are genetic findings with different clinical manifestations after birth not necessarily congruent with prenatal results.
Results: The 28-year-old healthy primipara was send to our hospital at the 31th week of pregnancy especially with distinct discrepancy of growth as well as sonomorphologic abnormalities (bulky stomach, conspicious legs with discrepancy between thigh and lower leg). The TORCH panel showed normal results. An amniocentesis was performed detecting low levels (12–15%) of a mosaic trisomy 2, karyotype 47, XX +2 (2)/ 46, XX (38). Due to nearly arrest of growth together with increasing polyhydramnion labor was induced in the 37th week of pregnancy and the child was born via spontaneous birth. APGAR 2/5/8, weight 1545 g (P <1, >3 SD), length 42 cm (P <1, >2.5 SD), head circumference 30.1 cm (P <1, >3 SD). Clinical examination showed a female newborn with several extensive skin lesions. Besides, hypoplastic extremities and dysplastic auricles on both sides could be detected. The suspected diagnosis of a Junctional epidermolysis bullosa with pyloric atresia could be confirmed by genetic findings (Integrin a6 gene defect). Furthermore we detected a maternal heterozygote mutation of the ITGA6 gene while the father was healthy. Additional genetic investigations had been performed revealing a uniparenteral disomy of the ITGA6 gene as cause of the letal homozygosity in the newborn child.
Discussion: Full trisomy 2 as well as high-level mosaicism would lead to spontaneous miscarriages or severe fetal malformations. Due to a very rare event of trisomy rescue a uniparental disomy can lead to the manifestation of a recessive condition in case of mutation transmission by only one parent. This case demonstrates uniparental disomy 2 as cause for a severe form of fatal junctional epidermolysis bullosa.

SGPP 62

Hb-F Sarajevo, a reason for low oxygen saturation and neonatal cyanosis – a case report

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Clinical presentation: This male infant was born at 41 0/7 weeks of gestation to a healthy 24-year-old G1P1 woman by vaginal delivery. The pregnancy was unremarkable and the infant adapted well with normal Apgar scores. Arterial and venous umbilical cord pH values were 7.28 and 7.38. The infant's birth weight was 3570 g (P75-90). One hour following delivery, the infant developed respiratory distress with a SpO_2 of 86% while breathing room air. No evidence was found for an infectious, pulmonary or cardiac cause, however, oxygen requirement to maintain a normal saturation (FiO_2 45%) persisted. A marked discrepancy between the SpO_2 and the pO_2, measured in arterial and capillary blood samples was noted. We therefore suspected a hemoglobinopathy and DNA-sequencing revealed a heterozygote mutation in the g-globin gene, called Hb-F Sarajevo. As the infant showed no clinical symptoms apart from a discrete intermittent cyanosis of the lips, the oxygen therapy was stopped and the infant was discharged home on the 23rd day of life.
Discussion: This case demonstrates the importance of including hemoglobinopathies in the differential diagnosis of low oxygen saturation. Hemoglobinopathies are hereditary disorders of globin chain synthesis. Structural changes in the hemoglobin protein can cause instability of the protein or change the oxygen affinity. The new hemoglobin could also have a new absorption spectra for light absorbance leading to spurious low SpO_2 measurements. We hypothesize a decreased oxygen affinity of the hemoglobin in this infant. Repeated measurements of the oxygen saturation by a peripheral arterial access with and without additional oxygen supply supported this hypothesis. To our knowledge, only two other cases with neonatal cyanosis and the Hb-F Sarajevo mutation have been described (2012 in Switzerland and 2016 in Slovenia) until now. Both infants were born at term and presented with severe cyanosis with SpO_2 measurements of 74% and 72% but without signs of tissue hypoxia. At the age of 6 months as with our patient, the SpO_2 was normal in both children.
Conclusion: A hemoglobinopathy should be suspected in a clinically asymptomatic child with a low SpO_2 reading after underlying cardiac or pulmonary disease have been excluded. In hemoglobin variants, oximetry readings do not adequately reflect the oxygenation-carrying properties of arterial blood – abnormal hemoglobins affect the oxyhemoglobin dissociation curve as well as the absorption spectra.

SGPP 63

"You can only give warmth to your baby when it's too late": parental bonding with their extremely preterm and dying child

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Introduction: Various factors hamper the bonding process between parents and their extremely premature baby. Parental bonding is especially difficult and complicated when babies do not survive and die either in the delivery room or in the neonatal intensive care unit (NICU). This process is strongly compressed and complicated by several factors addressed in this study.
Methods: In order to explore how parents experience the dying process of their extremely premature babies, we conducted 13 qualitative semi-structured interviews with 20 parents. We recruited parents of extremely premature infants who were born alive and died in the delivery room or in the NICU at the University Hospital Zurich in the years 2013–2015.
Results: Our study on end-of-life decisions in extremely preterm babies shows that parents of this group experience a multitude of stressors due to the immediate separation after birth, the alienating setting of the intensive care unit (NICU), the physical distance to the child, medical uncertainties, and upcoming decisions. Even though they are considered to be parents (assigned parenthood), the child's frail condition prevents them acting as primary caregivers. Instead,

they depend on professional instructions for access and care. Embodied parenthood can be experienced only at the end-of-life, i.e., during the dying trajectory and after the child's death.
Conclusion: Our study illustrates that parents of extremely preterm babies suffered from unpreparedness of becoming parents: They were considered parents (assigned parenthood) with the birth of their baby, but to actually feel like parents they needed to go through a process of biological and psychosocial bonding. This process of embodiment between child and parents through holding, touching, smelling, caring, and protecting could barely happen before dying because of the child's frail condition, which necessitated physical distance to enhance the chances of potential survival (distant parenthood). Thus, caring for their dying and deceased child enabled parents to become parents in an embodied sense.

SGPP 64

Distal humeral epiphyseal separation

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Distal humeral epiphyseal separation is a rare orthopedic condition in newborn age. Its diagnosis is challenging as it can be easily misinterpreted as an elbow dislocation, brachial plexus palsy or even overlooked. We report the case of a term newborn boy (3750 g, 92. centile) with a distal humeral epiphyseal separation and short-term outcome after surgical correction. Moderate swelling, painful and decreased movements of the right arm were observed since the first day of life (DOL). A traumatic nature of the symptoms was supposed but no delivery complication was reported. After radiographic (DOL 2) and subsequent ultrasound (DOL 4) exams as well as consultation with the pediatric orthopedist (DOL 4) the diagnosis of distal humeral epiphyseal separation was made. Given the displaced and acute nature of the fracture, closed reduction and percutaneous pinning was performed under arthrography. Postoperative clinical and radiographic follow-up examination showed early restoration of function and abundant bony healing, respectively. Despite very poor epidemiological data, distal humeral epiphyseal separation seems to be rare and associated with traumatic delivery with excessive traction and rotation of the forearm. Because of the skeletal immaturity in the newborn, it is mandatory to perform both radiographic and ultrasound assessments of a painful hypokinetic elbow in the newborn. This may allow early diagnosis and consequent treatment. While a consensus on the therapeutic approach is lacking, surgical reposition is indicated as the condition can lead to elbow cubitus varus deformity or elbow dysfunction.

SGPP 65

Too much of a good thing: a case report

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Neonatal hypernatremic dehydration is a rare clinical condition that can be life threatening. The few case reports reported in the literature are mainly of healthy newborns in the first week of life, associated with initial attempts of breast feeding. Early detection can be difficult because symptoms may be masked until neurological signs occur; features are often similar to those of sepsis. We report the case of a 24-day-old neonate who presented to the emergency department with fever, lethargy and signs of shock. An initial diagnosis of late-onset sepsis was made and treatment was immediately initiated with fluids and broad-spectrum antibiotics. Laboratory findings revealed severe metabolic acidosis with severe hypernatremia (Na+: 164 mmol/L). The patient was hypotensive with increasing drowsiness; he was admitted to the PICU, intubated and ventilated. Septic shock was ruled out by laboratory findings which confirmed a low value for C-reactive protein, Procalcitonine was negative as well as negative blood cultures. A careful history revealed that the hypernatremia was secondary to a feeding error, with concentrated infant formula beeing used to supplement breast milk. We report this case to raise awareness on this rare condition and to highlight the importance of early maternal support and follow-up visits of newborns soon after discharge to prevent neonatal hypernatremic dehydration.

SGPP 66

Reduction of the prevalence of necrotizing enterocolitis in preterm infants with the prophylactic administration of probiotics

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Background: Necrotizing enterocolitis (NEC) is the most common and most feared gastrointestinal illness in newborns. A correlation between the development of the disease with the altered microbial gut flora of preterm infants has been discussed. Therefore, in many neonatal units worldwide the prophylactic administration of probiotics to premature infants is common.
Objective: The effect of the prophylactic use of probiotics to reduce the prevalence of NEC in preterm infants with a gestational age (GA) of less than 32 0/7 weeks and/or a birth weight of less than 1'500 g was analyzed in the Department of Neonatology at the University Hospital Zurich. In addition, we searched for possible effects on the severity and the mortality of the disease and we analyzed whether the risk factors of the affected infants differed from the not-affected ones.
Methods: A retrospective cohort was compared to a historical control group. All infants with a GA of less than 32 0/7 weeks and/or a birth weight of less than 1'500 g were included. The infants in the study group received Infloran, which contains Lactobacillus acidophilus and Bifidobacterium infantis, usually from the first day of life twice a day for 14 days. The NEC diagnosis was made by means of the adapted Bell-criteria.
Results: The study included 1'054 infants, 573 in the control group and 481 in the study group. The prevalence of NEC was significantly lower in the study group (0.8% compared to 7.7%, 95% confidence interval 0.04–0.30). No adverse effects were observed. A reduction in the severity of the disease could not be detected, a decline in the mortality was suggested. There were no differences in the risk factors of the affected and the not-affected infants.
Conclusions: The prophylactic use of Infloran in preterm infants with a GA of less than 32 0/7 weeks and/or a birth weight of less than 1'500 g significantly reduced the prevalence of NEC. No significant effects on disease severity and mortality could be detected.

SGPP 67

Cervical spinal ischemia after minor head trauma

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Introduction: Spinal cord ischemia is rare. Etiology is heterogeneous and includes dissection, vasculitis, and embolic occlusion. Various cases of spinal cord ischemia following minor trauma in children have been reported. Hyperextension or flexion injuries may provoke a temporary occlusion of the vertebral arteries or the anterior spinal artery. Depending on the anatomical level and severity of spinal cord lesion, patients develop para-/tetraparesis or para-/tetraplegia, sensory deficits, and loss of bladder and bowel control within hours. Patients with sulcal artery syndrome typically develop hemiparesis.
Case report: A 9-year-old boy presented with headache, right sided nuchal pain, somnolence, involuntary loss of urine, and right sided hemiparesis without sensory deficit a few hours after hitting his head while playing football. CT scan of the brain and cervical spine was normal. MRI of the brain and cervical spinal cord including angiography, but without diffusion weighted (DW) imaging of the spinal cord, showed no abnormalities. Clinically, we suspected spinal cord ischemia and started a treatment with acetylsalicylic acid (ASA). During the next hours, the boy became tetraparetic and complained about vertigo. A MRI 36 hours after trauma, confirmed ischemia of the central spinal cord with restricted diffusion reaching from the medulla oblongata at the level of the foramen magnum to the second cervical vertebrae, signs of T2 demarcation, and a slightly reduced flow in the basilar artery without stenosis or dissection. Subsequently, we prescribed therapeutic anticoagulation with heparin for one week, followed again by prophylactic ASA. During the following days, neurological exam revealed incomplete C5 tetraparesis with neurogenic bladder and bowel dysfunction, up-beat nystagmus, and still no sensory loss. Our patient underwent an intensive rehabilitation program and luckily showed complete clinical recovery, which was confirmed 6 months later by a control MRI without residual lesions.
Conclusions: In patients presenting with pain, paralysis with or without sensory loss, and bladder or bowel dysfunction after minor head trauma, spinal cord ischemia should be considered. Since MR angiography can be normal if small arteries are affected or in transient vasospasm, DW-MRI, which is not routinely performed on spinal cord, should be obtained to rule out ischemia or infarction.

SGPP 68

A precocious neonatal pancytopenia revealing a cobalamin C deficiency

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A male newborn was born vaginally at 41+2 weeks of gestation after induced labour because of over-term. Intrauterine growth retardation (IUGR) was discovered over pregnancy. Mother serologies were negative as well as vagino-rectal specimen for group B streptococci. Neonatal adaptation was normal. The weight was 2840 gr (<3°p); the length 49 cm (3–10°p); the head circumference: 32 cm (<3°p). Due to the low birth weight periodic blood-glucose monitoring was performed, revealing a persistent hypoglicemia (2 mmol/l) that required parenteral glucose administration. At 18 hours of life the patient presented a global neurological deterioration. The C-reactive protein (CRP) was increased (39 mg/L) therefore an empirical intravenous antibiotic therapy was undertaken. Cytomegalovirus (CMV)-DNA on urine was negative as well as cerebral ultrasound scan. General condition improved and after 10 days the newborn was discharged. Blood culture was negative. Unfortunately the patient was readmitted at 18 days of life because of hypothermia (rectal temperature 34.4 °C) and poor general condition. CRP was once again increased (80 mg/L). A second cycle of empirical intravenous antibiotic therapy was started. The blood cell count revealed a pancytopenia (white blood cell: 1700/mmc; neutrophils: 390/mmc; platelet: 39000/mmc; haemoglobin: 91 g/L) that was interpreted as para-infectious. In a couple of days general clinical condition improved once again but pancytopenia, feeding difficulties and global hypotonia persisted. Viral serologies for Parvovirus B19, Epstein Barr Virus and CMV resulted negative. Abdominal ultrasound and ferritin levels were normal, therefore a macrophage activation syndrome was excluded. The patient developed polypnea that resulted to be compensatory of a progressive metabolic acidosis. A vitamin B12 deficiency or a metabolism disease was consequently suspected. Mother history was negative for chronic gastritis or vegan diet. The patient's Guthrie card was expanded to test other metabolic diseases. It was relieved that propionylcarnitine levels were elevated and methionine levels were reduced, confirming the suspect of a cobalamin disease. Intramuscular OH-cobalamin therapy was therefore started. The urine tests and the second Guthrie card revealed the final diagnosis of a cblC deficiency with an homozygotic c.271dupA mutation in the MMACHC gene. The patient is now 3 months old, and is following a specific neuro-developmental program.

SGPP 69

Neonatal brain arterio-venous malformation: a rare cause of early-onset congestive heart failure

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This full-term male neonate was born uneventfully by spontaneous delivery with normal Apgar scores. Routine fetal ultrasounds were normal. On day 3, he became hemodynamically and neurologically unstable, with tachypnea and increasing drowsiness. Physical examination showed a normal body temperature, tachypnea with a 3/6 systolic heart murmur, a hyperdynamic precordium, enlarged neck vessels, hepatosplenomegaly. Blood pressure in all four limbs and pre-post-ductal oxygen saturation were in the normal range. Laboratory findings ruled out infection and revealed hyperlactatemia with compensated metabolic acidosis. Chest radiography showed cardiomegaly. Echocardiogram revealed pulmonary hypertension due to overflow, tricuspid regurgitation with good biventricular functions, a closed DA (Ductus arteriosus), and ruled out coarctation of the aorta. Cranial ultrasound demonstrated a large anterior venous structure compatible with a high-flow frontal pial arteriovenous fistula (AVF), confirmed by MRI, with normal surrounding brain parenchyma. Careful physical examination revealed a loud, continuous high-pitch murmur over the cranial vault. Due to the hemodynamic situation, aggressive medical treatment of the cardiac failure was initiated, associating intubation for mechanical ventilation and continuous PGE1 infusion to allow reopening of the DA. After 24 hours the patient was hemodynamically stabilized and was urgently transferred to an expert center for endovascular embolization, which was safely performed on day 7. The AVF was partially reduced and the hemodynamics returned to normal. A successful second elective embolization was performed at seven months. Clinically, the child exhibits normal psychomotor development.This case illustrates a rare and unexpected non-cardiac cause of neonatal heart failure. Cranial auscultation should be a part of the neonatal physical examination. Being an extremely rare disorder, referral to an expert pediatric neurointerventional center is mandatory to ensure optimal outcome.

SGPP 70

Implementation of Pediatric Palliative Care (PPC) in a Neonatal Intensive Care Unit (NICU) in St.Gallen

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Introduction: The Children's Hospital of Eastern Switzerland (OKS) is a hospital with 76 beds. A multiprofessional PPC and ethic team was established in 2007. After nine years of intensive development our PPC program reached full size (notification D) of sanaCERT Suisse certification. Until presently, the OKS is the only children's hospital in Switzerland which is certified in PPC. Furthermore, our PPC team is a member of the Pediatric Palliative Care Network Switzerland (PPCNCH). In order to focus on neonates, the biggest group needing PPC, we decided to develop a special program for Neonatal Palliative Care (NPC).
Background: According to epidemiologic data, 400–500 children between the age of 0 and 18 years are dying in Switzerland each year. It`s known from the PELICAN study (Bergsträsser; Zimmermann et al., 2016), that about 50% of these children are dying in the first year of life, of which 40% in the neonatal period. Four out of five children are dying in an ICU, the majority of them after a decision-making process with the decision to withhold or withdraw further treatment. Apparently, the neonates represent an important group and therefore it is reasonable to concentrate on NPC in the NICU.
Case presentation: We present three neonatal patients suffering from severe, life threatening conditions: one newborn with trisomy 18, one premature of 32 week of gestation with a large intracerebral hemorrhage and a neonate with a life threatening conditions. The presentations demonstrate the difficulties and challenges and illustrate the importance of the involved teams networking in decision making and implementing care for these patients and their families under particular circumstances. The circumstances of death and the definitions of withholding or withdrawing therapy will be explained. Furthermore, the different requirements, discussions and the resources available in these cases will be presented. This indicates the possible improvements and developments in that area.
Conclusion: Neonates are an important group to consider in a PPC program. Considering that the neonatal period is a very special phase of life for the child and his/her family deserve particular consideration and structures in order to treat them adequately. Caring for a neonate and his family needing PPC is a challenging task for the family and the multiprofessional team. Clear structures and allocated resources are very important to fulfill this need in a meaningful way.

SGPP 71

Collodion Baby: a case of autosomal recessive congenital ichthyosis

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Case report: A term girl presented "collodion baby" aspect at birth. Her body was covered by a cellophane-like membrane. Skin was taut, shiny and translucent. Progressive epidermic detachment was observed leading to multiple areas of erythroderma. Extremities were oedematous. Dysmorphic signs were noted: ectropion, eclabium (fish-like mouth), low-set and poorly hemmed ears. Congenital ichthyosis was strongly suspected. Regular topical emollients were started and she was placed in a humidified incubator. Perfusion and nutrition by nasogastric tube were initiated. Antibiotics were started at 6 days of life due to positive blood cultures (Staphylococcus capitis), and anti-fungal treatment was added due to muco-cutaneous candidiasis. She was discharged home at 2 months of life, and topical treatment with emollients was continued. The clinical picture was consistent with a congenital ichthyosiform erythroderma of Brocq. Genetic testing revealed molecular biallelic heterozygous variant ALOX12B, in favour of autosomal recessive congenital ichthyosis (ARCI) type II.

Discussion: Congenital ichthyosis is a heterogeneous group of rare disorders of keratinization due to mutations in genes involved in skin barrier formation. ARCI refers to non-syndromic forms of ichthyosis, affecting only the skin and mainly includes harlequin ichthyosis (HI), lamellar ichthyosis and congenital ichthyosiform erythroderma. Symptoms are generalized scaling, hyperkeratosis and often erythema. Prevalence is around 1:200'000. Mutations in 9 different genes are associated with ARCI. Infants usually present at birth with a collodion membrane disappearing within the first four weeks of life, replaced by definitive ichthyosis phenotype. Dysfunction of skin barrier can lead to hypothermia, water loss and infections. With prompt admission in neonatal intensive care and supportive treatment, mortality has been considerably reduced, except for HI. Long term treatment is based on topical emollients (i.e petrolatum jelly). Systemic retinoids can be used in severe cases.
Conclusion: ARCI is a rare heterogeneous group of non-syndromic disorders of cornification. Management of collodion baby requires admission to a neonatal intensive unit to prevent and treat acute complications. Long term treatment is based on topical treatments aimed at skin hydration. Several mutations were found allowing accurate diagnosis and genetic counselling.

SGPP 72

Abnormal movements in a neonate: seizure or pain?

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Case report: A term newborn boy, with low Apgar score (3/5/6), presented presented with hyperreflexia and and hyperreactivity. At H1 of life, paroxystic episodes of apnea, bradycardia, and desaturation with tonic movements of the four limbs with jittering were noticed, suggesting neonatal seizures. Blood sugar level was 2.1 mmol/L and rapidly corrected. Paroxysmal tonic movements persisted despite phenobarbital, midazolam and phenytoin. Screening for infectious and metabolic disorders was negative. Three EEGs were normal, even during paroxystic episodes. Brain MRI showed biventricular hemorrhage without hydrocephalus. Dysautonomic symptoms (Harlequin phenomenon and transient pupillary asymmetry) led to the clinical diagnosis of Paroxysmal Extreme Pain Disorder (PEPD). Introduction of carbamazepine reduced the frequency of painful episodes. The clinical evolution was complicated by intestinal perforation requiring surgical treatment. High throughput sequencing of candidate genes (SCN9A, SCN10A, SCN11A and TRPA1) showed a de novo heterozygous missense SCN9A mutation, thus confirming the diagnosis.
Discussion: PEPD symptoms may begin at birth or early in infancy. It is a very rare debilitating disease, frequently misdiagnosed clinically as seizures, but with normal EEG. Metabolic work-up is unremarkable. Tonic attacks are not controlled by anti-convulsivants usually given for neonatal seizures. Autonomic nervous system is involved, explaining Harlequin and dysautonomic phenomenon. Feeding, perineal stimulation, colics and crying classically trigger pain crises. PEPD is an autosomal dominant channelopathy leading to a gain-of-function of the voltage-gated sodium ion channel Nav1.7, thus altering perception of pain in nociceptors of peripheral nervous system. In most patients, carbamazepine reduces both severity and frequency of attacks. Intestinal perforation has not been reported in PEPD and the potential causative link is still open to discussion.
Conclusion: PEPD is very rare and should be considered in case of paroxysmal neonatal movements with normal EEG. Association of dysautonomic features highly suggests the diagnosis, which can be confirmed by molecular analysis.
Carbamazepine, a Na+ voltage-gated channel blocker, is the first line treatment.

SGPP 73

Unusual neonatal onset of cardiomyopathy: a case report

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Introduction: Cardiomyopathies are defined by structural and functional abnormalities of the ventricular myocardium which are unexplained by the flow-limiting coronary artery disease or abnormal loading conditions. Up to 80–90% of cases, the disease is an autosomal dominant trait. Only very few cases are caused by other genetic disorders (AR, X-linked and maternally inherited traits). They are classified as: hypertrophic cardiomyophaty, dilated cardiomyophaty, restrictive cardiomyophaty and arythmogenic of RV.
Case Report: A male child was born at term after a smooth pregnancy, Apgar 9/10/10, perinatal disease indicators (meconium-stained amniotic fluid, maternal fever). Body weight was 3250 g. At 10 hours from birth, symptoms of tachypnea and respiratory distress were shown; x-ray prooved evidence of multiple thickened parenchymal, normal levels of cardiac silhouette instead. During suspected early-onset neonatal sepsis, we proceeded with antibiotic therapy with positive results, however symptoms of tachypnea recurred at the age of 8 days old. Inflammatory indexes were negative. X-ray showed an enlargement of cardiac silhouette. During an echocardiography were detected dilatation of left ventricular, septal hypokinesia, impairment of the LV systolic function (FE 35–40%) and slight pericardial effusion. Cardiac catheterization has ruled out a coronary anomaly. Myocardial biopsy showed a framework with initial cardiomyopathy hypertrophic and excluded an infectious or metabolic origin. The baby was treated with β-blockers and diuretics. Follow-up after one month: LV not dilated, FE always reduced, but improved (45–50%), mild mitral regurgitation (before severe), apical hypertrophy. No clinical signs of heart failure.
Conclusion: The onset of hypertrophic cardiomyopathy with dilatation and heart failure is particular since it's usually a late event which occurs after the fibrotic transformation of the hypertrophied myocardium. The definitive diagnosis is due by a biopsy. By newborn with tachypnea in which it was ruled out infectious, metabolic or pulmonary origin ,it must always been considerated a cardiogenic etiology underlying.

SGPP 74

Fortification of Expressed Breast Milk – preterm infants rarely receive recommended levels of energy and macronutrients

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Background: Expressed breast milk (EBM), whether mothers own or donor milk, usually fails to meet the macronutrient, micronutrient, electrolyte and caloric demands in very preterm infants. Therefore, fortification of EBM is considered standard care in nutritional support of these patients. Both exaggerated postpartal growth and growth restriction are associated with long-term negative consequences. Therefore, the composition of fortifiers must be adapted to provide energy and macronutrients within recommended limits for this patient population.
Methods: We analysed pooled, single mothers milk samples using the MIRIS® Human Milk Analyser to obtain values for protein, carbohydrates, fat and energy content. The values were used to extrapolate whether EBM with standard fortification (Nestlé FM85 5%) would comply with international recommendations on enteral nutrient supply for very preterm infants.
Results: 305 samples of EBM from 29 mothers were analysed. Mean nutritional values were comparable with those of previously published results: energy 72 ± 6 kcal/100 mls, protein 1.14 ± 0.26 g/100 mls, carbohydrates 6.5 ± 0.2 g/100 ml, fat 4.5 ± 0.7 g/100 mls. At volumes of 150 ml/kg/day 187 (61%) of samples would have provided recommended energy requirements, but only 37 (11%) samples provided recommended amount of protein. Increasing volumes to 180 ml/kg/day improved protein provision (53% of samples within recommended range), but 97% of samples provided energy above recommended level. Already at volumes of 150 ml/kg/day 303 (99%) samples would provide carbohydrates above recommended level.
Conclusion: Current composition of a standard multicomponent fortifier is insufficient when aiming to achieve recommended macronutrient delivery in very preterm infants. At volumes between 150–180 ml/kg/day a majority of infants will receive energy and carbohydrates levels above and protein levels below recommended intake.

SGPP 75

Maternal metastases to the placenta – recommended follow-up in the newborn

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Maternal cancer in pregnancy is fortunately rare and metastases to the placenta even more. Based on a case of a 33-year-old mother diagnosed with an adenocarcinoma of the colon stage 4 with proven metastases to the placenta we present an overview of maternal cancer types known to metastasize to the placenta. In addition we will describe our approach regarding the follow-up of the newborn. In our case the diagnosis of cancer was made at 34 weeks of gestation. The advanced stage necessitated a premature ceasarean delivery at 34 4/7 gestational age. The only neonatal complications after birth were a wet lung that required a positive pressure support (CPAP) for the first nine minutes of life and a feeding tube for the first two days of life because of feeding problems. In accord with our pediatric oncologists we controlled the tumor biomarkers carcinoembryonic antigen (CEA) and cancer antigen (CA) 125 both elevated in the mother in the newborn after 3 weeks. CEA was slightly elevated and CA-125 in the normal range. A follow-up is planned with our oncologists till the CEA tumor marker is in the normal range. We assume that CEA like any other IgG is transferred through the placenta during the pregnancy.

SGPP 76

Kratom – an understudied substance causing neonatal abstinence syndrome: a case report

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Background: The use of substances that augment human abilities is an old, common and transcultural phenomenon: be it illegal drugs, culturally accepted stimulants such as caffeine drinks, or unregulated drugs that have more recently become freely available via internet – such as Kratom. Kratom (Mitragyna speciosa) is available as leaves, powder or gum from a tropical tree in Southeast Asia. It is typically smoked or brewed into tea, traditionally used to fight fatigue and elevate work productivity, but also to treat opioid withdrawal. It can be bought legally on the internet (eg. Switzerland, USA). Kratom is falsely considered as a safe drug, or not as a drug at all, because it is legal and seen as a natural substance. It has dual properties of stimulation in low doses and analgesia in higher dosages. Opioid-like effects are induced by its two μ-opioid components. Kratom is an understudied drug and associated with dependency, withdrawal symptoms and serious adverse effects especially in multidrug-intoxicating scenarios. Our hospital has an established concept to treat neonates suffering from neonatal substance withdrawal, in particular opioids.
Case report: The mother of a term born neonate exhibits polydrug intake of Cocaine, Ritalin, Alcohol, Cannabis, Benzodiazepines and Kratom during pregnancy. The neonate is delivered with a perinatal and neonatal acidosis with a global respiratory insufficiency and requires intubation. The administered dosage of morphine to achieve sedation for intubation in this neonate is 5 times higher than the usual morphine dosage. The neonate requires ventilation for 2 days. On day 4 of life, the scores for neonatal abstinence syndrome (Finnegan) are significantly high (up to 14 points). Upon administering morphine, the symptoms are regressive and upon reduction of the morphine according to our concept, withdrawal symptoms occur again. The required maximum dosage of morphine to achieve a steady state of Kratom-withdrawal in this patient is 1.2 mg/kg/d, not higher than that of neonates with opioid-withdrawal (max. 3.0 mg/kg/d). The time to terminate the morphine in comparison to the heroine or methadone withdrawal is not yet evaluated as the newborn is still under substitution.
Conclusion: Kratom consumption in pregnancy causes a neonatal abstinence syndrome similar to that of opioids and can be treated identically. Kratom use, including its clinical pharmacology and toxicology, are not yet understood and require further research.

SGPP 77

Severe endocarditis presenting with acute renal failure

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A 13-year-old female patient with hyponatremia, oliguria and acute renal failure (ARF) was transferred to our PICU from a regional hospital. A direct transfer to the nearest PICU offering haemodialysis was not possible due to capacity issues in this unit. The girl had an influenza-like infection with fever during the 10 days prior to admission with profuse, watery diarrhoea and frequent vomiting over the last 2 days, so that dehydration/hypovolemia was initially suspected to be the cause of the ARF. As neonate she was diagnosed with Turner syndrome and had repair of coarctation of aorta with a relatively complicated course and a long hospital stay. On admission she was in a reduced shape with hypotension, but normal mental status. She was treated with repeated volume boluses of normal saline. Septical work-up was done, and she was commenced on antibiotics. She had notably low diastolic blood pressures not responding to volume support. The echocardiography revealed a severe aortic valve insufficiency, and rupture of a sinus of Valsalva aneurysm was suspected. The clinical situation worsened rapidly, and the patient needed intubation and catecholamine support. The need for transfer and the risk of transport to an ECMO-centre were discussed with the parents. Meanwhile the patient went into asystole and was resuscitated for 25 minutes. She returned to a sinus rhythm, but remained in severe shock. Parents declined transfer in view of the worsening situation. The patient died during a second asystole, following shortly, as the parents rejected further resuscitation. The autopsy revealed severe endocarditis involving the tricuspid-, mitral and aortic valves with perforation from the aortic root into the right ventricle accounting for the aortic insufficiency and low diastolic blood pressures. The blood cultures grew group B Streptococci. The AKI might be explained by a combination of severe dehydration and aortic insufficiency (forward failure).

SGPP 78

Newborn screening for Severe Combined Immunodeficiency (SCID) and severe T cell deficiency

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Introduction: Severe combined immunodeficiency (SCID) and severe T cell deficiency fulfil criteria for newborn screening (NBS) since these diseases are asymptomatic at birth and might be fatal within the first year of life, the confirmation of the disease is easy (numeration of lymphocyte subsets), and early hematopoietic stem cell transplantation (HSCT) is a curative treatment. Quantification of TRECs (T-cell receptor excision circles) from dried blood spots (DBS) is a sensitive and specific screening test for SCID and severe T cell deficiency. TRECs are a reliable marker of the number of circulating naïve T cells recently emigrated from the thymus and are undetectable or very low in infants with SCID or severe T cell deficiency.
Methods: In a retrospective study we analyzed TREC copy numbers from dried blood spots (DBS) of the original newborn screening cards of 7 babies with confirmed SCID as well as 57 controls. TREC copy numbers were measured from 1.5 mm punch using the newly developed EnLite Neonatal TREC assay. After an elution step, TRECs were directly amplified from the eluate and hybridised with a probe, detected by time-resolved fluorescence resonance energy transfer. Three standards with known TREC copies were measured to calculate TREC copies in the samples. Simultaneous amplification of beta-actin allowed to monitor the amplification of a sample.
Results: 68 TREC measurements from 7 SCID patients showed a mean of 0.6 copies/μl blood with a range from 0–9 copies/μl, while 131 measurements form 57 controls showed on average 136 copies/μl with a range from 17–350 copies/μl. Thus TREC copy numbers of the 7 SCID patients separated well from controls and were all below the 2.5th percentile of 36 copies/μl suggested as cut-off by the manufacturer.

Conclusion: The TREC assay is a reliable assay, easily to be implemented into NBS programs. NBS for SCID and severe T cell deficiency is already recommended in the US and a few other countries. Since early HSCT before the occurrence of irreversible organ damage can provide cure for these patients, a proposal to the Swiss Health Ministry (BAG) regarding inclusion in the routine NBS screening program in Switzerland is underway.

SGPP 79

Clinical Neurophysiology in child neurology – Indications, benefits and limits

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Neurophysiological techniques like evoked potentials and neurography are well established tools in adult neurology. These techniques also offer valuable information in child neurology but still are less frequently used. Especially in children who cannot verbalize there neurological condition sufficiently these techniques offer objective and quantitative information about the nervous system. In this work we give an overview about the most frequent used neurophysiological tests in our clinic. We summarize the technical aspects of the methods used and present clinical cases to show how these techniques can guide the diagnostic workup. We show that auditory evoked potentials are a reasonable method in evaluating hearing in context of screening for hearing disorder and for evaluating brain stem function. Visual evoked potentials are performed for evaluating visual function and for diagnosis of demyelinating diseases. Somato-sensory evoked potentials allow evaluation of the functional integrity of the somatosensory system from the peripheral nerve to the cerebral cortex and are helpful in determination of prognosis in coma and asphyxia. Nerve conduction studies are useful to determine a dysfunction of the peripheral nervous system and to separate between peripheral and central neuropathies. Electro myography is useful in evaluating the neuromuscular interplay and monitoring the recovery after traumatic nerve lesions. We conclude that neurophysiological examinations in children are valuable tools. In order to conduct these examinations successfully one has to consider the special technical needs for children at different ages. During the interpretation process one has to consider the developmental changes and has to apply age correlated normative values.

SGPP 80

Retrospective analysis of treatment strategy and outcome in infantile spasms in the neuropediatric department of St. Gallen Children's Hospital

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Background: Infantile spasms or West syndrome is an often harmful form of infantile epileptic encephalopathy which is difficult to treat and often causes developmental impairment. Typical EEG shows hypsarrhythmia. Three treatment modalities are mostly used: ACTH, Steroids and Vigabatrin. Many different schemes using these three drugs in variable dosages and combinations are practiced across neuropediatric centers.
Methods: We retrospectively evaluated treatment, etiology and developmental outcome of all children diagnosed with infantile spasms in children's hospital St. Gallen from January 2011 to January 2017 treated according to the scheme of Bahi-Buisson and Dulac, Paris. Therapy was initiated with Vigabatrin 100 mg/kg/d, increased to 150 mg/kg/d after 14 days when spasms and hypsarrhythmia were persisting, combined with hydrocortisone 15 mg/kg/d after another 14 days when symptoms remained and escalated from hydrocortisone to ACTH 0.05 mg/kg/d after another 14 days if spasms were still present. Clinical seizure activity, EEG pattern and developmental outcome were estimated regularly by neuropediatric examination.
Results: Between January 2011 and January 2017 13 infants presented with infantile spasms and hypsarrhythmia in EEG. Median age at onset of spasms was 6.2 months. 9 of 13 showed cessation of spasms and resolution of hypsarrhythmia receiving vigabatrin alone. In two infants vigabatrin and hydrocortisone were needed and two infants needed escalation from vigabatrin and hydrocortisone to ACTH to get seizure free. Three of 13 infants developed other seizure types later on. 5 of 13 showed genetic mutations associated with infantile spasms: three infants with Trisomie 21, one with Neurofibromatosis type 1 and one with STXBP-1 mutation. In our population three out of 13 infants showed normal development after cessation of spasms, five showed mild developmental impairment, three showed developmental impairment ascribed to Trisomie 21 and two had severe developmental impairment.
Conclusion: The treatment regimen starting with vigabatrin and escalating to hydrocortisone and ACTH is effective to achieve seizure freedom with tolerable side effects. Escalation should be performed rigorously. A quarter of infants showed normal development which is comparable to previous reports.

SGPP 81

Association between structural and functional thalamocortical connectivity in term-born and very preterm adolescents

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Background: Very preterm birth is associated with alterations in the development of the thalamocortical system as assessed by structural and functional MR imaging. We related sleep spindles, an electrophysiological characteristic of sleep which reflects the functional integrity of the thalamocortical system to structural properties of thalamocortical connections in very preterm and term-born adolescents.
Objective: To investigate the effect of very preterm birth on the association between structural and functional thalamocortical connectivity.
Methods: High-density sleep EEG (128 electrodes) was recorded in 27 very preterm (age [M ± SD] 12.7 ± 1.5 years) and 35 [12.8 ± 2.0 years] term-born participants. Sleep spindles were automatically detected and spindle density (no./min) was calculated for each electrode. Probabilistic tractography of diffusion tensor data was run to track fibers from the thalamus to different cortical regions of interest (ROI). Voxels connecting to the frontal, parietal or temporal ROI, respectively, were combined into clusters and their volume was calculated. Spindle density at each electrode was correlated with the volumes of the three clusters and Fisher r-to-z-transformation was used to investigate whether the correlation coefficients differed between the two groups.
Results: In term-born adolescents, spindle density measured in electrodes over fronto-temporal brain regions correlated with the volume of the thalamic cluster projecting to frontal brain regions (mean $r = .46 \pm .08$) and spindle density over parietal brain regions correlated with the volume of the thalamic cluster projecting to parietal brain regions (mean $r = .44 \pm .06$). In contrast, in very preterm adolescents, spindle density was not related to the volume of any of the three thalamic clusters. In a cluster of 9 electrodes over parietal brain regions, correlation coefficients were significantly different between the groups (all $p < .05$; mean $r = -.12 \pm .06$ and $.46 \pm .09$ in the very preterm and term-born group, respectively).
Conclusion: Region-specific associations between structural and functional measures of thalamocortical connections were identified in term-born adolescents, thus, providing evidence for their close relationship. The lack of similar associations in very preterm adolescents emphasizes the importance of a multimodal assessment of thalamocortical connectivity for a comprehensive understanding of the impact of very preterm birth on the integrity of the system.

SGPP 82

MERS caused by S. pneumoniae – misdiagnosed as acute schizophrenia

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MERS (mild encephalopathy with reversible splenial lesion) is a rare clinico-radiological syndrome presenting with encephalopathic symptoms 1–7 days after a prodromal viral infection and is characterized by a lesion in the splenium of corpus callosum detected by cranial MRI. Symptoms are usually reversible and the MRI normalizes within weeks. The pathophysiology of MERS is poorly

understood; various viruses have been identified as potential triggers. We only found one single case in the literature reporting an association with a S. pneumoniae infection.
Case Report: A previously healthy 15-year-old girl presented with a history of fever, headache and cough for the last 4 days. Parents noticed a slowing in thinking and motor activity, impaired speech production, hallucinations and derealisation. After parents contacted the general practicioner via phone the girl was admitted to adult psychiatry with the presumptive diagnosis of an acute schizophrenia. In the routine laboratory investigation at the psychiatric department an elevated CRP (110 mg/l) and white blood cell count (17.4 G/l) was noticed, therefore the girl was referred to our paediatric hospital. Clinical evaluation confirmed the encephalopathic symptoms noticed by her parents, her neurological examination was otherwise normal. No further signs of acute schizophrenia were present. MRI of the brain showed an increased T2-signal and diffusion restriction in the splenium of the corpus callosum leading to the diagnosis of MERS. Cell count and protein levels in the CSF were normal. EEG showed a mild slowing of background activity in frontal parts. The day after admission growth of streptococcus pneumoniae in the blood culture was reported. Pneumonia was confirmed on chest x-ray and appropriate intravenous treatment with amoxicillin was initiated. Overt psychiatric symptoms improved within days; a neuropsychological assessment 4 weeks after admission revealed mild deficits in attention, verbal memory and executive functions assumed to improve further.
Conclusion: Among the infection-triggered encephalopathies, MERS can present with acute psychiatric symptoms. For adequate diagnosis, management and counselling, standard cMRI is necessary. Besides well-known viral triggers, it can be caused by pneumococcal infection. The case shows the importance of history taking and appropriate clinical investigation to distinguish between idiopathic psychiatric conditions and para-infectious inflammatory encephalopathies.

SGPP 83

Sudden postnatal collapse – a case too early to be screened

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Introduction: Sudden unexplained postnatal collapse (SUPC) in an apparently healthy newborn is a rare event carrying a high risk of mortality or persisting neurological sequelae. Despite intensive investigations, the aetiology of SUPC remains often obscure. MCAD deficiency is an inborn error of fatty acid oxidation with potential severe complications in infants during catabolic conditions. Detection of MCAD deficiency is part of the swiss neonatal screening program aiming to prevent adverse events. According to the national program, screening is performed at the age of 72–96 hours. We present a case of a newborn with a sudden neonatal collapse due to hypoglycaemic multi-organ failure caused by MCAD deficiency at the age of 36 hours.
Clinical case: A female infant was born at 38 6/7 weeks of gestation following an uneventful pregnancy. Initial clinical evaluation was normal and the baby was regularly breastfed. At the age of 36 hours, the girl became somnolent, hypopnoeic and required emergency resuscitation. A hypoglycaemia of 0.6 mmol/l was detected and treatment with i.v. glucose was initiated. After successful cardiopulmonal resuscitation, she was transferred to the intensive care unit for further treatment. Blood chemistry revealed metabolic acidosis with elevated CK and hyperuricaemia. Analysis of urine organic acid showed an abnormal pattern of dicarboxylic aciduria and the analysis of acylcarnitines in a blood spot revealed a characteristic pattern of elevated hexanoylcarnitine (C6), octanoylcarnitine (C8) and decanoylcarnitine (C10) leading to the diagnosis of MCAD-deficiency. A supplementation of L-Carnitine was initiated. The results of a cMRI and EEG unfortunately were indicative for an unfavourable neuro-developmental outcome.
Conclusion: Prior to neonatal screening for MCAD deficiency, the disease was usually diagnosed after a sudden multi-organ failure or even death during a period of catabolism in early infancy. Infants who have been identified prior to the onset of symptoms have an excellent prognosis. In the case presented, sudden metabolic failure occurred at the age of 36 hours, too early to be detected by screening. The reason for this very early presentation remains unclear. The case demonstrates the importance of monitoring blood glucose followed by metabolic investigations in every new-born with sudden, unexplained collapse.

SGPP 84

Rare disease in a busy pediatric immigrant heath outpatient clinic: the case of Duchenne muscular dystrophy

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Duchenne muscular dystrophie (DMD), a leading childhood myopathy, is caused by mutations of the dystrophin gene on the X chromosome with weakness as the principal symptom, due to muscle fiber degeneration. It is a rare disease, affecting 1.3 to 2 per 10,000 boys. The clinical onset of weakness usually occurs between two and three years of age, with difficulty running, jumping, and walking up steps. Patients are usually wheelchair bound by the age of twelve and primary dilated cardiomyopathy and conduction abnormalities develop as well as orthopedic complications and impaired pulmonary function. Most patients usually die in their twenties from respiratory insufficiency or cardiomyopathy. A four-year-old boy originating from Sri-Lanka, in Switzerland since six months was seen at the migrant health consultation, with a proximal weakness of the lower extremities noted during the examination. The parents reported long-standing weakness and tiredness during exercises compared to his sisters and peers, which was falsely attributed to the fact that the family was hidden in a small room for 9 months before their arrive in Switzerland. The boy could not move as freely as he wanted in this space. Improvement was however observed since their arrival in Switzerland. The diagnostic retained was either a chronic under-utilization of the muscles, or an hypovitaminosis D, a newly recognized cause of muscle weakness among immigrants, but to rule out a dystrophinopathy, a creatine kinase level was planned for the next appointment. The blood tests revealed a diminished vitamin D level and an elevated serum creatine kinase concentration (29 634 U/L), consistent with DMD, which was confirmed by molecular genetics. After an update of his immunization status, a treatment by oral corticosteroid was introduced, associated with physiotherapy. A baseline heart ultrasound was performed which shows no cardiac involvements as well as a test of pulmonary fonctions, related to the numbers of pulmonary infections the boy had. Clinicians might encounter in pediatric migrant health clinics undiagnosed rare disorders that should not be overlooked. The diagnostic of DMD should always be excluded by a creatine kinase level, in a boy with muscle weakness.

SGPP 85

Pontine tegmental cap dysplasia

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Introduction: Pontine tegmental cap dysplasia is a very rare brainstem malformation causing a wide range of clinical symptoms such as variable cranial nerve dysfunction and motor and cognitive impairments. Only 25 cases had been described until now. The common MRI findings include pontine hypoplasia and a tegmental cap, which contains ectopic nerve bundles.
Case presentation: We report the first diagnosed case of pontine tegmental cap dysplasia in Switzerland. We describe the case of a preterm boy born at 35 5/7 gestational weeks. Postnatal, he presented with persistent feeding problems in the first week of life. In the physical examination we found a muscular truncal hypotonia, a facial nerve palsy on the right side and a bilateral absence of corneal reflexes. The chest x-ray showed a wedge-shaped vertebra BWK5 and the otoacoustic emissions were negative on the right side. The cranial ultrasound was normal. At the age of one week, an MRI of the brain was performed. It showed the typical findings for a pontine tegmental cap dysplasia (pontine dysplasia, asymmetric cerebellar hemisphere, aplasia of the right facial and vestibulocochlear nerve).
Discussion: The prognosis remains unsecure. As described earlier, clinical manifestations vary from severe cognitive impairment to mild learning disability. The early diagnosis helps to prevent blindness due to bilateral corneal opacity by the frequent use of vitamin A-eye-ointment and oral scalding from hot liquids. In addition, physiotherapy, ophthalmological and neuropediatric examinations on a regular basis could improve the outcome.
Conclusion: In newborns with (focal) neurological abnormalities, a cranial MRI should be considered. Early diagnosis of a brain malformation might improve the outcome due to supportive therapy.

SGPP 86

Northern Epilepsy – not so northern anymore

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Background: Neuronal ceroid-lipofuscinoses (NCL) are a clinically heterogeneous group of neurodegenerative disorders, characterized by seizures, progressive neurocognitive decline, motor function problems and vision loss. The predominant symptoms vary among the different phenotypes. We present a case of a Northern Epilepsy variant due to a newly described homozygous mutation in the CLN8 gene.
Case presentation: A now 13-year-old girl of Turkish descent was diagnosed with focal epilepsy at the age of 6 years. Neurocognitive testing and MRI of the brain were unremarkable. Treatment with valproic acid was initiated. Regular follow up revealed normal EEGs and seizure freedom. A marked decline in school performance at the age of 12 years prompted a diagnostic follow up. Neurocognitive decline of 20 IQ points and progressive cerebellar atrophy were noted. During sleep EEG a secondary generalized seizure occurred, followed by a prolonged postictal asystole of 27 seconds, leading to a pacemaker implantation. Genetic testing by targeted panel analysis revealed a homozygous missense mutation in the CLN8 gene (Exon 3, c.677T>C p.[Leu226Pro]), first published in November 2016 in members of a Turkish family with similar clinical presentation. Subsequent ophthalmological examination revealed a beginning retinopathy without giant potentials during low frequency photic stimulation in the EEG.
Discussion: Mutations in the CLN8 gene lead to different phenotypes, depending on the functional effects of the underlying mutations. The clinical presentation of our patient corresponds to the phenotype of Northern Epilepsy – an entity until recently known to be restricted to a Finnish cohort only, presenting with a milder phenotype compared to the classical NCL forms. The cause of the prolonged asystole might be related; however, conduction deficits are usually known to occur in >20 years old affected patients with late-infantile NCL.
Conclusion: Regular neuropsychological testing is important in patients with epilepsy to detect early neurocognitive decline to prompt further diagnostic investigations regarding possible underlying neurodegenerative disease. The identification of the underlying cause for the neurocognitive decline (hidden nocturnal epileptic activity versus underlying neurodegenerative disease) is important for the clinical management of the patient. Genetic epilepsy panel testing is a crucial diagnostic tool in widening the spectrum of neuro-metabolic disorders.

SGPP 87

Severe vitamin B12 deficiency in an exclusively breast fed girl

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Background: Vitamin B12 deficiency of the mother can lead to severe vitamin B12 deficiency in exclusively breastfed infants. This leads to ineffective erythropoiesis with megaloblastic anaemia, neurological abnormalities, microcephaly and failure to thrive in the infant. Early substitution may improve the outcome. Although restricted vegan diet of the mother is an oncoming trend, other causes of vitamin B12 have to be considered.
Case report: A 7-month-old girl was sent by her paediatrician because of failure to thrive. Percentiles showed a stagnation of weight with a drop below P3 since 3 months and a deceleration of growth in body length and head circumference. Clinical examination revealed a muscular hypotonia of the trunk and a developmental delay with a corrected developmental age of 3 to 4 months. Blood analysis showed a normochromic macrocytic anaemia and a severe deficit of vitamin B12. Metabolic urine analysis approved the vitamin B12 deficiency, showing an elevated excretion of Methylmalonacid and Methylcitronacid. The girl was exclusively breast fed. Although the mother's alimentation was a balanced diet including animal products, she also had a severe deficiency of vitamin B12 since puberty with a positive family history. Substitution of the mother was stopped over 5 years ago. The first two children (aged 15 and 5) showed normal development. Treatment consisted of intramuscular substitution of vitamin B12 (and oral folate substitution) for child and mother. Additionally the girl's alimentation was changed to formula milk. She already showed progress in neuro-development within the first week of treatment and a general improvement of alertness and curiosity. Further investigations to evaluate the reason for the mother's deficiency will be will be performed by her GP.
Conclusion: Screening of vitamin B12 deficiency is indicated in all exclusively breastfed infants with failure to thrive; especially if in combination with megaloblastic anaemia, developmental delay or muscular hypotonia, even if there is no restricted diet of the mother. An early diagnosis and therapy of the infant is very important for the further neuro-development and health.

SGPP 88

Transient ischemic attack (TIA) – a problem not to miss

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Background: Infections, anemia and arteriopathies are known risk factors in pediatric arterial ischemic stroke (AIS). TIAs often precede AIS in patients with arteriopathies and should be promptly recognized in order to prevent AIS.
Case presentation: A 6-year-old girl known with a beta-thalassemia minor (β-TM) presented with acute right sided hemiparesis and motor aphasia. She reported rhinitis, fever and reduced appetite during the previous three days. Suspicion of stroke prompted immediate cerebral Angio-MRI showing occlusion of the left middle cerebral artery (MCA). There were no signs of ischemic infarction on the diffusion weighted images. The focal neurologic deficit disappeared within a few hours. Conventional angiography confirmed occlusion of the left middle and anterior cerebral artery (M1 and A1 segment) and additionally revealed stenosis of the left internal carotid artery (ICA); collaterals were present, consistent with moyamoya like arteriopathy. Laboratory investigations showed pancytopenia (Hb 58 g/L, Tc 133 G/L, Lc 2.5 G/L) in the context of an acute Parvovirus B19 infection. There were no signs of cell lysis, increased hematopoiesis or malignant cells. Anemia was treated with an Ec-concentrate. Prophylactic ASS-therapy was installed and no further stroke-suspicious events occurred.
Discussion: Our patient suffered from a transient aplastic crisis (TAC) due to Parvovirus B19 in the context of preexisting β-TM, resulting in reduced cerebral blood flow, further aggravated by the moyamoya like arteriopathy. Immediate neuroimaging followed by appropriate acute treatment strategies most likely prevented ischemic infarction in this child. Moyamoya syndrome is a well described arteriopathy increasing the risk of AIS in patients with thalassemia major and sickle cell disease. So far it has never been described in patients with β-TM.
Conclusion: This case illustrates the frequently described multifactorial etiology in pediatric arterial ischemic disease. Furthermore, it highlights the importance of immediate neuroimaging in case of acute focal neurologic deficits, especially in children with underlying hematological disorders.

SGPP 89

When the merry-go-round never stops: "à propos" of a case of "post-traumatic" benign paroxysmal positional vertigo and its treatment

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Introduction: We present a case of "post-traumatic" (trauma being circular acceleration on a merry-go-round) benign paroxysmal positional vertigo (BPPV), an uncommon cause of vertigo in childhood, and demonstrate the diagnosis and therapeutic manoeuvers.
Case report: During a trip on a merry-go-round, a 10 y.o. girl experiences a sudden malaise with ocular revulsion and loss of consciousness. After the urgent stop she recovers quickly, and is brought home in car. During the trip, the malaise recurs several times. During the next days, she signals violent vertigo, when sitting up from bed, in car or elevator, and during different postural changes. General and neurological examination is normal besides a positive Dix-Hallpike test*. This presentation being highly suggestive of BPPV, a trial of

reduction by Epley manoeuver* is performed, which elicits the vertigo and allows its definitive disappearance.
Discussion: In contrast to the benign paroxysmal vertigo (migraine variant), BPPV is rare in childhood (3% of vertigo's causes). Its commonly (although not universally) accepted pathophysiology is a displacement of otoliths, disloged from the utricular macula to a semicircular canal, usually (90%) the posterior one. This should make it included more commonly in the differential diagnosis of post-traumatic vertigo in children (as it has been reported in adults), besides more serious causes: vestibular concussion, vestibular nerve lesion following temporal bone fracture, and perilymphatic fistula. In our case, the clear clinical presentation, with absence or other neurologic signs and the "benign" type of trauma precluded the need for any other imaging or functional investigations, which would have been otherwise warranted. Functional investigations could have included Dix-Hallpike test with Frenzel lenses, or video nystagmography. In cases of BPPV of the posterior canal, the Eppley or Semont manoeuvres* are curative in 80% of cases in adult series, but recurs in up to 50% of cases in these series. It has to be pointed out that spontaneous resolution also occurs over time with different frequency depending on series.
Conclusion: BPPV is rare in childhood, but should not be forgotten in the diffential diagnosis of vertigo, especially if post traumatic, as diagnosis and treatment are basically easy.
*videos at: https://www.dropbox.com/s/fi9wuucxtwbobla/BPPV.mp4?dl=0

SGPP 90

Frequency and characteristics of aggressive incidents in a paediatric rehabilitation setting: a pilot study

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Background: Aggressive incidents are a serious concern in health care and have various effects on the physical and emotional well-being of the staff. Little is known about it in somatic paediatric settings. The goal of this study was to determine the frequency and characteristics of aggressive incidents in a paediatric rehabilitation setting. Furthermore, it aimed to explore risk factors for aggressive behaviour in order to manage it more efficiently.
Methods: The study was carried out at the 47-bed rehabilitation centre Affoltern am Albis of the University Children's Hospital of Zurich. During six months all aggressive incidents were recorded prospectively by the staff using a specific form (EVA-form). In addition, all patients under the age of 18 years who were hospitalised during that time were divided into two groups: (i) study group – patients who were involved in aggressive incidents and (ii) control group – all other patients. Their demographic, anamnestic and clinical data was retrospectively collected and compared.
Results: 14 of 105 patients (13%) were involved in a total of 79 aggressive incidents. On average 0.4 incidents per day occurred and 0.9 after a second reminder of the staff to record all the incidents. Most often the incidents occurred on Mondays. 98% of the incidents included physical and 22% verbal aggression. In 80% of the incidents staff members (43% nurses, 32% therapists) were the targets of the aggression. Twenty-nine (37%) incidents resulted in pain, 9 (11%) in a visible injury and in 21 (27%) incidents there were no effects. Following triggers for aggressive behaviour were frequent: patients were urged to do something (32%) and physical proximity (29%). The study group has significantly higher scores in the Cumulative Illness Rating Scale (CIRS) compared to the control group and previous aggressive behaviour was more often documented.
Conclusion: These findings emphasize the magnitude of aggressive incidents in paediatric rehabilitation and thus the importance of raising awareness and educating the staff. Further studies with bigger study populations are needed to identify risk factors for aggressive behaviour in paediatrics.

SGPP 91

Intense asthenia and lower limb pain: think of ADEM

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Introduction: ADEM (Acute Disseminated EncephaloMyelitis) is a rare disease (0.1–0.3/100'000 children per year), mostly post-infectious and rarely post-vaccination. Clinical presentation can be misleading depending on location of lesions.
Case report: A 3 y.o. boy presented important asthenia for more than a week with diffuse pain, and weakness of the lower limb. Initial laboratory results (hematology, CRP and VS) were normal. Because of weakness on lower limbs without loss of reflexes and of pain on the palpation of the lumbosacral spine, spinal and brain MRI was performed. MRI showed multiple lesions in white matter, left thalamus and putamen and in the medulla with slight contrast enhancement. Cerebrospinal showed fluid, pleiocytosis (29 µL) and oligoclonal bands. ADEM was diagnosed and methyl-prednisolon (500 mg/m^2) given. We excluded an optic nevritis by fundus examination. Evolution was favorable without relapse until now and normalization of MRI.
Discussion: ADEM is an immune-mediated demyelinating CNS disorder with predilection to early childhood, most of case occurring before the age of 10. Frequently after an upper respiratory illness. Diagnosis criteria for ADEM are acute onset of encephalopathy (alteration of consciousness and fever), a first polyfocal clinical CNS event and brain MRI abnormalities consistent with demyelination. ADEM lesions typically involve white matter and cortical gray-white matter junction, sometimes basal ganglia, cerebellum and brainstem. Neurological signs are dependent of the localization of brain/medullar lesions. They rapidly progress with maximal deficit in 2–5 days. In this case, pain and asthenia are related to thalamic lesions. A follow-up imaging is necessary to demonstrate the full recovery at 3 months. CSF examination can be normal or shows pleiocytosis. New seric antibodies, anti-MOG, can predict a relapsing course. Treatment of ADEM is methylprednisolone iv for 2–5 days and then oral prednisolone for 1–2 weeks. In refractory case, intravenous immunoglobulins for 2–5 days are used. Full recovery is achieved in 50–70% of cases.
Conclusion: This case is an atypical presentation of ADEM with predominant asthenia. Radiologic and clinical follow-up is necessary, because other diseases (multiple sclerosis, neuromyelitis optica) can present initially as an ADEM.

SGPP 92

Outcome after transverse myelitis – a case series

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Introduction: Transverse myelitis (TM) is caused by local inflammation of the spinal cord. Corresponding to the spinal level and extension of the lesion it manifests with different neurological (motor, sensory) deficits. TM is a rare (incidence is 2/million children/year), often disabling condition. We report the rehabilitation outcome of 4 cases after an episode of acute TM observed at the RCA from 2012–16.
Case 1: 4-year-old girl initially diagnosed paresis of both legs and left arm, no sensory impairment or bladder dysfunction. Spinal MRI showed TM in cervical spine level C3–C7. Enterovirus, a possible causative, was found in stool sample and nasopharyngeal secret, not in liquor. After 5 months of rehabilitation, she regained ambulatory function, paresis in the left arm persisted (ASIA D).
Case 2: 7-year-old girl initially complaints of symmetrical leg weakness, complete sensory loss below Th10, no bladder- or bowel control. Diagnosis confirmed by spinal MRI. Etiology remains unclear as no infectious pathogen was found. After the course of 7 months, persistent paraplegia (ASIA A), neuropathic bladder- and bowel function needing catheterization.
Case 3: 6-year-old boy, initially presented with gait difficulties, sensible deficits below Th1, retention of urine and feces at the age of 3. At diagnosis, MRI showed affection of the terminal cone, no infectious agent found. 4 yrs after he requires assistance to walk (ASIA C). There was full recovery of the sensibility and bowel control but persisting bladder dysfunction.
Case 4: 4-year-old boy, diagnosed with encephalomyelitis presented with complete paresis of the upper limbs, loss of trunk stability, leg weakness, distinct dysarthria. MRI revealed longitudinal hyperintensity along spine and affection of brainstem. Enterovirus as causative agent detected in stool sample and nasopharyngeal secretion. During rehabilitation the patient regained mobility, except persistent armparesis (ASIA D).
Conclusion: TM is a condition with varying outcomes from good recovery to residual disability. Spinal MRI (imaging will be discussed) is seen as major tool for diagnosis but TM remains a diagnosis of exclusion. Infectiological work-up has to be established within the first few days in order to detect a causative agent.

SGPP 93

Acute impairment of consciousness in an infant: think to cerebral venous sinus thrombosis (and brain imaging before lumbar puncture)

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Introduction: Paediatric cerebral venous sinus thrombosis (CVST) is an uncommon condition in infant and its polymorphic presentation often delays the diagnosis potentially impacting the prognosis.
Case report: A 21-month-old girl consulted a first time for a febrile episode without other signs nor symptoms. Laboratory evaluation revealed only an iron-deficiency anaemia (74 g/L). A viral infection was diagnosed and iron prescribed. Six days after, she presented five episodes of vomiting and persistent irritability without diarrhoea nor fever. A trial of oral rehydration was successful, improved the irritability and she was discharged to home. The day after she displayed general deterioration and unusual crying without fever nor vomiting. Vital signs were normal except for hypertension (130/76 mm Hg) in an uncooperative child. Clinical findings included irritability, axial hypotonia, ROT hyperactives without clonus. Blood tests confirmed anaemia (70 g/L), inflammatory markers were negative. Given the suspicion of central nervous system infectious a lumbar puncture (LP) was performed, showing hyperproteinorrhachia (1435 mg/l) and a mild hypoglycorrhachia (3.21 mmol/l) without pleocytosis. She then experienced an acute neurological deterioration with increased irritability, upper limbs hypertonia and a right mydriasis which warranted a CT scan showing a widespread CVST (inferior sagittal sinus, straight sinus and left sigmoid sinus) and acute hydrocephalus. MRI showed in addition bi-thalamic and left midbrain infarctions. She was treated with external ventricular derivation and anticoagulation with rapid improvement of her consciousness but right hemiparesis. One year after, she has mild motor deficit of her right upper limb.
Discussion: Massive CVST can occur in infancy. In this case, investigations showed no hypercoagulabilty but the combination of anaemia and dehydration were major risk factors for CVST. Nonspecific signs can lead to diagnosis delay, with a risk of brain injury. In case of acute or progressive impaired consciousness, a brain imaging should be performed first, LP being potentially dangerous.
Conclusion: Although rare, cerebral venous sinus thrombosis should not be forgotten in the differential diagnosis of acute impaired consciousness. The traditional rule to carry out the LP without delay in acute meningitis seems still warranted. However, facing a likely alternative diagnosis (as in absence of fever) is an indication to perform a brain imaging first.

SGPP 94

Intramedullary spinal cavenoma with hematomyelia in a 10-year-old girl – a case report

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Background: Cavernous malformations are uncommon vascular malformations of the central nervous system (CNS) characterized by abnormally dilated blood vessels, lined by a thin endothelium without intervening normal nervous tissue. Intramedullary cavernomas are very rare in children. There are only a few cases described in English speaking literature. The reported risk for hemorrhage in intramedullary carvenomas is about 1.6%.
Case report: A 10-year-old girl presented in our emergency department with sudden lower back pain. Within hours she developed lower body dysaesthesia and paraparesis, which progressed to paraplegia from L1 downwards with bladder and bowl dysfunction. Magnetic resonance imaging (MRI) showed an intramedullar cavernoma with hematomyelia and perifocal edema in the conus medullaris. The girl was treated with corticosteroids to decrease edema. As paresis and in MRI bleeding were not progredient, microsurgical excision of the malformation with laminectomy T11–T12 was performed six days later. Postoperative paraplegia was unchanged. Intensive neurorehabilitation was started.
Conclusion: Intramedullary spinal cavernoma can cause acute severe neurological symptoms, related to an acute hemorrhage within the spinal cord parenchyma even in children. Longterm outcome is variable and not well characterized.

SGPP 95

Prevalence and treatment of headache and migraine in children and adolescents in a single center of eastern Switzerland

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Background: Migraine and headache in children and adolecents are increasing conditions which negatively affects childrens home, school and social activities. It has been shown that compared to placebo there is no overall effective preventive medication for headache in children and adolescents. Therefore behavioural treatment, individualized medical treatment and combination of both therapies becomes more important.
Methods: Over the period of one year 117 patients with headache were assigned in our headache consultation composed of a consultant and a psychologist. Out of these, 100 were diagnosed and followed in our clinic. We record the number of tension type headache, migraine and other headache conditions, as well as the type of therapy we implemented. Depending on treatment response, patients were transfered back to general practitioner or other institutions.
Results: Of all patients 47% were diagnosed with tension type headache, 41% with migraine an 12% with other types of headache, like postcontusional headache. Gender ratio was balanced. Median age of girls was sligthly older than in boys (11,4 years vs 10,8 years). Only six patients were treated with preventive medications (Flunarizin, Topamax, ASS), whereas 28% received multimodal cognitive behavioral therapy. Three patients needed subsequent psychologic hospital treatment, four patients were transfered to an ambulant psychological treatment. For the others sporadic medical consultations were sufficiant.
Conclusion: In our treatment group we saw a good response to our double track system, with the possibility of multimodal headache treatment individualized to the patient and a low imperative of preventive medication. The behavioral treatment herefore serves also as a connector to a potentially inevitable following psychologic therapy.

SGPP 96

Status epilepticus and acute limbs ischemia in a 6-year-old girl: be ware of CAPS!

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Case report: A 6 y.o. African girl, recently treated with carbamazepine and ethosuximide for refractory atypical focal epilepsy, was admitted for status epilepticus and right limbs pain, following 3 days of flu without fever. Midazolam and clonazepam stopped seizures. A few hours later her right limbs were cold, pale, pulseless. Doppler Ultrasound confirmed multiple occlusive arterial clots. She also developed left hemiparesis and brain MRI showed right parietal ischemic lesions. Clinical course included rapid (<6 hours) progressive disease. She was treated with unfractionated heparin and transferred to intensive care unit. Given thrombus progression with limb-threatening conditions despite adequate anticoagulation she required systemic thrombolysis, multiple embolectomies and fasciotomy due to compartment syndrome. Initial laboratory investigations showed elevated inflammatory parameters (fibrinogen 6.8 g/l and sedimentation rate 90 mm/h). A cardiac condition, inherited thrombophilia, vascular anomaly and sickle cell disease were excluded. Interestingly, homogenous antinuclear, anti-DNA, anti-nucleosome, anti-histones and antiphospholipid antibodies (aPL) were positives, suggesting an autoimmune-mediated conditions. Catastrophic antiphospholipid syndrome (CAPS), the most severe form of the antiphospholipid syndrome (APS) with multiple thrombotic events leading to organ failure, was evoked, possibly triggered by primary or drug-induced lupus. Complications included amputation of 5 toes, staphylococcus septicaemia, rhabdomyolysis with acute renal failure. Immunsuppression with steroids and azathioprine was added to the anticoagulation with rapid improvement.
Discussion: Thrombotic events in children are rare and usually secondary to clinical conditions, including catheters, sepsis and other inherited or acquired prothrombotic conditions, such as APS. CAPS represents 1% of cases, with female predominance and is a rapid (<7 days) life-threatening condition with thrombotic microangiopathy, multiple (≥3) organ involvement, and persistent (>12 weeks) aPL with a high mortality rate up to 37%. Rapid aggressive anticoagulation and immunosuppression are mandatory.

Conclusion: CAPS is a rare life-threatening autoimmune disease with disseminated intravascular thrombosis and multiple organ failure. Awareness of this condition may enable prompt diagnostic and treatment initiation with anticoagulation and immunotherapy.

SGPP 97

Stridor – not always what it's supposed to be

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Introduction: Stridor is a frequent finding and indicates an extrathoracal airway obstruction. The phase of the stridor may point to the anatomical level of airway obstruction. The most frequent cause is laryngomalacia, which usually resolves spontaneously over time without requiring further diagnostics or treatment. However other causes must be considered when clinical symptoms and signs are not typical and in the case of either severe or progressive airway obstruction.
Case report: We report on an 11-week-old female infant presenting to the ED with biphasic stridor for further assessment. The stridor developed briefly after birth and was initially judged as laryngomalacia, but became louder and distinctly biphasic over time. The stridor was more pronounced during and right after drinking. Up to the consultation, she was developing normally, without significant respiratory distress and showed no failure to thrive. A suspected gastroesophageal reflux was treated with anti-reflux formula. Given the biphasic feature of the stridor a laryngotracheoscopy was performed showing an extrinsic compression of the trachea for more than two-thirds of the diameter and an inspiratory subtotal collapse. Subsequently an Angio-MRI and an echocardiography were performed resulting in the diagnosis of an innominate artery compression syndrome. Because of the extent of the compression and unlikelihood of spontaneous resolution, cardiovascular surgery was performed with complete resolution of the symptoms.
Conclusion: A careful history and examination of stridor helps the clinician in distinguishing between the more frequent laryngomalacia and other causes needing further assessment. In particular, a stridor of biphasic nature in contrast to an exclusively inspiratory one, must prompt the clinician to consider a broader differential diagnosis.

SGPP 98

Bilateral bronchiectasis and normal sweat test – which differential diagnoses to consider?

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A 6-year-old boy was referred to our clinic with a suspected diagnosis of asthma. The mother described breathlessness on exertion and a productive cough especially in the mornings. There was no failure to thrive. The patient had already been hospitalized four times with episodes of wheezing with partial respiratory insufficiency. Those episodes had been diagnosed as atypical pneumonia, due to their clinical presentation and typical radiological findings. The physical examination showed signs of chronic hypoxia. Consequently a chest CT was performed, which showed bilateral bronchiectasis. Two separate sweat test samples were negative. Nasal nitric oxid measurements for screening of primary ciliary dyskinesia were twice below 200 ppb. The ciliary function and ultrastructure (HSVM) was normal. The patient showed no evidence of immune deficiency. Multiple etiologies for bronchiectasis are known, nevertheless non-CF bronchiectasis are rare in children.

SGPP 99

Necrotizing pneumonia as the first manifestation of primary intestinal lymphangiectasia

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Background: Primary intestinal lymphangiectasia (PIL) is a rare disease of the lymphatic system characterized by abnormally dilated lymphatic vessels in the intestinal wall, resulting in protein-losing enteropathy. Children typically present with abdominal pain, diarrhea and oedema or ascites caused by hypoalbuminemia. Rare cases can present with signs of immune deficiency.
Case description: A 6-year-old girl presented with Streptococcus A necrotizing pneumonia. A history of multiple infections such as complicated pneumonia, recurrent bronchitis and erysipelas, lead to the suspicion of primary immunodeficiency. Repeated extensive immunologic workups only revealed fluctuating, slightly decreased IgG levels. A year later, the girl presented with oedema, ascites and persistent pleural effusion, and a slight hypoalbuminemia. Protein-losing enteropathy was identified and the diagnosis of PIL was made by albumin-marked scintigraphy showing small bowel and left colonic protein leakage and widened lymphatic vessels on duodenal biopsies. The patient recovered quickly with a low-fat diet enriched with medium chain triglycerides (MCT), resulting in normalization of albumin and IgG levels and showed no recurrence of severe infection since then.
Discussion and conclusion: Diagnosis of PIL may be challenging in patients with atypical manifestations. As shown in this case, PIL can present with repeated bacterial infections, suggestive of primary immunodeficiency. Nevertheless, in this case, the persistent pleural effusion was more likely due to a concomitant pleural lymphatic malformation rather than to the unfavorable course of the necrotizing pneumonia. Although only few cases are reported with relevant hypogammaglobulinemia, primary intestinal lymphangiectasia leads to intermittent intestinal loss of immunoglobulins resulting in a higher risk of infection. Immunoglobulin replacement therapy is not required in these cases, as hypoalbuminemia and hypogammaglobulinemia rapidly resolve with a low-fat MCT-enriched diet allowing regression of swollen abnormal intestinal lymphatics and decreased enteric leakage of plasma proteins, including immunoglobulins.

SGPP 100

Feasibility and normal values of an integrated conductivity (Nanoduct) sweat test system in healthy newborns

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Background: Nanoduct is a simple and practical sweat analysis system to measure conductivity in situ. It requires only three microlitres of sweat, which makes it especially applicable to newborns.
Methods: We measured conductivity in 260 healthy term infants at the age of four days, and again at four weeks to determine the proportion of successful tests, test duration, and normal values for sweat conductivity in newborns.
Results: Sufficient sweat was collected in 159/260 of four-day olds (61%), and in 225/239 of four-week olds (94%). Mean (sd) test duration was 27 (5) and 25 (5) minutes. Mean (sd, range) conductivity was 53 mmol/L (16, 8–114) at age four days, and 36 (9, 12–64) at four weeks.
Conclusions: Determination of sweat conductivity using Nanoduct is not very successful in four-day old newborns. However, at the age of four weeks the success rate is high (94%), and conductivity results at that age are comparable to what has been reported for older healthy children.

SGPP 101

The expansion and performance of national newborn screening programmes for cystic fibrosis in Europe

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Background: Newborn screening (NBS) for cystic fibrosis (CF) is a well-established public health strategy with international standards. The aim of this study was to provide an update on NBS for CF in Europe and assess performance against the standards.
Methods: Three questionnaires were sent to key workers in each European country, each specific to countries without NBS, national NBS programmes and regional NBS programmes.
Results: End 2016, there were 18 national programmes, 3 countries with regional programmes and 25 countries not screening in Europe. All national programmes employed different protocols, with IRT-DNA the most common strategy. Five countries were not using DNA analysis. In addition, the processing and structure of programmes varied considerably. Most programmes were achieving the ECFS standards with respect to timeliness, but were less successful with respect to sensitivity and specificity.
Conclusions: There has been a steady increase in national CF NBS programmes across Europe with variable strategies and outcomes that reflect the different approaches.

SGPP 102

Very early development of bronchopulmonary dysplasia in a premature infant; is it Wilson-Mikity Syndrome?

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Introduction: A few decades ago, bronchopulmonary dysplasia (BPD) was the end result of mechanical ventilation with oxygen toxicity and barotrauma in premature newborns with surfactant deficiency. Nowadays BPD (often referred to as "new BPD") describes a disruption of lung development in premature newborns, which may be affected by antenatal or early postnatal inflammation. Nearly 50 years ago, Wilson and Mikity described a syndrome of BPD in premature infants, characterized by early development of cystic interstitial emphysema despite minimal ventilator support. It is currently unclear whether Wilson-Mikity Syndrome (WMS) exists as a distinct entity.
Case report: A male infant was born through caesarean section at 25 weeks gestational age with a birth weight of 535 g because of placenta praevia and premature rupture of membranes (PROM). The effective date of PROM was not possible to evaluate, but an oligohydramnion was observed over a period of 4 weeks. The infant developed respiratory distress in the first minutes of life requiring intubation, administration of surfactant and high-frequency oscillation ventilation. On the second day of life, he could be switched to conventional ventilation, which had to be continued for a total of 5 weeks before the infant was extubated and put on non-invasive respiratory support. On chest X-ray, cystic interstitial emphysema was identified already on the third day of life and progressed to a picture of full-blown radiological BPD on the fifth day of life. Ureaplasma urealyticum was detected in tracheal secretions and treated with a 5-day course of azithromycin. Subsequently, the child showed progressive improvement and could be discharged at the age of almost 2 months on low flow oxygen support.
Conclusion/Discussion: This infant developed a very atypical form of severe BPD within the first few days of life, suggesting a diagnosis of Wilson-Mikity Syndrome. The cause of Wilson-Mikity Syndrome is unknown, but it is hypothesized that it represents the extreme end of a spectrum of atypical bronchopulmonary disease caused by individual susceptibility to mechanical stress, possibly triggered by inflammatory processes such as ureaplasma infection.

SGPP 103

A girl with Bernard-Soulier Syndrome without a hemorrhagic diathesis, demonstrating different severity of the bleeding phenotype

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We report on a 15-year-old Swiss girl who was referred to our clinic with suspected chronic immunothrombopenia due to prolonged, moderately severe isolated thrombocytopenia, without neither a clinical bleeding tendency nor a hemorrhagic history. Blood tests revealed thrombocytopenia with scattered giant platelets as well as a proteinuria, suggesting a MYH9 associated disorder. However, immunofluorescence analysis and the fluorescence activated cell sorter (FACS) of the peripheral blood smear excluded a MYH9 disorder, but showed a reduced expression of the membrane glycoprotein GPIbIX, which is highly suspecting a Bernard-Soulier-Syndrom (BSS). BSS was confirmed by a lack of platelet aggregation in response to ristocetin and a molecular genetic testing, which determined a heterozygous GP9 gene mutation in exon 3. This gene encodes a small membrane glycoprotein found on the surface of human platelets. Defects in this gene are a cause of Bernard-Soulier syndrome, which is an extremely rare congenital bleeding disorder, usually presenting with a von Willebrand deficiency like bleeding tendency at the mucous membranes, but sometimes without a hemorrhagic diathesis demonstrated in our case.

SGPP 104

Failure to thrive in an infant or a young child? Think of diencephalic syndrome associated with a hypothalamic/chiasmatic tumor!

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Background: Diencephalic syndrome (DS), a rare cause of failure to thrive in infants or young children, is caused by hypothalamic/chiasmatic tumors (most commonly low-grade gliomas [LGGs]). DS is characterized by severe emaciation despite average caloric intake, often combined with normal linear growth, loss of subcutaneous adipose tissue, hyperkinesia, euphoric mood, hyperthermia, vomiting, and/or nystagmus. Little is known about its pathophysiological mechanisms.
Case Description: A 5-month-old girl presented with repetitive vomiting, cachexia, generalized loss of subcutaneous adipose tissue, drinking refusal, hyperkinesis, euphoria and relative hyperthermia. Magnetic resonance imaging showed a hypothalamic/chiasmatic tumor compatible with a LGG. Treatment with carboplatin/vincristine was started. Due to rapid progression and hydrocephalus after 2 months, partial resection was performed (histology: pilomyxoid astrocytoma) and followed by treatment with vinblastine. After further progression 5 months later, therapy was switched to irinotecan/bevacizumab, subsequently leading to stabilization of tumor volume. – At diagnosis, ghrelin and growth hormone serum levels were significantly increased, whereas insulin and leptin were normal. However, later during a period of tumor progression, serum and cerebrospinal fluid ghrelin were normal, thereby not supporting a causative function of grehlin in DS. Fibroblast growth factor 21 (FGF21) plasma concentration was increased at diagnosis; however, no evidence for increased activity of brown adipose tissue as a potential cause of a hypermetabolic state was found on FDG-PET.
Conclusions: In infants and young children with failure to thrive, DS caused by a brain tumor should be considered as a possible cause. We could not identify a pivotal role for the metabolism-regulating hormones ghrelin, leptin, insulin, and growth hormone, or for FGF21 for the pathogenesis of DS. Chemotherapy is the mainstay of tumor treatment, as complete resection of hypothalamic/chiasmatic LGGs is most often not feasible.

SGPP 105

If the biopsy sets the outcome – an unusual evolution in a girl with hepatoblastoma

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Introduction: Hepatoblastoma (HB) is the most common primary liver tumor in childhood with peak age between 6 months and 3 years and the hallmark of high levels of a-Fetoprotein (AFP). By now the cure rate has improved to 80%. Risk estimation is based on the pre-treatment assessment of tumor extension (PRETEXT). According to the standards of the international childhood liver tumors strategy group SIOPEL, all patients receive platin-based chemotherapy prior to and after surgery. Diagnostic tumor biopsy is mandatory, the pfreferred method is a percutaneous ultrasound guided needle biopsy, using a "true cut" needle to avoid seeding tumor cells into uninvolved liver segments, the abdominal wall or peritoneal cavity.
Case report: We describe a girl with HB diagnosed at the age of 5 years, PRETEXT III. Diagnostics and therapy were performed according to the SIOPEL standards, for the biopsy using a "true

cut"-needle. Complete first remission was achieved, follow-up examinations were regularly performed. After eight months AFP-levels rose continuously, initially without other signs of relapse, 2 months later with a painful cutaneous swelling exactly at the site of the former biopsy. The tumor in the abdominal wall was resected, intraoperative exploration revealed another suspicious nodule in the great omentum. Both proved to be relapses of the primary HB, showing lymphovascular infiltration, the latter being the rationale for intensified postoperative chemotherapy. The AFP showed timely normalisation and had since remained low.
Discussion: Tumor seeding by "true cut"-needle biopsy seems to be rare, case reports and therapeutic evidence are lacking. Our case shows that "vaccination metastases" do occur, furthermore demonstrating that standard Platin-chemotherapy may not be sufficient to eradicate spreaded tumor cells. Rescue with second complete remission is possible, yet at the price of intensified toxic chemotherapy with increasing risk of long term sequelae. This raises the question if the standard procedure of percutaneous biopsy should be revised.
Conclusion: "True cut"-needle biopsy does not fully protect from seeding tumor cells into neighbour tissue. To avoid the burden of a second line therapy the standard procedure of percutaneous biopsy should be questioned in favour of biopsies by laparascopy or minilaparatomy, providing sufficient material for histological classification as well as biological research to identify more precise risk profiles and treatment.

SGPP 106

Methemoglobinemia in children reported to Tox Info Suisse: a retrospective case series

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Objectives: Pediatric acquired methemoglobinemia is rare nowadays. However, it has to be taken in consideration in case of acute cyanosis. Therefore, a methemoblobin (Methb) level should be measured, and an accurate medical history including dietary factors should be obtained to reveal the triggering agent. The purpose of this study was to assess epidemiologic and clinical data of children with increased MetHb levels.
Methods: Retrospective analysis of pediatric cases (<16 y) with MetHb >3% reported by physicians to Tox Info Suisse between 1996 and 2016. The severity of symptoms was graded according to the Poisoning Severity Score.
Results: 16 cases were included (9 females and 7 males). The mean age was 0.8 y (range 0.6–15 y). MetHb level ranged between 10% and 57% (median 25%). An asymptomatic course was reported in 1 case, minor sings in 4 cases, moderate signs in 6 cases and severe signs in 4 cases. In 1 case information regarding the clinical course was lacking. There were no fatalities. Additional to the methemoglobinemia, recorded signs and symptoms included cyanosis (13 cases), tachycardia (4), vomiting (3), somnolence (3), tachypnea (1), vertigo (1), and hemolysis (1). 8 cases (age 8–12 months) were attributed to the consumption of homemade vegetable purée: Brassica oleacea (n = 3), Cucurbita pepo (n = 2), Foeniculum vulgare (n = 2) and Spinacia oleraca (n = 1). Other offending agents were: lidocaine/prilocaine (n = 3), dapsone (n = 2), and amyl nitrite (n = 1, poppers abuse by a 15-y-old boy). In 2 cases no causing agent was identified. 8 children (MetHb 15–57%, median 36.5%) were treated with a single dose of methylene blue (1–1.5 mg/kg iv) with rapid improvement without relaps of methemoglobinemia. 1 child (MetHb 44.6%) with severe dapsone poisoning with hemolysis received methylene blue in repeated doses. In 7 cases (MetHb 9.8–25.4%, median 12%) the methemoglobinemia was of short duration and resolved spontaneously without treatment. Further investigation for pathological enzyme activity or hemoglobin variants was performed in 3 children with negative results.
Conclusion: Consumption of homemade vegetable purée by toddlers was the main cause of methemoglobinemia in this series. In case of prompt improvement after a single dose of methylene blue or spontaneous recovery, and the identification of the triggering agent, further investigations regarding inborn factors for methemoglobinemia seem not to be necessary after a single episode of methemoglobinemia.

SGPP 107

M-ficolin in pediatric patients at diagnosis of acute lymphoblastic leukemia

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Background: M-ficolin (MF) is a pattern-recognition molecule of the lectin pathway of complement activation produced by phagocytes (neutrophils, monocytes). Its serum concentration is correlated with absolute phagocyte counts (APC) in peripheral blood (PB). However, five pediatric patients with B-cell acute lymphoblastic leukemia (B-ALL) and disproportionately high serum MF (>1.5 µg/mL) despite very low PB APC (≤0.5 G/L) have been described. This retrospective single-center cohort study aimed to describe serum MF in pediatric patients with ALL, and to explore potential associations with MF positive ALL blasts.
Methods: Remnants of sera and air-dried bone marrow (BM) smears collected at ALL diagnosis in pediatric patients ≤17 years were used. MF serum concentration was measured externally by TRIFMA using the monoclonal MF antibody 7G1. We developed a protocol for manual immunostaining BM smears with this antibody and transferred it into a protocol for automated double-immunostaining with additional blast staining (anti CD79a for B- or mature B-ALL, anti CD3 for T-ALL). We determined the proportion of double positive cells, i.e., MF positive blasts, and used uni- and multivariate linear regression to analyze associations of serum MF with different clinical and laboratory parameters.
Results: We retrieved serum remnants in 55 of 61 (90%) newly diagnosed pediatric patients with ALL. The median serum MF concentration was 0.47 ug/mL (range, 0.02 to 6.97; IQR, 0.18 to 1.11). The median APC was 0.65 G/L (range, 0.00 to 13.62; IQR, 0.29 to 2.01). We found no disproportionately high serum MF in patients with very low APC (0 of 55; 0%; 95% exact CI, 0 to 6). We retrieved BM smears in 50 of these 55 patients and reached acceptable quality of double-immunostaining in 38. We found low proportions (0.5% to 5%) of MF positive blasts in 8 (21%) of these 38 BMs. Serum MF was significantly and independently associated with PB APC, PB platelets and BM segmented neutrophils (total variance explained, 68%), but not with the presence or the proportion of MF positive blasts.
Conclusion: We could not confirm an earlier finding of disproportionately high serum MF concentrations despite low PB APCs in some pediatric patients with ALL. As expected, serum MF reflected different parameters of remaining intact hematopoiesis. MF positive leukemic blasts were rarely found, without evidence of a relevant role in the production of MF detectable in serum.

SGPP 108

Methemoglobinemia ans hemolysis after fava beans ingestion: uncommon G6PD deficiency dianosis

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Introduction: Methemoglobinemia is a rare disease. In children, it is most commonly due to toxic levels of oxidant drugs. The association between methemoglobinemia and glucose 6 phosphate dehydrogenase (G6PD) deficiency is rarely described in the literature.
Case report: A healthy 10-month-old boy presented to the emergency department with a history of malaise, perioral cyanosis, fatigue, jaundice, mild tachypnea, and an oxygen saturation (SpO_2) of 70%, resistant to supplemental oxygen. There were no other signs or symptoms and no abnormalities on chest X-ray. A day prior, he had eaten fava beans. The blood analysis revealed normal gas tensions and increased methemoglobin fraction (13%) associated with a severe hemolytic anemia. A red blood cell transfusion improved the anemia, decreased the methemoglobinemia level and increased the SpO_2 to above 90%. The initial G6PD activity assay was normal. No toxins nor drugs were found in the blood analysis and haemoglobin (Hb) electrophoresis was normal. Four months after the illness, repeat assays confirmed the diagnosis of G6PD deficiency.

Discussion: Methemoglobin is an altered state of hemoglobin in which the ferrous (Fe++) iron of the heme group is oxidized to the ferric (Fe+++) ion, making it unable to bind oxygen. This occurs when oxidative stress is important, and the pathways for its reduction are overwhelmed. G6PD plays a key role in the protection of erythrocytes against oxidative stress. In G6PD deficiency, the oxidizing products of fava beans cannot be reduced by the G6PD-dependant hexose monophosphate shunt, inducing a severe oxidative stress that leads to hemolysis and increased methemoglobinemia. The only treatment for our patient was red-blood-cell transfusion, as methylene blue, the usual treatment, may increase hemolysis since the capacity to reduce the drug to active leucomethylene blue is limited in G6PD. The first negative G6PD test in our patient was thought to be secondary to the important reticulocytosis at the time of the illness.
Conclusion: G6PD deficiency is a condition that makes erythrocytes susceptible to hemolysis under conditions of oxidative stress, such an ingestion of fava beans. The association with methemoglobinemia is uncommon and rarely described in the literature. Our case is a reminder that the simultaneous occurrence of methemoglobinemia and hemolysis in a symptomatic patient with a history of fava beans ingestion, should raise the possibility of G6PD deficiency.

SGPP 109

Whole exome sequencing for the identification of primary immunodeficiencies in a national cohort of children with bacterial sepsis

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Background: Many primary immunodeficiencies (PIDs) are associated with an increased susceptibility to bacterial infection. However, the presence of underlying PIDs among pediatric sepsis cases has not been systematically evaluated. We hypothesized that community-acquired sepsis may represent the first manifestation of an underlying PID and performed whole exome sequencing (WES) of samples collected from a national cohort of children with bacterial sepsis.
Methods: Eligible children were previously healthy children admitted to the ten largest children's hospitals in Switzerland between 01.09.2011 and 31.12.2015 with community-acquired sepsis caused by S. aureus, S. pneumoniae, S. pyogenes, H. influenzae, or E. coli. Analysis of WES data was restricted to rare variants (<1% and <0.1% MAF for homozygous/hemizygous and heterozygous variants, respectively) in 182 PID genes for which an association with increased susceptibility to bacterial infection has been described in the literature.
Results: A total of 23 rare homozygous/hemizygous variants were found in 23/154 patients (15%). There was a larger number of very rare monoallelic variants in genes for which heterozygous mutations have previously been associated with immunodeficiency and susceptibility to bacterial infection. No major differences between infections caused by the different pathogens or sepsis severity and the likelihood of detecting mutations in PID genes were seen.
Conclusion: WES allowed to detect potentially pathogenic variants in previously reported PID genes. While functional confirmation of these variants is pending, the findings suggest that PIDs might be more common than previously thought among apparently healthy children experiencing a first sepsis episode. WES represents a promising approach to diagnose PID in children with sepsis.

SGPP 110

Food introduction with focus on prevention of peanut allergy: A Swiss Pediatric Immunologist and Allergist group position paper

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Background: Food allergy and especially peanut allergy are reported to increase worldwide. Therefore, clinicians and researchers are frequently asked about prevention strategies for food allergy. Nutrition is a crucial environmental factor in early life, influencing the development of the child's immune system and hence potential strategies based on nutrition are currently explored. It is already agreed internationally that there is no benefit in allergy prevention by avoiding or delaying introduction of any specific solid food in the healthy baby during the first year of life. These aggreements resulted in the current guidelines of the Swiss Society of Pediatrics and the Federal Commission for Nutrition, that solid foods, without any dietary restrictions shall be introduced in the diet of a healthy baby around the age of 4 to 6 months, regardless of the risk of atopy. Based on currently available data, introduction of solid foods earlier than after 4 months of life is not recommended. Recently, a randomized clinical trial, the Learning Early About Peanut Allergy (LEAP) study conducted in the UK, has shown that, among high risk children, planned early introduction of peanut and continued with regular intake decreases the risk of peanut allergy at 5 years of age. Based on these results, a consensus was released and stated that early consumption (between 4 and 11 months of age) of peanut-containing products should be recommended among high risk infants, especially in countries with high prevalence of peanut allergy. Although food allergies are believed to be on the rise, data on their prevalence are lacking in many countries, which is also the case in Switzerland.
Conclusion and recommendation: The Swiss Pediatric Immunologist and Allergist group (PIA-CH) recommends the introduction of solid food between 4 and 6 months and that allergenic solids, such as peanut, do not need to be avoided. However, as peanut is not a staple food for young children and recent data on prevalence of peanut allergy are lacking in Switzerland, we currently do not recommend specifically early introduction of peanut as a primary allergy prevention for peanut allergy among high-risk infants. Furthermore, we conclude that epidemiologic studies are needed to examine de prevalence of food allergies in Switzerland.

SGPP 111

Susceptibility to infection and lack of NETosis in severe G6PDH deficiency – a phenotype similar to chronic granulomatous disease

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Glucose-6-phosphate dehydrogenase (G6PD) deficiency is well known to cause hemolytic anemia, linked to impaired nicotinamide adenine dinucleotide phosphate (NADPH) production and imbalanced redox homeostasis in erythrocytes. As G6PD is expressed by a variety of hematologic and non-hematologic cells, a broader clinical phenotype could however be postulated in G6PD deficient patients. We describe three brothers with severe G6PD deficiency and susceptibility to bacterial infection, linked to strongly reduced NADPH oxidase function and consequently impaired formation of neutrophil extracellular traps (NETs). Defective NET formation has so far been only observed in patients with the NADPH oxidase deficiency chronic granulomatous disease (CGD) who require antibiotic and antimycotic prophylaxis to prevent life-threatening bacterial and fungal infection. As severe G6PD deficiency can be a phenocopy of CGD with regard to the cellular and clinical phenotype, careful evaluation of neutrophil function seems mandatory in these patients to decide on appropriate anti-infective preventive measures.

SGPP 112

Allergic/atopic disease due to milk-protein allergy associated protein-losing enteropathy

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Case: A five-month-old boy was referred by his GP because of a chronic generalized rash, perianal dermatitis and failure to thrive. During the previous weeks, he also showed loose, frequent stools. He had been diagnosed with therapy-refractory eczema at 2 months of age. At presentation, he showed a maculopapular rash/eczema with dry skin predominantly on head, trunk and perineum. He appeared dystrophic, yet alert; clinical examination revealed reduced general muscle tone and mild hepatomegaly. Anthropometrics showed weight <P3 (5.0 kg), length P3-10 and head circumference P10-25. There were no clinical warning signs for immune deficiency. He had been fully breast fed since birth; formula milk had been added a few days ago, exacerbating the rash and loose stools. His mother is neither vegetarian nor vegan. Further tests revealed eosinophilia, elevated IgE, hypoalbuminemia, very low alkaline phosphatase and elevated thyroid-stimulating hormone with normal T3/4. Zinc deficiency secondary to protein allergy was presumed with protein-losing enteropathy leading to digestive malabsorption and malnutrition, later confirmed by highly positive IgE (class 5) against milk proteins. An extensively hydrolysed formula was introduced (Alfamino). Zinc deficiency was confirmed with low serum level (6.7 mmol/l). Further laboratory evaluation also showed deficiency of Vitamin D and iron (with normal haemoglobin). Oral supplementation with zinc (4 mg/kg/d) and Vitamin D (1000 IE/d) was started. As the skin lesions improved rather slowly on oral Zinc supplementation (which is at variance with isolated zinc deficiency), a coexistent atopic dermatitis was suspected. Introduction of topical steroid led to rapid improvement of the skin lesions. After two weeks in hospital, the general condition and the stool pattern had normalized, and the patient had significantly gained weight (5.9 kg).
Conclusion: The child was diagnosed with severe allergic/atopic disease due to milk-protein allergy associated protein-losing enteropathy leading to digestive malabsorption and malnutrition and dermatitis. The severe dermatologic manifestation is caused by a combination of severe zinc deficiency and atopic dermatitis. This case shows the importance of extending the differential diagnosis and re-evaluating a patient with therapy-refractory eczema, in particular in association with failure to thrive.

SGPP 113

Hematopoietic stem cell transplantation for purine nucleoside phosphorylase deficiency patients cures combined immunodeficiency and prevents progression of neurological symptoms

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Purine nucleoside phosphorylase (PNP) deficiency is a rare autosomal recessive disorder of purine metabolism causing severe combined immunodeficiency (SCID) and progressive neurological disease. The only treatment is allogeneic hematopoietic stem cell transplantation (HSCT) but the current knowledge about clinical presentation and outcome is still scarce. We applied HCST to 36 children and followed their outcome at our institutions. Their median age at transplantation was 2.3 years (2 mo–17.1 yrs). The patients presented with neurological (n = 30), infectious (n = 28) and autoimmune (n = 7) complications. 13 patients received transplants from HLA-matched family/sibling, 11 patients from matched unrelated donors, other 12 received transplants from HLA-mismatched family (n = 3), unrelated (n = 1), or mismatched cord blood donors (n = 8). The first conditioning regimen (n = 12) comprised Fludarabine, Melphalan and rabbit ATG. The second regimen (n = 19) consisted of intravenous (one case oral) Busulfan, Cyclophosphamide, rabbit ATG. The third regimen (n = 4) comprised Treosulfan and Fludarabine. Stem cell sources comprised peripheral blood stem cells, bone marrow and cord blood. One patient was transplanted without conditioning. A stem cell boost, donor lymphocyte infusion or re-transplantation was required in 6 patients. At a median follow-up period of 8.5 years (6 mo–14.8 yrs), n = 30 patients are alive with stable and complete immune reconstitution, 21 patients with stable myeloid donor cell chimerism of ≥90% and in 7 of 25–89%. N = 28 of the surviving patients showed ameliorated or residual and stable neurologic abnormalities, including motor dysfunction, ataxia, mental retardation, and sensorineural deficits, whereas 2 progressed and stabilized thereafter. The interindividual differences of neurological sequelae was broad. Five exhibited acute GvHD stage ≥2 and seven chronic GvHD. Six patients died of severe infectious (n = 3), GvHD (n = 2) or neurological (n = 1) complications. In this worldwide largest cohort of PNP patients, myeloablative and low toxicity conditioning regimens followed by related and alternative donor transplants led to long-term full or partial myeloid donor chimerism sufficient to cure immunodeficiency/autoimmunity and to mostly abolish progression of neurological symptoms. Autologous reconstitution remained a problem, but could be overcome by additional cellular therapies.

SGPP 114

Cernnunos disease: a syndromic, radiosensitive primary immunodeficiency syndrome

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Introduction: We present a patient with severe autoimmune hemolytic anemia (AIHA), albinism and growth retardation where immunological investigations revealed severe combined immunodeficiency disease (SCID) with radiosensitivity. Genetic analyses showed a homozygous mutation in the XLF-gene, compatible with Cernnunos syndrome. Mutations in Cernunnos/XLF lead to defects in the non-homologous end joining (NHEJ) pathways of DNA repair and have been identified as responsible for radiosensitive SCID. NHEJ is the major DNA double strand break (DBS) repair pathway in mammalian cells. Defects in this pathway impair process of V(D)J recombination functions and class switch recombination, which are important for immune response and T and B cell diversity.
Case Report: A 13-month-old twin girl was referred to our hospital with an acute episode of Coombs positive AIHA. At that time, she had been treated with high dose steroids and intravenous immunoglobulins (IVIG). Growth retardation with microcephaly and thumb malformation were noted. Since the age of 6 months she had developed partial albinism of the scalp hair and cilia. Except for mild rhinitis almost from birth, she did not suffer from severe infections. Besides anemia, mild thrombocytopenia, lymphocytopenia was noted. Immunological work up showed almost absent naïve CD4+ helper T cells and absent CD31+ recent thymic emigrants. T cell receptor analyses revealed polyclonal but sparse TCR rearrangement, maternal T cell engraftment was ruled out. Mitogen proliferation rate was reduced and there was marked radiosensitivity. Analyses of neonatal dried blood spots showed absent T cell receptor excision circles (TRECs), compatible with SCID. For the moment, the girl is treated with low-dose steroids for AIHA, regular IVIG and antibiotic prophylaxis. Unrelated donor hematopoietic stem cell transplantation is planned, as no matched sibling donor is available.
Conclusion: In conclusion, patients with a combination of unusual symptoms including autoimmunity, microcephaly, albinism, and lymphopenia should be evaluated for SCID.

SGPP 115

Cyclic neutropenia: a rare cause of recurrent fever and infection in childhood

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A 6-year-old girl was referred for assessment of 'chronic idiopathic neutropenia' and recurrent episodes of fever, mucositis/gingivitis, and skin abscesses. Additional symptoms included headache, arthralgia, and sometimes diarrhoea. Episodes were associated with markedly raised CRP. The administration of oral antibiotics resulted in a more rapid clinical improvement but prophylactic antibiotics did not prevent episodes. She had been admitted twice to hospital for administration of IV antibiotics and/or surgical intervention of abscesses. Neutropenia had first been noticed when she was 1.5 years old. Over the past years, absolute neutrophil counts (ANC) ranged between 0–4 G/l. No obvious cyclic pattern was seen during former serial blood count testing. Previous anti-neutrophil antibody testing was negative and bone marrow (BM) investigation had shown normal cellularity with reduced number of mature granulocytes (peripheral ANC 0.27 G/l). The diagnostic immunological work-up in our department was largely normal apart from low T cell numbers, which had already been noted in the past. A repeated BM investigation showed normal cellularity with this time a reduced number of granulocyte precursors (peripheral ANC 3.5 G/l). During serial blood count testing over 6 weeks, ANCs were generally low showing only one peak while the patient was well. However, she suffered from 2 febrile episodes during periods of low ANCs. By extending the observation period and measuring CRP, a clear cyclic pattern was observed with neutropenic episodes and symptoms occurring every 19 days. Cyclic neutropenia was finally confirmed by the detection of a heterozygous variant in the ELANE gene, which has previously been associated with both congenital and cyclic neutropenia. This variant in the intron 4 leads to a loss of the splice site, the use of a cryptic splice site upstream and, consequently, to a deletion in exon 4 (NP_001963.1:p.Val190_Phe199del). The patient is now being treated with s.c. G-CSF resulting in an increase and a shorter duration of ANC nadirs associated with an almost complete disappearance of symptoms. Cyclic neutropenia is a rare form of congenital neutropenia, which can be challenging to diagnose. In cases where diagnosis is suspected and the cycling pattern is not obvious extending serial blood count testing beyond the usually recommended 6 weeks and/or combine it with repeated CRP measurements may reveal the diagnosis.

SGPP 116

Causes of low neonatal T-cell receptor excision circles (TRECs): a systematic review

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The amount of research addressing TREC screening in newborns in order to identify immunodeficiency has increased dramatically in recent years, making it challenging for clinicians and researchers alike to have an overview over all the recent progress in the field. In this Systematic Review we aim to provide a systematic overview of studies describing patients with low TRECs and the associated diseases, which range form genetically caused Severe Combined Immunodeficiency to idiopathic lymphopenia. We aim to provide clinicians with guide on what diseases to assess when evaluating neonates with low TRECs and aid them in the management of such patients until a diagnosis is made. Furthermore we would like to highlight the need further research into the origins of lymphopenia at birth. Systematic Review: Medline, Embase and Scopus were searched, checking reference lists of included studies and review articles complemented searches. Papers were selected by one reviewer, and assessed and extracted in duplicate. Our search retrieved 1'747 studies, the 42 included papers investigated 24 different genes in 6'093'942 individuals. One additional genetic cause for low TRECs was identified in included abstracts. The largest single study enrolled almost half of all included newborns. The most common genetic cause for low TRECs was 22q11.2 deletion syndrome followed by Interleukin-2 receptor gamma (IL2RG) and adenosine deaminase (ADA) deficiency. Furthermore 12 syndromes were associated with low TRECs at birth as were diseases with no apparent genetic cause, such as ileal atresia.There was a large variability in the design, execution and reporting between studies, thus making large statistical analysis impossible. We recommend following cut of values and screening algorithms as proposed in large prior studies. Based on the published data we can encourage screening for specific genetic mutations associated with Primary Immunodeficiencies (PID). Furthermore we provide a list of syndromes and other diseases that should be excluded in with low TRECs. During the assessment of patients we recommend stringent management. Finally our research reveals the very high percentage of patients left undiagnosed after a positive TREC screening, highlighting the much needed research in the field of PIDs.

SGPP 117

More than monocytopenia: phenotype of 7 patients with GATA2 deficiency

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Immunodeficiency driven by heterozygous germline GATA2 mutations is marked by a wide variety of phenotypes. First published as a syndrome of monocytopenia and nontuberculous mycobacterial infection (MonoMAC) in 2010, more recent data suggests a broader spectrum ranging from a mildly increased susceptibility to infection [JT1] to life-threatening inflammation, malignancy and thrombosis. We present a series of 7 affected patients seen at our institution since 2014 with different pheno-/genotypes. Most of our patients presented with classical findings such as recurrent warts, monocytopenia and myelodysplastic syndrome. However, one patient was diagnosed with Emberger syndrome (primary lymphedema with myelodysplasia), one with pulmonary alveolar proteinosis, one had suffered from nontuberculous mycobacterial infection, one patient had severe aphthosis and recurrent episodes of fever and one patient had experienced an episode of severe varicella infection. Our aim is to highlight possible clinical features described in the current literature in relation to our patients. Since diagnosis is often substantially delayed, we would like to emphasize the clinical and laboratory findings that should prompt the consulting physician to think of this genetic disorder and thereby help to raise awareness of GATA2 deficiency.

SGPP 118

Delayed rash during penicillin treatment: not always a benign condition!

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Background: Delayed skin rashes are frequently observed in children with ongoing penicillin treatment. They are in a gran majority of cases benign and due to the underlying infection rather than a true drug allergy. The oral provocation test (OPT) without prior allergy testing have been shown to be a secure procedure and is now proposed as a first line diagnostic tool in these children. Prior to considering OPT, a precise clinical history is mandatory in order to classify the reaction, as severe cutaneous drug reactions (SCAR) have been described in children with an associated high morbidity and mortality rate.
Case report: We present the case of a healthy 15-year-old girl who reached our consultation with a history of adverse drug reaction to penicillin 3 years earlier. At the 5th day of treatment with phenoxymethylpenicillin for a Streptococcal angina, she started with a progressively generalizing urticarial rash evolving in bluish and yellowish lesions together with high fever, facial edema, arthralgia, conjunctivitis with photophobia, dyspnea, thoracic pain, buccal aphthae and cheleitis. Symptoms persisted for 1 week without bullous lesions, cutaneous desquamation or scars. No allergy work-up and no

specific avoidance measures have been initially proposed and a penicillin treatment was again prescribed but its intake refused by the mother who subsequently reached our consultation. The clinical history being highly suspicious for a drug allergy with severity sings (mucosal involvement), we recommended strict avoidance of all penicillins. In order to provide a safe alternative in the β-lactam family, we performed path test (PT) to cefuroxime, ceftriaxone but also amoxicillin. PT was only positive for amoxicillin confirming the diagnosis of penicillin allergy. 5 days after PT, the patient developed an isolated maculo-papular rash lasting 4 days.
Conclusion: With this case report we want to highlight the importance of an accurate clinical history searching for severity signs suggestive for SCAR while evaluating a child who experienced a delayed skin rash during penicillin treatment. These signs include a prolonged rash, fever, mucosa involvement, bullous lesions, cutaneous desquamation, facial edema, icterus, hepatosplenomegaly and diffuse lymphadenopathies. As these allergic reactions are potentially life-threatening, the child should be referred to an allergologist for further evaluation prior to considering diagnostic OPT.

SGPP 119

Pre- and probiotic interventions in neonate piglets to support vaccination efficacy against Salmonella Typhimurium

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Long-chain inulin type fructan (lc inulin) is a fermentable fibre demonstrated to have direct immunogenic properties, and known to enhance T helper cell skewing towards Th1. Lactobacillus acidophilus (L. acidophilus) is a commonly used probiotic thought to mainly benefit health by its production of vitamin K and lactate. Here we tested whether Frutafit® lc inulin alone or combined with the L. acidophilus strain W37 can enhance efficacy of vaccination against Salmonella Typhimurium. Piglets were used as a model for early life in humans. Piglets were cross-fostered 24 hours after birth, dietary intervention started 24 hours later and continued until sacrifice. Piglets were assigned to 4 groups i. placebo non-vaccinated, ii. placebo vaccinated iii. lc inulin (Frutafit®) vaccinated and iv. lc inulin/L. acidophilus W37 (Winclove B.V.) vaccinated. Animals were weaned on day 24, and vaccinated with a single doses of Salmoporc STM® (IDT Biologika) 24 hours after. To analyse the effect on protection against Salmonella, animals were challenged with this pathogen, daily, for 3 consecutive days before sacrifice, on day 55. All zootechnical parameters were measured daily to study animals well-being, it included diarrhoea occurrence and severity. Blood was sampled prior and post vaccination, 3 weeks post vaccination, prior and after challenge. Plasma was used for anti-body titre measurements. Finally, fresh whole blood was used for staining and flow cytometry analysis of innate cells (granulocytes, monocytes and NK cells), T cells (cytotoxic and helpers) and memory T cells. Animals were healthier in the groups that received a dietary intervention compared to placebo groups. Moreover, diarrhoea occurrence and severity was significantly lower in lc inulin and lc inulin/L. acidophilus W37 groups compared to placebo groups. Four weeks after vaccination, prior challenge, the animals treated with the combination lc inulin/L. acidophilus W37 showed an increase of anti-body production, markedly increased post-challenge. T helper cells were significantly enhanced in the lc inulin treated group, but not when lc inulin was combined with L. acidophilus W37. Finally, trends towards increased frequency of memory T cells was observed in both lc inulin alone and lc inulin/L. acidophilus W37 treated groups compared to vaccinated placebo groups. Piglets benefited from both treatments, and the combination lc inulin/L. acidophilus W37 strongly enhanced anti-body titre production.

SGPP 120

Recurrent headache in a girl with autoinflammatory syndrome: evidence of unexpected idiopathic intracranial hypertension as a contributive component

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Introduction: We report, here, for the first time the association of autoinflammatory syndrome (AS) with chronic idiopathic intracranial hypertension (IH) and normal ophthalmic examination (OE). The diagnosis of TRAPS is discussed. A recent report from the Eurofever/EUROTRAPS international registry pointed out the genetic heterogeneity of the tumor necrosis factor receptor-associated periodic syndrome (TRAPS) accompanied by a variable disease phenotype at presentation.
Case report: A 4-year-old girl born full-term with uncomplicated pregnancy and delivery, presented recurrent episodes of fever by the age of 2 years, every 2 weeks, lasting 1–3 days and sometimes associated with febrile generalized seizures. Growth and psychomotor development were normal. Extensive biological investigation was performed to look for a monogenic AS. Immunodeficiency was excluded and investigation of a genetic defect associated with hyperimmunoglobulin D syndrome (HIDS), cryopyrin-associated periodic syndrome (CAPS) and the tumor necrosis factor receptor-associated periodic syndrome (TRAPS) was performed. The heterozygous R92Q variant in the gene for tumour necrosis factor receptor superfamily member 1A (TNFRSF1A) was identified, raising the suspicion of TRAPS. A treatment with an interleukin-1 beta-blocker (Canakinumab, Ilaris®) was started at the age of 3 years old. At the age of four, she was referred to our paediatric emergency clinic for a severe headache with some characteristics of migraine. To exclude meningitis, lumbar puncture (LP) was performed and the migraine was unexpectedly abolished. Some weeks later, in front of a relapse of headache, assessment of intracranial pressure by LP technique showed a severe IH (44 cm H_2O [15–20]), while eye fundus examination and brain MRI were normal. Despite switching from canakinumab (interleukin-1 antagonist) to interleukin-1 receptor antagonist (anakinra, Kineret®), systemic steroid and acetazolamide treatment, she needs now every four weeks a LP because of severe headache: it shows constant IH, with normal OE.
Conclusion: This case report suggests that assessment of intracranial pressure by lumbar puncture technique should be performed in all patients with AS and headache, even in the absence of any signs of IH by OE. The etiology of IH in this case is still not known. Differential diagnosis after exclusion of the other known causes of IH, is 1) part of the clinical phenotype of AS or 2) a secondary effect of IL-1β blockers.

SGPP 121

Changes in HSDS, BMI, bone age and Tanner stage in response to GH treatment over 3 years in children with IGHD or born SGA

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Background: Approximately one-third of children born small for gestational age (SGA) enrolled in the American Norditropin Studies: Web-Enabled Research (ANSWER) Program have growth hormone deficiency (GHD).
Objective: To evaluate the impact of GHD status on changes in height SDS (HSDS), BMI, bone age per chronological age (BA/CA), and Tanner stage in patients with isolated GHD (IGHD) and children born SGA in response to 3 years of Norditropin® treatment (somatropin rDNA origin, Novo Nordisk A/S, Denmark).
Design/Methods: GH-treatment-naïve patients aged <18 years with SGA or IGHD participating in the ANSWER Program. SGA patients were further stratified by peak GH levels to stimulation (<10 or ≥10 ng/mL). Rate of Tanner shift per year included patients who were stage 1 at baseline and stage 2+ post-baseline. Mean change in BMI SDS (DBMI SDS), and HSDS (DHSDS) from baseline and BA/CA were compared between patient groups from Y1 to Y3 using analysis of covariance with Tukey-Kramer adjustment.
Results: Mean GH dose per group was 0.05 mg/kg/day both at baseline and at Y3. Although SGA patients w/o GHD responded well to treatment, ΔHSDS in this group was significantly lower vs IGHD patients ($p \leq 0.0032$; Y1 to Y3) and SGA patients with GHD at Y1 ($p < 0.05$). SGA patients w/o GHD achieved greater bone maturity compared to those with GHD (BA/CA Y1 to Y3: 0.86 to 0.98 vs 0.84 to 0.88). A lower proportion of SGA patients w/o GHD shifted from Tanner stage 1 to 2+ by Y3 compared to those with GHD and IGHD patients.
Conclusions: These results suggest that 1) growth response to GH treatment in children born SGA may be influenced by the patients' GH secretory status and 2) SGA patients treated with GH with or w/o GHD enter puberty at a rate similar to that observed for IGHD patients.

SGPP 122

Impact of celiac disease on children's growth

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Objectives: Growth is a major concern in pediatrics, especially in children with chronic disease. Failure to thrive can develop as the consequence of malabsorption in children with celiac disease but can also be the sole disease manifestation. The goal of this retrospective study was to assess growth evolution in children and adolescents with celiac disease under a gluten-free diet, and determine if there is a correlation between the evolution of these parameters and the age at diagnosis or adherence to diet.
Methods: 141 patients (103 girls, median age 6.8 ± 4.1) with a histologically confirmed diagnosis of celiac disease have been included. Growth parameters (weight, height, body mass index (BMI) and growth velocity) expressed as z-scores, were collected at time of diagnosis and after one, two and five years of follow-up under gluten free diet, as well as antitransglutaminase IgA antibodies levels when available.
Results: Only a few children had failure to thrive for weight (10%) and/or height (6%) at diagnosis. However, after introduction of a gluten-free diet, a significant increase in all growth parameters was observed after one year, persisting after two years for height. No correlation could be found between the evolution of growth parameters and the age at diagnosis. Surprisingly, a negative correlation has been established between these parameters and adherence to the diet.
Conclusion: Newly diagnosed children with celiac disease develop catch-up growth over the first year of a gluten-free diet, with height increase persisting over the second year. Age at diagnosis did not influence evolution of growth.

SGPP 123

Severe hypokalaemia due to diuretic abuse in a teen girl with eating disorder

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Background: Hypokalaemia (HK) is found in up to 20% of people with eating disorders and is a clinically relevant electrolyte abnormality, since it can cause life-threatening conditions, such as cardiac arrhythmias, myopathy, rhabdomyolysis, and nephropathy. HK in eating disorders usually develops as a result of diuretic improper use and gastrointestinal losses. This report describes a teen girl with a severe HK. A thorough medical history and proper laboratory tests allowed an early and accurate diagnosis and treatment.
Clinical case: A 15-year-old female patient was admitted to our emergency department with a four month history of weakness, dizziness, light-headedness, nausea and abdominal pain, and mild myalgia. On admission, she was fully responsive. Physical examination was unremarkable, blood pressure was 115/78 mm Hg, heart rate 81 bpm. Blood tests revealed severe HK (1.9 mmol/l), hyponatremia (125 mmol/l), hypochloraemia (77 mmol/l), hypomagnesaemia (0.68 mmol/l), metabolic alkalosis (HCO3– 28.2 mmol/l) and pre-renal acute kidney insufficiency (creatinine 71 µmol/l). ECG showed severe abnormalities of ventricular repolarization (QTc max 0.52 msec). Intravenous K+ supplementation, together with rehydration was immediately undertaken. Over the following 48–72 hours, electrolytes, metabolic alkalosis, kidney insufficiency and the ECG intervals gradually normalised. The adolescent denied diarrhoea, vomiting, and any ingestion of laxatives, and diuretics. Nevertheless, finally urine screening for diuretics showed a large amount of furosemide. On ultrasound scan signs of mild nephrocalcinosis were found as well, indicating chronic abuse of diuretics. When faced with the positive result of diuretic intake, the patient finally admitted the drug abuse and self-dietary restrictions over the last months. The patient agreed to stop taking diuretics and to undergo a psychiatric evaluation.
Conclusions: HK is the most relevant electrolyte abnormality in patients with eating disorders. Only a minority of adolescents with eating disorders abuse diuretics and correct and prompt diagnosis can be very difficult, as these patients often deny diuretic intake. Concealed diuretic abuse, associated or not with surreptitious vomiting and laxative abuse, should always be taken into account in young women with eating disorders. Urine screening for diuretics should be performed in patients who deny diuretic intake.

SGPP 124

Acute kidney injury in primary Epstein-Barr virus infectious mononucleosis: systematic review

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Background: textbooks and reviews do not mention the possible association of primary symptomatic Epstein-Barr virus infectious mononucleosis with acute kidney injury in subjects without immunodeficiency or autoimmunity. Methods: stimulated by our experience with two cases, we performed a systematic review of the literature according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. Results: the literature documents 38 cases (26 male and 12 female individuals ranging in age from 0.3 to 51, median 18 years) of primary Epstein-Barr virus infectious mononucleosis complicated by acute kidney injury: 27 acute interstitial nephritides, 1 jaundice-associated nephropathy, 7 myositides and 3 hemolytic uremic syndromes. Acute kidney injury requiring renal replacement therapy was observed in 18 (47%) cases. Acute kidney injury did not resolve in one patient with acute interstitial nephritis. Two patients died because of systemic complications. The remaining 35 fully recovered. Conclusions: in individuals with acute Epstein-Barr virus infectious mononucleosis, a relevant kidney injury is rare but the outcome potentially fatal. It results from interstitial nephritis, myositis-associated acute kidney injury, hemolytic uremic syndrome or jaundice-associated nephropathy.

SGPP 125

Acute idiopathic scrotal edema – systematic review of the literature

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Background: Acute idiopathic scrotal edema is an uncommon and likely under-recognized cause of acute scrotum in childhood.
Methods: We systematically reviewed the literature according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. We searched reports with no date limits in subjects 1 to 20 years of age with acute idiopathic scrotal edema.
Results: A total of 37 studies were included. Sixteen case series addressed the prevalence of acute idiopathic scrotal edema among males with acute scrotum: among 3403 cases, the diagnosis of acute idiopathic scrotal edema was made in 413 cases (12%). Twenty-four reports addressed history, clinical and laboratory findings, management and course of acute idiopathic scrotal edema in 311 subjects. The patients mostly ranged in age from 5 to 8 years, presented with acute scrotal redness and swelling, associated or not with mild pain. Ninety percent or more of the cases developed in subjects without atopic diathesis, were not preceded by inguinoscrotal surgery, acute febrile illnesses or scrotal trauma. They were afebrile, in good general condition and presented without pruritus, nausea, vomiting or abdominal pain. The lesions were bilateral in 2/3 and unilateral in 1/3 of the cases. The condition resolved spontaneously within 2–3 days without sequelae. Approximately 10% of patients experienced a recurrence.
Conclusions: Acute idiopathic scrotal edema is a self-limiting condition that accounts for ≥10% of cases of acute scrotum in prepubertal boys. It remains a diagnosis of exclusion and a pediatric urological evaluation is mostly recommended.

SGPP 126

Acute pyelonephritis: always benign?

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Background: Renal abscesses (RA) are defined as an infective fluid collection in the kidney tissue and is a rare complication of acute pyelonephritis, where acute vasospasm and inflammation can occasionally lead to liquefactive necrosis and abscess formation. In most cases pediatric RA are small and E. coli being the most often detected causative infectious pathogen. The size of the renal abscess define the therapeutical approach. A conservative management with intravenous antibiotics is often effective in RA <3 cm, whereas in cases where the size exceed 3 cm, a drainage of the RA may be considered.
Case description: We report the case of a previous healthy 6-year-old boy who presented to our emergency departement (ED) with fever and dysuria. The urine examination confirmed the diagnosis of acute pyelonephritis and an empirical oral antibiotic treatment with a third generation cephalosporine was initiated. The patient remained febrile and although urine culture showed a sensitive E. coli and perfect compliance, renal ultrasound (RU) showed formation of multiple RA. An intravenous broad-spectrum antibiotic treatment was started. Fever persisted and RA size increased in RU. We then performed and MRI to better assess the size of the RA, which showed three lesions, the biggest being >3 cm in the superior renal lobe. The patient underwent percutaneous drainage and cultures confirmed E. coli as causative pathogen. The intravenous broad-spectrum antibiotic treatment was continued for 6 weeks and the RA showed a progressive organization and resolution of fever after several weeks.
Conclusion: RA are a very rare complication of acute pyelonephritis in children and can develop even if correct initial management. The symptoms are non-specific and renal abscess should be suspected in case of persistent fever in patients treated for acute pyelonephritis. The intravenous broad-spectrum antibiotic treatment is necessary for the recovery, but depending on the size of RA a surgical approach may be necessary. An interdisciplinary approach is important for the management of these patients. The patient will undergo further investigation to better assess eventually pre-existing conditions which could favorise the formation of RA.

SGPP 127

A typical-atypical Kawasaki Disease?

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Introduction: Kawasaki Disease (KD) is a self-limiting vasculitis with inflammation of the medium-sized arteries. Major criteria of KD are: oral mucosa and lips erythema, rash, bilateral nonexudative conjunctivitis, palmar and plantar erythema and cervical lymphadenopathy accompanied by fever for more than five days.
In addition the Kobayashi score helps defining patients at risk for complications, so that conventional therapy can be intensified. Cardiovascular complications are coronary artery dilatation and aneurysm. Treatment consists of intravenous immunoglobulines (IVIG) and oral acetylsalicylic acid (ASA). In high risk patients prednisolon intravenously is added to the therapy. Children who do not fulfill the criteria at presentation but still develop coronary artery changes have a so called atypical KD. Infants under 6 months are at increased risk for atypical KD with delayed start of treatment and coronary artery aneurysm formation.
Case Report: A 3-month-old boy was admitted to our hospital with fever >40 °C for six days and increasing CRP despite of antibiotic therapy. He presented in good general condition with uneventful personal history and normal physical examination. The laboratory findings on admission were: increased CRP, slightly elevated liver enzymes and neutrophilia. He fulfilled only three of six KD criteria. Suspecting an atypical KD in a young infant a therapy with IVIG and ASA was initiated. An echocardiography showed a significant coronary artery dilatation. Also, slight dilatation of the superior mesenteric artery was seen on abdominal sonography. Therefore additional treatment with prednisolon was introduced. During the hospitalization the patient developed plantar and palmar erythema as an additional KD criterium. The boy was discharged from hospital afebrile and with normalized CRP after 8 days of treatment.
Conclusion: KD should be in differential diagnosis of any child with unexplained fever lasting for more than five days, even if the KD criteria are not fulfilled. Young infants are at an increased risk for atypical KD. The Kobayashi score supports the risk estimation of KD. Timely treatment is able to minimize cardiac complications and improve clinical outcome.

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SGPP 128

A high-performance analysis pipeline to find disease-causing mutations in patients with primary immunodeficiencies

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The emergence of Next Generation Sequencing (NGS) has greatly modified the landscape of human genetics and very few branches have done this more than Whole Exome Sequencing (WES) which focuses on the exonic regions of human genomes. As the protein coding region of the genome, exomes often harbor disease causing genetic mutations that can be linked to a phenotype in Mendelian diseases. Based on this knowledge we present an ongoing genetic study and give an overview of results obtained so far. The designated aim of our project on Immunodeficiencies is to create a workflow, initiated by WES results. This should allow the identification of patients with unusual or novel genetic forms of Primary Immunodeficiency diseases (PID) and is aided by a thorough clinical characterization. Patients included in our studies present with symptoms of immunodeficiency or -dysregulation of unknown origin. High throughput genetics including WES and transcriptomics are initially performed on patients and guide the choice of functional immunological assays conducted afterwards. These choices are hinged on the prior data analysis which involves several steps. These steps need to be suitably designed and assembled into an efficient pipeline, the huge amount of data produced requires IT skills as well as computational power. So far, we have included 25 PID patients in this pipeline. We have found four patients with well-known PIDs, who were not diagnosed previously due to their unusual clinical and immunological presentation, three further patients were found to have mutations associated with recently described PIDs and we are currently working on the characterization of two novel PIDs. The functional immunological assays confirming these mutations are underway and presented in other abstracts at this conference. These initial results are very encouraging, yet as there are very few patients in our cohort with the same disease WES results can be difficult to interpret this especially if a mutation has not yet been described yet. To overcome these barriers, we are now starting to perform phenotypic profiling of patients' blood cells using high-throughput multiplexed imaging, cellular mass cytometry, and computer vision. Using advanced statistics, machine-learning, and network theory, these datasets will be turned into powerful means to link the data acquired in our genetic analysis to the molecular networks underlying cellular phenotypes.

SSAIO 1

Evidence for a role of eosinophils in blister formation in bullous pemphigoid

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Background: Bullous pemphigoid (BP) is an autoimmune bullous disease of the skin characterized by subepidermal blister formation due to tissue-bound and circulating autoantibodies to the hemidesmosomal antigens BP180 and BP230. Although eosinophils and their toxic mediators are found abundantly in BP lesions, their role in blister formation has remained unclear. This study aimed at investigating the role of eosinophils in the pathogenesis of BP with a specific focus on blister formation and to define conditions inducing dermal-epidermal separation (DES).
Methods: In an ex vivo human model of BP, normal human skin cryosections were incubated with purified human peripheral blood eosinophils with or without activation in the presence or absence of BP autoantibodies, brefeldin A, diphenyleneiodonium (DPI), DNase, or blocking F(ab')2 fragments to CD16, CD18, CD32 and CD64. DES was assessed by light microscopy studies and quantified using Fiji software.
Results: Following activation with IL-5 and in the presence of BP autoantibodies, eosinophils induced separation along the dermal-epidermal junction of ex vivo skin. DES was significantly reduced by blocking any of the following: Fcg receptor binding ($p = 0.048$), eosinophil adhesion ($p = 0.046$), reactive oxygen species (ROS) production ($p = 0.002$), degranulation ($p < 0.0001$), or eosinophil extracellular trap (EET) formation ($p = 0.048$).
Conclusions: Our results provide evidence that IL-5-activated eosinophils directly contribute to BP blister formation in the presence of BP autoantibodies. DES by IL-5-activated eosinophils depends on adhesion and Fcg receptor activation, requires elevated ROS production and degranulation, and involves EET formation. Thus, targeting eosinophils may be a promising therapeutic approach for BP.

SSAIO 2

Basophil-derived interleukin-4 promotes epicutaneous antigen sensitization concomitant with the development of food allergy

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Background: Exaggerated TSLP production and infiltration of basophils are associated with the pathogenesis of atopic dermatitis (AD), a recognized risk factor for the development of food allergies. While TSLP and basophils have been implicated to promote food-induced allergic disorders in response to epicutaneous sensitization, the mechanisms by which TSLP-elicited basophils guide the progression of allergic inflammation in the skin to distant mucosal sites such as the gastrointestinal tract are poorly understood.
Objective: We sought to test the role of basophil-intrinsic IL-4 production in TH2 sensitization to food antigens in the skin and effector food allergic responses in the gut.
Methods: Mice were epicutaneously sensitized with ovalbumin on an AD-like skin lesion, followed by intra-gastric antigen challenge to induce IgE-mediated food allergy. The requirement for basophil-derived IL-4 production for TH2 polarization and the pathogenesis of IgE-mediated food allergy was assessed in vitro by co-culture experiments with naïve T cells and in vivo using IL-4 3'UTR mice that selectively lack IL-4 production in basophils.
Results: Epicutaneous food antigen sensitization is associated with the infiltration of IL-4 competent innate immune cells to the skin with basophils and eosinophils representing the predominant populations. In contrast to basophils, absence of eosinophils did not alter disease outcome. Co-culture of IL-4 competent basophils together with dendritic cells and naïve T cells was sufficient to promote TH2 polarization in an IL-4 dependent manner in vitro, while absence of basophil-intrinsic IL-4 production in vivo was associated with reduced food allergic responses.
Conclusion: TSLP-elicited basophils promote epicutaneous sensitization to food antigens and subsequent IgE-mediated food allergy via IL-4. Strategies to target the TSLP-basophil-IL4 axis in patients with AD may lead to innovative therapies that can prevent the progression of allergies to distant mucosal sites.

SSAIO 3

Characterization of immune modulatory B cells in melanoma patients

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Immunotherapy aims at activating high numbers of tumor-antigen specific CD8+ T cells and CD4+ Th1 cells in order to combat tumors, however the desired immune protection is not always obtained. The role of other lymphocytes is less clear and can potentially be detrimental. In mouse models, B cells have been shown to play a role in the regulation and/or inhibition of T cell responses. Our preliminary data show that there are less switched memory cells present in PBMC of melanoma patients than healthy donors. Differentiation and activation as characterized by CD38 and HLA-DR as well as CD20 expression on B cells is very heterogeneous in patients. Patients with B cells expressing lower levels of HLA-DR and CD38 have a higher prevalence of multiple lymph node metastases. Lower expression levels of CD20 correlates with more distant metastases in patients. Interestingly, decreased expression levels of these markers can be induced by culturing purified B cells with the supernatants obtained from different melanoma cell lines suggesting the presence of immune modulatory compounds. Our aims are to further characterize the B cell subsets in periphery and tumor microenvironment of melanoma patients enrolled in different clinical studies. Second we would like to identify clinically relevant mechanisms by which tumor cells inhibit, mediated by B cells, T cells in cancer patients. We are combining RNA sequencing and protein expression analysis with direct ex vivo assessments of functional properties. We hope that our results may extend basic scientific knowledge, and contribute to improve diagnosis and treatment decisions for cancer patients.

SSAIO 4

Short-chain fatty acids in prevention of the development of allergies in children and mice

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Background: Consumption of milk products such as yogurt or butter in the first year of life is associated with fewer incidences of atopic dermatitis and asthma later in life. Short-chain fatty acids (SCFA) such as butyrate are present in these milk products but are also produced by fermentation of fiber by the gut microbiota. They are a critical energy source of colonocytes and have immune-regulatory function in the host.
Aim: To investigate the role of exposure to SCFA by diet in preventing allergic diseases and colitis in children and mice.

Methods: SCFA levels were assessed by UPLC in fecal samples of one year old children of the European birth cohort study PASTURE/EFRAIM and related to allergic health outcomes later in life. Furthermore, mice were gavaged by SCFA in the context of disease models for allergic-airway inflammation and colitis.
Results: Children with very high levels of butyrate and propionate in fecal samples of the first year of life were protected against asthma and food allergy later in life. Mice that received SCFA during sensitization suffered less from allergic-airway inflammation compared to the controls. Airway resistance in response to methacholine or total cells and eosinophils in bronchoalveolar lavage were reduced. By contrast, lung regulatory T cells were increased. Finally, weight loss and symptom score was improved by SCFA in a colitis model.
Conclusion: SCFA are promising anti-inflammatory compounds to prevent the development of diseases such as allergies and colitis.

SSAIO 5

T cell-induced CSF1 promotes resistance to immunotherapy in melanoma

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Colony stimulating factor-1 (CSF1) is a key regulator of monocyte and macrophage differentiation, and promotes their pro-tumorigenic functions. Overexpression of CSF1 has been correlated with poor prognosis in cancers of the reproductive system. In human melanomas, several studies showed a correlation of melanoma thickness with macrophage infiltration. We describe here that CSF1 secretion by melanoma cells acts as a resistance mechanism towards activated T cells and might particularly affect responses to T cell based immunotherapies. We found increased levels of CSF1 in plasma of patients with advanced malignant melanoma, which correlated with disease progression. Histological analysis of primary melanoma biopsies as well as transcriptomic analysis from skin cancer TCGA datasets substantiated a positive correlation of CD8 with CSF1 and other markers (CSF1R and CD163) associated with immunosuppressive macrophages. Mechanistically, as shown in in vitro studies, IFNγ and TNFα released by activated tumor-specific CTLs triggered melanoma cells to secrete CSF1. Interestingly, high CD8A gene expression was associated with high CSF1R, CD68, and CD163 expression in pretreatment tumors from non-responders to anti-PD1 therapy. Furthermore, a shift in the balance between CD8+ T cells and macrophages in the tumor in favor of CD8+ T cells, depicted by a high CD8A/CSF1R ratio, correlated with prolonged overall survival in melanoma patients. Finally, in a preclinical mouse model of melanoma, concomitant PD1 and CSF1R blockade was superior to single agent treatments and induced complete regression of all tumors. These results substantiate the hypothesis that blockade of CSF1-mediated resistance increases the efficacy of immune checkpoint inhibition in melanoma.

SSAIO 6

Dual angiopoietin-2/VEGFA inhibition elicits anti-tumor immunity that is enhanced by PD1 blockade

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Pathological angiogenesis is a hallmark of cancer and therapeutic target. Vascular-endothelial growth factor-A (VEGFA) and angiopoietin-2 (ANGPT2) sustain tumor angiogenesis. Here we show that combined ANGPT2/VEGFA blockade by a bi-specific antibody (A2V) provided synergistic anti-tumoral benefits in both genetically engineered and transplant tumor models, including metastatic breast cancer (MMTV-PyMT), pancreatic neuroendocrine tumor (RIP1-Tag2), and melanoma. Mechanistically, ANGPT2/VEGFA blockade promoted vascular regression, cancer cell death, and tumor-antigen presentation by intratumoral phagocytes. A2V also "normalized" the remaining tumor blood vessels and induced the upregulation of T cell-recruiting and adhesion-associated molecules, such as CXCL10 and VCAM, on tumor endothelial cells. This facilitated the extravasation and perivascular accumulation of activated, interferon-γ (IFNγ)-expressing cytotoxic T lymphocytes (CTLs). In turn, CTL-derived IFNγ upregulated PDL1 expression in the vascular endothelial cells of A2V-treated tumors, possibly blunting the anti-tumoral functions of the CTLs. An anti-PD1 antibody suppressed this adaptive response and improved tumor control by A2V in MMTV-PyMT and other tumor models, while it had dismal therapeutic activity in monotherapy. These findings support the rationale for co-targeting tumor angiogenesis and immune checkpoints in cancer therapy.

SSAIO 7

T cell migration from inflamed skin to draining lymph nodes requires intralymphatic crawling supported by ICAM-1 / LFA1 interactions

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T cells are the most abundant cell type found in afferent lymph, but the mechanisms of their migration through afferent lymphatic vessels are poorly understood. Performing intravital microscopy in murine ear skin, we imaged T cell migration through afferent lymphatic vessels in vivo. T cells entered into and actively migrated within lymphatic capillaries but were passively transported in contractile collecting vessels. Contact hypersensitivity-induced inflammation increased lymphatic endothelial expressed ICAM-1 and, correspondingly, intralymphatic T cell motility. Antibody-mediated blockade of ICAM-1 or its integrin ligand LFA-1 equally reduced intralymphatic T cell motility. In vitro, blockade of lymphatic endothelial cell-expressed ICAM-1 significantly reduced Th1 cell adhesion, crawling and transmigration across lymphatic endothelium and decreased T cell advancement from lymphatic capillaries into collectors in skin explants. In vivo, antibody-mediated blockade of ICAM-1 or LFA-1 significantly reduced the migration of adoptively transferred T cells from the skin to draining lymph node. Overall, our findings demonstrate that T cell migration through afferent lymphatic vessels occurs in distinct steps and reveal a key role for ICAM-1-LFA-1 interactions in this process.

SSAIO 8

Non-linear scaling of CD8+ T cell responses by bystander DCs

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Naive CD8+ T cells integrate signals from peptide antigen-MHC complex (pMHC), costimulatory molecules, and cytokines upon their encounter with antigen-presenting dendritic cells (DCs). Despite the vast knowledge on molecular regulators of effector CD8+ T cell responses, it remains largely elusive how many DCs are required to induce exponential expansion of CD8+ T cells and how this requirement is modulated. Here, we addressed this question by combining conventional flow cytometry with intravital two-photon microscopy (2PM) and whole-organ imaging of lymph nodes (LNs) using light sheet fluorescence microscopy (LSFM) in a DC vaccination model. Subcutaneous injection of more than 20,000 DCs induced exponential expansion of a starting population of 15–25 OT-I cells in a popliteal LN on day 7 post-vaccination. Using LSFM to precisely enumerate OT-I cells and injected DCs, we found that on average 370 DCs migrated to the draining LN by 24 h after injection of 20,000 DCs.

Next, we investigated whether all of these 370 DCs have to carry cognate pMHC. To this end, we substituted varying number of pMHC-presenting DCs with non-presenting "bystander" DCs while keeping the total number of injecting DCs to 20,000. Indeed, supplementary injection of bystander DCs substantially rescued the suboptimal expansion of OT-I cells after vaccination with <20,000 pMHC-carrying DCs. In the presence of bystander DCs, as few as 2,500 pMHC-carrying DCs (corresponding to 50 cells per popliteal LN) sufficed exponential expansion and effector differentiation of OT-I cells, although OT-I cells expanded more when all the injected DCs carried pMHC. This effect of bystander DCs was independent of direct interactions between reacting OT-I cells and bystander DCs. In contrast, Il12a–/– bystander DCs did not potentiate expansion of OT-I cells, indicating that bystander DCs augment antigen-specific CD8+ T cell response largely by secreting inflammatory cytokines. In sum, our results suggest that activated DCs that do not present cognate pMHC significantly lower the pMHC requirement for exponential expansion of responding CD8+ T cells.

SSAIO 9

Neutrophil extracellular traps participate in both helminth killing and host damage

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Hookworms are skin-penetrating parasites infecting about 2 billion people, principally in the south hemisphere. The skin has recently been shown to be an important bulwark against parasite establishment in a memory setting. However, the initial interaction between host and parasite in the skin during a primary infection is still poorly characterized. Here, we present the fate of the larvae from their skin penetration to their migration to the lungs using intravital microscopy. We observe that neutrophils are rapidly recruited to the site of infection and adhere to the larvae. Surprisingly however, neutrophils are not sufficient to cause parasite killing. We further show that the parasite adjusts its development to the neutrophils presence by an evasion demarche: on one hand, the parasite delays its exsheathment to benefit from an additional layer of cuticle protection; on the other, in response to bleach induced by the neutrophils, the parasite secretes specific Excretory-Secretory (ES) products with anti-neutrophil activity. Building on these observations, we show that vaccination with those ES products thus renders the parasite susceptible to killing by netosis. Altogether, this study demonstrates that hookworms can sense the presence of neutrophils and respond by secreting products that degrade neutrophil extracellular traps to avoid killing.

SSAIO 10

Tissue resident memory T cells and resident macrophages cooperate for immune protection of exocrine glands

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After clearing of infections, distinct memory T cell populations persist in the host for efficient protection upon pathogen re-encounter. Re-activation of resident CD8+ memory T cells (TRM) is particularly relevant for a fast response to viral and microbial infections on a tissue level. However, the requirements for efficient tissue protection by TRM are not fully understood. Here, we used multiphoton intravital imaging, confocal imaging and light sheet microscopy of submandibular salivary glands (SMG) to dissect memory-mediated tissue surveillance during acute and memory phase of lymphocytic choriomeningitis virus (LCMV) infections in mice. SMG is an exocrine gland with ductal and acinar structures, which are anchored via extracellular matrix (ECM) sheets. We discovered in SMG and lacrimal glands an extended tissue macrophage network, which facilitates migration of TRM between different epithelial and stromal compartments by extending protrusions through ECM and densely packed epithelial cells. These protrusions create gaps in the ECM layer that are used by TRM as guidance cues for efficient scanning of ductal and acinar structures. Depletion of the macrophage network resulted in significantly reduced TRM motility and patrolling ability. After viral re-challenge of the SMG, we found that the macrophage network was essential for fast clearance of infected cells by TRM. In sum, we describe a close cooperation between the resident macrophage network and TRM in exocrine glands that ensures effective tissue surveillance by granting T cell access to epithelial structures sheathed by ECM.

SSAIO 11

Antigen recognition avidity dependent miR-155 upregulation in melanoma tumors correlates with increased CD8+ T cell infiltrates

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MicroRNAs (miRs) are noncoding small RNAs that regulate protein expression at the post-transcriptional level in all cells, including those forming the immune system. We previously showed that a single miR, miR-155, promotes effector CD8+ T cell responses in viral infection, vaccination and adoptive cell transfer for tumor therapy in mice. However, little is known yet about miR-155 expression regulation in tumor infiltrating CD8+ T cells. We report that in situ antigen recognition and T cell avidity are major determinants in the regulation of miR.-155 expression in CD8+ T cells. In fact, tumor specific mouse effector CD8+ T cells showed T cell avidity dependent increased miR-155 expression levels in melanoma tumors than in the spleen. Interestingly, miR-155 expression levels correlated with increased tumor specific CD8+ T cell infiltrates and tumor control. In agreement with these observations in mouse model systems, human effector memory (EM) CD8+ T cells from melanoma patients showed increased miR-155 expression levels in melanoma tumors and tumor infiltrated lymph nodes (TILNs) compared to T cells from tumor-free areas. Moreover, miR-155 expression levels in patients' EM CD8+ T cells positively correlated with their frequencies in TILNs raising the possibility that miR-155 overexpression might be therapeutic by increasing effector T cell numbers and/or enhancing resistance against the immunosuppressive tumor microenvironment. Indeed, T cell transfer of miR-155 overexpressing mouse tumor specific CD8+ T cells enhanced tumor control of low but not high affinity antigen expressing tumors. Thus, miR-155 overexpression may be particularly useful to enhance tumor control of cancer patients' CD8+ T cells as most of them are low affinity CD8+ T cells.

SSAIO 12

ASC-dependent inflammasomes do not shape the commensal gut microbiota composition

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The gut microbiota regulate susceptibility to multiple human diseases. The Nlrp6/ASC inflammasome is widely regarded as a hallmark host innate immune axis that shapes the gut microbiota composition. This notion stems from studies reporting dysbiosis in mice lacking these inflammasome components when compared with unrelated wild-type animals. Here, we describe the first gut microbial analyses in inflammasome-deficient mice while minimizing non-genetic confounders using littermate-controlled and ex-germfree ASC-deficient mice that were all allowed to shape their gut microbiota naturally after birth. Careful microbial phylogenetic analyses of these littermate-controlled cohorts failed to reveal regulation of the gut microbiota composition by the ASC-dependent inflammasomes. Our results dismiss a generalizable impact of ASC-dependent inflammasomes on the composition of the commensal gut microbiota, and highlight the necessity for littermate-controlled experimental design in assessing the influence of host immunity on gut microbial ecology. In addition, we investigated how the intestinal inflammasome expression is modulated depending on changes in the microbiota. To address this second point, we have performed in vivo experiments colonising germ-free (GF) mice with limited and defined or diverse but undefined commensal bacteria to investigate how the intestinal inflammasome

components are regulated under different hygiene conditions. Interestingly we observed that inflammasome expression is the same in GF and stable colonised animals but it shows an early and transiet upregulation shortly upon colonisation. More over, this phenomenon was not induced by any type of commensal bacteria (such as B. fragilis or L.murinus) but it was more peculiar for pathogens (e.g. C. rodentium). To conclude, we tried also to address if a lack of inflammasome expression was impacting on DSS-induced colitis susceptibility. In both SPF (specific-pathogen free) and gnotobiotic conditions, ASC–/– mice resulted to be less susceptible than their WT counterpart, showing how the ASC signaling promotes colitis susceptibility.

POSTERS SSAI

SSAIP 1

Allergic disease in 8-year-old children is preceded by delayed B-cell maturation

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Background: We previously reported that exposure to a farming environment is allergy-protective, while high proportions of neonatal immature/naïve CD5+ B cells and putative regulatory T cells (Tregs) are risk factors for development of allergic disease and sensitization up to 3 years of age. Objective: To examine if B- and T-cell maturation are associated with allergic disease and farming environment over the first 8 years in life. Methods: In the prospective FARMFLORA study, including both farming and non-farming families, 48 out of 65 children took part in the 8-year follow-up study. Various B- and T-cell maturation variables were examined in blood samples obtained at several occasions from birth to 8 years of age and related to doctors' diagnosed allergic disease and sensitization, and to farming environment. Results: We found that the incidence of allergic disease was lower among farmers' compared to non-farmers' children during the 8-years follow-up period, and that farmers' children had higher proportions of memory B cells at 8 years of age. Moreover, a high proportion of neonatal CD5+ B cells was a risk factor for and may predict development of allergic disease at 8 years of age. A high proportion of Tregs was not protective against development of these conditions. Conclusion & Clinical Relevance: High proportions of neonatal naïve B cells remained as a risk factor for allergic disease in school-aged children. Thus, the accelerated B-cell maturation observed among farmers' children may be crucial for the allergy-protective effect of a farming environment.

SSAIP 2

Investigation of a flagellin:Betv1 conjugate protein on the regulation of group 2 innate lymphoid cell responses in a mouse model of birch pollen allergy

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Background: Inhalant allergies caused by common airborne allergens such as Bet v 1 from birch pollen, are a significant cause of morbidity in Europe. Through the use of recombinant protein technology, we developed a fusion construct of Bet v 1 and the toll-like receptor 5 (TLR5) ligand flagellin (rFlaA:Betv1) in order to increase the immunogenicity of an allergen for immunotherapy. Group 2 innate lymphoid cells (ILC2) are believed to be important initiators of allergic immune responses at mucosal barriers. Therefore their likely role in birch pollen allergy, as well as their possible restrain by fusion constructs such as rFlaA:Betv1, could be an interesting avenue of exploration in mouse models of allergy.
Objective: To investigate early ILC2 recruitment and activation in an inhalant mouse model of birch allergy, and their modulation by rFlaA:Betv1 vaccination.
Method: In order to observe early ILC2 recruitment, mice were challenged intranasally with high dose birch pollen extract (BPE) on two subsequent days. Two weeks prior to challenge, mice were prophylactically vaccinated with either PBS or rFlaA:Betv1. Lungs were harvested and IL-5+ and IL-13+ ILC2 were quantified by intracellular cytokine staining following PMA and Ionomycin restimulation ex vivo. ILC2 cells were lineage negative (CD3, B220, TCR-β, TCRγδ, CD11b, CD11c, CD27, CD5, GR-1, CD49b) as well as CD127+ and CD25+. To quantify the protease activity of BPE, a 0.1% gelatin substrate gel assay was performed.
Results: We observed that ILC2 harvested from mice vaccinated with rFlaA:Betv1, but not with PBS, showed a small reduction of IL-5 and IL-13 positivity. Counter intuitively, prophylactic vaccination with rFlaA:Betv1 increased both lymphocyte and ILC2 recruitment to the lungs. In addition, BPE given to mice intranasally on 2 consecutive days was not a sufficiently strong stimulus to recruit additional ILC2. These findings correlated with the observation that BPE, as compared to other allergen extracts, shows weak protease activity.
Conclusion: Through these preliminary studies we observed that a TLR ligand: allergen construct such as rFlaA:Betv1 may have the potential to modulate ILC2 recruitment, while dampening IL-5 and IL-13 production in the lungs in a mouse model of birch pollen allergy. Further studies are required to confirm these results.

SSAIP 3

Personalized and rapid food allergy test using natural allergenic extracts

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In clinical allergy, alongside with skin prick tests, in vitro determination of specific IgE for a particular patient contributes to the diagnosis and helps to estimate the risk associated with different food allergens. However, with commercial methods of specific IgE antibodies detection (component-resolved diagnosis, CRD), the clinician is typically limited by the list of the available allergens. To overcome this limitation, we developed two component-resolved diagnostic tests for food allergy in which natural extracts can be used. In the first developed method, the CRD is performed using immunoaffinity capillary electrophoresis (IACE) coupled with matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS). Meanwhile, the second method is based on in-tube immunomagnetic separation (IMS) with mass spectrometry identification (MALDI MS or peptide mass fingerprinting). In both techniques, magnetic beads coated with antihuman IgE antibodies are used to extract the IgE antibodies from the blood serum of the allergic patient. Then, the immunocomplex, obtained on the magnetic beads, is used to quantify the total IgE level or to probe the IgE binding with standard allergens or natural allergenic extracts. Afterwards, the identification of the extracted proteins, i.e. potential allergens, is performed by MALDI MS with or without CE separation. After optimisation, the proposed methods have been successfully applied to a commercial blood sample of a patient with a known allergy to cow's milk, with results confirmed by standard tests. As a proof-of-concept, the sensitization profile of a patient suffering from protein contact dermatitis to the cow's whey fraction has been determined. We confirmed the presence of circulating IgE antibodies binding lactoferrin and bovine serum albumin. Cross-reactivity tests were also performed using goat and sheep milk and revealed the patient sensitivity to serum albumins from these two

milks. Such approaches open the possibility for direct identification of IgE-bound allergens molecular mass and structure. These methods allow the discovery of yet unknown allergens and could be useful for precise personalized allergy diagnosis, allergens epitope mapping, and cross-reactivity studies.

SSAIP 4

Post orgasmic illness syndrome: is there a place for an immunologist?

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Introduction: Post orgasmic illness syndrome (POIS) is a less recognized chronic disorder, first described in 2002 [1]. It manifests with paroxysmal symptoms that occur within minutes to hours after ejaculation, and disappear spontaneously after 3 to 7 days. The symptoms are variable and have been classified in 7 clusters [2]. It has been hypothesized that POIS may be triggered by an immune reaction to the man's ejaculate. The antigens involved may be present in the seminal fluid, as persistence of the disease is described after vasectomy [2, 3]. However the real physiopathology of POIS remains unclear. Therefore there is no validated treatment, even if in the past years a desensitization approach with autologous semen has been applied [4].
Methods: We report the case of a 33-year-old male who experienced flu-like symptoms with burning eyes and extreme fatigue occurring few minutes after ejaculation. The allergy work-up performed included skin-prick tests (SPT) and intradermal injections (IDR) with autologous seminal fluid prepared by centrifugation of ejaculate, determination of specific IgE and tryptase.
Results: The symptoms recurred since puberty with the same clinical pattern. They were triggered by ejaculation and even after sexual arousal with production of preejaculatory fluid. The symptoms decreased over 5 days and were accompanied by cognitive disturbances. The patient had no atopic diseases, which was corroborated by negative SPT to the current aeroallergens. Total IgE was slightly elevated at 58 kU/l (N <50). Prick-to-prick with seminal fluid was equivocal (wheal of 4 mm without erythema) and IDR was negative at the suggested dilution of 1/40'000, but positive at 1/10'000. Tryptase levels were normal at baseline (3.85 µg/l, n >15.5) and did not change significantly one hour after ejaculation (3.75 µg/l). Specific IgE (ImmunoCAP) to seminal fluid and to rCan f5 were negative (<0.35 kU/l).
Conclusions: In this case of POIS with prominent ocular symptoms corresponding to a cluster 4 (eye cluster) according to one study group [3], the IDR to autologous seminal fluid was positive, while the undiluted SPT was equivocal. Although a false positive IDR due to the presence of proteolytic enzymes in seminal fluid cannot be excluded, the findings are suggestive of an immune-mediated mechanism, which has been proposed by others, with claims of successful desensitization procedures. Further investigations are needed to better evaluate the underlying mechanisms.

SSAIP 5

Concomitant immediate and delayed type hypersensitivity to Amoxicillin in the same patient: two case reports

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Case report: Adverse drug reactions to amoxicillin (AMX) usually present with urticaria or exanthema resulting either from type I or type IV sensitization. We present two cases showing clinical manifestations of both immediate and delayed type hypersensitivity to AMX with corresponding skin test reactivity.
Patient 1: A 54-year-old female patient developed a generalized pruritic, partly maculopapular exanthema after two doses of AMX/clavulanic acid (AMX/CL) administered for an erysipelas on the abdomen. Antibiotic therapy was switched to clindamycin for 14 days. The exanthema persisted and was accentuated in the large folds, and facial angioedema occurred despite administration of antihistamines and prednisone. Subsequently, the patient showed disseminated desquamation. A diagnosis of Symmetric Drug Related Intertriginous Flexural Exanthema (SDRIFE) was retained. Skin tests with AMX and AMX/CL were positive after 20 min and at 24h. PPL and MDM were negative, benzylpenicillin and piperacillin/tazobactam were positive after 24 hours only, clindamycin was positive in the immediate reading only. In the basophil activation test (BAT) to AMX the patient was a non responder, the lymphocyte transformation test (LTT) proved positive to AMX. Oral reexposure with aztreonam and cefuroxime was tolerated. SDRIFE was attributed to amoxicillin, angioedema may have been caused by clindamycin.
Patient 2: A 23-year-old female patient was treated for helicobacter pylori gastritis with AMX/CL, clarithromycin and pantoprazole for 7 days. On day 8 she developed a maculopapular exanthema persisting for 3 days. Dizziness, facial swelling and dyspnea occurred at the same time for one day only. Skin tests were positive for AMX and AMX/CL after 20 min and persisted afterwards for more than a week. Skin tests with clarithromycin and pantoprazole were negative. In the BAT to AMX the patient was a non responder, the LTT proved negative to AMX. Both patients had an unusual both immediate and delayed skin test reactivity to AMX. In both patients clinical manifestations represent more likely a T cell mediated mechanism, although also immediate type symptoms were present. So far it is unclear whether one single epitope or two different determinants on the AMX molecule are responsible for this unusual concomitant sensitization pattern.

SSAIP 6

Developing a passive basophil sensitization assay for the diagnosis of immediate type drug hypersensitivity reactions

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Introduction: Besides skin testing, basophil activation test (BAT) with patient basophils, named direct BAT, is used in the diagnosis of immediate type drug hypersensitivity reactions. However, the need for functionally active basophils limits its use and does not allow for storage or batch analysis. Here we present a serum-based test, "indirect BAT": we use healthy donor basophils sensitized with patients' sera in vitro.
Method: PBMCs of well-characterized basophil donors were isolated and donor basophils were stripped of their IgE by lactic acid pre-treatment and resensitized with patients' sera in vitro. Basophils were then stimulated with serial protein or drug dilutions and controls. Basophil activation (CD63 upregulation) was measured by flow cytometry.
Results: Basophil resensitization with sera of pollen allergic patients containing Bet v 1 specific IgE and subsequent stimulation with the protein allergen was successful and reproducible with different patients' sera and different donor basophils. Divalent drugs (e.g. chlorhexidine) were also able to activate passively sensitized basophils depending on the amount of drug specific IgE. Indirect BAT with monovalent drugs (e.g. betalactams) was more challenging: while some sera and in particular cephalosporins worked fine, other drugs and sera failed to elicit a basophil activation.
Conclusion: We were successful in improving and standardizing the conditions of indirect BAT using patients' sera on donor basophils. The assay is reproducible with standard protein allergens or divalent drugs. The concentration of drug specific IgE was crucial for basophil activation (cut-off around 1.0 kU/l). On the other hand, the use of indirect BAT for monovalent drugs still requires a better understanding of drug-protein interactions and conditions for IgE cross-linking by drugs to optimize the test conditions.

SSAIP 7

Is Alexidine cross-reactive with chlorhexidine specific IgE?

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Background: Chlorhexidine (CHX) is a widely utilized disinfectant for skin and mucosal surfaces as well as medical devices. CHX is a biguanide compound with two chlorophenyl endings linked by a hexamethylene chain. CHX can cause rare IgE-mediated anaphylaxis. Alexidine (ALX), also a biguanide with similar hexamethylene center but without aromatic endings, has similar bactericidal properties and represents a potential substitute for CHX. The allergic potential of ALX is unknown.
Methods: We investigated whether patients with IgE to CHX also react with ALX. We performed inhibition assays with CHX, chlorguanide (CG) and ALX using a commercial IgE assay for CHX (ImmunoCAP,

ThermoFisher Scientific, Uppsala). In addition, we performed basophil activation tests (BAT, CD63 and CD203a as activation markers) with CHX and ALX.
Results: 24 patients from Switzerland with allergic reactions to CHX and 9 sera with elevated CHX-specific IgE from Australian patients were included. In 22 patients with CHX-specific IgE >0.7 kU/l CAP inhibition studies were performed. CG showed a strong inhibitory effect (>60%) in 19/22 and CHX in 8/22 tested sera, while ALX (>60%) inhibited CHX positivity in 2/22 sera when it was used at 67 times higher concentration than CHX. 10/21 patients showed a positive BAT with CHX. ALX was stimulatory only if basophils were pretreated with IL-3 (3/22), but not in normal BAT (0/22). One patient was positive for ALX but not for CHX.
Conclusion: The IgE response to CHX appears to be polyclonal: CG seems to be the main epitope, and is best accessible as free CG, followed by the bivalent CHX. ALX with its biguanide epitopes appears to react with some of the CHX specific IgE, but both inhibition-tests and BAT assays show that this cross-reactivity is weak and of questionable functional relevance.

SSAIP 8

Emollient therapy alters barrier function and skin microbes in infants at risk for developing atopic dermatitis

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Background: Atopic dermatitis (AD) begins in early childhood and is characterized by impaired skin barrier and shifts in the skin microbiome. Skin emollients are commonly used to protect and repair the defective skin barrier in AD.
Objective: The goal of this study was to investigate whether long-term emollient therapy is associated with alterations of skin barrier function and shifts of the skin microbiome in infants at high risk for developing AD.
Methods: We prospectively enrolled newborns with a family history of AD to be randomized to either emollient treatment group or control group. At 6 months of age, we tested the skin barrier (transepidermal water loss/TEWL, water capacitance/CAP, pH) and skin microbiome (16S rDNA sequencing of skin swabs from cheek, dorsal and volar forearm).
Results: The emollient group (n = 10) had significantly lower skin pH compared to controls (n = 9) (p = 0.02), but without a statistically significant difference in TEWL or CAP. The emollient group had higher numbers of different bacterial taxa (Chao richness) at cheeks (p = 0.003), dorsal forearms (p = 0.008), and volar forearms (p = 0.003) as compared to controls. Both *Streptococcus pneumoniae* and *S. salivarius* statistically significantly contributed to the observed skin microbiome differences between patient groups. *S. salivarius* was significantly more abundant in emollient subjects at all sampling sites (p = 0.02). We then analyzed our previous larger cohort of older children with AD and also observed higher *S. salivarius* proportions in AD patients with treated and less severe disease (p = 0.01).
Conclusions: Long-term emollient therapy is associated with altered skin barrier function and shifts of skin microbiome in infants with a high risk for AD. Additional studies are needed to understand how alterations in streptococci may contribute to the therapeutic effects of emollients in AD.

SSAIP 9

Molecular aspects of sensitization to skin colonizing *Malassezia* spp. in atopic dermatitis

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Malassezia spp. is a genus of lipophilic yeasts and comprise the most common fungi on healthy human skin. This genus currently encompasses 14 species, and 9 of these species are frequently isolated from human skin. Despite its role as a commensal on healthy human skin, *Malassezia* spp. is attributed a pathogenic role in atopic dermatitis (AD). Here we report the latest findings on the molecular mechanisms by which *Malassezia* spp. may contribute to skin inflammation in AD.
Three *Malassezia* species, namely *M. furfur, M. sympodialis* and *M. globosa*, produce 14 currently characterized immunogenic proteins (allergens). These allergens elicit a specific IgE response. Furthermore, some of these allergens interact with human immune cells such as dendritic cells or T cells, supposedly through Toll-like receptors 2 and 4, and elicit a pro-inflammatory immune response. For example, the allergen Mala s 11 from *M. sympodialis* is a manganese-dependent superoxide dismutase (MnSOD). The IgE-mediated sensitization to this protein correlates to the severity of AD, and this protein induces the release of pro-inflammatory cytokines such as Interleukin (IL-)6, IL-8, IL-12p70 and TNF-alpha by dendritic cells. Mala s 11 also activates auto-reactive T cells that may react against its human homologue. Another species, *M. globosa*, produces the very recently characterized allergen MGL_1304, that induces the degranulation of mast cells and the release of IL-4 by basophils.
In canine atopic dermatitis, also *M. pachydermatis* plays an important pathogenetic role; allergen-specific immunotherapy with *Malassezia* extracts is even very successfully used in veterinary dermatology.
In conclusion, these *Malassezia* spp. allergens may be involved in the molecular mechanisms that lead to skin inflammation and may therefore be of significance for the course of AD. Sensitization to *Malassezia* can be determined by specific IgE to *Malassezia* spp. (m227) and in case of possible autoreactivity by determing IgE to Asp f 6 (MnSOD from *Aspergillus fumigatus*, m222), which is strongly crossreacting with Mala s 11.

SSAIP 10

Accelerated growth of *Malassezia* species in optimized culture conditions

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Malassezia spp. is a genus of lipophilic yeasts, comprising 14 species. It is the most common fungal genus of the healthy human skin microbiome but it is also attributed a pathogenic role in skin diseases such as atopic dermatitis (AD). AD is a frequent, recurrent eczematous skin disease that commonly associates with other atopic diseases such as hay fever, asthma and food allergies. To date, 14 immunogenic proteins of *Malassezia* spp. are characterized, that are predominantly produced by potentially pathogenic species, such as *M. sympodialis*. These proteins induce an IgE- or T cell-mediated immune response in humans that may contribute to skin inflammation in AD. Little is known on the exact pathogenic mechanisms, and if *Malassezia* species change protein production in the altered environment of human atopic skin. Cultivation of *Malassezia* spp. is a desirable tool to investigate these disease-contributing mechanisms. However, *Malassezia* is a fastidious fungus that barely growths on standard agars, what hampers research. We aimed to (i) examine the lipid preferences and (ii) to optimize culture conditions of *Malassezia* species. 28 strains isolated from human skin were used for the analysis. The strains represented five species with a supposed pathogenic role in skin diseases: *M. sympodialis*, *M. restricta*, *M. globosa*, *M. slooffiae* and *M. furfur*. Species identity was confirmed by sequencing. Strains were plated on various agar media (Leeming Notman, Sabouraud Dextrose, Tween 60-Esculin, Cremofur EL, mDixon) to determine their media preferences. To assess lipid preferences, commonly used culture media components (e.g., tweens, olive oil, oleic acid) and human sebum components (e.g. squalene, cholesterol) were added in varying concentrations. The mono-unsaturated lipid, oleic acid was the single most effective lipid to improve growth of all investigated *Malassezia* species. Other additives provided non-essential growth enhancement. Incubation time could be shortened to 2–4 days (versus 2–4 weeks) with only slight differences between species. In summary, we optimized cultivation of all relevant *Malassezia* species with reduced cultivation time on a single agar. This will enable (i) proteomic analyzes of *Malassezia* species under culture conditions resembling atopic skin, and (ii) the investigation of interaction between *Malassezia* spp. and human immune cells to elucidate the pathogenic contribution of *Malassezia* spp. to AD and other atopic diseases.

SSAIP 11

Parechovirus meningoencephalitis in 1-month-old infant

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Case report: 1-month-old infant born at term by urgent C-section due to pathological cardiotocography with an excellent newborn adaptation to extrauterine life was admitted to the emergency room for fever (38.8 °C), signs of dehydration, irritation and vomits. She did not present any prenatal risk factor for infection. No clinical focus of infection was observed, despite sepsis-like appearance. A large etiological work-up was performed and a treatment of Amoxicilline and Garamycine was started. During the hospitalization we noticed initial clinical deterioration. She remained febrile at 40.2 °C and didn't respond to acetaminophen treatment. We changed the anti-biotherapy to Meropenem. The initial laboratory tests showed normal cell count with the C-reactive protein at 4.2 mg/L. The result of the urine culture was negative. The lumbar puncture revealed: xanthochrome cerebrospinal fluid (CSF), proteins 1210 mg/L, leukocytes 3/µl, 92.5% mononuclear cells and 7.5% polynuclear cells. The PCR of the CSF was positive for Parechovirus. Hemocultures were negative. The diagnosis of Parechovirus meningoencephalitis was retained and the antibiotherapy stopped. Finally the patient had a satisfactory clinical evolution.
Discussion: Human parechoviruses(HPeVs) are RNA viruses, members of the large and growing family of Picornaviridae. Although 16 types have been described; most reports relate HPeV1-8 to children. HPeV1 and HPeV2 cause mainly gastrointestinal or respiratory illness and HPeV3 more severe illness in young infants, including sepsis like illness, meningitis and encephalitis. HPeV3 disease can be presented mostly as an irritable infant less than 90 days old with high fever for several days without focus (sepsis/meningitis presentation). No antiviral medication is available, but in severe infections monoclonal antibodies and immunoglobulins are under discussion. Mortality and neurological sequelae (cerebral palsy, learning disability, epilepsy and developmental abnormalities) are rare.
Conclusion: HPeV should be considered in the pediatric differential diagnosis as potential cause of severe viral sepsis and meningoencephalitis mostly in young children. Rapid identification of HPeV by PCR could contribute to shorter duration of both antibiotic use and hospital stay.

SSAIP 12

Unusual presentation of a child with severe combined immunodeficiency: The value of whole exome sequencing and potential benefit of newborn screening

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Severe combined immunodeficiency in infancy is characterized by opportunistic infections, failure to thrive and sometimes also by erythroderma, caused by maternal T-cells or by oligoclonal expansion of dysfunctional autoreactive T-cells (Omenn Syndrome, OS). OS comprises also lymphadenopathy hepatosplenomegaly, loss of hair and eye brows, eosinophilia and elevated serum IgE. Differential diagnoses of erythroderma include disorders/deficiencies such as atopic dermatitis, Netherton's syndrome, hyper-IgE syndrome, Wiskott-Aldrich syndrome and Graft-versus-host-disease. Along with immunological investigations, a skin biopsy is a part of the diagnostic work-up. Here, we describe the case of a girl who presented with erythroderma and axillary lymphadenopathies at the age of 2 months, developing hepatosplenomegaly over the next few months, followed by leukocytosis with elevated lymphocyte and eosinophil counts. IgE was elevated but IgM was initially normal. A skin biopsy was inconclusive, since it showed acute spongiform dermatitis with lymphohistiocytic infiltration of the dermis but none of the features required for the other differential diagnoses. Blood analyses revealed the absence of naive T cells in the presence of NK- and B-cells. A few maternal T cells were found transiently in the patient's blood – indicating severe combined immunodeficiency (SCID). The initially normal IgM levels started to fall at the age of 9 months. Failure to thrive, persistent viral infections and autoimmunity became apparent at the age of 11 months and prompted the decision to perform haematopoietic stem cell transplantation. We performed Whole exome sequencing (WES) and identified a homozygous C118Y mutation in the third exon of the gene encoding IL-7R?; this mutation is known to cause defective IL-7R expression and thus explained the SCID and OS observed in our patient. This was further confirmed by retrospective analysis of the newborn screening dried blood spots from the patient revealing zero T-cell receptor excision circles (TRECs) per mg DNA. Inconclusive skin biopsy results and the late onset of increased susceptibility to infection considerably delayed proper and timely diagnosis. WES turned out to be helpful in evaluating this unusual presentation of a SCID. The diagnostic delay could have been avoided by systematic screening for SCID in newborns.

SSAIP 13

Comparison of infusion duration and number of infusion sites for Subcutaneous Immunoglobulins (SCIG)

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Introduction: Patients with primary immunodeficiencies (PID) have several SCIG options with differing concentrations and administration parameters. A calculator to estimate the infusion duration and infusion site number was developed based on the administration parameters for the new SCIG 20% and non-facilitated SCIGs on the US market: HIZENTRA (IgPro20), GAMMAGARD LIQUID (SCIG 10%-GGL), and GAMUNEX-C (SCIG 10%-C).
Methods: The calculator estimated the total monthly dose derived from assumptions for patient weight, dose/kg, and weekly administration. Infusion site number was determined using the maximum volume/site specified in the label or the new SCIG 20% study protocol. The infusion duration was calculated based on the recommended maximum infusion rate, assuming that the maximum rate was used for the entire infusion duration.
Results: Calculating the number of infusion sites and infusion duration per treatment for each SCIG product, the infusion parameters for a hypothetical patient with PID weighing 70 kg receiving 500 mg/kg/month are:
New SCIG 20%: 1 infusion site/treatment; 0.73 hours/treatment
IgPro20: 2 infusion sites/treatment; 0.88 hours/treatment
SCIG 10%-GGL: 3 infusion sites/treatment; 0.97 hours/treatment
SCIG 10%-C: 3 infusion sites/treatment; 1.46 hours/treatment
Conclusion: Patients with PID have several SCIG options, requiring varying numbers of infusion sites and infusion duration; patients can opt for more infusion sites to speed-up infusions or slow infusion rates for tolerability reasons. For the hypothetical patient, infusing at the respective maximum infusion rates and volumes/site, the new SCIG 20% had the shortest infusion duration and required only one infusion site. Previous studies have demonstrated that the new SCIG 20% is well-tolerated at these rates.

SSAIP 14

Tolerability of a new human immune globulin subcutaneous, 20% preparation in patients with primary immunodeficiency diseases

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Introduction: Conventional subcutaneous immunoglobulin administration has disadvantages, including limited volumes that can be infused at a single site and the need for multiple infusion sites. Human immune globulin subcutaneous, 20% (SCIG 20%) is a ready-for-use, liquid preparation of highly purified human immunoglobulin G. Higher protein concentrations allow for increased doses/site, but with potential risk for increased adverse reaction rates. In a phase 2/3 study in patients with primary immunodeficiency diseases (PID) in North America, patients could infuse SCIG 20% up to 12 grams immunoglobulin (60 mL)/site at a rate of 60 mL/hr/site. Herein, local adverse events (AEs) were investigated for potential association with increasing SCIG 20% infusion volumes and rates from the phase 2/3 study in North America.

Methods: Patients received weekly SCIG 20% infusions using a T34L syringe driver and a high flow 24-gauge needle set – for up to ~1.3 years. Up to 60 mL was administered per site, as tolerated. For patients weighing <40 kg, for the first two infusions the recommended infusion volumes were ≤20 mL/site, and then ≤60 mL/site for subsequent infusions as tolerated. The recommended infusion rate for the first two infusions was 10–20 mL/hr/site; subsequent infusions could be increased up to 60 mL/hr/site, as tolerated.
Results: Overall, 74 patients aged 3–83 years received 4327 SCIG 20% infusions; most (98.2%) were not associated with a local adverse reaction. There was no association between the increasing volume/site (30–39, 40–49, 50–59, and ≥60 mL/site) and the rates of causally-related local AEs (0.4%, 1.4%, 1.1%, and 0.3%, respectively). In all, 72% of patients reached 60 mL/hr/site, for a median total infusion time of 0.95 hr (53% and 85% of infusions were delivered in <1, and <1.5 hr, respectively). More than half (57%) of infusions were delivered at ≥60 mL/hr/site, with no association between the increasing infusion rates (30-39, 40-49, 50-59, and ≥60 mL/hr/site) and the rates of casually-related local AEs (0.8%, 0.9%, 4.5%, and 0.4%, respectively). Patients (53/74) reached 60 mL/hr/site after a mean 5.7 (95% CI: 3.3–8.2) and median 3.0 (95% CI: 3.0–3.0) infusions. Most (99.8%) infusions were completed without administration changes.
Conclusions: SCIG 20% infusions were well-tolerated, irrespective of relatively high infusion volumes and fast infusion rates.

SSAIP 15

Is the NLRP3 variant Q703K a gain of function mutation that induces auto-inflammatory manifestations?

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Introduction: PFAPA is an auto-inflammatory disease (AID) of unknown etiology. Recently, we showed a dysregulated IL-1β secretion in PFAPA patients and we found NLRP3 variants in 20% of them. In this study, we aimed to investigate the potential implication of the Q703K NLRP3 variant as a gain of function mutation in AID. We describe the phenotype of our patients with recurrent fever presenting this variant and in the second part of our study we compare the cytokine profile of Q703K+ versus Q703K- asymptomatic adults.
Materials and Methods: 1) We reviewed all our patients presenting with recurrent fever suspected to be of auto-inflammatory origin and where the NLRP3 Q703K variant was found. 2) Monocytes of 6 PFAPA families whereby only one of the two parents was carrying the Q703K variant were isolated by MACS and stimulated with LPSup. Levels of IL-1β, TNF-α and IL-6 produced by monocytes of Q703K+ and Q703K- parents have been compared by ELISA.
Results: We report 13 patients with the Q703K NLRP3 variant: 10 were PFAPA patients among 99 from our cohort, 1 had a CAPS phenotype and 2 an undefined AID. The clinical presentation in the Q703K+ PFAPA patients was similar to Q703K-PFAPA patients. The patient with CAPS phenotype presented with urticarial rash, recurrent fever, deafness and arthralgia. One patient with undefined AID presented recurrent fever with neurological symptoms, and the second with fever flares and angioedema. The production of IL-1β, TNF-α or IL-6 was not significantly different between monocytes of Q703K positive and Q703K negative parents.
Discussion & Conclusion: NLRP3 Q703K variant may be found in patients with various AID, suggesting a potential role of this variant in the phenotype. However, in-vitro, the presence of the Q703K variant did not lead to increased IL-1β production. These findings suggest that the Q703K variant alone is not sufficient to induce auto-inflammatory manifestations, as well as a polygenic origin for PFAPA syndrome.

SSAIP 16

A non-malignant tumoral presentation of a rare primary-immunodeficiency

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Many primary immuno-deficiencies typically associate non-malignant or malignant lymphoproliferations with recurrent infections. We report a 7-year-old patient presenting with chronic abdominal symptoms since early infancy (bloating, pain, diarrhoea and vomiting) along with recurrent ear nose and throat (ENT) infections. He was multi-investigated for his abdominal symptoms by echography and CTscan which both revealed multiple intra-abdominal adenopathies, mainly localised in the right iliac fossae region and multiple nodules infiltrating the gut wall. These multiple masses were highly metabolically active on PETscan. Colonoscopy confirmed several nodules disseminating along the colon with masses in the caecum. Lymphoma was first suspected. However the non occlusive nature of the lesions and the respect of the normal gut wall appearance at echography made this hypothesis unlikely. Moreover, histology demonstrated a dense pleomorphic non-clonal T and B cell lymphoproliferation, together with eosinophilis; germinal center architecture was blurred. These features were not consistent with a diagnosis of lymphoma. Immunological investigations showed normal IgG while IgG2 sub-class was decreased, normal IgA and increased IgM. Post-vaccine antibodies showed good responses for protein antigens, but insufficient antibody response for pneumococcal antigens even after extra doses of 13-valent conjugate and 23-valent plain polysaccharide pneumococcal vaccines. Lymphocyte sub-populations showed decreased CD4+ T cells (included recent thymic emigrants) and B cells with normal distribution. T lymphocytes proliferated well after stimulation with mitogens. After lymphoma and PIDs classically prone to lymphoproliferation were ruled out we undertook to explore further our patient with a whole exome sequencing screen. Data analysis based on a panel of genes enriched in "immunodeficiency related genes", revealed that our patient harbored an heterozygous c.3061G>A, p.Glu1021Lys, PI3K delta chain mutation. This mutation causes activated PI3K delta syndrome (APDS), a rare autologous dominant PID recently described. This PID displays variable phenotype but typically combined non-malignant lymphoproliferation to ENT and broncho-pulmonary recurrent bacterial infections and patients are prone to lymphoma. At time of diagnosis the patient had no bronchiectasis. He started on intravenous polyvalent immunoglobulin substitution with the plan to assess the effect after 6 months.

SSAIP 17

Early versus late administration of icatibant in patients with hereditary angioedema

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Rationale: Relationship of the timing of icatibant self-treatment to demographic and treated-attack characteristics for patients with hereditary angioedema due to C1-inhibitor deficiency are poorly understood.

Methods: Data from the Icatibant Outcome Survey was used to evaluate early versus late icatibant self-treatment (patients with median time-to-first injection <1hr versus ≥1hr from attack onset, respectively).
Results: Of 229 patients analyzed, 89 (38.9%) had median time-to-first injection <1hr (median [Q1, Q3] for 482 icatibant-treated attacks, 0.25h [0.0, 0.5]) with no gender differences. Early self-treatment varied across countries, ranging from 77.1% (Germany/Austria) to 11.6% (France). Early (versus late)-treaters treated skin attacks at a higher rate (50.2% vs 34.6% respectively, P = 0.0098); conversely, late (versus early) treaters treated abdominal attacks at a higher rate (66.6% vs 49.7% respectively, P = 0.0078). Laryngeal attack frequency was not significantly different (P = 0.6064), nor was grouped attack severity (very mild/mild/moderate vs severe/very severe; P = 0.313). Significant reduction (P <0.001) in median (Q1, Q3) time to resolution [3hrs (0.8, 9.3) versus 7hrs (3, 19.3)] and attack duration [4hrs (1, 10.3) versus 12.5hrs (6.0, 26.0)] was observed between early versus late treatment, respectively (206 patients; 913 attacks).
Conclusion: Early treaters had shorter time to resolution and attack duration compared to late treaters, possibly indicating the importance of early access to icatibant in the face of HAE attacks. Differences in local practice patterns, icatibant availability, and tendency of early treaters to treat any symptoms without delay may drive prevalence of early use across countries. These and other findings from this analysis are hypothesis generating and should be further evaluated.

SSAIP 18

Dissecting the crosstalk between epithelial and mesenchymal cells in the presence of IL-17A within systemic sclerosis

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Background: Novel data suggest that keratinocytes may be involved in the pathogenesis of fibrosis within systemic sclerosis (SSc), a condition characterized by vasculopathy, fibrosis and immuno-inflammatory events [1]. Epithelial cells are preferential targets of IL-17A, which levels are increased in SSc [2]. Taking into perspective the fibrotic process, we aimed at investigating the crosstalk between keratinocytes and fibroblasts in the presence of IL-17A.
Material and methods: Conditioned-media of primary human keratinocytes primed with IL-17A, TNF and/or TGF-β were used to stimulate healthy donors (HD) and SSc fibroblasts. Alternatively, organotypic cultures of full human skin were treated with these cytokines. Responses were assessed by quantifying inflammatory mediators. The factors produced by keratinocytes were identified by a proteomic approach.
Results: Keratinocytes conditioned-media enhanced fibroblasts responses. Priming of keratinocytes with IL-17A increased the fibroblast production of IL-8, IL-6, MCP-1 and MMP-1, but not of type I collagen. However, IL-17A significantly decreased type I collagen production induced by TGF-β. Pretreatment of keratinocytes with TGF-β alone did not or only marginally affected IL-8, IL-6, CCL2 and MMP-1 production by fibroblasts. Vice versa, keratinocyte activation in the joint presence of IL-17A and TGF-β surprisingly resulted in a synergistic positive effect on fibroblast production of these mediators. By proteomic approach, we identified GM-CSF and TGF-α as mediators produced by keratinocytes under the synergistic effect of IL-17A and TGF-β. In full human skin, IL-17A promoted pro-inflammatory responses by inducing 2- to 4-fold increase of IL-8, IL-6, MCP-1 and MMP-1 levels, while showing direct anti-fibrotic effects and decreasing by 2-fold collagen production triggered by TGF-β (p = 0.02).
Conclusions: Keratinocytes profoundly influence dermal fibroblasts responses which are further modulated in the presence of IL-17A favoring their anti-fibrotic and pro-inflammatory phenotype. These data support a role for keratinocytes in the pathogenesis of SSc. To better understand the relevance of these circuitries in SSc we will perform experiments to explore the role of GM-CSF, TGF-α and other factors released by keratinocytes that activate fibroblasts.

1 Takahashi T, et al. J Exp Med. 2017 Feb 23, DOI: 10.1084/jem.2016024.
2 Truchetet ME, et al. Arthritis & Rheumatol. 2013;65:1347–56, DOI: 10.1002/art.37860

SSAIP 19

Tumor infiltrating lymphocytes (TILs) in lymph node metastases of stage III melanoma correlate with response and survival in patients treated with ipilimumab at the time of stage IV disease

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Prognosis of metastatic melanoma improved with the development of checkpoint inhibitors. The role of tumor infiltrating lymphocytes (TILs) in lymph node metastases of stage III melanoma remains unclear. We retrospectively characterized TILs in primary melanomas and matched lymph node metastases (stage III melanoma) of patients treated with the checkpoint inhibitor ipilimumab. Tumor infiltrating lymphocytes were characterized using immunohistochemistry for CD3, CD4, CD8 and FoxP3. 4/9 patients (44%) responded to treatment with ipilimumab (1 complete and 2 partial remissions, 1 stable disease). All responders exhibited CD4 and CD8 T-cell infiltration in their lymph node metastases, whereas all non-responders did not show an infiltration of the lymph node metastasis with TILs. The correlation between presence or absence of TILs in responders vs. non-responders was statistically significant (p = 0.008). Median distant metastases free survival, i.e. progression from stage III to stage IV melanoma was similar in responders and non-responders (22.1 vs. 19.3 months; p = 0.462). Median progression free and overall survival show a trend in favor of the patients having TIL rich lymph node metastases (6.8 vs. 3.3 months and 41.8 vs. 8.2 months respectively, p = 0.086). Our data suggest a correlation between the T-cell infiltration of the lymph node metastases in stage III melanoma and the response to ipilimumab once these patients progress to stage IV disease. Our findings may help in selecting patients with completely resected high-risk stage III melanoma for adjuvant treatment with checkpoint inhibitors.

SSAIP 20

Development of a unique platform for pediatric immuno-rheumatologic diseases (JIRcohorte): inclusion of 1316 patients in Switzerland

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Introduction: Pediatric immuno-rheumatologic diseases are rare, characterized by chronic course and significant impact on patient's life. Recent developments have significantly improved the prognosis of these diseases, but a close follow-up of patients' cohorts is essential to evaluate the long-term outcome. The JIRcohorte is an international platform developed to follow pediatric immuno-rheumatologic diseases, and evaluate the long-term tolerance and efficacy of immunosuppressive and biological therapies. The challenge was to develop a tool with items both common for all patients and specific for each disease.
Objective: Describe the multi-module tool implemented in the JIRcohorte platform and the collective of patients included in the different modules.
Methods: For each of the eCRF, an expert group has defined the items to be collected for prospective follow-up of patients with a specific disease. A first comparison was done to highlight the identical items from the different eCRF and the items specific to each one. For all the items which were reported in more than one module in a similar but not identical way, a negotiation between the experts made it

possible either to find a common item or to clearly define the difference between both items. We describe the patients of 8 Swiss centers included in the JIRcohorte between February 2014 and February 2017.
Results: Thanks to the development of a multi-module tool, we were able to reduce the number of items to insert in the JIRcohorte from 3860 to 2188, by keeping the same level of information. A total of 1316 patients and 5561 visits were collected. The number of patients and visits per module are as follows: Juvenile Idiopathic Arthritis (677 patients, 2899 visits), Temporomandibular Arthritis (60, 139), Juvenile Dermatomyositis (6, 32), Juvenile Systemic Lupus (18, 78), Juvenile Periodic Fever Syndrome (148, 345), Still Disease (45, 161), Uveitis (103, 1200) and Vaccination (1169, 3663).
Conclusion: JIRcohorte collects follow-up data on pediatric patients with different immuno-rheumatologic pathologies. Thanks to its structure, with both common and specific items in each module, it can be used as a valuable tool to compare pediatric patients with different inflammatory rheumatic diseases.

SSAIP 21

Autoantibody quantity and affinity analysis in chronic autoimmune urticaria patients

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Introduction: Ca. 35–50% of patients with chronic urticaria (CU) have functional IgG autoantibodies (autoAbs) against the α-subunit of the high affinity IgE receptor (anti-FcεRIα) and/or against mast cell and basophil surface bound IgE (anti-IgE). These presumably autoimmune forms of CU can be identified by functional tests like autologous serum skin tests (ASST) or by CU-basophil activation tests (CU-BAT), which, however, are both cumbersome and not always reliable. We aim to improve and complement the CU-BAT on a blood donor free basis. Thus, we investigate the characteristics of anti-FcεRIα and anti-IgE autoAbs in CU measuring their quantity as well as their affinity and compare it to the CU-BAT.
Method: We established a chaotropic ELISA measuring autoAb titer combined with antibody affinity. In a first step, autoAbs of CU-patients and control sera were quantified using a human anti-FcεRIα ELISA and human anti-IgE ELISA. In a second step, autoAb quantified serum samples were normalized to a titer in the standard range. Diluted serum samples were incubated in presence and absence of the chaotropic agent ammonium thiocyanate, which is able to disturb intermolecular forces such as antibody-antigen binding sites. Antibody affinity was determined as the percentage of still bound autoAbs quantity after ammonium thiocyanate addition.
Results: Anti-FcεRIα as well as anti-IgE autoAbs, which are found in CU-patient sera, differ greatly in their quantity and quality. Hence, based on autoAbs quantity and quality CU-patients can be divided in different subgroups: anti-FcεRIα respectively anti-IgE low affinity/low quantity, high affinity/low quantity, low affinity/high quantity, and high affinity/high quantity.
Conclusion: Subdivision in affinity/quantity groups showed a better correlation to CU-BAT patient data compared to quantity analysis alone. Determination of quantity and affinity may be helpful in determining functionally relevant autoAbs and thus substitute CU-BAT and ASST as diagnostic tests. However, the value of affinity determination still needs to be analyzed in larger patient cohorts and to be correlated to the severity of CU. As a simple ELISA test the presented CU-serum tests are promising to follow the highly variable course of CU and to monitor the response and persistence of therapeutic interventions such as anti-IgE therapy.

SSAIP 22

Fingolimod in multiple sclerosis: impact on tumor-infiltrating lymphocytes and immunotherapy

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Fingolimod (FTY720) is an orally administered sphingosine-1 phosphate receptor modulator used for the treatment of relapsing forms of multiple sclerosis (MS). It exerts its effects by sequestering lymphocytes in the lymph nodes, which leads to reduced trafficking of these cells to the central nervous system. Clinical studies carried out during the approval of fingolimod did not show an increase in cancer incidence among treated patients. However, its effects on anti-cancer therapies and clinical outcome were not investigated, and currently there is limited knowledge on how to continue treatment in fingolimod-treated MS patients who develop cancer. Due to its immunomodulating effects, it is likely that fingolimod affects the efficacy of anti-cancer immunotherapy. A histological investigation of the tumors of patients treated with fingolimod suggests that the tumors of these patients have lower numbers of tumor-infiltrating lymphocytes (TILs) compared to organ-matched tumor samples from non-fingolimod treated patients. A high density of TILs has been associated with better response rates and better survival outcome in patients treated with immunotherapy, highlighting the importance of TILs in the tumor microenvironment. In addition, preliminary data from B16F10-bearing mice treated with fingolimod and adoptive T cell transfer also suggests that fingolimod affects the number of TILs.

SSAIP 23

Treatment preference on the new Subcutaneous Immunoglobulin 20% (SCIG 20%) treatment in patients with Primary Immunodeficiency Diseases (PID) in Europe (EU)

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Rationale: SCIG offers an opportunity for patients with PIDD to self-infuse at home, potentially reducing treatment burden and improving satisfaction. This analysis assessed treatment preference with CUVITRU, the new SCIG 20%.
Methods: Treatment preference was assessed with a questionnaire within a phase 2/3 study in 48 EU patients with PID treated with IVIG 10% for 3 months followed by SCIG 20% for ≥12 months. Questionnaires administered at the end of the study evaluated preferences about treatment aspects using a 5-point Likert scale and included questions about whether a patient preferred to continue SCIG 20% and preferred location of therapy. Questionnaires were completed by their caregiver/parent (≤13 years) or patient (≥14 years).
Results: Overall, 88% of all patients stated that they would prefer to receive SCIG 20% rather than other Ig treatments with 84% of younger (≤13 years) and 91% of older (≥14 years) patients preferring SCIG 20%. Home infusion was preferred by 88% of all patients. The aspects of treatment with the highest proportion of 'like'/'like very much' responses were "ability to fit treatment into my own schedule" (96%) and "ability to self-administer without medical supervision" (94%).
Conclusions: Overall, 88% of patients preferred to continue receiving the recently approved SCIG 20%; they liked the ability to have more control over self-administration of their Ig treatment.

SSAIP 24

Cellulitis after two-step excision of a basal cell carcinoma favored by rituximab induced hypogammaglobulinemia

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A 78-year-old multimorbid man attended our hospital for complete excision of an ulcerated basal cell carcinoma on his right temple. On the third postoperative day he presented with a painful, warm and erythematous oedema around his right eye spreading to the temple. A cranial MRI showed a diffuse inflammation of the soft tissue. Bacterial culture of a wound swab revealed an infection with staphylococcus aureus and confirmed the diagnosis of a cellulitis. Further blood tests detected a significant hypogammaglobulinemia. Extended patient history indicated that he was diagnosed with a small cell lymphocytic B-cell non Hodgkin lymphoma 6 years ago and treated with an R-CHOP regimen. Due to recent progression he had been kept on a maintenance therapy with rituximab (RTX), which is a fully humanized monoclonal antibody targeting the CD20 receptor on the surface of B-cells. We started treatment with amoxicillin and clavulanic acid and achieved immunoglobulin restitution via application of i.v. immunoglobulins, which lead to full recovery. Very little is known about

superficial postoperative complications in RTX patients. Current guidelines addressing perioperative care in RTX in patients do not give clear recommendations other than consideration of prophylactic antibiotic treatment. To our knowledge this is the first detailed case report of a severe soft tissue infection after superficial surgery in a RTX patient, despite that it represents a very common clinical situation. We intend to raise awareness among physicians and particularly surgeons confronted with RTX-patients to take additional diagnostic measures, e.g. extended laboratory, to identify high-risk groups for infections.

SSAIP 25

A personalized medicine approach to identifying and treating a patient with a novel STAT3 gain of function mutation

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Mutations in signal transducer and activator of transcription (STAT) 3 can cause either an autosomal-dominant loss of function or gain of function (LOF or GOF) in humans. Germline GOF mutations have only recently been described in patients with immune dysregulation presenting as severe autoimmunity, increased susceptibility to infections and short stature. STAT3 GOF mutations have been shown to reduce STAT5 and STAT1 phosphorylation, which are key to various intracellular pathways. We postulate that erythropoietin signaling, which heavily depends on STAT5 activation via the erythropoietin-receptor, is defective in patients with germline STAT3 GOF mutations. Using whole exome sequencing, we identified a hitherto undescribed germline heterozygous mutation in STAT3, c.2144C>T (p.P715L), in a patient with severe autoimmunity, aplastic anaemia and short stature. STAT3 activity caused by this mutation was evaluated by transcriptome analysis and luciferase reporter assay. Increased activation of STAT3 was attributable to hyperphosphorylation as evidenced by increased levels of pSTAT3 in mutated cells and restoration of STAT3 hyperactivity after treatment with STAT3 phosphorylation inhibitors. Phospho-proteome analysis and immunoprecipitation experiments are conducted to look at the phosphorylation state of signalling molecules in cells expressing mutant vs unmutated STAT3 and to define differences in multimeric complexing involving STAT3. Using peripheral blood mononuclear cell cultures as a basis for reticulocyte differentiation assays, we could demonstrate the poor differentiation response of cells derived from the patient to stimulation with various growth factors including erythropoietin. In these cultures and in bone marrow cultures we could show an arrest of cells in the pre-erythroblastic state of hematopoiesis (CD235a intermediate stages). Using curcumin, a natural STAT3 inhibitor and Actemra, an IL-6 receptor antagonist, we could increase the number of cells generating colony-forming units and maturing to erythroblasts. We conclude that anemia in STAT3 GOF patients can additionally be due to signaling defects during erythropoiesis aside from the autoimmune component. Thus, this personalized medicine approach has led us to identify promising therapeutics that are now being administered to treat the patient's anaemia, the effects of which have yet to be evaluated.

SSAIP 26

Tumor-derived PGD2 and NKp30-B7H6 engagement drives an immunosuppressive ILC2-MDSC axis

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Group 2 innate lymphoid cells (ILC2) are involved in different human diseases, such as allergy, atopic dermatitis and nasal polyposis. Yet, their role in human cancer remains unknown. We demonstrate that, in acute promyelocytic leukemia (APL), ILC2 are increased and hyper-activated through the interaction of CRTH2 and NKp30 with tumor-derived PGD2 and B7H6, respectively. ILC2, in turn, via IL-13 secretion activate monocytic myeloid derived suppressor cells (M-MDSC). Upon APL treatment with all-trans retinoic acid (ATRA) and achievement of complete remission, the levels of PGD2, NKp30, ILC2, IL-13 and M-MDSC are restored. Similarly, disruption of this novel tumor immunosuppressive axis by specifically blocking PGD2, IL-13 and NKp30 partially normalizes ILC2 and M-MDSC levels and results in increased survival. Thus, using APL as a model, we uncovered a novel tolerogenic pathway that might represent a relevant and universal immunosuppressive mechanism operating in various human tumor types, as supported by our observations in prostate cancer. Hence, this axis offers new targets for immune intervention in human non-APL malignancies.

SSAIP 27

Tocilizumab in refractory Graves' ophthalmopathy

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Introduction: Graves' ophthalmopathy (GO) affects about 25% of patients with Graves' disease. Main features are eyelid retraction, uni- or bilateral proptosis, and impaired ocular motility. During the acute phase, clinical inflammatory signs are spontaneous orbital pain, painful eye movements, redness of the eye or eyelids and conjunctival, caruncular or eyelid edema. These signs form the clinical activity score (CAS), which allows for monitoring treatment response. Intravenous methylprednisolone pulses (MP) are the mainstay of therapy. Patients unresponsive to glucocorticosteroids (GC) or with contraindications may benefit from immunobiologics. Tocilizumab (TCZ), a humanized monoclonal antibody against the interleukin-6 receptor, has shown promising results in an open-label study [1].
Methods: We report three cases with refractory GO seen at Jules-Gonin Eye Hospital and CHUV between June 2016 and February 2017. All patients had persistent unilateral or bilateral proptosis with inflammatory signs, which had not responded to six MP. Two also had type I diabetes, which precluded further use of GC. All received monthly TCZ infusion at the dosage of 8 mg/kg body weight. All underwent ophthalmologic examination before and after 3 months of treatment, with CAS assessment.
Results: After 3 months, GO improved in all patients (#1: 3 mm reduction of proptosis and CAS 3/7 reduced to 1/7 in both eyes; #2: no change in proptosis, but reduction of CAS 4/7 to 2/7 in OD and 1/7 to 0/7 in OS; #3: 1 mm reduction of proptosis and change of CAS 3/7 to 0/7 in OD, 4/7 to 1/7 in OS, and improvement of eye movements). One patient with latent tuberculosis received concomitant rifampicine. One patient developed herpetic keratitis after the first TCZ infusion and was treated with valacyclovir. There were no other adverse events.
Conclusion: In these three cases with severe Graves' ophthalmopathy refractory to conventional therapy, tocilizumab improved symptoms within 3 months. Treatment was generally well tolerated. Controlled studies are urgently needed to assess whether IL-6 receptor blockade could benefit patients with early severe ophthalmopathy.

Reference:
1 Pérez-Moreiras JV, Alvarez-López A, Gómez EC. Treatment of active corticosteroid-resistant Graves' orbitopathy. Ophthal Plast Reconstr Surg. 2014;30:162–7.

SSAIP 28

Generation of a human p47phox-deficient chronic granulomatous disease cell line using CRISPR/Cas9 for fast gene therapy vector testing

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Chronic granulomatous disease (CGD) comprises a group of hereditary monogenetic immunodeficiencies characterized by defective respiratory burst and microbicidal activity of phagocytes leading to recurrent life-threatening infections. Mutations in gp91phox, p47phox, p67phox, p40phox or p22phox subunits of the phagocytic nicotinamide adenine dinucleotide phosphate (NADPH) oxidase may result in CGD. The gp91phox subunit is the most frequently mutated in CGD (65%), and the mutations are scattered throughout the whole cytochrome b-245 beta chain (CYBB) gene. Conversely, the p47phox-deficient CGD, which is the second most common form of CGD (25%) is almost exclusively caused by a single GT-dinucleotide deletion (ΔGT) in exon 2 of neutrophil cytosolic factor 1 (NCF1) gene. The ΔGT mutation causes a frameshift and premature translation interruption. Furthermore, it is shared with two pseudogenes, NCF1B and NCF1C, which are located on the same chromosome. The pseudogenes are extraordinarily homologous to the NCF1 gene (99.5%). Presumably, homologous recombination between the NCF1 gene and its pseudogenes results in the predominance of the ΔGT mutation among the p47phox-deficient CGD patients. At present, the development of gene therapy vectors for p47phox-deficient CGD is hampered by the absence of human cell lines allowing for rapid gene therapy vector testing. The existing p47phox –/– mouse models cannot replace vector testing on human cells. Previously, we established human p47phox-deficient induced pluripotent stem cell (iPSC) lines harboring the ΔGT within the NCF1 gene. These iPSC-based cell lines reflect the genetic background of the most common mutation in CGD. However, maintenance and differentiation of iPSC lines is laborious and in many aspects impractical. As an alternative to the iPSC-based cell lines, we established a novel model for ΔGT p47phox-deficient CGD based on a human acute myeloid leukemia PLB-985 cell line. We utilized the CRISPR/Cas9 technology to introduce the ΔGT mutation in p47phox encoding NCF1 gene. The established PLB-985 NCF1 ΔGT cell line reflects the most frequent form of p47phox-deficient CGD genetically and functionally. The cells can be differentiated to granulocytes in seven days and are correctable by γ-retroviral vectors. The PLB-985 NCF1 ΔGT cell line creates an attractive alternative to currently used iPSC models for rapid testing of novel gene therapy approaches.

SSAIP 29

Regulation of Rgs1, a modulator of G-protein linked receptor signaling in intestinal intraepithelial T cells

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Regulators of G-protein signaling (Rgs) represent a diverse protein family characterized by the presence of a conserved RGS domain, which physically interacts with specific receptor linked G-protein subunits to enhance the intrinsic GTPase activity of heterotrimeric G-proteins. Immune cells predominantly express R4 Rgs subfamily protein members, and differential Rgs expression patterns appear to be involved in modulating cell migration. Particularly, Rgs1 was shown to modulate the chemotactic behavior of immune cells (T-, B cells, monocyte/macrophages) in vitro and in vivo. Consistent with previous findings, we observed highly elevated Rgs1 expression in conventional and unconventional T cell subsets in the intestinal epithelium of mice when compared with circulating T cells. In vitro experiments revealed that TGF-β and IL-15 synergistically induce elevated Rgs1 expression, which was further enhanced by concomitant anti-CD3/-CD28 mediated activation of CD8αβ TCRαβ T cells. Intriguingly, we observed in vivo a rapid down-regulation of Rgs1 expression in unconventional CD8αα TCRαβ intraepithelial lymphocytes (IEL) during onset of experimental colitis concomitant with the appearance of this enigmatic T cells at extra-epithelial locations. Subsequently, we defined the expression patterns of the R4 Rgs subfamily members in conventional and unconventional T cell subsets from various anatomical locations. These findings suggest that Rgs1 is likely regulated by micro-environmental cues enriched in the intestinal mucosa. We now investigate the functional relevance of this tightly regulated, differential Rgs1 expression for the positioning of conventional and unconventional T cell subsets in specific intestinal niches. Preliminary data obtained upon analysis of Rgs1-deficient, vs. wildtype mice, and the analysis of congenic Rgs1–/–, and Rgs1+/+, mixed bone marrow chimeras suggest that absence of Rgs1 may indeed directly affect the distribution pattern of various T cell subsets in vivo.

SSAIP 30

The immunoproteasome subunit LMP7 is required in the thymus for filling up a hole in the T cell repertoire

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In the context of IFN-γ or TNF-α the proteolytically active β1c, β2c, and β5c subunits of the constitutive proteasome are replaced by β1i, β2i, and β5i (LMP7) building the so called immunoproteasome, which is critically involved in the processing of ligands for MHC class I presentation. As compared to wild type mice, infection of LMP7-deficient mice with the lymphocytic choriomeningitis virus (LCMV) yielded a strongly reduced CTL response to the LCMV glycoprotein (GP) derived T cell epitope GP118-125. However, the class I mediated presentation of GP118-125 was not dependent on LMP7. Using bone marrow chimeras and adoptive transfer of LMP7-deficient CD8+ T cells into RAG1-deficient mice we showed that LMP7-deficient mice lack GP118-125-specific T cell precursors and that LMP7 is required in radioresistant cells of the thymus – most likely thymic epithelial cells – to enable their selection. Since LMP7 is expressed in negatively selecting medullary thymic epithelial cells but not in positively selecting cortical thymic epithelial cells it appears that LMP7 is required to avoid excessive negative selection of GP118-125-specific T cell precursors. Taken together, this study demonstrates that the immunoproteasome is a crucial factor for filling up holes within the cytotoxic T cell repertoire.

SSAIP 31

T cell-expressed Liver Receptor Homolog-1 (LRH-1/NR5a2) regulates anti-viral immune responses

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Nuclear receptors (NR) regulate a large spectrum of developmental and physiological processes in different organs and tissues. They are also involved in the regulation of development and activation of immune cells. Liver receptor homolog-1 (LRH-1, NR5a2) is one of the members of the NR subfamily NR5, which is mainly expressed in organs and tissues from endodermal origins, such as liver, ovary, pancreas and intestine. LRH-1 has a broad spectrum of functions in these tissues, including the regulation of metabolism, steroidogenesis, cell cycle progression and inflammation. However LRH-1 is also expressed in cells of hematopoietic origin. In mature T cells LRH-1 has been implicated in the activation-induced cell cycle entry and proliferation. In this study we investigated the impact of LRH-1 deficiency in the cytotoxic T cell-mediated regulation of lymphocytic choriomengitis virus (LCMV) infection. LRH-1 was deleted using the Cre-Lox system using a CD4 promoter driven Cre expression, which leads to LRH-1 deletion also in CD8+ T cells. Interestingly, although LRH-1 deletion strongly reduces the number of peripheral CD8+ T cells, CD8+ T cells expanded normally after LCMV infection, and were comparable to control mice. Moreover, the activation markers CD25 and CD69 were induced normally in LRH-1-deficient T cells, and even higher than in LRH-1+/+ T cells. However, while LCMV was able to expand in both genotypes and all tissues, only LRH-1 wildtype T cells were able to eliminate the virus, whereas virus titers remained high in mice with LRH-1-deficient T cells. These data demonstrate a novel role of the nuclear receptor LRH-1 in the control of T cell-mediated anti-viral immune responses.

SSAIP 32

Dual roles of the nuclear receptors LRH-1 and SHP in the regulation of inflammation-driven intestinal tumor development

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Liver Receptor Homolog-1 (LRH-1/NR5a2) is a nuclear receptor that regulates metabolism, development, proliferation and inflammation. In the intestinal mucosa LRH-1 is expressed in the intestinal crypts, where it regulates the renewal of the epithelial layer by controlling stem and progenitor cell proliferation, as well as immune homeostasis and inflammation by the transcriptional control of the synthesis of immunoregulatory glucocorticoids (GC). Therefore, deletion of LRH-1 in the intestinal mucosa results in reduced intestinal GC synthesis and renders mice more susceptible to intestinal inflammation. Interestingly, LRH-1 has been associated with the development of colorectal tumors by controlling tumor cell proliferation as well as suppressing tumor surveillance via the release of immunosuppressive GC. Indeed, intestinal epithelium-specific LRH-1-deficient mice show reduced colonic tumor formation in response to chronic intestinal inflammation after azoxymethane (AOM) and dextran sodium sulphate (DSS) treatment (mouse model of colon carcinogenesis) compared to wildtype mice. Small heterodimer partner (SHP/NR0B2) is a nuclear receptor with no DNA binding domain. SHP is a transcriptional target of LRH-1, and at the same time a potent inhibitor by forming a transcriptionally inactive heterodimer with LRH-1. As absence of SHP could result in enhanced LRH-1 activity, including increased proliferation and GC synthesis, and thereby immune escape by intestinal tumor cells, we investigated the impact of SHP deletion on the regulation of LRH-1 activity in the intestinal epithelium and colorectal tumor formation using the AOM/DSS model. Surprisingly, we observed that absence of SHP resulted in significantly reduced numbers and size of intestinal tumors paralleled by reduced weight loss during the three cycles of DSS treatment indicating reduced inflammation. Moreover, in line with the role of intestinal GC synthesis in controlling inflammation regulated via LRH-1 and the proposed role of SHP in the regulation of LRH-1, we observed increased steroidogenic enzymes expression and higher levels of GC in colonic tissue from SHP-deficient compared to wildtype mice paralleled by reduced colonic inflammation. These data support an unexpected role of SHP in the control of inflammation-driven intestinal tumor development, not via the control of LRH-1-promoted tumor cell proliferation, but rather via the regulation of intestinal inflammation and associated tumor development.

SSAIP 33

Major role of the AAA-ATPase p97 in direct antigen processing and presentation on MHC class I

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Direct antigen presentation on major histocompatibility complex (MHC) class I molecules allows immunosurveillance of proteins synthesized within cells. Generally, epitopes presented derive from proteasomal degradation of proteins into small peptides. One source of antigenic peptides is most likely a fraction of rapidly degraded proteins termed defective ribosomal products (DRiPs). Up to now, little is known about factors influencing DRiP formation and processing. The AAA-ATPase p97 is involved in different cellular protein control pathways and supports substrate degradation via the ubiquitin-proteasome system. By chemical inhibition of p97 or expression of a dominant negative p97 mutant we show that MHC class I restricted presentation of virus-derived and endogenous epitopes as well as bulk MHC class I surface expression is dependent on p97 activity. Rapid accumulation of poly-ubiquitylated proteins in cells with disrupted p97 activity points towards a role upstream of the proteasome. Taken together, we identify p97 as an essential factor for MHC class I antigen processing which further extends the repertoire of p97-dependent cellular functions.

SSAIP 34

Peripheral tolerance restricts immune responses against melanoma-associated self-antigens

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Immune tolerance limits the efficacy of tumor vaccination against self-antigens. To elucidate the mechanism underlying peripheral tolerance in melanoma, we used Dct-deficient mice lacking the tyrosinase-related protein-2 (TRP-2), which is expressed in melanocytes but not in the thymus. To assess whether peripheral tolerance against this self-antigen can be overcome with a potent vaccine strategy, we used a recently developed viral vaccine vector expressing TRP-2. After immunization Dct–/– mice showed significantly increased frequencies and improved functionality of TRP-2-specific CD8+ T cells compared to Dct-proficient mice. In addition, therapeutic immunization of B16F10-tumor-bearing Dct-deficient mice resulted in complete eradication of the tumors and long-term survival whereas wild-type mice only showed a delay in tumor growth. Therefore, our data suggest that peripheral tolerance is responsible for failures of vaccination therapy against melanoma-associated self-antigens.

SSAIP 35

Resident intestinal eosinophils maintain small intestine structure and function

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The adult small intestine is the largest reservoir of resident eosinophils, however functional roles for these cells remain enigmatic. Whole-mount immunostaining allowed thorough analysis and detailed localization of Siglec-F+ intestinal villus eosinophils in 3D. Furthermore, 3D imaging revealed that eosinophils contribute to intestinal maintenance as ΔdblGata-1 eosinophil-deficient mice have significantly altered villus morphology compared to wild-type animals. A similar altered villus morphology was observed in Nod Scid gamma KO mice, which also have decreased numbers of intestinal eosinophils. Altered villus structure has functional consequences as ΔdblGata-1 mice exhibit impaired fat absorption. In all we show a novel role for eosinophils in intestinal maintenance and function.

SSAIP 36

Lymphadenopathy driven by TCR-Vγ8Vδ1 T-cell expansion in FAS-related autoimmune lymphoproliferative syndrome

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Mutations in the FAS gene are linked to autoimmune lymphoproliferative syndrome (FAS-related ALPS, also referred to as ALPS-FAS). This disease is associated with abnormally high counts of αβTCR+, CD4– CD8– double negative (DN) T-cells. Here, we report on two patients with ALPS-FAS, who respectively carried a novel

(c.657delA) mutation and a previously described in FAS. Whereas the DN T cells in ALPS patients usually express αβTCRs, those isolated from our patients' lymph nodes expressed γδTCRs. These γδ T cells were highly proliferative and had a cytotoxic phenotype. Furthermore, the T cells within enlarged lymph nodes were restricted to Vγ8Vδ1-TCR usage, and had oligoclonal complementary-determining region 3 repertoires. We also established that Vδ1-T cell expansion is controlled by FAS-dependent apoptosis in vitro; this finding suggests that the massive accumulation of Vδ1-T cells in these two patients was linked to their FAS mutations. Lastly, we evidenced elevated levels of in vitro methylprednisolone, rapamycin and pyrimethamine resistance in Vδ1-T cells (relative to αβ-T cells). We believe that these features of Vδ1-T cells are associated with the partial treatment resistance observed in one of the two patients.

SSAIP 37

Inducible abrogation of the skin-specific synthesis of immunoregulatory glucocorticoids results in spontaneous skin inflammation

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Glucocorticoids (GC) are known for their immunosuppressive effects and are clinically used to treat inflammatory diseases. So far, GC synthesis is well described not only in the adrenal cortex but also in extra-adrenal organs, such as the intestine, skin, and lung. The de novo GC synthesis in human skin is already well explored and is regulated by a local network similar to the hypothalamus-pituitary-adrenal axis. GC synthesis involves several cytochrome P450 enzymes, including the side-chain cleavage enzyme P450scc (CYP11A1) for the conversion of cholesterol to pregnenolone or the 11-beta-hydroxylase (CYP11B1) catalyzing the final conversion to active cortisol/corticosterone. However, the role and function of cutaneous de novo-derived GC in the regulation of local inflammatory processes remains thus far unexplored. Here, we introduce an inducible model allowing a keratin-14-specific deletion of the Cyp11b1 gene. Genomic deletion of Cyp11b1 in keratinocytes reduced mRNA expression and biosynthesis of active corticosterone in the whole skin indicating the importance of GC synthesis in keratinocytes for the integumentary system. Moreover, Cyp11b1-deficient skin is prone to skin sensitization as we observed spontaneous induction of Th1/Th17 type pro-inflammatory cytokine expression, dendritic cell (DC) activation and lymph node (LN) migration. In fact, knockout mice develop spontaneous dermatitis-like skin inflammation with pro-inflammatory immune cell infiltration. Using FITC painting and migration assay, our preliminary results indicate that skin GC also modulate the immune response type as skin sensitization with FITC, a Th2 type immune response inducer, resulted in decreased skin-derived DC numbers in draining LN of knockout mice, which is possibly regulated by an antagonistic Th1/Th17 immune response. Furthermore, human skin biopsies showed decreased expression of P450scc in lesions of patients with atopic dermatitis (AD) and psoriasis. Similarly, preliminary results revealed decreased gene expression of CYP11B1 in human skin lesions from psoriasis and AD patients indicating a deregulated skin GC synthesis. Our findings demonstrate the importance of de novo-produced skin GC in regulating local skin inflammation, contributing thereby to the skin barrier homeostasis. Thus, this highlights the biological significance of cutaneous GC in the skin, and may offer novel understandings and targets in the pathogenesis of skin inflammatory diseases.

SSAIP 38

NLRP3 inflammasome activation by glufosinate causing IL-1β dependent lung inflammation

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Glufosinate-ammonium (GLA), a herbicide, shares structural analogy with glutamate and inhibits glutamine synthetase (GS) and increases glutamate levels. A single GLA exposure induces inflammatory cell recruitment in the broncho-alveolar space, increased glutamate; MPO and lung inflammation within 24h. Chronic exposure causes increased airway resistance. We find that blockade of the glutamate receptor by MK801 prevents GLA-induced lung inflammation. Further, increased glutamate levels activated the ASC-NLRP3 inflammasome. In vitro GLA activated ASC speckle formation and IL-1β production in macrophages. GLA induced inflammation depends on IL-1 receptor 1 (IL-1R1) signaling in myeloid cells. In conclusion, GLA aerosol exposure causes glutamate dependent NLRP3 activation with IL-1beta driven inflammation and airway hyperreactivity in mice.

SSAIP 39

Neutrophil infiltration in inflammatory disease models and the treatment with intravenous immunoglobulins (IVIG)

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Neutrophils are the most abundant white blood cells in the human body. Being one of the first infiltrators at sites of infection, they recruit other components of the innate and adaptive immune system by releasing granules, producing ROS and NETosis. By creating an inflammatory environment, neutrophils stay one of the prominent immune cell types until resolving of the inflammation, as can be seen eg. in appendicitis. Unusually high neutrophil counts are observed in autoimmune diseases such as Kawasaki's disease. Kawasaki's disease affects children of young age, causing vasculitis symptoms and particularly affecting the coronary arteries. Intravenous immunoglobulin (IVIG), usually used as treatment for patients with immunodeficiencies, has been used off label in Kawasaki's disease, resulting in drastic drops in neutrophil counts. Nevertheless, some patients do not respond to this IVIG treatment. Since the exact mechanism of action of IVIG on neutrophil apoptosis or resistance is not yet clear, we would like to understand more about the molecular mechanism of IVIG. By characterizing neutrophils at inflammatory sites and analyze their behavior upon IVIG treatment, we aim to get more insight into the field.

SSAIP 40

Siglec-7 and -9 receptors on cytotoxic T cells and their impact on anti-tumor immune responses

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In the past few years, the role of cytotoxic T lymphocytes (CTL) in the fight against cancer has been put into light and is playing a growing importance into the development of check-point inhibitor therapies against cancer. Siglecs are inhibitory receptors recognizing sialoglycan ligands and are able to trigger inhibitory functions similarly to the well described CTLA-4, PD-1 or Tim-3, which regulate the activity of immune cells. Ligands of Siglec receptors have been shown to be highly upregulated in various types of tumors. Therefore, we aim to investigate the Siglec-7+ and -9+ CTL pools characteristics in healthy donors as well as in different diseases. Ultimately, we want to assess the potentiality of Siglec receptors in tumor pathogenesis and treatments. CTL were isolated and sorted from patients and healthy donors to be analyzed and characterized using FACSverse. Cells have been screened for various membrane receptors expression, cytokine production, proliferation capacity and fundamental tissue quantification for a total overview of the Siglec+ CTL pools characteristics. Moreover, functional assays measuring the migration capacity and the cytotoxic potential towards tumors were also performed. All together, the study of Siglec+ CTL in different diseases have shown an increased Siglec+ pool in the peripheral blood of the patients. The Siglec+ CTL represent a more differentiated, more cytotoxic, more migratory and more proliferative subset of CTL. Finally, Siglec receptors appeared to be highly co-expressed with various other regulatory receptors among activated CTL of healthy donors. Siglecs on CTL may represent further potential therapeutic targets for immune check-point therapy of malignancies with high expression of sialoglycans.

SSAIP 41

The Lin28 / let-7miRNA feedback axis regulates development and maintenance of thymic epithelia and their capacity for regular T cell selection

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The thymus provides the physiological microenvironment for the development of the majority of T lymphocytes. Its function is therefore critical for the successful establishment of the immune system's capacity to distinguish between vital self and injurious non-self. This competence is primarily instructed by thymic epithelial cells (TECs), whose differentiation, growth and function depend on the transcription factor Foxn1. TECs adopt during their maturation either a cortical (c) or medullary (m) identity. cTEC mediate immigration of early thymic progenitors (ETP), their commitment to the T cell lineage and positive selection of TCR specificities able to recognize MHC with intermediate avidity. mTEC negatively select thymocytes bearing self-reactive TCRs by providing co-stimulatory signals and antigens derived from otherwise tissue restricted proteins through promiscuous gene expression. Micro RNA (miRNA) represent an essential class of small (19–25 nucleotides), noncoding RNAs indispensable for biological processes including cell fate determination, self-renewal, differentiation, proliferation, apoptosis and cellular homeostasis. Cell- and tissue-specific miRNA expression patterns have been identified, suggesting unique biological roles for specific miRNAs. The most abundant family of miRNAs in mammals is lethal-7 (let-7). Most of the let-7 family members are bound to the RNA binding protein Lin28 which is present in two paralogues, A and B with an amino acid identity of 77%. Differentially transcribed, Lin28A and Lin28B differ regarding their subcellular localisation and biological function. The specific regulatory roles of each of the Lin28 paralogues in the context of TEC biology, however, are unknown. We therefore investigated TEC in which either Lin28A or Lin28B are heterochronically expressed using tissue-directed transgenesis. Lin28B expression results in thymus hypercellularity and promotes an increase in cTEC numbers. Lin28A expressing mice, however, feature a hypoplastic thymus despite the presence of a regular cTEC compartment, and display a reduction in mTEC numbers. Furthermore, the ability of the mTEC compartment to instruct functional competence to SP thymocytes is severely compromised by Lin28A expression. The TEC compartment is to our knowledge the first example where Lin28A and B exert opposing roles in regulating organ size. Whether this is related solely to TEC cellularity or includes cell intrinsic changes is now investigated.

SSAIP 42

The intraspecies diversity of C. albicans triggers qualitatively and temporally distinct host responses that determine the balance between commensalism and pathogenicity

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Candida albicans is a member of the normal human microbiota, but as an opportunistic pathogen it can also cause severe infections in immunocompromised individuals. It is generally believed that the host immune status alone determines the outcome of the interaction between the commensal fungus and the host, resulting in either health or disease. Interleukin 17 (IL-17)-mediated immunity has emerged as a critical mechanism of the host to regulate the antimicrobial response, thereby limiting fungal overgrowth at the epithelial barriers. Complementarily, neutrophils contribute to host defense by preventing systemic dissemination of the fungus. Whether in addition to host factors, differences in C. albicans that exist between individuals may also contribute to disease development remains unclear. We used the well-established mouse model of oropharyngeal candidiasis (OPC) to probe the host response to diverse natural isolates of C. albicans in a uniform and Candida-naïve host environment. The isolates displayed no gross differences in growth, hyphenation, drug resistance or cell wall composition in vitro, but they triggered highly variable degrees of inflammation in vivo. The impaired neutrophil response and the delayed induction of IL-17 and antimicrobial peptides by some isolates correlated with their persistence in the mucosal epithelium. Importantly, however, the role of IL-17 in preventing uncontrolled fungal growth was conserved with all isolates tested, highlighting the essential functionn of this cytokine in host protection from C. albicans. Differences in the host response induced by the diverse isolates in vivo was reflected by their capacity to induce the release of 'alarmins' such as IL-1α from keratinocytes. This supports the notion that the epithelium can sense variations in the fungus and translate them into host signals that mediate fungal clearance or persistence. Together, this study demonstrates the relevance of the natural diversity of C. albicans for determining the fine balance between commensalism and pathogenicity in vivo.

SSAIP 43

BH3 mimetics are counteracting cytokine driven increase in basophil survival, opening novel treatment strategies in basophil dependent diseases

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Basophils constitute the least frequent granulocyte population within the blood, shearing many similar features with mast cells. Even though mast cells are tissue resident cells, basophils were long thought to be only their circulating, redundant relatives. However, basophils were recently recognized to be essential in protective immunity against certain parasites such as helminths but are also pivotal in the initiation of the pathological features of IgE-mediated chronic allergic inflammation. Nevertheless, due to their rarity and rather short lifespan, functional analysis of basophils is a challenging issue. We have lately established a novel method using conditional Hoxb8 to generate IL-3 driven bone marrow-derived basophils in vitro after massive expansion of committed progenitors. With this so-called IL-3condHoxb8 basophil cell model as well as with bone marrow derived mast cells we are investigating the potential of the newly developed BH3 mimetic compounds to regulate their fate decision of cell death. This revealed the distinct and critical importance of specific Bcl-2 family members like Bcl-2, Bcl-xL and Mcl-2 in basophil viability, distinct from mast cells. Exploring the functional mechanisms of basophil and mast cell survival we reassured the vital impact of IL-3 as a major regulator of the Bcl-2 family member expression. Thereby we found that IL-3 leads to the increase of anti-apoptotic Bcl-2, Bcl-xL as well as Mcl-1, whereas the pro-apoptotic BH3-only protein Bim and Puma rather become down regulated. However, the IL-3 induced increase of the survival potential could be counteracted by various BH3 mimetics suspecting novel treatment strategies in basophil or mast cell dependent diseases.

SSAIP 44

Role of intestinal resident CD4+ T cells in colitis and disease relapse

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Long-term immunity to pathogens is conferred by the adaptive immune system with memory functions. Memory T lymphocytes are classically divided between effector and central memory with the capacity to recirculate between non-lymphoid tissues and to home to secondary lymphoid organs, respectively. Recent evidence however shows the existence of non-migratory memory T lymphocytes which persist locally at epithelial barrier tissues. Given their long-term persistence at epithelial barrier tissues such as the intestine, we took advantage of our reversible mouse model of colitis with predictable onset of remission and disease relapse to investigate the role of tissue resident CD4+ T cells in the induction of remission and disease relapse. In this model, colitis mediated by adoptive transfer of naïve CD4+ T cells in lymphopenic RAG KO mice can be reversed by the depletion of CD4+ T cells (using depleting anti-CD4 antibody) resulting in short-term remission. We hypothesized that naïve CD4+ T cells following priming become tissue resident in the intestine and are responsible in driving the onset of relapsing disease. To distinguish between circulating and resident CD4+ T cells, mice were labeled in vivo with a fluorescent antibody followed by perfusion. We found that following priming, naïve CD4+ T cells become tissue resident (non-labeled) in the intestine and express CD69 and CD103 (signature markers of tissue resident memory CD8+ T cells) as well as anti-apoptotic marker Bcl-2. These resident CD4+ T cells are not depleted during remission induction by

anti-CD4 mAb treatment. Importantly, during remission, we observed the emergence of a subset of intestinal resident CD4+ T cells that express S1PR1 and the transcription factor KFL2, which are critical for tissue egress, suggesting that these cells may leave the intestine and contribute to the circulating effector CD4+ T cell pool. Taken together, our data suggests that in chronic relapsing inflammatory diseases circulating effector CD4+ T cells are critical mediators of sustained disease since their depletion results in short-term remission and that tissue resident CD4+ T cells in the intestinal mucosa critically contribute to relapsing inflammatory disease in the colon.

SSAIP 45

Patients with Primary Antibody Deficiency exhibit a qualitative defect in carbohydrates recognition by circulating immunoglobulins

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Primary immunodeficiency (PID) constitutes a group of more than 130 immunological disorders associated to different defects in the adaptive or innate immunity. Among the PIDs, Primary Antibody Deficiency (PAD) disorders constitute approximately the 50% of all the diagnoses. Despite the consensus on the decreased antibody titters in PAD patients, the quality of their binding capacity has been poorly studied. Using glycan array technology we observed a general defect in the carbohydrate recognition by PAD patients, with a marked decrease in the binding to bacterial and tumor carbohydrate determinants. The analysis of particular structural features of glycans revealed a specific loss of recognition for carbohydrates with Gala in their terminal position, associated with a reduced xenogeneic reactivity. We as well discovered a similar glycan recognition pattern between patients with IgG subclass deficiency and other PADs; reinforcing the clinical relevance of this diagnosis. Our study suggests that not only the quantity but the quality of the circulating antibodies might influence the pathogenesis of Primary Antibody Deficiency syndromes.

SSAIP 46

MAF-dependent ROR-γt+ Treg cells control the development of mouse inflammatory bowel disease through IL-10 production

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Foxp3-expressing regulatory T (Treg) cells play a key role to maintain self-tolerance and therefore prevent autoimmunity. Indeed, in the absence of these cells, humans and mice develop life threatening lymphoproliferation and inflammation. Recently, a new subset of Treg cells expressing both Foxp3 and ROR-γt – the master transcription factor of Th17 cells – has been described. Largely expressed in the gut, ROR-γt+ Treg cells have an enhanced suppressive activity compared to ROR-γt– Treg cells, especially in an in vivo context of gut inflammation such as colitis. We found that the transcription factor MAF, well known for inducing the transactivation of cytokines-related genes (il10, il4, …) in effector CD4+ T cell subsets, is highly expressed in some Treg cells, particularly in ROR-γt+ Treg cells. To study the role of MAF in this population of Treg cells, we generated mice deficient for MAF in T cells. Interestingly, these mice developed late onset colitis correlating with the absence of ROR-γt+ Treg cells in colon and mesenteric LNs. While IL-10 production was reduced, TNF-α and IL-17A expression was increased in the colon. We found that in vitro induced Treg cells derived from Maf KO CD4+ T cells produced less IL-10 and had impaired suppressive capacity compared to those derived from WT animals. Taken together, our data suggest that MAF expression in Treg cells, especially ROR-γt+ Treg cells, is essential to maintain their suppressive activity and thereby prevent inflammatory bowel disease by inhibiting Th1 and Th17 polarization. Further experiments will elucidate the implication of maf in the development and/or the maintenance of ROR-γt+ Treg cells.

SSAIP 47

Regulation of iNKT cell responsiveness

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Invariant NKT cells (iNKT) represent a unique sublineage of T lymphocytes activated by self- and microbial-derived glycolipids in the context of the MHC-related molecule CD1d. Their significance in the transactivation of innate and adaptive immune responses is well described, as well as their potent antitumor activity. The glycosphingolipid α-GalactosylCeramide (αGalCer) is a strong iNKT TCR agonist leading to the rapid secretion of Th1 and Th2 cytokines shortly after in vivo injection. However, this initial activation is followed by several weeks of unresponsiveness to antigen re-challenge and by a skewed polarization towards IL-10 production. We have demonstrated that several CD1d-expressing APCs including monocytes, DCs, B cells or CD1d transfected EL-4 cell line, are able to induce iNKT cell anergy. However, we found that the solubility of αGalCer analogs, which can be modified by discrete chemical modifications in their lipid tail, greatly influences the induction of iNKT cell anergy. Indeed, highly hydrophobic αGalCer analogs, which require their intracellular processing by APCs, induce stronger iNKT cell anergy after a single stimulation, while more polar αGalCer analogs, which mainly load on the APC cell surface, are able to maintain iNKT cells responsiveness to multiple restimulations. At this point it is not yet clear whether an active mechanism in the APC is induced during glycolipid processing, or if the prolonged presentation of αGalCer analogs via the endocytic pathway is enough to dictate the induction of anergy in the iNKT cells. Importantly, the intracellular processing of αGalCer analogs is also required to optimally promote the transactivation properties of iNKT cells, such as NK cell activation and the maturation of pro-inflammatory DCs, which lead to efficient T cell priming. In this context, we found that the combination of αGalCer analogs and anti-PD1 checkpoint blockade result in synergistic antitumor effects even against CD1d-negative tumors. These data clearly illustrate the strong adjuvant effect of iNKT cells on the antitumor adaptive immune response which is unleashed upon PD-1 blockade. These results should rekindle the interest in the manipulation of iNKT cells for therapeutic cancer vaccination combined with checkpoint blockade.

SSAIP 48

mRNA splicing and epithelial integrity

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Intestinal epithelial cells (IECs) have co-evolved with its neighbouring microbiota and the mucosal immune system to form a sophisticated and tightly regulated network. When this tripartite network is dysregulated, resulting chronic inflammation can promote intestinal diseases such as inflammatory bowel disease (IBD). The role of alternative mRNA splicing for intestinal homeostasis and pathology is poorly understood. Epithelial splicing regulatory protein 1 (ESRP1) is an epithelial-specific central regulator of mRNA splicing. The Triaka mouse model has a point mutation in Esrp1 leading to a hypomorphic function of ESRP1 splicing activity. As Esrp1–/– mice are neonatally lethal but Triaka mice develop normally, we can investigate the physiological role of Esrp1 in adult mice. We aim to assess whether dysregulated mRNA splicing impacts on the intestinal epithelial barrier, and if this modifies the composition of the adjacent microbiota and mucosal immune system to promote intestinal immunopathology. At homeostasis, Triaka mice have no overt intestinal pathology but have greatly increased CD8αβ+ TCRαβ+ intestinal epithelial lymphocytes (CD8 IELs) compared to wild-type (WT) mice as assessed by flow cytometry. WT mice CD8 IELs reach similarly high frequencies to that of homeostatic Triaka mice following 3 days of 2% Dextran Sodium Sulfate (DSS) treatment in drinking water. The increase of CD8 IELs is dependent on commensals as germ-free Triaka and WT mice have similarly low frequencies. In a DSS-chronic colitis model, weight loss is

more pronounced in Triaka compared to WT mice. Our results suggest that mRNA splicing in IECs modifies the mucosal immune compartment. Future work will include elucidating how this mechanistically influences intestinal immunopathology.

SSAIP 49

The role of autophagy in eosinophils

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Background: Autophagy is a highly regulated catabolic process by which cell constituents are digested by lysosomal degradation to primarily assure energy and molecules for cell survival. It is also incorporated in various biological mechanisms including multiple innate and adaptive immune pathways. Our research group demonstrated that autophagic pathway negatively affects neutrophil generation and is as well involved in lipid metabolism, where it specifically degrades lipid contents in murine hepatocytes.
Methods: To study the role of autophagy in eosinophils, we generated an eosinophil-specific Atg5 knockout mice (designated Atg5E?). Because eosinophils in mice comprise only 1–3% of circulating leukocytes, we provoke eosinophilia by crossbreeding our genetically modified mice with IL-5 transgenic mice.
Results: Atg5 is efficiently deleted within eosinophilic lineage and deletion is eosinophil specific. We measured eosinophil numbers and discovered decreased absolute ($p = 0.0085$) and relative ($p = 0.0271$) numbers in the peripheral blood of Atg5E? IL-5 transgenic mice as compared with control IL-5 transgenic mice. We found that Atg5E? IL-5 transgenic mice have greater amount of immature eosinophils than their counterpart controls (mean relative numbers of Siglec-FintCCR3- cells: bone marrow, 32.9% versus 24.8%; spleen, 21.5% versus 12.9%). Lack of Atg5 does not compromise in vitro viability of eosinophils, isolated from the bone marrow. Next, in Atg5-deficient eosinophils we detected an increased amount of lipid bodies as compared with control eosinophils ($p = 0.0004$). Upon physiologic stimulation with C5a following GM-CSF priming, eosinophils deficient for Atg5 exhibit an impaired ability to de novo synthesise lipid bodies.
Conclusions: Decreased numbers of Atg5-deficient eosinophils in the peripheral blood and higher numbers of immature Atg5-deficient eosinophils at the sites of their eosinophilopoesis indicate that autophagic process is required for regular eosinophil production. Higher amount of lipid bodies in eosinophils, deficient for Atg5, demonstrates involvement of autophagy in lipid regulation.

SSAIP 50

Regulation of cytoskeletal dynamics by post-translational glutathionylation: implications for NET formation

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Neutrophils are the most abundant cells in blood and their antimicrobial defense capabilities are defined, at least partially, by their formation of neutrophil extracellular traps (NETs). For the past decade, efforts have been made to elucidate the molecular mechanisms of NET formation. In this study, we demonstrate that inhibiting cytoskeletal dynamics using pharmacological inhibitors or in knockout mouse neutrophils having defects in genes regulating the actin and tubulin networks, prevents the degranulation and DNA release both required for NET formation. Wiskott-Aldrich syndrome protein (WASP)-deficient mouse (Was–/–) neutrophils, which are unable to polymerize actin, exhibit a block in degranulation and DNA release after stimulation. In addition, activation of mouse and human neutrophils with a genetic defect in NADPH oxidase failed to induce actin and tubulin polymerization or NET formation. Moreover, neutrophils deficient in glutaredoxin 1 (Grx1), an enzyme required for de-glutathionylation of actin and tubulin, were unable to polymerize either cytoskeletal network and failed to degranulate or release DNA. Taken together, cytoskeletal dynamics are achieved as a balance between ROS-regulated effects on polymerization, and glutathionylation on the one hand, and the Grx1-mediated de-glutathionylation that is required for NET formation, on the other. Thus, these findings inform us about the molecular mechanisms involved in NET formation and provide new potential strategies for increasing the anti-microbial activity of neutrophils in patients with defects in the innate immune system.

SSAIP 51

Shp-2 modulates aspects of T cell exhaustion differently from PD-1

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T cell exhaustion is defined as a dysfunctional state of T lymphocytes that occurs during chronic antigen stimulation, such as chronic viral infection or cancer. The exhausted phenotype is characterized by the hierarchical loss of T cell effector functions and the acquisition of inhibitory receptors expression. Blockade of these inhibitory receptors has become a breakthrough in cancer immunotherapy, enabling to reinvigorate anti-tumoral exhausted T cells and better control disease. Among different inhibitory receptors expressed on exhausted T cells, Programmed cell death 1 (PD-1) is one of best characterized and affects T cell receptor (TCR) and co-stimulatory signalling. Interestingly, Src-homology domain-containing phosphatase 2 (Shp-2) has been proposed to a play pivotal role most prominently downstream of PD-1. It was shown that Shp-2 is recruited to the phosphorylated immunoreceptor tyrosine-based switch motif (ITSM) present in the cytosolic tail of PD-1. Yet, the relevance of Shp-2 in exhausted T cells has never been addressed. Using a conditional knockout mouse for Shp-2 in T cells (Ptpn11fl/fl CD4cre mouse), we were able to show that during chronic viral infection, Ptpn11fl/fl CD4cre mice show increased percentages of virus-specific T cells as compared to control mice. In addition, we identified that this phenomenon is CD8+ T cell-intrinsic. Despite qualitatively improved, these cells still presented hallmarks of exhaustion, suggesting that Shp-2 removal is not sufficient to revert T cell exhaustion, despite partially ameliorating the anti-viral response. We are currently testing whether Shp-2 is required for inhibitory receptor signalling, PD-1 in particular. To this end, we are treating chronically infected or cancer-bearing Ptpn11fl/fl CD4cre and control mice with anti-PD-1 blocking antibody. Altogether these data suggest that Shp-2 contributes to selected aspects of T cell exhaustion. Furthermore, our data will indicate whether Shp-2 is required for PD-1-mediated signaling, advancing our understanding of signaling downstream of inhibitory receptors and potentially opening novel opportunities for improved combined therapies concomitantly targeting PD1 and Shp-2.

SSAIP 52

Effector T helper cell recruitment to inflammatory sites via CCR2

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Migration of CD4+ T helper (Th) cells to extralymphoid sites of inflammation is pivotal for execution of their effector function. Differentiation of distinct Th cell subsets is coupled with induction of subset-specific and tissue-tropic chemokine receptors that drive their recruitment into diverse inflammatory lesions. However, increasing evidence indicates that trafficking of Th cell subsets to inflammatory sites can occur independently of subset-specific chemokine receptors. Thus, coordinated migration is likely to involve multiple integrated receptor inputs that are spatiotemporally regulated, but knowledge of this complexity in migration is limited. Recently, we demonstrated that homing of IL-23-driven pathogenic Th17 cells to the central nervous system was coordinated through the chemokine receptor CCR2 in experimental autoimmune encephalomyelitis, a murine model of the human autoimmune disease multiple sclerosis. Our more recent data indicates that functional CCR2 is induced on various effector Th cell subsets under disparate inflammatory settings. CCR2 ligands are not present at homeostasis, but are rapidly elicited by numerous cell types in response to pan-inflammatory stimuli including IL-1 and TNF. Here, we present evidence that CCR2 serves as a pan-inflammatory chemokine receptor driving Th cell trafficking to effector sites. Understanding how CCR2 functions in collaboration with subset-specific and tissue-tropic receptors to coordinate Th cell trafficking will inform the rational design of therapeutic strategies to intervene in this process in Th cell-driven pathology.

SSAIP 53

Characterization of replication-defective lymphocytic choriomeningitis virus induced CD8+ T cell immunity

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The Lymphocytic choriomeningitis virus-based recombinant vaccination vector system (rLCMV) has the capacity to elicit strong CD8+ T cell responses. Substitution of the gene encoding the glycoprotein, which is crucial for the viral entry process, renders the virus propagation-deficient. The single-round replication cycle of rLCMV hence uncouples viral replication and dissemination from innate immune recognition and viral elimination by CD8+ T cells. Using either self of foreign antigens encoded by rLCMV, we have assessed the impact of innate immunologic recognition and different immune cell subsets that underpin the high efficacy of this vaccination approach. We found that ablation of TLR7, MDA5 or MyD88 had no impact on the CD8+ T cell response in C57BL/6 mice immunized with rLCMV expressing ovalbumin (rLCMV-OVA). In contrast, type I IFN receptor-deficient *(Ifnar$^{-/-}$)* mice immunized with the same vector developed an initially delayed CD8+ T cell response, but showed equally efficient responses after 28 days. Moreover, *Ifnar$^{-/-}$* mice vaccinated with rLCMV swiftly eliminated even fast replicating LCMV strains during challenge infection providing further evidence for the high protective capacity of this novel viral vector system.

SSAIP 54

The effect of inflammatory cytokines on chemokine receptor and adhesion molecule expression in breast cancer cell lines and tissue

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Intercellular Adhesion Molecule-1 (ICAM-1, CD54), a cell surface glycoprotein that functions as an integrin, has a well-established role in leukocyte migration during inflammation. Recent studies have indicated a role for this protein in cancer development and metastasis. Proinflammatory cytokines are commonly encountered in the breast cancer microenvironment and the expression of chemokine receptors and adhesion molecules may influence the cancer cells ability to become invasive. In this study three breast cancer cell lines were stimulated with the cytokines interferon gamma (IFNγ), Tumour Necrosis Factor alpha (TNFα), Interleukin 1 beta (IL-1β) and Interferon alpha (IFNα). The breast cancer cell lines MCF7, SK-BR-3 and MDA-MB-468 were chosen to reflect the hormone positive, HER-2 enriched and triple negative subtypes of breast cancer, respectively. Relative gene expression was measured using quantitative real time polymerase chain reaction for the genes: CXCR4, CXCR5, CCR5, CCR7, ICAM-1 and LTβR. ICAM-1 protein was measured by Western Blot and localised by immunofluorescence. In addition, CXCR4, CXCR5, CCR5, CCR7, ICAM-1, LTβR, CXCL13, CCL19, LTβ, IL-1β, TNFα, INFα and INFγ gene expression was measured in human breast cancer samples, and ICAM-1 and macrophage (CD68) immunohistochemistry was performed on formalin-fixed, paraffin embedded tumour tissue. ICAM-1 expression was induced in all of the cell lines by IFNγ, TNFα and IL-1β with increased ICAM-1 protein production compared to time-matched controls. IFNγ appeared to induce ICAM-1 gene expression but no protein was detected. Membranous and para-nuclear ICAM-1 staining was identified after 24 hours in unstimulated MCF7 and MDA-MB-468 cells and the same cell lines stimulated with IFNγ. ICAM-1 expression was increased in the human tumour tissue compared to normal tissue. However, this did not correspond with ICAM-1 positivity in tissue sections or numbers of macrophages in the tumour. The expression of CCR7 and LTβ were significantly increased in tumor samples compared to normal breast tissue. The study highlights a mechanism that cancer cells may use to enhance their invasive and metastatic potential by making use of a protein normally involved in cell migration.

SHORT ORAL COMMUNICATIONS LTM4

LTMO 1

Germinal center formation and prognostic power of tumor-associated tertiary lymphoid structures are hampered by corticosteroids

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Tertiary lymphoid structures (TLSs) are associated with survival in various cancers, but how TLSs develop in this context is poorly understood. We used multi-spectral microscopy, quantitative pathology and gene expression profiling to investigate TLS formation in human lung squamous cell carcinoma (LSCC). We identified a niche of CXCL13+ perivascular and CXCL12+LTB+ and PD-L1+ epithelial cells that support TLS formation. We characterized sequential stages of TLS maturation that culminate in germinal center (GC) formation. The number of GC-positive TLS independently predicts survival of LSCC patients. In chemotherapy-treated patients, however, GC formation was significantly impaired and the prognostic value of TLSs was lost. We show that corticosteroids that are administered concomitantly with chemotherapy inhibit the development of TLSs and GCs, suggesting that steroids impair the immune control of cancer. Based on our findings, we propose that deliberate induction of TLSs may be a novel immunotherapy for LSCC.

LTMO 2

Stromal cells of gut-draining lymph nodes stably attain tolerogenic properties and modulate functional modules of incoming dendritic cells

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Peripherally induced Foxp3+ regulatory T cells (Tregs) contribute to tolerance towards commensals and food-born antigens. Previous works of others and us has shown that the microenvironment of gut-draining lymph nodes (LN) promotes peripheral Treg induction relying on resident LN stromal cells and dendritic cells (DCs). Furthermore, several interaction nodes between LN stromal cells and incoming DCs have been identified to modulate immune responses under steady-state and inflammatory conditions. We aim to identify the microenvironmental factors that educate LN stromal cells for their respective site-specific function and to elucidate the stable functional properties by which LN stromal cells contribute to peripheral tolerance induction by modulating incoming DCs and shaping the LN microenvironment. We transplanted gut- as well as skin-draining LNs from normally housed, germfree, selectively colonized, chronically inflamed and previously infected mice to assess LN stromal cell contribution to immune-modulatory functions. Within the transplant setting, we dissected the contribution of LN stromal cells to Treg induction and to the modulation of incoming DCs, by not only measuring the frequency of de novo induced Foxp3+ Tregs, but also performing RNA-seq based transcriptomics of fibroblastic reticular cells (FRC) and DCs re-isolated from transplanted LNs. Here, we identified that microbiota are demanded to imprint tolerogenic properties in mesenteric LN (mLN) stromal cells, a process that generally happens during the neonatal phase. Imprinting of tolerogenic properties, which is stable and can even resist inflammatory perturbations, is independent of commensal composition, short-chain fatty acids and IgA. Importantly, mLN-FRCs stably retain multiple functional modules subsequent to transplantation to the skin-draining popliteal fossa and shape pre-dominantly blood-derived DCs to acquire gut-associated functional properties. Commensals are required to long-lastingly and stably imprint tolerogenic properties in mLN stromal cells during the neonatal phase. This early functional stabilization is potentially required to re-equilibrate tolerogenic properties within mLNs after inflammatory perturbations by contributing to the tolerogenic microenvironment of the mLN and modulation of functional properties of incoming blood-derived DCs.

LTMO 3

Lymphotoxin-beta receptor signalling during fetal life shapes gut stromal cell populations and orchestrates mucosal B cell response in adulthood

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Lymphotoxin-beta Receptor (LTβR) signalling is required for the development of secondary lymphoid organs, including Peyer's patches, and for maintaining the immune system homoestasis during adulthood. Intestinal IgA levels are also severely reduced in LT-deficient mice, however the underlying mechanisms are unclear. Using genetic and pharmacological approaches, we found that LTβR signalling is dispensable for mucosal IgA responses in mice where the LT pathway is blocked during adult life. In contrast, we discovered that LTβR signalling is critically required during fetal life for the generation of IgA-producing cells specific for a T-dependent antigen (Rotavirus). We also found that while an altered microbiome contributed to defective homeostatic fecal IgA in LT-deficient mice, fecal anti-rotavirus IgA responses in LT-deficient mice were reduced independent of animal husbandry practices that affect the microbiome. Specifically, in WT–>LTβ–/– chimeras, plasma cell differentiation and generation of rotavirus-specific antibody-secreting cells (ASC) in the mesenteric lymph nodes were modestly reduced concomitant with a severe defect of ASC accumulation in the lamina propria, suggesting ASC migration or/and ASC survival in the lamina propria may be dependent on in utero LTβR signaling. Lastly, mice treated in utero with LTβR-Ig, a blocking agent, recapitulated the phenotype of WT–>LTβ–/– chimeras, and this treatment led to the alterations in gene expression of lamina propria stromal cells in adulthood. Collectively, our data demonstrate that in utero LTβR signalling shapes gut stromal cell populations and has an impact on mucosal B cell response during adulthood.

LTMO 4

IL-7 dependent maintenance of ILC3s is required for normal entry of lymphocytes into lymph nodes

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Interleukin 7 (IL-7) is a key developmental cue throughout the lymphoid compartment. It is essential for the development and homeostasis of both T and B lymphocytes, and also plays a fundamental role in neonatal lymph node organogenesis, and Il7–/– mice lack normal lymph nodes. Whether IL-7 is a continued requirement for the maintenance of lymph node structure and function in adults, however is unknown. To address this, we generated mice in which IL-7 function was ablated in normal adult hosts. Inducible Il7 gene deletion in adults resulted in a rapid loss of lymph node cellularity beyond that predicted by the expected loss of naive T cells. Homing assays revealed a defect in lymphocyte entry to lymph nodes following IL-7 ablation. Stromal and dendritic cell components of lymph nodes were present in normal number and representation following IL-7 ablation. In contrast, ILC subpopulations were substantially decreased after IL-7 ablation. In confirmation, IL-7R blockade in T cell deficient hosts caused in a similar loss of ILC populations and resulted in impaired lymphocyte homing to lymph nodes. Testing lymphocyte homing in bone marrow chimeras reconstituted with Rorc–/– bone marrow confirmed that ILC3 in lymph nodes are required for normal lymphocyte homing. Taken together, our data suggest that maintenance of intact lymph nodes relies upon IL-7 dependent maintenance ILC3 cells.

LTMO 5

Chronic viral infections induce major disruption of bone marrow stromal cell networks and persistent loss of hematopoietic stem cell function

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Hematopoiesis is the highly dynamic and tightly regulated process of continuous blood cell production, sustained by a rare population of self-renewing, multipotent hematopoietic stem and progenitor cells (HSPCs), which reside in specialized microenvironments within bone marrow (BM) cavities. The basic tissue infrastructure of the BM is provided by stromal cellular networks of mesenchymal, neural and vascular origin, which are critically involved in the fine regulation of different stages of hematopoiesis. Viral infections act as major stressors to the hematopoietic system, inducing massive and adaptive responses in cellular output. Albeit the effects of viral challenge and ensuing inflammatory responses on hematopoietic cells have been studied in detail, how viral infections alter BM stromal scaffolds and thus shape hematopoietic responses remains poorly defined. By combining conventional in vitro and in vivo assays with 3D confocal imaging, we herein investigated the structural and functional alterations imposed on the BM after a chronic infection with Lymphocytic Choriomeningitis Virus (LCMV). Our data shows that chronic LCMV infections result in a substantial loss of the BM endothelial and mesenchymal stromal progenitor cell populations and a decrease in their capacity to produce HSPC-sustaining factors. Upon viral challenge, conspicuous vasodilation of BM sinusoidal vessels is induced and followed by intense vascular remodeling and substantial disruption of extracellular matrix networks throughout the BM cavity. Major damage to BM stromal integrity is accompanied by a profound and sustained reduction in the number of both hematopoietic multipotent progenitors as well as hematopoietic stem cells by phenotype. Competitive repopulation assays further revealed that remaining HSCs are additionally impaired in their repopulation capacity for prolonged times after LCMV infection. Finally, our results indicate that the observed alterations in the composition and functionality of cells in the BM are, at least partially, mediated by activated virus-specific CD8 T cells. The molecular and cellular mechanisms by which the competence of the BM microenvironment is compromised during infection are currently under investigation.

LTMO 6

Dissecting the stromal cell origin in Peyer's patches

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Peyer's patches (PPs) are lymphoid organs located in the small intestine, playing an important role in gut immunity. B and T lymphocytes are the main cell populations which are segregated into different lymphoid areas driven by the presence of three major stromal cell populations: fibroblastic reticular cells in the T cell area, and follicular dendritic cells (FDCs) and marginal reticular cells (MRCs) in the B cell area. Previously, our group has shown that Collagen VI (ColVI)-Cre mice are a useful new tool for PP MRC and FDC analysis. Here, we used multicolor fate mapping systems in combination with confocal and light sheet fluorescent microscopy, to dissect the ontogeny and dynamics of these cell populations. Analysis of adult PP from ColVI-Cre Confetti mice showed the presence of monocolored cell columns connecting MRC and FDC networks, and pointing to an ontogenetic relation between them. To discern the directionality of this relation, we studied PP organogenesis during embryonic development. We found that at embryonic day 18.5 ColVI-Cre+ cells appeared as a single cell layer in the MRC area underneath the epithelium. During the first week of life, these cells proliferated, migrated and differentiated into FDCs. Using conditional and complete knockout mice, we also showed that the MRC development is TnfR1 independent, while their differentiation into FDCs is TnfR1 dependent. To summarize, we demonstrate that during PP development, FDCs arise from MRCs in a TnfR1 dependent manner.

LTMO 7

High-endothelial cell-derived sphingosine-1-phosphate regulates dendritic cell localization and vascular integrity in the lymph node

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While the sphingosine-1-phosphate (S1P)/sphingosine-1-phosphate receptor-1 (S1PR1) axis is critically important in lymphocyte egress from lymphoid organs, S1PR1 activation also occurs in vascular endothelial cells (ECs) including those of the high-endothelial venules (HEVs) that mediate lymphocyte immigration into lymph nodes (LNs). To reveal the functional significance of S1P signaling involving HEVs, we generated conditional knockout mice for the S1P-transporter Spinster-homologue-2 (Spns2), as HEVs are shown to express Lyve1 during development. In Lyve1-Spns2Δ/Δ mice, dendritic cells (DCs) were unable to interact with HEVs, and HEVs were severely impaired in function, morphology and size, leading to markedly hypotrophic peripheral LNs. Impaired co-localization of HEVs and DCs was also observed in wildtpe mice treated with S1PR1 antagonists. Additionally, release of the DC-chemoattractant CCL21 from HEVs was reduced in Lyve1-Spns2Δ/Δ mice. Together, our results reveal an unexpected role of EC-derived S1P in maintaining HEV integrity, facilitating HEV-DC interactions through the control of S1PR1-dependent CCL21-secretion from HEVs.

LTMO 8

Marginal reticular cell RANKL regulates B cell-associated stromal cell differentiation in the steady state

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RANKL (receptor activator of NF-κB ligand) is member of the Tumor necrosis factor (TNF) super-family (SF) and signals via RANK. It plays an important role for immune cells by promoting bone marrow hematopoiesis (by inducing the differentiation of the bone matrix degrading osteoclasts), mobilization of hematopoietic stem cells and lymph node development. In the adult, RANKL is constitutively expressed by MRCs, while, under conditions of inflammation, keratinocytes and T cells also express RANKL. Our aim is to better understand the role of RANKL expressed by marginal reticular cells (MRCs) in the lymph node under steady state conditions. Because MRCs are positioned in close vicinity to B cells and may be precursors of follicular dendritic cells (FDCs), it is possible that RANKL plays a role in the differentiation of B cell-associated stroma. To approach this question, we generated mice with conditional RANKL deficiency in the stromal compartment using the Cre recombinase under control of the CCL19 promoter, active in embryonic lymphoid tissue organizers and adult stroma. These mice lost RANKL expression in lymphoid tissue organizers and in adult MRCs. We found that the proper segregation of the B cell follicle from the T cell zone was disrupted and that FDCs were missing. In addition, although RANKL was not required for MRCs formation (on the grounds of MAdCAM-1/VCAM-1 expression), the TNFSF member is necessary for CXCL13 expression by MRCs. To understand the mechanism underlying MRC activation and FDC formation by RANKL, we have measured the expression of TNFα, LTα, LTβ, and their receptors TNFR1, TNFR2 and LTβR by qRT-PCR in whole lymph nodes. Strikingly, we found that the expression of TNFR1 was significantly reduced, whereas the expression of all other genes was normal. We are now in the process of identifying the cells whose TNFR1 expression is affected by the lack of MRC RANKL. Taken together, RANKL may therefore present a novel therapeutic strategy against B cell-mediated immunopathologies by acting on its stroma.

LTMO 9

Fluid absorption modulates Peyer's patch homeostasis and mucosal antibody responses

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Peyer's patches (PPs) are B cell-rich lymphoid tissues situated throughout the small intestine which play an important role in steady-state antigen acquisition and mucosal antibody responses. PP architecture and stromal cell composition closely resemble that of peripheral lymph nodes despite geographical and functional differences. Notably, fibroblastic stromal cells located in small intestinal PPs form a network of collagen-rich reticular fibers similar to the network of conduits found in lymph nodes. In the PP, these conduits extend from the basement membrane of the intestinal epithelial cell lining into the PP follicle, and terminate along both blood and efferent lymphatic vessels. Unlike lymph nodes, PPs lack a conventional source of afferent lymph that would normally contribute fluid flow through the conduit network. Instead directional fluid flow through PP conduits appears to depend largely on water absorbed across the overlying intestinal epithelium. Water absorption across the intestinal epithelium is a process regulated by the maintenance of osmotic gradients. We find that by disrupting water absorption (either through blockade of sodium/hydrogen exchangers or by introducing a non-absorbable osmotic agent into the lumen), we can limit or prevent the contribution of absorbed lumenal fluids to the flow of PP conduits. Surprisingly, this alteration to conduit flow did not result in significant functional changes to PP stroma (as assessed by RNAseq). However, disruption of fluid absorption over the course of at least 2 days has profound effects on the structural integrity of the high endothelial venule and surrounding perivascular FRCs. These alterations correlate with a striking defect in the recruitment of naïve recirculating

lymphocytes to the PP which then cumulatively results in decreases to total naive B and T cell numbers. Interestingly, we find a contrasting increase in naive lymphocyte numbers in peripheral lymph nodes. Finally, we find that prolonged blockade of water absorption significantly impacts mucosal antibody responses. Induction of antigen-specific B cells and fecal IgA titers are reduced and germinal center responses are decreased in scale. We anticipate that further investigation of the mechanisms by which these changes occur will provide useful insight to the means by which immune functions are affected during conditions of impaired water absorption and altered conduit flow.

LTMO 10

The thymic medulla: how is it built and maintained?

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Intrathymic T-cell development and selection are critically dependent on both cortical and medullary thymic epithelial cells (TECs) which originate from a common precursor expressing the thymoproteasome subunit β5t, a molecular marker typically used to identify cortical TECs. Lineage tracing studies at single cell resolution in 1 week old mice have demonstrated that the medullary TEC compartment is largely derived from the progeny of individual β5t+ cortical precursors located at the interface of the cortex with the medulla. However, the precise phenotype of the immediate progeny of these precursors and thus their developmental path remains unknown. To identify individual cell stages during the transition from β5t+ precursors to lineage committed medullary TEC, we employed single cell transcriptome analysis and ordered the individual profiles by pseudotiming, which assigns individual cells to a "finite place" along a developmental trajectory without previous knowledge of the exact variance of markers that identify sequential maturational stages. The single cell RNA-Seq analysis by pseudotime reconstruction revealed three distinct cell populations. Two cell clusters positioned at either extremity of the trajectory displayed multiple transcriptomic features typical for cortical and medullary TECs, respectively, identifying "starting" and "finishing" points of the development from β5t+ precursors to medullary TEC. Positioned between these two stable states were cells that progressively changed their gene expression profiles representing cells in a gradual transition from a β5t+ precursor to a medullary identity. To probe the contribution of this developmental pathway to the steady-state maintenance of the medullary TEC compartment in adult mice, we next followed the progeny of β5t+ precursors in an in vivo lineage fate mapping experiment. Analysis of medullary TEC after labeling β5t+ TECs at a single time point revealed an increasing contribution of these cells to the mTEC compartment. As many as a third of all mTECs had derived twenty weeks later from β5t+ TEC precursors, a frequency only mildly lower than that observed 8 weeks after labeling of 1 week old β5t+ TEC precursors, possibly reflecting the significantly increased half-life of mTECs in 4 week and older mice. In aggregate, these studies shed light on early mTEC developmental stages and demonstrate the contribution of adult β5t+ cortical progenitors to the maintenance of the thymic medulla.

LTMO 11

Regulation of plasma cell output from germinal centre by a subset of fibroblastic reticular cells

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Germinal centers (GCs) are the sites where B cells undergo affinity maturation. The regulation of output from the GC is not well understood. We show that from the earliest stages of the GC response, GCs produce plasmablasts that emerge at the interface between the GC dark zone and the T zone (GTI). Plasma cells generated in the GTI may provide an early source of affinity matured antibody that may provide antibody feedback regulation of GC B cell selection. We show that IL-21 produced by follicular help T cells supports plasmablast output in the GTI. Further, we define a new fibroblatic reticular cell population that is located inside the GTI and shares expression markers with T zone reticular cells (CD45–, Ter119–, EpCAM–, MadCAM–, CD31–, gp38+), but expresses high levels of BP3. These gp38+, BP3high FRC (GTIRC) associate closely with plasmablasts. GTIRC produce high levels of mRNA for TNFSF13 (APRIL), IL-6, CXCL12, CCL19 and CCL21 mRNA. We compared cytokine and chemokine mRNA production of GTIRC to myeloid cells, dendritic cells, and different stromal cell subpopulations. GTIRC share highest production of APRIL and IL-6 mRNA with BP3-, GP38+ medullary FRC, while CXCL12, CCL19 and CCL21 mRNA level is as high as gp38+ BP3int central T zone FRC. Functional significance of APRIL produced by GTIRC was shown by blocking signals from TACI ligands in vivo, which inhibits GC-associated plasmablast generation in the GTI within 24 h.

LTMO 12

Lymphocytes Program the differentiation of lymphoid stroma in the periphery during tertiary lymphoid structure formation

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In secondary lymphoid organs (SLO), stromal cell derived chemokines establish B/T cell organization into distinct areas that facilitates effective immune responses. Tertiary lymphoid structures (TLS) are assemblies of lymphocytes, dendritic cells and fibroblasts that resemble SLO, but harbor at sites of inflammation and cancer. TLS formation and ectopic production of lymphoid chemokines correlates with systemic manifestations of disease and worse clinical outcome. To dissect the signals required for stromal cell maturation we used an inducible mouse model of TLS formation by cannulation of the salivary glands with a replication deficient adenovirus in RORgt–/–, lymphotoxin (LT)βR–/–, LTα–/–, Rag2–/–, IgMnull (B cell deficient) and CD3εtg (T cell deficient) mice. Analysis of TLS formation revealed that LTα or LTbR deletion inhibited stromal cell activation and chemokine production thereby preventing TLS organization. Lymphocytes (in particular T cells) emerged as major sources of LTβ in TLS, with a small contribution from RORgt+ cells. As such, RORgt–/– mice displayed a milder phenotype compared to LTbR–/– mice with partly organized TLS and low lymphoid chemokine expression. In contrast, Rag2–/– mice infected with adenovirus, failed to induce production of homeostatic chemokines. Analysis of IgMnull and CD3εtg mice demonstrated that T cells were required for CXCL13 and CCL19 production. Conversely, B cell deficiency caused defective CXCL13 expression but no change in CCL19 production. To analyze relative contributions of B and T cells towards stromal cell maturation in humans we developed an in vitro co-culture system of activated lymphocytes with adipose derived stromal cells (ADSC) or rheumatoid arthritis (RA) synovial fibroblasts. Both stromal cell lines responded to T cell stimulation by upregulating CCL19 whilst B/T cell co-culture promoted CXCL13 induction to a greater extent than with B cells alone. Lymphocyte co-culture with RA fibroblasts induced significantly higher chemokine expression compared to lymphocyte/ADSCs co-culture suggesting that the RA joint microenvironment imprints stromal cells with a capacity for elevated lymphoid chemokine expression under pathogenic conditions. These data suggest that stromal cells respond to direct T and B cell contact by differentially upregulating T and B cell zone chemokines. This is partly regulated by LT, however, the specific signals regulating CCL19 versus CXCL13 production are still under investigation.

LTMP 1

Topological small-world organization of the fibroblastic reticular cell network determines lymph node functionality

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Fibroblastic reticular cells (FRCs) form the cellular scaffold of lymph nodes (LNs) and establish distinct microenvironmental niches to provide key molecules that drive innate and adaptive immune responses and control immune regulatory processes. Here, we have used a graph theory-based systems biology approach to determine topological properties and robustness of the LN FRC network in mice. We found that the FRC network exhibits an imprinted small-world topology that is fully regenerated within 4 wk after complete FRC ablation. Moreover, in silico perturbation analysis and in vivo validation revealed that LNs can tolerate a loss of approximately 50% of their FRCs without substantial impairment of immune cell recruitment, intranodal T cell migration, and dendritic cell-mediated activation of antiviral CD8+ T cells. Overall, our study reveals the high topological robustness of the FRC network and the critical role of the network integrity for the activation of adaptive immune responses.

LTMP 2

Lymphatic expansion and contraction modulates the immune response during infection and inflammation

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Lymphatic vessels are comprised of lymphatic endothelial cells (LECs). These vessels were originally characterized for their ability to transport lymph, maintain fluid homeostasis and transport immune cells. Recently, advances in molecular markers for LECs have brought these cells to the forefront of scientific discovery and have opened up exciting new areas of research. Our recent discovery highlighting the importance of lymphatic endothelial cells in influencing protective immunity became one of these new areas. Despite these advances there remains a significant gap in our understanding of how lymphatic endothelial cells modulate immunity and drive disease pathogenesis. We have discovered at least one of the mechanisms by which LECs program the immune response during the resolution of inflammation in the lymph node. LECs act as the repository for viral antigens or soluble antigens during immune challenge. LECs are unable to present their archived antigens to CD8+ T cells, and instead transfer their antigen via a previously unknown mechanism to hematopoietically derived antigen presenting cells (APCs). Our new data show that acquisition of LEC-archived antigen by APCs is increased during periods of lymph node contraction 3–5 weeks after vaccination or viral challenge. During this period, we identified BatF3-sensitive migratory dendritic cells (DCs) as the APC subset predominantly responsible for the acquisition of antigen from LECs and cross presentation to CD8 T cells. Furthermore, the loss of apoptotic cell receptors such as Clec9a or migratory receptor CCR7 on DCs compromises antigen capture and cross presentation. Collectively these data suggest a model whereby LEC apoptosis during lymph node contraction facilitates exchange of archived antigen with a migratory APC, ultimately influencing cross presentation of antigen to circulating memory CD8+ T cells and enhancing protective immunity.

LTMP 4

Tekcre+/Lyve1cre+ endothelial cell specific LTbR is not essential for lymph node organogenesis but maturation and function

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Lymphotoxin (LT) ab – lymphotoxin beta receptor (LTbR) signaling mediated cellular crosstalk plays a critical role for lymph node (LN) development. However, the involved cellular compartment remains unclear. The major role of LTbR signaling has been long considered to exert in the mesenchymal lymphoid tissue organizer (LTo) cells. A recent paper reported that endothelial LTbR signaling is also critical for the formation of LNs using VE-cadherincreLtbrfl/fl mouse model, but the detailed mechanisms is still unclear. Here, we have utilized Tekcre and Lyve1cre mice, together with Ltbrfl/fl mouse to further investigate this issue. Surprisingly, we found neither Tekcre nor Lyve1cre, nor even double cre mediated LTbR deletion abrogates LN organogenesis. Interestingly, however, TekcreLtbrfl/fl mice showed reduced lymphoid tissue inducer (LTi) cell recruitment at the embryonic LN anlagen, impaired LN maturation and HEV formation and function. Thus, together with the previous report, our current data argues that Tekcre+/Lyve1cre+ endothelial LTbR is not essential for initial LN organogenesis, but required for hematopoietic cell recruitment at later stage and LN full maturation and function, suggests a special endothelial cell population for LN formation.

LTMP 5

Mechanisms underlying the development of tertiary lymphoid structures in cancer

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Lymphoid aggregates are able to form upon infiltration of lymphocytes into tumours. Some of such aggregates form an architecture that resembles that of secondary lymphoid organ (SLO) follicles. These are referred to as tertiary lymphoid structures (TLSs). TLSs also induce adaptive immunity and, hence, could be beneficial in cancers by driving anti-tumour immunity at the tumour site. This hypothesis is supported by the observation that cancer patients with high density of tumour-associated TLS have better prognosis. The aim of this project is to identify the factors that are involved in TLS formation and to investigate function of TLS in mouse models of cancer. By studying models of spontaneous lung metastasis from Lewis lung carcinoma (LLC) and 4T1 breast carcinoma, we observed organized lymphoid aggregates in metastatic lungs derived from 4T1 but not from LLC tumours. Furthermore, we observed that in an intravenous model of LLC where C57BL/6 mice were also exposed to LPS as an additional inflammatory stimulus, LLC tumours still did not encourage TLS formation at the tumour border. In the future, RNA from 4T1 lung metastases will be analysed at different time points for expression of potential factors implicated in TLS formation – to capture different phases of TLS formation – and later the cell types responsible for expression of such factors will be studied and their roles functionally investigated. Lastly, LLC tumours were genetically modified to overproduce CXCL13 or lymphotoxins, which are molecules implicated in secondary lymphoid organ formation, to investigate if they are sufficient to promote TLS formation by this tumour in vivo.

LTMP 6

Role of CD112 in vascular biology

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Lymphatic vessels (LVs) are central for fluid drainage and for transporting leukocytes and antigens to draining lymph nodes. Performing a transcriptional analysis of endothelial cells isolated from murine skin, we have recently identified CD112 (nectin-2), an adherent junction molecule, to be highly expressed in lymphatic endothelial cells (LECs) and, to a lesser extent, in blood vascular endothelial cells. CD112 belongs to the immunoglobulin superfamily and can interact homophilically or heterophilically with other nectin family members to mediate cell-cell adhesion. Moreover, CD112 binds immune receptors (e.g. TIGIT, DNAM-1, CD112R) on specialized immune cells to modulate immune function. In this project, we confirmed expression

of CD112 protein by human and murine LECs in vitro and in lymphatic and blood vessels of murine tissues in vivo. In functional in vitro studies, blockade of CD112 reduced human LEC migration and enhanced permeability of LEC monolayers, suggesting the involvement of CD112 in stabilizing LEC-LEC interactions. Moreover, CD112 blockade significantly reduced in vitro transmigration of human T cells across LEC monolayers, suggesting a potential role in lymphatic trafficking. In vivo, mice pre-treated with a CD112 blocking antibody displayed reduced drainage of a lymphatic-specific dye from the skin. Surprisingly, no drainage defect was observed in CD112-deficient (CD112–/–) mice, possibly due to compensatory mechanisms: In fact, we found that CD112-knockout mice displayed higher expression levels of the junctional molecule VE-cadherin, which might have compensated for the absence of CD112. Interestingly though, preliminary data indicate significant differences in the lymphatic and blood vascular network in CD112–/– as compared to WT pups: Specifically, absence of CD112 appears to enhance maturation of the vascular network in various tissues. Collectively our data reveal a novel role for CD112 in stabilizing LEC-LEC interactions and in supporting lymphatic function.

LTMP 7

Fibroblastic reticular cells regulate intestinal inflammation via IL-15-mediated control of group 1 ILCs

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Immune processes in intestinal tissues are tightly regulated in order to prevent exaggerated immunity against commensal microbiota while maintaining the capacity to respond against pathogens. Fibroblastic reticular cells (FRCs) build the cellular scaffold of secondary lymphoid organs and control immune responsiveness through regulation of T cell migration and survival. While FRC phenotype and function in lymph nodes are well-characterized, knowledge about Peyer patch (PP) is still limited. To assess the function of PP FRCs, we specifically deleted the innate immune sensing adaptor molecule MyD88 in these cells using the Ccl19-Cre mouse model. We found that genetic ablation of MyD88-dependent signal transduction in FRCs did not affect PP formation, structure and composition under homeostatic conditions. However, during infection with an enteropathogenic virus, cell-specific MyD88 ablation unleashed IL-15 production by PP FRCs leading to uncontrolled group 1 innate lymphoid cells (ILC) expansion and activation with substantially elevated production of IFN-γ. Enhanced group 1 ILC activity fostered accelerated viral clearance. However, such heightened antiviral immunity came at the cost of severe intestinal inflammatory disease with commensal dysbiosis, loss of intestinal barrier function and decreased colonization resistance. Thus, FRCs of PPs function as immune regulatory cells that restrain activation of innate immune cells and thereby prevent immunopathological damage in the intestine.

LTMP 8

Selective deletion of S1PR1 in Lyve1 lineage cells induces T cell accumulation in the thymus

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T cell egress from the lymphoid tissues is essential for immunological homeostasis. While stromal cells-produced sphingosine-1-phosphate (S1P) has been shown to promote T cell egress by acting on the S1P receptor S1PR1 on T cells, the significance of S1P/S1PR1 signaling in the stromal cells remains unclear. To address this issue, we developed conditional knockout mice (Lyve1-CRE/ S1pr1f/f mice) in which S1pr1 was selectively targeted in cells expressing the lymphatic endothelial cell marker, Lyve1. In these mice, T cells were significantly reduced in secondary lymphoid tissues, and CD62L+ mature CD4 and CD8 single-positive (SP) T cells accumulated in the medulla failed to undergo thymus egress. Using a Lyve1 reporter strain in which Lyve1 lineage cells expressed tdTomato fluorescent protein, we unexpectedly found that a considerable proportion of the thymocytes were fluorescently labeled, indicating that they belonged to the Lyve1 lineage. The CD4 and CD8 SP thymocytes in Lyve1-CRE/ S1pr1f/f mice exhibited an egress-competent phenotype (HSAlow, CD62Lhigh, and Qa-2high), but were CD69high and lacked S1PR1 expression. In addition, CD4 SP thymocytes from these mice were unable to migrate to the periphery after their intra-thymic injection into wild-type (WT) mice. In contrast, WT T cells could migrate to the periphery in both WT and Lyve1-CRE/ S1pr1f/f thymuses. These results demonstrated that thymocyte egress is mediated by T cell-, but not stromal cell-expressed S1PR1 and caution against using the Lyve1-CRE system for selectively gene deletion in lymphatic endothelial cells.

LTMP 9

NKp46 calibrates tumoricidal potential of type 1 innate lymphocytes

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Innate lymphoid cells (ILCs) are functionally related to effector T cells, but similar to NK cells do not express somatically rearranged antigen receptors. Among ILC subsets, ILC1s are most alike NK cells functionally, but the exact relationship between the two subsets remains unclear. During the course of the development NK cells acquire a repertoire of activating and inhibitory receptors, which ultimately defines their reactivity against target cells. The array of receptors during early developmental stages will control and imprint functional properties of NK cells, a process known as "NK cell education". It is yet unclear which receptors mediate cell recognition by ILCs, and whether they undergo an education-like process during their development. Prototypic type 1 ILCs reside in the liver, and a large part of their function is attributed to the expression of TRAIL, a TNF superfamily member with a well-documented anti-tumour activity. Here we show that TRAIL expression on ILC1 is controlled by an activating NKp46, which has been previously shown to control NK cell education. In the absence of NKp46 ILC1 fail to express normal levels of TRAIL on the surface which results in diminished cytotoxicity toward TRAIL receptor positive targets. These findings provide the first evidence of an education-like process in ILCs that calibrates their anti-tumour response.

LTMP 10

Dendritic cell migration to lymph nodes is controlled by dermatan sulfate and defines the efficiency of the adaptive immunity

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For full activation of naïve adaptive lymphocytes in antigen draining lymph nodes (LNs), sequential presentation of peptide: MHC complexes by LN resident and tissue derived dendritic cells (DCs) is an absolute prerequisite. To get to the draining LN, skin derived DCs need to migrate from the infection site to the afferent lymphatics, which can only be reached by traversing a collagen dense network located in the dermis of the skin. Here, we show that mice deficient for dermatan sulfate epimerase 1 (Dse), an enzyme strictly controlling collagen fibrillogenesis in connective tissues through chondroitin/dermatan sulfate glycosaminoglycans, have an altered network of collagen fibers and consequently display an impaired DC migration from the skin towards draining LNs. Consequently, the initiation of the cellular and humoral immune response was dramatically reduced, ultimately leading to a severe decrement of antigen specific serum immunoglobulin levels. Hence, we postulate dermatan sulfate epimerase 1 as a novel and critical regulator for the commencement of the adaptive immune response, by controlling antigen bearing DC migration to skin draining LNs.

LTMP 11

Impaired lymph node stromal cell function during the earliest phases of rheumatoid arthritis

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Objective: Rheumatoid arthritis (RA) is an autoimmune disease with unknown etiopathogenesis where systemic autoimmunity precedes clinical onset of disease. Adaptive immunity is initiated in lymphoid tissue where lymph node stromal cells (LNSCs) regulate immune responses through their intimate connection with immune cells. Malfunctioning of LNSCs creates a microenvironment in which normal immune responses are not properly controlled possibly leading to the development of RA. Here we developed an experimental model for studying the functional capacities of human LNSCs during the earliest phases of RA.
Methods: 24 patients with RA, 23 individuals positive for autoantibodies but without clinical disease (RA-risk group) and 14 seronegative healthy controls underwent ultrasound-guided inguinal lymph node (LN) biopsy. Human LNSCs were isolated and expanded in vitro for functional analyses. In analogous cocultures consisting of LNSC and peripheral blood mononuclear cells, αCD3/αCD28 induced T cell proliferation was measured using CSFE dilution.
Results: Fibroblast-like cells grown out of the biopsy consisted of fibroblastic reticular cells (FRCs: Podoplanin+CD31-) and double negative cells (DN: gp38-CD31–). Cultured LNSCs expressed characteristic adhesion molecules and cytokines with stable expression during culture. Basal expression of CXCL12 was lower in LNSCs from RA-risk individuals compared with healthy controls. Key LN chemokines CCL19, CCL21 and CXCL13 were induced in LNSCs upon stimulation with TNFα and lymphotoxin α1β2, but to a lesser extent in LNSCs from RA patients. Human LNSCs inhibited T cell proliferation in a ratio-dependent manner.
Conclusions: Using this innovative experimental model we show that the LN stromal environment is altered during the earliest phases of RA possibly leading to deregulated immune responses.

LTMP 12

Distinctive expression of T cell homing molecules in human autoimmune lymph node stromal cells upon TLR3 triggering

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Infections are implicated in autoimmunity. Autoantibodies are produced in lymphoid tissue where lymph node stromal cells (LNSCs) regulate lymphocyte function. Infections can alter the interaction between LNSCs and lymphocytes resulting in defective immune responses. Rheumatoid arthritis (RA) is a prototypic autoimmune disease in which autoantibody production precedes overt synovial tissue inflammation and clinical disease, allowing the identification of at risk individuals. The etiology of RA is still unknown and systemic autoimmunity is probably initiated by an unidentified mechanism outside the synovial joints. We investigated the ability of human LNSCs derived from RA, RA-risk and healthy individuals to sense and respond to pathogens. Human LNSCs cultured directly from freshly collected lymph node biopsies expressed TLR1-9 with exception of TLR7 and the strongest expression was observed for TLR3. In all donors TLR3 triggering induced expression of ISGs, IL-6 and adhesion molecules like VCAM-1 and ICAM-1. Strikingly, T cell attracting chemokines CCL19 and IL-8 as well as the antiviral gene MxA were less induced upon TLR3 triggering in autoimmune LNSCs. This observed decrease, found already in LNSCs of RA-risk individuals, may lead to incorrect positioning of lymphocytes and aberrant immune responses during viral infections.

LTMP 13

Mathematical modelling of the impact of fibrosis on the immune responses

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Tissue fibrosis is a characteristic feature of many chronic inflammatory processes. In chronic hepatitis infections the liver fibrosis takes place, a chronic pneumonia leads to the lung fibrosis and in HIV infection progressive fibrosis of lymphoid organs is observed. The development of fibrosis in various organs has many common features in terms of the regulatory mechanisms of inflammatory processes which lead to an increased deposition of collagen in connective tissues interfering with a normal functioning of the organs. Recent clinical and experimental studies are focused on the analysis of anti-fibrotic therapy as an additional component of conventional antiviral treatments with a final aim to restore the functioning of the target organ. In HIV infection the objective is to stop the destruction of lymphoid organs, exhaustion of T cell and the development of AIDS. In our study we introduce a mathematical model describing the lymph node structural organization and the mechanisms regulating the collagen deposition. In particular, we present an algorithm based on extended formulation of Cellular Potts Model to generate voxel-based geometrical approximation of the reticular network – the scaffold of T cell zone, consisting of fibroblastic reticular cells enwrapping the conduit system, from given conduit system topology. In addition, we develop a mathematical model of stromal homeostasis regulation during the chronic phase of HIV infection formulated with ODEs. It describes the interactions between fibroblastic reticular cells (FRC) and CD4+ T cells via their survival factors: IL-7 and LT-beta, the activation of increased collagen production through the signaling of TGF-beta produced by Treg cells, death of virus-infected CD4+ T cells and FRCs, and inhibitory influence of deposited collagen on the availability of survival factors. The parameters of the model are estimated to ensure homeostatic steady state for uninfected patients and progressive CD4+ T cells depletion, lymphoid tissue fibrosis, and reticular cell network destruction for chronic HIV patients. Finally, we formulate a hybrid agent-based model to integrate the structural and functional aspects of immune regulation mechanisms in lymphoid tissues.
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LTMP 14

Transcriptional analysis of adhesion molecule and chemokine expression along the afferent lymphatic vessel tree

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Leukocyte migration through afferent lymphatic vessels (LVs) to draining lymph nodes (dLNs) is important for initiating or regulating adaptive immune responses. Intravital microscopy has recently revealed that this migratory process consists of entry into lymphatic capillaries, followed by active, intralymphatic crawling within capillaries. Only once leukocytes reach the downstream, contracting collectors do they detach and are freely transported to the dLNs by lymph flow. Lymphatic capillaries and lymphatic collectors display great morphologic and functional differences, but these have so far not been studied at the transcriptional level. The aim of this work was to perform a transcriptional analysis of lymphatic endothelial cells (LECs) isolated from murine dermal capillaries or collectors (capLECs and colLECs, respectively), in order to better understand leukocyte migration through LVs and to identify new genes involved in this process. Since we expected several migration-relevant genes to be upregulated in inflammation, we FACS-sorted capLECS and colLECS from inflamed and uninflamed murine skin. Furthermore, since blood endothelial cells (BECs) are a well-studied cell type, these were also isolated to serve as a reliable control. RNA-sequencing results revealed three distinct transcriptomic signatures for capLECs, colLECs and BECs. Characteristic vascular markers were specifically expressed among

the different cell populations, with highest reads (FPKM values) of vWF and VEGFR1 in BECs; Prox1, PDPN and VEGFR3 in colLECs and capLECs, and highest reads of Lyve-1 in capLECs. Interestingly, we found that capLECs and colLECs differentially express several genes reportedly or presumably involved in leukocyte migration, and in many cases their expression levels are modulated by inflammation. Overall, our results shed light on the gene expression differences between capLECs, colLECs and BECs and will serve as the starting point for functional studies investigating the molecular control of leukocyte migration through LVs.

LTMP 15

ILC3 direct Yap1-mediated regeneration of small intestinal crypts after stem cell damage

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Epithelial cells of the intestine combine uptake of nutrients and water with providing a physical barrier to prevent translocation of microorganisms from in the intestinal lumen. Intestinal injury provokes an epithelial response aimed at rapidly restoring the intestinal barrier by expansion and differentiation of epithelial stem cells enclosed within the small intestinal crypts. Intestinal repair was long thought of as an epithelial-autonomous process, yet recently we identified a role for group 3 innate lymphoid cells (ILC3) in crypt regeneration after stem cell damage. ILC3-derived IL-22 controls maintenance of intestinal stem cells, however the underlying mechanisms inducing stem cell renewal and differentiation remain largely unidentified. Here, using IL-22-deficient mice, IL-22 neutralizing antibodies and Stat3 inhibition we show that IL-22 is dispensable for epithelial proliferation and intestinal pathology following stem cell injury. Based on these findings we hypothesised that ILC3 modulate damage-driven epithelial stem cell-specific signalling pathways and thereby control expansion and differentiation of stem cells independently of IL-22. To identify ILC3-regulated intestinal stem cell responses upon injury we generated ILC3-deficient Lgr5-reporter mice, induced stem cell damage and performed RNA-sequencing of purified stem cells. In the absence of ILC3, intestinal stem cells fail to activate Yap1 signalling, a critical pathway involved in early stem cell responses to injury. Moreover, in wildtype mice, pharmacological inhibition of Yap1-TEAD interactions and subsequent activation of downstream effector genes aggravates pathology after stem cell damage. Yap1 is known to transiently reprogram Lgr5-expressing stem cells and temporally drive differentiation at the expense of stemness. In ILC3-deficient mice, this reprograming is distorted and stem cells fail to down regulate Wnt signalling, an essential step in inducing the early boost in differentiation. Together this results in diminished crypt output, altered epithelial differentiation, defective regeneration and increased intestinal pathology. In sum, our findings reveal an important role for ILC3 in controlling the evolutionary conserved Yap1 pathway of intestinal crypt regeneration. This highlights a previously unappreciated layer of epithelial regulation by innate immune cells and identifies ILC3 as potential therapeutic targets to induce tissue healing after injury or during inflammation.

LTMP 16

Lymphatic endothelial cells control initiation of lymph node organogenesis

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Lymph nodes (LN) are strategically situated throughout the body at junctures of the blood vascular and lymphatic systems to direct immune responses against antigens draining from peripheral tissues. The current paradigm describes LN development as a programmed process that is governed through the interaction between mesenchymal lymphoid tissue organizer cells and hematopoietic lymphoid tissue inducer (LTi) cells. Using cell type-specific ablation of key molecules involved in lymphoid organogenesis, we found that initiation of LN development is dependent on LTi cell-mediated activation of lymphatic endothelial cells (LECs) and that involvement of mesenchymal stromal cells is a succeeding event. Endothelial cell activation was mediated mainly by signaling through the non-canonical NF-κB pathway and steered by sphingosine- 1-phosphate receptor-dependent retention of LTi cells in the LN anlage. Finally, the finding that pharmacologically enforced LTi cell-LEC interaction promotes ectopic LN formation underscores the central lymphoid tissue organizer function of LECs.

LTMP 17

Intestinal fibroblasts – Do they form a survival niche for IgA-producing plasma cells?

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One of the most active sites of immune defense in our body is the intestinal mucosa where we harbor billions of commensals and several potential pathogens. Secretory immunoglobulin A (sIgA) serves as a first-line defense that limits the access of microbes to the lamina propria (LP) and plays critical roles in the regulation of host-commensal interactions. The amount of secreted IgA exceeds all other isotypes (>70%) with most IgA being produced by plasma cells (PCs) residing in the LP of the small and large intestine. Recent work indicates that at least a subpopulation of intestinal PCs can survive and secrete IgA for more than 9 months in vivo. PCs themselves are not intrinsically long-lived, but are thought to depend on signals provided by their environment, in so-called survival niches. The current model states that hematopoietic cells form that niche along with epithelial cells. We describe in this study a dense and highly organized network of fibroblastic stromal cells that we call intestinal fibroblastic reticular cells (iFRC), which localize next to IgA+ cells. Given the role fibroblasts play in PC survival within the bone marrow we asked whether iFRC have an important function in PC survival within the lamina propria. To this end we have developed an isolation protocol for iFRC allowing to look for expression of PC survival genes. Indeed, iFRC were found to be a major source of baff, april and also cxcl12 transcripts. In coculture experiments we observed that purified iFRC as well as myeloid cells are able to promote IgA+ PC survival in vitro. Ongoing experiments aim to establish whether other cell types contribute to the formation of the IgA+ PC survival niche and which factors are involved in that process.

LTMP 18

Depletion of a subset of activated stromal cells impairs ectopic lymphoneogenesis in inflammation

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Background: Crucial structural and immunological functions of stromal cells have been recently shown by stromal cell deletion both in the context of cancer [1] and secondary lymphoid organ (SLO) homeostatic responses [2]. In the lymph node, FAP+gp38+ lymphoid stromal cells regulate lymphocyte homing and homeostasis; and depletion of FAP+gp38+ cells results in aberrant expression of lymphoid survival factors, disrupted lymph node organization and decreased ability to mount efficient immune responses [2]. We previously demonstrated that, during tertiary lymphoid structure (TLS) formation, salivary gland gp38+ stromal cells provide signals involved in the recruitment and organization of lymphocytes in inflamed tissue [3]. With this work we aimed to specifically dissect the role of FAP+ cells and to investigate the consequences of targeting this subset of stromal cells in an animal model of inducible ectopic lymphoneogenesis in murine salivary glands.
Methods: FAP-DTR mice were treated with Diphtheria Toxin (DTx) and used as a model of conditional depletion of FAP-expressing stromal cells. Following depletion, submandibular salivary glands of

FAP-DTR mice and littermate controls were intra-ductally cannulated with luciferase-encoding replication-deficient adenovirus to induce TLS formation as previously described [4]. A combination of immunofluorescence, quantitative RT-PCR and flow cytometry on enzymatically digested salivary glands were used.
Results: During inflammation and TLS formation in wild-type salivary glands, FAP is upregulated on a subset of activated stromal cells that also expresses gp38 and produces lymphoid chemokines. In inflamed DTx-treated FAP-DTR salivary glands, we observed significantly reduced numbers of gp38+ stromal cells and the presence of smaller lymphocytic aggregates with defective lymphocyte infiltration and lymphoid chemokine production. Furthermore, selective depletion of FAP+ cells induced loss of anatomical segregation of lymphoid aggregates and profoundly compromised TLS assembly.
Conclusions: Collectively, we provide evidence that, in inflammation, salivary gland stromal cells upregulate FAP and play a critical role in the establishment and persistence of TLS. Moreover, our work establishes stromal cells as a novel therapeutic target in TLS-associated diseases.

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LTMP 19

Human lymph node stromal cells constrain T cell activation through four molecular mechanisms

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Fibroblastic reticular cells (FRCs) are immunologically specialised myofibroblasts that organize the internal structure of lymph nodes. Mouse studies reveal rich supportive relationships between FRCs and T cells. In mice, FRCs suppress the proliferation of newly activated T cells by producing nitric oxide, reducing their production of pro-inflammatory cytokines. This function is thought to prevent immunopathology of the lymph node architecture during infection. In humans, this function, if it exists, may take on added clinical importance, because when anti-viral T cells are unable to clear virus within lymph nodes of humans, FRCs become fibrotic and, unlike mice, lose the ability to support the survival of T cells. This leaves patients severely T cell immunodeficient. Here we show that primary human FRCs strongly regulate the activation and proliferation of human T cells, putting a brake on proliferation and acquisition of early activation markers. We show that they utilise an entirely different mechanism of action to mice, via provision of prostaglandin E2 (PGE2), adenosine 2a receptor (Adora2a), transforming growth factor beta (TGFb) and indoleamine 2,3 dioxygenase (IDO). Suppression required feedback from T cells, and DNA synthesis and certain SOCS signalling pathways were inhibited, but suppressed T cells were not permanently anergised. We have successfully inhibited human FRC suppression of T cells using small molecule inhibitors. This is the first demonstration that human FRCs suppress T cell proliferation. We show that human FRCs utilise four mechanisms of action to reduce T cell activity post-activation. The results are of interest biologically as well as for future therapeutic development.

LTMP 20

Platelet-derived CLEC-2 and its ligand Podoplanin (Gp38) inhibit synovial inflammation?

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Background and objectives: During synovial inflammation, platelets and their associated microparticles escape from the synovial microvasculature and provide pro-inflammatory factors leading to the activation of synovial fibroblasts (SF) that actively contribute to joint damage [1]. In patients with rheumatoid arthritis (RA), SF up-express the surface protein Podoplanin (PDPN) while its ligand, CLEC-2, is brought into the synovial membrane by platelets [2, 3]. Despite these observations, clear experimental approaches that explore the role of PDPN/CLEC-2 interactions in RA pathogenesis are lacking.
Results: Our FACS and quantitative PCR analyses on freshly isolated mouse synoviocytes demonstrate that joint inflammation triggered PDPN up-expression on a pro-inflammatory SF subset with concurrent accumulation of PDPN+ anti-inflammatory macrophages. These populations disappeared with the resolution of inflammation. Joint inflammation was more pronounced during the disease onset in the complete absence of CLEC-2 (i.e.: Rosa26-Ert2Cre x Clec1bFlox/Flox mice) and in the absence of platelet-derived CLEC-2 (i.e.: Pf4-Cre x Clec1bFlox/Flox mice). Wild-type mice treated with an agonist anti-PDPN antibody were partially protected from induced auto-immune arthritis as demonstrated by their clinical features, their reduced leucocyte and non-haematopoietic cell accumulations into the joints as well as their attenuated bone erosion and remodelling.
Conclusions: We provide the first in vivo evidence that the PDPN/CLEC-2 interactions inhibit early phases of joint inflammation as ablation of platelet-derived CLEC-2 leads to worse arthritis without affecting its resolution. Accordingly, mimicking PDPN/CLEC-2 interactions with an agonist anti-PDPN antibody restrains auto-immune arthritis in mice. These observations suggest that platelets, known for promoting joint inflammation, also contribute to the suppression of arthritis in a CLEC-2 dependent manner. The mechanisms underlying this anti-inflammatory process are currently under investigation.

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LTMP 21

Roles for lymph node fibroblasts in dampening T cell responses

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Secondary lymphoid organs are sites where adaptive immune cells meet antigen-presenting cells to induce T cell tolerance or immunity. The backbone of these specialized organs consists of different types of non-hematopoietic cells that compartmentalize these organs into specific areas, like the T cell zone where the clonal selection and amplification of T cells occurs. Fibroblastic reticular cells (FRCs) are the main non-hematopoietic cell type found in T zones, where they form a sponge like network. This cell type has been shown to be able to "communicate" with T cells and dendritic cells in order to maintain immune homeostasis but also to modulate immune responses. We and others have shown that FRCs attenuate antigen-specific T cell responses by the IFNγ-dependent upregulation of inducible nitric oxide synthase (iNOS) followed by the release of nitric oxide (NO). Further we observed that FRC provide a second mechanism to dampen T cell responses by expressing cyclooxygenase 2 (COX2). The aim of the current study is to dissect the immune modulatory role of FRCs in more detail by focusing on the role of these two attenuating factors. Our data show that the level of iNOS expression within FRCs and thus the amount of NO production correlates with the strength of the ongoing immune response. In contrast to the inducible iNOS expression our data show that COX-2 is constitutively expressed in FRC. We will report the identity of a Cox2-dependent lipid mediator produced by FRC and the corresponding receptors necessary on T cells to mediate this suppressive effect. By using a FRC specific COX-2 deficient mouse model we will show data that this pathway is able to modulate antiviral T cell responses during chronic infection. These findings suggest that the use of common non-steroidal anti-inflammatory drugs like aspirin and ibuprofen that block Cox1/2 enzymes may enhance adaptive immunity in chronic viral infection by acting on FRC and inhibiting their suppressive function.

LTMP 22

CXCL13 modulates B cell trafficking into the lung early during influenza A virus infection and initiates tertiary lymphoid organ formation

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Tertiary lymphoid organs (TLOs) are generated in peripheral tissues during infection and in chronic inflammatory diseases, in particular autoimmune disease. While there is an association between disease severity and the formation of TLOs, the mechanisms that govern the formation of these structures are not well known. We have studied the formation of lung the TLO, inducible bronchus-associated lymphoid tissue (iBALT), during influenza A virus infection. Here we demonstrate that formation of germinal centre (GC)-containing iBALT requires T follicular helper (Tfh) cells and CXCL13 signalling. Lung Tfh cells are phenotypically similar to lymph node Tfh cells, requiring SAP and Bcl-6 expression, but less dependent on CXCR5. Rather, CXCR5 signalling is essential in B cells for iBALT formation. CXCL13 induction in the lung is maximal 5 days post infection, prior to iBALT organisation and formation of GCs. Using both genetic manipulation and interventional systems, we have shown that CXCL13 is required for B cell, but not T cell, entry into the lung parenchyma from the vasculature. CXCL13 is induced chiefly in stromal cells, and we hypothesise that inflammation-induced CXCL13 expression is a common mechanism regulating trafficking of B cells into inflamed tissues and the initiation of TLO formation. Thus, these early events offer a therapeutic target to limit TLO formation in infection and/or autoimmunity.

LTMP 23

Activation of skin and lymph nodes antigen-presenting cells induced by Salmonella Typhi porins

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Introduction: Salmonella Typhi (S. Typhi) porins are important targets of the mice and humans immune protective immune response, these proteins are also potent immunogens capable of generate long lasting antibody response for more than 11 years post-immunization in humans. In mice, porins induce life-lasting bactericidal antibody responses and protection against 500 lethal dose challenge with S. Typhi. The mechanisms involved in the induction such atypical antibody responses remain poorly understood. Here we report the initial characterization of the activation, migration and T cell activation induction capacity of antigen-presenting cells (APC) in skin and lymph nodes in mice immunized with S. typhi porins.
Methods: C57/BL6-MHCII-GFP mice were immunized intradermally with porins. Epidermis of the skin was obtained 12h post-immunization and stained with MHC-II, CD86, CD40 and PD-L1. Tissue sections were analysed by confocal microscopy. Cervical lymph nodes were obtained and prepared for a flow cytometry staining to identify dendritic cell subsets (resident and migratory) and its activation. The capacity of porins activated APC to activate T cell responses was evaluated co-immunising porins with inactivated Sporothrix schenkii conidia. Conidia specific memory T CD4+ cells in lymph nodes were analysed by flow citometry and in skin by a delayed-type hypersensivity test.
Results: S. Typhi porins induced a higher expression of MHC-II and CD40 in skin than controls, in contrast, CD86 and PD-L1 expression were not increased by porins. Porins induced an increased number of CD86+ cells in skin despite CD40+ and PD-L1+ cells were not found in greater amount. Porins induced an increased number of migratory dendritic cells in lymph nodes which had an activated phenotype. Conidia specific total T CD4+ cells, central memory T CD4+ cells and effector memory T CD4+ cells, were increased in lymph nodes by co-immunization of conidia with porins. The cellular response in skin induced by conidia-porins was higher than the controls.
Conclusion: Intradermal immunization with S. typhi porins induced early activation of epidermal dendritic cells and recruitment of antigen-presenting cells to skin, also promoted migration of skin dendritic cells that are able to generate memory T CD4+ cells in lymph nodes and skin, inducing systemic immune responses.

LTMP 24

An organ-wide transcriptome of stromal cells identifies the core signature of steady-state murine fibroblasts

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Tissue-resident fibroblasts play important roles in the maintenance of tissue architecture, scar resolution, tumor progression, immune regulation and fibrosis progression through extracellular matrix production. The identification of organ-independent fibroblast-specific marker genes will facilitate the investigation of the biological roles of fibroblasts, the origin of activated fibroblasts and the identification of fibroblasts from single-cell transcriptome data in various pathological conditions. However, such marker genes have not been identified. To address this issue, we purified tissue-resident fibroblasts and smooth muscle cells from steady-state murine lung, liver, heart, skin, kidney, small intestine, thymus and bone marrow by fluorescent-activated cell sorting and performed global transcriptome analyses using a next-generation sequencer. We identified 233 fibroblast core signature genes and 432 smooth muscle core signature genes that are highly conserved across multiple organs and differentially expressed between fibroblasts and smooth muscle cells. Gene ontology analysis revealed that the fibroblast core genes included extracellular matrix-related genes and the smooth muscle core genes included cytoskeleton-related genes. We selected 36 fibroblast-marker genes and 68 smooth muscle-marker genes that were more highly expressed in those cells than in other tissue cell subsets, including endothelial cells and epithelial cells. Expression of these 36 fibroblast-marker genes was also highly conserved in cancer-associated fibroblast derived from B16 and LLC subcutaneous tumors. Using these new marker genes, we identified fibroblast population based on public single-cell transcriptome data of human melanoma (GSE72056). In addition, we found that already-known single fibroblast-marker genes could not cover all of the fibroblast population, and fibroblast-activation marker genes showed several distinct expression patterns in fibroblast population. Because the single-cell transcriptome data involves various background melanoma, our marker genes might be robust in various pathological conditions. Overall, our data might provide useful resource for further investigation of fibroblast biology.

LTMP 25

Notch signaling in stromal cells of secondary lymphoid organs

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Spleen and lymph nodes display functionally distinct compartments, which optimize antigen capture and presentation thereby leading to efficient lymphocyte activation and differentiation. These microenvironments are formed to a large extent by fibroblasts: Follicular Dendritic Cells (FDC) in the B cell follicles with the remaining fibroblasts being collectively termed Fibroblastic Reticular Cells (FRC). My goal is to understand the role of Notch signaling in FRC development and function. The Notch pathway is evolutionary conserved and important for developmental processes, including cell fate decisions. Mammals possess four receptors (Notch 1–4) that are bound by five ligands of the Jagged family and Delta-like (DLL) family. When analyzing gene array data, we observed that lymph node FRCs express three Notch receptors (Notch1, 2 and 3) as well as Hes1, one of the main Notch target gene. Fibroblasts in non-lymphoid sites also express Notch signaling and are able to overexpress it under specific conditions such as systemic sclerosis for dermal fibroblasts or rheumatoid arthritis for synovial fibroblasts. It has been shown that in vitro stimulation of this signaling can induce the release of collagen protein, but also could affect cytokine production or fibroblast differentiation. Therefore Notch signaling seems to play multiple functions in fibroblasts of non-lymphoid sites. By genetic loss-of-function experiments, we are investigating the function of Notch1 and Notch2 genes selectively in CCL19 Cre recombinase expressing fibroblasts of secondary lymphoid organs, both during homeostasis and immune response. My studies show that while the general organization of spleen and lymph nodes is preserved, including the presence of all FRC subsets, combined deletion of Notch 1 and 2 in

FRC reduces markedly their matrix and chemokine production, which is associated with alterations in T and B lymphocyte retention. Therefore Notch 1 and 2 are not needed for cell fate specification but for their function. We plan to dissect these findings in more detail, including their impact on adaptive immunity.

LTMP 26

Innate immunological sensing by CCL19-expressing fibroblastic stromal cells secures peritoneal immunity

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Fibroblastic stromal cells (FSC) expressing homeostatic chemokines such as CCL19 or CXCL13 not only determine the structure and function of classical secondary lymphoid organs (SLOs), but are also present in non-classical SLOs of tissues that line the body cavities. To determine whether immune-stimulating FSC contribute to innate and adaptive immune responses in body cavities, we ablated the innate immunological sensing molecule MyD88 in CCL19-expressing FSC. We found that a particular population of FSC present in both omentum and mesentery expresses canonical fibroblastic reticular cells markers co-localized preferentially with B cells in fat-associated lymphoid clusters. FSC-specific deficiency of MyD88 strongly reduced CCL2-dependent myeloid cell recruitment and subsequent CD4+ T cell-dependent B cell activation following intraperitoneal exposure to bacterial antigens. Containment of bacterial in the peritoneal cavity was compromised in conditionally MyD88-deficient mice indicating that a particular FSC subset present in non-classical SLOs determines the swift activation of optimal immune responses in the peritoneal cavity.

LTMP 27

Interleukin-7-expressing fibroblasts promote breast tumor growth

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Fibroblastic stromal cells represent a highly abundant component of the tumor microenvironment (TME) and comprise both tumor-promoting and -suppressive subsets. However, the phenotype-function relation of such cancer-associated fibroblasts (CAFs) is still largely unexplored. Here, we investigated a genetically-defined population of CAFs using a syngeneic orthotopic model of breast cancer. We found that CAFs expressing the cytokine interleukin-7 (IL-7) represented a minor fibroblast fraction in the breast cancer TME. These cells expressed a particular set of surface markers and were found to co-localize with both tumor cells and vascular endothelial cells. Functionally, toxin-mediated ablation of IL-7-producing CAFs led to a significant reduction in tumor growth suggesting that this particular fibroblast subset generates a critical niche for tumor cell growth. Transcriptomic profiling uncovered a characteristic signature of IL-7-positive fibroblasts with high expression of various growth factors, stem cell ligands, and immune modulators. Taken together, we here report the identification of a novel subset of breast tumor-promoting CAFs. Functional dissection of the observed phenotype using cell type-specific knockouts is currently in progress.

LTMP 28

Origin and differentiation trajectory of splenic white pulp fibroblastic stromal cells

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The prevailing paradigm of splenic white pulp development assigns an indispensable LTβR signaling in lymphoid tissue organizer (LTo) cells to organize the white pulp. However, the identification and function of splenic LTo cells in vivo has not been feasible owing to the lack of a suitable mouse model. Using the Ccl19-Creeyfp mouse model, we found that the transgene marks distinct white pulp fibroblastic stromal cells, including T cell zone reticular cells (TRC), follicular dendritic cells (FDC), and marginal reticular cells (MRC) and perivascular stromal cells (PSCs). Moreover, conditional ablation of the Ltbr gene precipitated disorganization of the splenic white pulp and an impairment of fibroblastic stromal cell subsets specification. Cell fate mapping of embryonic LTo cells with the novel Ccl19-tTAeyfp inducible Cre mouse model revealed that splenic fibroblastic stromal cells descend from a common perivascular progenitor cell. In addition, cell fate mapping of embryonic LTo cells deficient for the Ltbr gene confirmed that differentiation of fibroblast populations in the adult spleen relies on this signaling pathways. Taken together, our results unveil that LTo cells residing in perivascular niches of fetal spleen can give rise to different fibroblastic stromal cell populations and that these cells regulate splenic compartmentalization in a LTβR-dependent manner.

LTMP 29

Targeting follicular dendritic cells: The CTA1-DD adjuvant modulates germinal center responses by directly influencing gene-transcription in follicular dendritic cells and potentiating follicular T helper cell functions

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The non-toxic CTA1-DD adjuvant, carries the ADP-ribosylating CTA1-enzyme from cholera toxin, which acts on Gsa in the cell membrane of target cells. CTA1-DD binds and modulates classical dendritic cells (DC), but is also targeted to follicular dendritic cells (FDC) via the DD-fragment in a complement receptor 2 (CD21)-mediated fashion, leading to enlarged and more numerous germinal center (GC) reactions following mucosal or systemic immunizations. To analyze the adjuvant's impact on FDC in greater detail, we developed a mouse model expressing the GFP-reporter gene under the CD21 promotor to better detect the FDC network and allow for sorting of these cells. In dissecting the mechanism of action, we observed that CTA1-DD greatly augmented gene transcription of several GC promoting factors in FDC, in particular CXCL13, IL-6 and IL-1β. These effects were dependent CTA1-enzyme-activity and preceded immune complex or germinal center formation, indicating direct binding and modulation of FDC by CTA1-DD. Additionally, CTA1-DD adjuvant potentiated Tfh responses with increased expression of Bcl-6, and associated with stronger GC and memory B cell responses. Importantly, the effect on GC-functions was also seen in infant mice, which paves the way for exploitation of the CTA1-DD adjuvant for neonatal vaccine development. Thus, CTA1-DD modulated the follicular environment and appeared to circumvent the intrinsic GC B cell and Tfh impairment seen in infants. Because of the dual effects of CTA1-DD on targeted FDC and DC our system adds to a better understanding of how to modulate GC functions for the benefit of more powerful and safer vaccines, suitable even for neonatal vaccination.

LTMP 30

Assessing the role of CXCL13-expressing lymphoid tissue organizer cells in the development of secondary lymphoid organs

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Fibroblastic stromal cells (FSC) play an important role in the recruitment, activation and maintenance of lymphocytes in secondary lymphoid organs (SLOs). Moreover, it has been proposed that embryonic FSC present in the lymph node anlage are critical for the development of lymph nodes and that lymphotoxin-b receptor (LTβR)-dependent activation of CXCL13-expressing fibroblasts is key for this process. CXCL13-expressing embryonic FSC are therefore commonly referred to as mesenchymal lymphoid tissue organizer (LTo) cells. To determine whether and to which extent CXCL13-expressing mLTo cells direct formation lymph nodes and other SLOs, we have crossed Ltbrfl/fl mice with Cxcl13-Cre/tdTomato; R26R-EYFP dual reporter mice. While the absence of LTβR on CXCL13-expressing cells

completely blocked formation of the splenic white pulp and substantially reduced the formation of Peyer's patches, all lymph nodes could be found at the same locations as in Ltbr-competent litter mate controls. These data suggest that CXCL13-expressing mesenchymal LTo cells contribute to SLO formation through distinct mechanisms.

LTMP 31

Foxc2 is essential for organ-specific lymphatic vessel maintenance and function

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The lymphatic vasculature is present in virtually every organ, where it plays various roles in order to support its function. Capillaries take up interstitial lymph, whereas collecting vessels, displaying intraluminal valves, transport the lymph to lymph nodes (LNs) and back to the blood vascular system. Recent studies have shed light on the complexity of this system and organ-specific structural organization of lymphatic vessels, however our knowledge of molecular mechanisms ensuring development and stability of tissue-specific lymphatic vessels, such as in LN, is still fragmentary. Heterozygous loss-of-function of the forkead transcription factor Foxc2 leads to lymphedema-distichiasis (LD), a debilitating disease with impaired capillary and collecting vessel specification and lymphatic vessel dysfunction. Recently, we have described the role of Foxc2 as a critical transcription factor necessary for postnatal lymphatic vascular function. Foxc2 deletion after birth in lymphatic endothelial cells leads to chylothorax and rapid 100% lethality, as a result of degeneration of intraluminal valves, loss of lymphatic endothelium integrity and overall aberrant lymph transport. Here we investigated the role of lymphatic vessels in embryonic development of lymph node anlagen in wildtype and in Foxc2f/f; Prox1-CreERT2 mice, in which inactivation of Foxc2 in lymphatic endothelial cells is achieved by administration of tamoxifen from the age of E13.5 and E14.5. We will present data that demonstrate the importance of a close interaction and interdependency between embryonic LN anlagen and lymphatic collecting vessel remodeling for the formation of a functional LN.

LTMP 32

CXCL13-expressing fibroblastic stromal cells govern the germinal center reaction during viral infection

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Production of CXCL13 by fibroblastic stromal cells (FSC) is important for B cell clustering in secondary lymphoid organs. Although follicular dendritic cells (FDC) are proposed to be the main CXCL13-expressing fibroblastic cell type, other, yet undefined, FSC are known to underpin the B cell follicle. The characterization and tracking of these fibroblasts in primary and secondary B cell follicles remains hindered due to poor resolution of cell-specific chemokine staining. In order to elucidate the fibroblastic cell composition of the B cell follicle under homeostatic conditions as well as following viral infection, we have generated a novel Cxcl13-Cre/tdTomato; R26R-EYFP reporter mouse that permits the cell-specific tracing of CXCL13-expressing fibroblasts. The dual reporter mouse expresses tdTomato and Cre recombinase directly under the control of the Cxcl13 promoter (marking cells currently expressing CXCL13 with red fluorescence), and EYFP under the control of the constitutive Rosa26 promoter in a Cre-dependent manner (marking cells with current and past CXCL13 expression with EYFP). Here, utilising this novel mouse model, we delineate distinct subsets of CXCL13-expressing fibroblasts under both homeostatic and inflammatory conditions. Our data illuminate the fibroblastic stromal cell composition of the B cell follicle and highlight the Cxcl13-Cre/tdTomato; R26R-EYFP model as an important tool for the phenotypic and functional characterization of CXCL13-expressing FSC.

LTMP 33

CCL19-expressing fibroblastic stromal cells form an essential niche for intestinal innate lymphoid cell development

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Immune responses against intestinal pathogens are initiated in gut-associated lymphoid tissues. In addition to the embryonically imprinted development of mesenteric lymph nodes and Peyer's patches (PPs), cryptopatches (CPs) and isolated lymphoid follicles (ILFs) develop postnatally in the lamina propria within the first weeks of life in response to microbial colonization. Using an inducible Cre recombinase mouse model based on doxycyclin-dependent silencing of gene expression, we found that ILF formation and maintenance in the lamina propria was controlled by CCL19-expressing fibroblastic stromal cells (FSCs) through integration of lymphotoxin-β receptor (LTβR) signals. Ablation of LTβR on CCL19-expressing FSCs revealed that these cells drive ILF maturation and thereby form a particular microenvironment that facilitates development and homeostasis of innate lymphoid cells (ILCs). Profoundly altered ILC development precipitated a significant decrease in the production of the ILC3-derived cytokine IL22 in the lamina propria and an increased susceptibility to Citrobacter rodentium infection. In summary, CCL19-expressing FSCs contribute to the maintenance of epithelial barrier integrity in the intestine through the provision of the essential niche for ILC differentiation.

LTMP 34

Fibroblastic reticular cells promote enhanced metabolism and survival in activated T lymphocytes via epigenetic alterations

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Lymph node fibroblastic reticular cells (FRCs) organize and regulate several aspects of T cell biology. They support lymphocyte recruitment and compartmentalization in the lymph node and facilitate encounters between antigen-presenting dendritic cells and T cells. Upon sensing T cell activation, FRCs release nitric oxide, which restricts proliferation to regulate the size of the activated T cell pool. Therefore, to date, FRCs were primarily thought to negatively regulate the functions of newly activated T cells. We now show that FRCs also express immunostimulatory molecules in response to activated T cells. Specifically, FRC-derived IL-6 creates a supportive niche for activated T cells by enhancing their expression of IL-2 and TNF-alpha. We used epigenetic profiling to more deeply explore this stimulatory communication and found that activated T cells significantly remodel their chromatin landscape in response to being cultured with FRCs or recombinant IL-6. We noted a number of differentially open regions of chromatin nearby several metabolic and pro-survival genes such as hexokinase and Bcl-2. Importantly, the epigenetic changes positively

correlated with gene expression and were also accompanied by enhanced metabolic flux and lipid biosynthesis during functional assays. Compared to T cells activated alone, FRC-conditioned T cells persist significantly longer upon adoptive transfer into virally infected animals with IL-6 being necessary and sufficient to induce this enhanced longevity. Furthermore, FRC-conditioned T cells preferentially differentiate into tissue-resident memory T cells during influenza infection. Although it remains to be determined if the suppressive and stimulatory programs run simultaneously, temporally, or contextually in FRCs, this study demonstrates a novel capacity of FRCs to enhance various functions of activated T lymphocytes.

LTMP 35

Mapping the spatial distribution and dynamics of CXCL13 in lymphoid tissues

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CXCL13 is a key determinant of humoral immune responses, regulating the precise localisation of lymphocytes within lymphoid tissues. Due to a complex and dynamic array of molecular interactions, mapping the precise spatial distribution of CXCL13 in situ is challenging. To address this, we have quantified, using an ensemble of imaging modalities, key factors that regulate CXCL13 bioavailability. These experiments are complemented by simulations of CXCL13 field formation and associated B-cell responses within a high fidelity in silico B-follicle. We have mapped the 3-Dimensional organisation of CXCL13+ stroma in situ using a CXCL13 reporter system, identifying three distinct but interconnected stromal subsets that are unique in their network properties.To quantify the dynamics of CXCL13 we use high-speed narrowfield microscopy, a technique capable of sub-millisecond sampling, single particle tracking with ~40 nm spatial precision, and quantitative stoichiometry determination. Using this approach we image Alexa-647 labelled CXCL13 and CCL19 in collagen matrix and lymph node tissue sections. Our results suggest that chemokines are heterogeneous in their diffusion and binding characteristics with multimerisation effects altering the rate of diffusivity. This data has informed the development and validation of a 3D computational model of the primary follicle. In silico migration is consistent with in vivo measurements, obtained using two-photon microscopy, for both wild-type and CXCR5–/– B-cells. Simulation analyses suggest that chemokine fields within the follicle are dynamic and non-uniform, identifying the CXCL13 diffusion constant, secretion rate and decay rate as key parameters governing the efficacy of B-cell antigen scanning. In silico CXCR5 expression on the cell surface is oscillatory and location-dependent with complete loss of the receptor leading to reduced network scanning rates. To summarise, we have quantified key molecular, cellular and tissue components of functional CXCL13 expression. We consolidate this data into an executable software platform capable of visualising chemokine-mediated cross-talk between CXCL13+ stroma and B cells, and generating novel hypotheses regarding the spatial distribution of chemokines within lymphoid tissues.

LTMP 36

Control of lymphoid stromal cell responses during virus infections. Control of lymphoid stromal cell responses during virus infections

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Subsets of lymphoid stromal cells (LSC), including fibroblastic reticular cells (FRC) construct the lymphoid organs and support immune responses in several ways. Yet, relatively little is known about the behaviour of LSC during infections, or to what degree pathogens influence LSC responses and functions. We found that local infection with herpes simplex virus (HSV) induced remodelling of the LSC network in the draining lymph node (dLN), characterised by expansion of FRC and a substantial increase in MadCAM-1+ marginal reticular cells (MRC) within 1 week after infection. In contrast, local infection with lymphocytic choriomeningitis virus (LCMV) induced poor LSC expansion. Co-infection of mice with LCMV and HSV, or induction of systemic inflammation by poly(I:C) or LPS injection during HSV infection, inhibited LSC expansion in the dLN. LCMV infection induced a systemic lymphopenia and reduced lymphocyte recruitment to the dLN. Reduced lymphadenopathy induced by inflammation or following FTY720 treatment during HSV infection did not alter antigen-specific T cell responses. In contrast, B cell responses to HSV infection were suppressed by systemic inflammation. Blockade of type I interferon receptor partially restored LSC expansion in dLN during co-infection. These data suggest that systemic inflammation alters local lymph node responses, suppressing stromal cell expansion and inhibiting B cell responses.

LTMP 37

Stromal-derived WNT-5A is increased in poor-prognosis colorectal cancer

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Persistent inflammation in the tumor microenvironment can benefit cancer cell growth and therapy resistance. To maintain inflammation, cancers require the interaction with other cell types in their proximity, most notably fibroblasts, leukocytes and endothelial cells. Recently, four molecular subtypes of colorectal cancer (CRC) have been defined, one of them (CMS4) being characterised by a prominent mesenchymal signature and poor outcome. Tumor-promoting effects of cancer-associated fibroblasts (CAFs) are well-established, and modulating their interaction with leukocytes, cancer cells and endothelial cells might therefore represent a valuable therapeutic approach to treat CMS4-type CRCs. Our analysis of published CRC datasets identified KRAS-mutated proximal CRC with high expression of IL-22R to be associated with poor-prognosis. Further pathway analysis demonstrated that these cancers are enriched in the expression of mesenchymal signature genes (e.g., collagens). Strikingly, elevated expression of WNT5A was associated with the mesenchymal signature in these tumors. In CRC patients, WNT5A expression is increased in tissue sampled from the tumor core, as opposed to histologically normal adjacent tissue. Podoplanin(gp38)-positive fibroblasts are the main source of WNT5A expression, as determined by the comparison of sorted mesenchymal, epithelial, endothelial and hematopoietic cells in mice. In cultured primary human fibroblasts and the fibroblast line Ccd18-Co, WNT5A expression can be further increased by the stimulation with inflammatory mediators, such as TNF-alpha and IL-17A. Together with reports demonstrating effects of WNT-5A on epithelial, myeloid and lymphoid cells, we hypothesise that WNT-5A inhibition might represent a valuable approach to disrupt tumor-promoting effects of CAFs on cancer cells and tumor-associated lymphocytes. We are currently investigating effects of WNT-5A on various myeloid and lymphoid subsets, as well as primary colorectal epithelial and cancer organoids. Results from these experiments and additional experimental CRC models will clarify whether the WNT-5A pathway represents a possible target to treat poor-prognosis CRC.

LTMP 38

Role of ACKR3 in leukocyte migration and the microarchitecture of secondary lymphoid organs

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Trafficking and segregation of immune cells in secondary lymphoid organs (SLOs) is essential under normal homeostasis and, even more importantly, during immune responses. The localization of leukocytes in SLOs is not mediated through physical barriers, rather through a network of different stroma cells, chemokine gradients and lipid agonists of G-protein coupled receptors. Localized specific stroma cells produce chemoattractants to recruit selectively T and B-cells, as well as myeloid cells, which express cognate receptors. In order to locally confine chemotactic gradients attractants have to be eliminated at distance to the source according to the “source and sink” model proposed by F. Crick. Atypical chemokine receptors constitute a family of scavenger receptors which efficiently eliminate chemokines and thereby markedly contribute to the maintenance of gradients. ACKR3

is a scavenger for the chemokines CXCL12 and CXCL11. The receptor is upregulated on plasma blasts and presumably licenses the cells to egress from CXCL12-rich germinal centres. In spleen ACKR3 is expressed on marginal zone (MZ) B-cells and inhibition of ACKR3 was reported to destroy the microarchitecture. ACKR3GFP/WT reporter mice confirmed expression in MZ B-cells. The MZ was visualizes after injection of encapsulated attenuated and fluorescently labelled S. pneumonia or with a brief i.v. pulse of CD19 targeting fluorescent antibodies before sacrifice of the mice. In addition, a strong GFP expression (ACKR3) was observed in a vascular structure located in the red pulp surrounding the white pulp. The structures appear to be distinct from endothelial cells of blood vessels. ACKR3 expressed on these vessels actively scavenges an ACKR3-selective chimeric chemokine suggesting depletion of CXCL12 in its near surroundings.

LTMP 39

Mesenchymal organizer cell-derived RANKL induces terminal differentiation of LTi cell in the lymph node anlagen

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Lymph node (LN) is a mammal specific secondary lymphoid tissue which develops during fetal stage. Lymphoid Tissue inducer (LTi) cell is a member of group 3 innate lymphoid cells (ILC3) originated from lineage negative (Lin-), IL-7Rα+ and Integrin α4+β+7 precursor cells in the fetal liver. Once LTi cells migrate into the lymph node anlagen, local mesenchymal stromal cells termed Lymphid Tissue organizer (LTo) cell got activated to produce chemokines and further recruitment LTi cells occur. All of these processes form positive feedback loop and contribute to the complete formation of complex lymph node structure. However, precise characterization of LTo cell nor investigation for molecular mechanism underlying LTi-LTo cell interplay were not completely performed. RANKL is a TNF family cytokine indispensable for the lymph node development. In this study, we found that RANKL expressed on LTo cell was indispensable for the lymph node development and that RANK, a receptor of RANKL was expressed on LTi precursor. Transcriptome analysis revealed downregulation of several LN-organogenesis-related genes in the absence of RANK in both LTi precursor and LTi cells. Finally, in the absence of RANK on LTi cell, lymph node formation was completely impaired. All these results suggest that locally expressed RANKL in the LTo cell induce terminal differentiation of LTi cell and contribute to the lymph node organogenesis.

LTMP 40

Elucidating the role of NDFIP1 in regulating "Innate like B cell" development and function

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Nedd4 family-interacting protein 1 (NDFIP1) is an adaptor for ubiquitin ligase ITCH. Genetic mutations in NDFIP1 are linked to allergic and autoimmune diseases due to an intrinsic function of NDFIP1 in dampening pro-inflammatory Th2 immune response. However, the role of NDFIP1 in regulating B cell development and function has not been tested. We found that NDFIP1-deficient mice lacked marginal zone B (MZB) cells and exhibited marked alterations to marginal zone (MZ) architecture. Mixed bone-marrow chimeras demonstrated that the MZ defect was due to a cell-extrinsic deficiency of NDFIP1 in hematopoietic cells. Loss of MZB cells and MZ architecture occurred in CD4.Cre NDFIP1fl/fl mice suggesting that these alterations were CD4 T cell dependent. Furthermore, CD4 T cell depletion in the NDFIP1 deficient mice restored MZB cells and MZ architecture confirming the role of CD4 T cells in mediating loss of MZB cells and altering the MZ architecture. NDFIP1-deficient CD4 T cells were Th2 biased and produced excessive IL-4. We found that NDFIP1 IL-4 DKO mice were protected from loss of MZB cells and MZ architecture, identifying IL-4 as one of the factors inducing the loss of MZB cells and MZ architecture. Ongoing studies will elucidate the mechanism by which chronic Th2 inflammation and particularly CD4 T cells and IL-4 drive the loss of marginal zone function and integrity.

LTMP 41

The autoimmune regulator, AIRE, is expressed in the synovium in rheumatoid arthritis and induced by pro-inflammatory mediators in fibroblast-like synoviocytes in vitro

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Background: AIRE is a transcriptional regulator of tissue specific antigens in medullary thymic epithelial cells (mTEC). AIRE orchestrates the negative selection of self-reactive T cells as well as the induction of regulatory T cells in the thymus. AIRE expression in mTEC is induced by RANKL and TNF. Extra thymic expression has been described in lymph nodes. AIRE was recently identified as a risk gene in rheumatoid arthritis (RA) by GWAS. Integrative analysis using omics data on fibroblast-like synoviocytes (FLS) from RA patients and controls points to a role of AIRE in RA-FLS. Activated FLS are key effector cells in RA, mediating persistent inflammation and tissue destruction. In addition, MHC class II is induced on FLS in the RA joint suggesting a role in antigen presentation.
Objectives: To investigate if AIRE is expressed in human synovial tissue in RA and in primary RA-FLS and if the expression in vitro is modified by RANKL or cytokines known to activate FLS.
Methods: Fixed paraffin-embedded synovial tissues from RA and control osteoarthritis (OA) patients were subjected to immunofluorescence (IF) and confocal microscopy using anti-human AIRE ab. Primary FLS were serum starved ON and then stimulated with IL-1β, TNF or RANKL or PBS for 12 hours before RNA isolation and qPCR or IF for AIRE expression.
Results: Occasional AIRE expressing cells were present in RA synovium (lining and sublining layer) but not in the OA specimens (n = 3). The staining pattern was rather perinuclear but otherwise similar to AIRE staining of mTEC. No AIRE expression was detected in unstimulated FLS. However, AIRE mRNA expression was induced up to 222 ± 102 fold in RA-FLS compared with unstimulated ($p = 0.009$, n = 3) by IL-1β but not by RANKL. In OA-FLS AIRE was induced 39 ± 9 fold ($p < 0.0001$, n = 6) by IL-1β and 10 ± 5 fold ($p = 0.011$) by TNF compared with unstimulated. A synergistic effect was seen using IL-1β + TNF (66 ± 33 fold, $p = 0.009$). The AIRE induction was significantly higher in RA than OA-FLS ($p = 0.035$). No mRNA expression of PADI4 or INS was detected in the stimulated samples. A perinuclear AIRE protein expression was detected in IL-1β + TNF stimulated RA FLS.
Conclusion: A strong dose dependent AIRE expression is induced by TNF and IL-1β in primary RA-FLS and AIRE is expressed in the RA synovium, which supports a role of AIRE in arthritis. Stimulation did not induce the known AIRE dependent genes PADI4 and INS in FLS. Studies are ongoing to identify AIRE dependent genes in RA-FLS.

LTMP 42

Composite fibroin-gelatin scaffolds in bioengineering of artificial lymphoid organs

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Functional artificial analogues of lymphoid tissue seem to be a promising tool in therapeutic modulation of the immune response in several diseases such as cancer, autoimmunity, primary and secondary immunodeficiencies. The goal of our study is to generate a functional analogue of lymphoid tissue with a potential for applications in patients. For this purpose we used 3D matrices made of fibroin from B. mori modified with gelatin as a 3D-scaffold for artificial tissue bioengineering. To reproduce the stromal compartment we initially used primary mouse embryonic fibroblasts (MEF). Interestingly, without any additional stimulation MEFs in fibroin-gelatin scaffolds in vitro overexpressed adhesion molecules ICAM-1 and VCAM-1 that are important for lymphocyte-stromal interaction. To test the ability of this system to promote lymphoneogenesis in vivo we used the model of kidney subcapsular implantation in mice. Following implantation even empty fibroin-gelatin scaffolds could induce immigration and clustering of lymphocytes, mainly B-cells. To exclude the impact of surgery-induced inflammation we implanted collagen scaffolds and found no

significant immigration of lymphocytes. At the same time, we observed immigrated lymphocytes only in the outer part of the fibroin-gelatin implants, while very few cells immigrated deep inside the scaffolds. Similarly, we did not observe blood vessel ingrowth, as indicated by CD31 staining, in the center of the implanted scaffolds. Addition of MEF to the scaffolds prior to implantation did not affect either vessel ingrowth or immigration of the cells to the inner part of the implants. In summary, we found that fibroin scaffolds affected fibroblasts in vitro by upregulation of adhesion molecules ICAM-1 and VCAM-1 and induced immigration of lymphocytes in vivo upon implantation in mice. Activation of LTbR with recently described single-chain ligands, especially LTa1b2, on MEF prior to implantation could be used to further improve immigration of lymphocytes to the implants and promote blood vessel ingrowth, thus, creating a tool for bioengineering of artificial lymphoid organs. Supported by the Russian Science Foundation grant #14-50-00060.

LTMP 43

Dual role of natural killer cells in response to influenza vaccine

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NK cells are innate immune cells capable of killing virus-infected and stressed cells without prior sensitization. They can also act as regulatory cells in balancing the immune response by eliminating wrongly activated T cells and/or DCs via the recognition of stress ligands. In addition, NK cells have been shown to induce Th1 differentiation by secretion of IFNγ. Previous studies restricted the time window of NK cell functions on initial days post treatment, ignoring the very early (hrs) and late responses post treatment. Through this study, using UV-inactivated influenza virus as an antigen and without any ex vivo stimulation we show that NK cells are activated by lymph node macrophages in an IFNAR-dependent manner. IFNγ is detected only at 12hrs post vaccination and produced solely by NK cells. Selective depletion of 'early' NK cells as well as blocking of initial IFNγ indicated that early IFNγ plays role in DC activation, survival and their production of IL-6. This depicts the regulatory role of NK cell at early time points post vaccination. Furthermore, on day 5 post vaccination NK cells act more as killers and significantly upregulate CD107a surface expression in addition to intracellular perforin levels. In vivo imaging studies signify the differences between early and late NK cell movement and interaction with other cells. Elimination of both IFNγ-producing regulatory and effector NK cells decrease TFH frequencies and affect the overall antibody responses to UV-PR8. In conclusion, this study elucidates the dual role of NK cells in maintaining and regulating immune responses against UV-PR8.

LTMP 44

Robo4 controls B cell migration – a new regulator for B cell egress in Peyer's patches

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Lymphocytes migrate into lymph nodes in a constant search of foreign antigens. They can enter the lymph node via two different routes: the blood circulation and the afferent lymphatics. When lymphocytes exit the lymph node they migrate into the cortical sinuses and egress via the efferent lymphatics. Although several regulators of lymphocyte entry and exit have been discovered, the molecular differences between the afferent and efferent lymphatics have not been distinguished. We performed a genome-wide microarray study to describe the molecular differences between the two arms of the lymphatic vasculature. Our studies revealed a vast number of molecules differentially expressed in the afferent and efferent lymphatics. Robo4 (magic roundabout) was selected for further functional analyses as a molecule predominantly expressed by the efferent lymphatics. Originally Robo4 has been described as an endothelial specific member of the roundabout family. Robo4 expression has been reported on vascular endothelial cells, and it has been shown to control endothelial cell migration. Our studies suggest Robo4 to be specifically expressed by the efferent arm of the lymphatic vasculature in comparison to the afferent one. To investigate the role of Robo4 regarding lymphocyte migration, we performed a set of in vivo adoptive transfer assays. We analyzed the effect of Robo4 in lymphocyte entry via the blood vasculature by injecting labeled lymphocytes intravenously into Robo4 deficient and control mice. As a result, Robo4 deficient mice had a slightly diminished amount of transferred B cells in their secondary lymphoid organs. This suggests Robo4 to specifically regulate the entry of B cells via the blood circulation. To study the role of Robo4 in the lymphatics we studied the egress of in situ labeled lymphocytes in the intestinal Peyer's patches. Surprisingly, B cells accumulated in the Peyer's patches of Robo4 deficient mice suggesting a defect in the egress of B cells. As Robo4 seems to only regulate the migration of B cells, we hypothesize B cells to express a specific binding partner for Robo4. Further studies are still necessary to dissect the mechanism of how Robo4 specifically regulates the entry and egress of B cells in secondary lymphoid organs.

LTMP 45

Mesenchymal stromal cells stimulate the proliferation and IL-22 production by type 3 innate lymphoid cells

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Infusion of mesenchymal stromal cells (MSCs) is a promising and increasingly applied therapy for patients who suffer from graft-versus-host disease (GvHD), a common and life-threatening complication of allogeneic stem-cell transplantations (ASCT). The therapeutic effect of MSCs is mainly ascribed to their suppression of (alloreactive) lymphocyte proliferation and enhancement of tissue-repair activity. However, only about half of the GvHD patients benefit from MSC therapy, and which factors determine MSC responsiveness is unclear. We recently observed that relatively high frequencies of activated type 3 innate lymphoid cells (ILC3s) before and/or after ASCT were associated with a lower risk to develop GvHD, which may be related to the production of tissue-protective IL-22 by ILC3s. To investigate whether ILC3s can contribute to the therapeutic effect of MSCs, we studied the interaction between MSCs and ILC3s in vitro. ILC3s isolated from human tonsils were CellTrace-labeled and co-cultured with human bone-marrow derived MSCs for 5 days in the presence of IL-2. Co-culture with MSCs significantly enhanced the proliferation of ILC3s and their IL-22 production. Reciprocally, ILC3s promoted ICAM-1 and VCAM-1 expression on MSCs. Transwell experiments revealed that for both directions, the interaction is mainly dependent on cell-cell contact or close proximity of MSCs and ILC3s. Addition of blocking antibodies against ICAM-1, VCAM-1, or their integrin ligands, did not affect ILC3 proliferation, suggesting that ILC3 stimulation is ICAM/VCAM independent. Soluble factors also contributed to the interaction, as ILC3s proliferated slightly better in the presence of MSC culture supernatant compared to IL-2 only. Based on experiments with blocking antibodies, we found IL-7 and AhR stimuli to be likely candidates for this effect. In conclusion, we show that via cell-cell contact and soluble factors, MSCs promote the proliferation and IL-22 production by ILC3s in vitro, suggesting ILC3s may play a role in the control of GvHD upon MSC therapy.

LTMP 46

Reticular stromal phenotypes correlate with the composition of perivascular lymphocytic infiltration in discoid lupus erythematosus and systemic sclerosis

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Perivascular leukocyte infiltrates occur in many human inflammatory conditions including autoimmune diseases such as lupus. Why leukocytes accumulate in the perivascular space is poorly understood.

Infiltrates range from disorganized, conventional perivascular cuffs to highly-organized tertiary lymphoid tissues. Tertiary lymphoid tissue architecture involves reticular stromal cells that resemble T cell zone fibroblastic reticular cells (FRC) and follicular dendritic cells (FDC) from B cell follicles. Roles for reticular stromal cells in less organized infiltrates are unexplored. We hypothesized that the appearance of specialized reticular stromal cells akin to FRC or FDC correlates with the lymphocytic composition of the infiltrate. Using skin samples from healthy donors, Systemic Sclerosis (SSc) and Discoid Lupus Erythematosus (DLE) patients we characterized the perivascular adventitial reticular cell phenotype using the markers CD90, Vascular cell adhesion molecule-1 (VCAM), and Podoplanin (Pdpn). Adventitial fibroblasts constitutively express CD90 in healthy skin. The area covered by CD90-expressing cells increases in both SSc and DLE skin, which we interpret as an expanded vascular adventitia. T cells accumulated when the adventitial reticular stroma expressed VCAM. In contrast, Pdpn expression by the adventitial reticular stroma was accompanied by B cell infiltrates. Human FRC express a mix of VCAM, Pdpn, and CD90 while human FDC express VCAM and Pdpn without CD90. These data suggest that following extravasation, specialized adventitial reticular cells in these infiltrates are involved in retaining lymphocytes.

LTMP 47

Interferon-gamma impairs expansion and alters hematopoietic support of bone marrow mesenchymal stromal cells

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The bone marrow (BM) is responsible for continuous blood cell formation, but also acts as a secondary lymphoid organ, where primary immune responses can occur. Importantly, these inflammatory reactions can influence the local hematopoietic process and skew the formation of particular blood cells, which can also lead to anemia and BM failure if the response persists. We have previously shown that interferon-gamma (IFNγ) plays a major role in this respect, as it directly affects the maintenance and differentiation of hematopoietic stem and progenitor cells (HSPCs). Here, we question whether IFNγ also affects the multipotent mesenchymal stromal cells (MSCs) in the BM. These cells are of key importance, as they provide hematopoietic support to hematopoietic stem cells (HSCs), both in vivo and upon expansion ex vivo. MSCs are also clinically relevant, as culture-expanded MSCs are currently exploited as cellular therapy for their immunomodulatory property, which is boosted by IFNγ. We treated primary human BM MSCs with IFNγ and found that it reduces their expansion capacity and viability, which is associated with upregulation of the pro-apoptotic molecule Noxa. When assessing the hematopoietic support function of MSCs in a co-culture assay with CD34+ HSPCs, we observed that pretreatment of MSCs with IFNγ increased the fraction of myeloid-committed HSPCs (CD34+CD13/33+), but strongly impaired the differentiation to monocytic (CD14+) or erythroid (CD36+) cells. To study the impact of IFNγ in vivo, we examined ARE-Del mice, which have elevated IFNγ production due to the lack of regulatory AU-rich elements in the IFNγ-3' untranslated region. BM lymphocytes in ARE-Del mice produced more IFNγ, which remodeled the BM stromal compartment and strongly reduced the number of MSCs (identified as CD45-Ter119-CD31-CD51+PDGFRα+ cells). Importantly, this reduction of BM MSCs coincided with a loss of quiescent HSCs, as only 30% of long-term HSCs in ARE-Del mice were quiescent, compared to 70% in WT mice. Loss of quiescence in long-term HSCs did not lead to increased self-renewal, but rather increased the differentiation towards short-term HSCs and multi-potent progenitors. Thus, we demonstrate that IFNγ has a negative impact on expansion and hematopoietic support of BM MSCs in vitro and in vivo, in mouse and human, leading to impaired HSC maintenance. This enhances our understanding on how (chronic) inflammation can affect hematopoiesis and thereby contribute to BM failure.

LTMP 48

Functional and structural dynamics of the bone marrow stromal microenvironment after cytoreductive therapies

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Bone marrow (BM) cavities are the primary sites of high throughput, continuous and tightly regulated production of mature blood cells during adulthood. Hematopoiesis is sustained by the proliferation and differentiation of hematopoietic stem and progenitor cells (HSPCs), which are maintained by signals emanating from stromal networks of mesenchymal, endothelial and neural origin. BM tissues are highly sensitive to cytoreductive treatments such as ionizing irradiation and chemotherapeutic agents, which are the treatment of choice for multiple malignancies and employed as conditioning regiments in BM transplantations. The cytotoxic effects and killing of rapidly cycling HPCs induced by myeloablative therapies have been extensively characterized. However, it is still largely unknown whether and to what extent these treatments target BM stromal cells. By combining conventional flow cytometry protocols and advanced 3D-imaging techniques we have investigated the structural and functional alterations triggered in the BM stromal infrastructure upon myeloablation with ionizing radiation or 5-fluorouracil administration. Furthermore, we have characterized the kinetics of regeneration of a fully competent BM microenvironment post-injury. As previously reported, cytoreductive treatments led to a severe loss of HSPCs. Notably, hematopoietic defects were accompanied by a similar profound decrease in sinusoidal endothelial and mesenchymal, CXCL12-abundant reticular (CAR) cells. Decline in stromal cell numbers was apparent 7 days after treatment and encompassed a major loss of structural integrity of the BM microenvironment. 3D imaging revealed massive sinusoidal dilation followed by appearance of ruptures in vessel walls. Partial restoration of tissue integrity was observed by day 14 post-treatment and was almost completely achieved by day 28. We observed massive de novo differentiation of mese.nchymal progenitors into adipocytes leading to adipogenic infiltration of large regions of the BM during the phases of acute damage and tissue regeneration. Of note, this process was fully reversible as virtually almost all adipocytes were cleared from BM tissues 56 days after treatment. Our observations demonstrate that while the stromal BM microenvironment is highly sensitive to myeloablative therapies, BM tissues are endowed with a robust intrinsic regenerative and self-organizing capacity that enables rebuilding of a fully functional tissue microenvironment after severe damage.

LTMP 49

Artery tertiary lymphoid organs organize aberrant atherosclerosis B cell responses in hyperlipidemic mice

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Atherosclerosis is a chronic inflammatory disease of medium-sized and large arteries. Genetic studies in mice have shown that various B cell subsets play opposing roles in atherosclerosis. However, it is not clear whether, where, and when atherosclerosis-specific autoimmune B cells are generated. Our recent studies identified artery tertiary lymphoid organs (ATLOs) in the aorta adventitia of hyperlipidemic ApoE-/- mice. ATLOs contain separate T cell areas and B cell follicles with activated germinal centers. Using transcript maps, FACS, immunofluorescence analyses, cell transfers, and Ig-ELISPOT assays, we observed that ATLOs organize multi-layered aberrant B cell responses in atherosclerosis: ATLOs showed upregulation of multiple B cell-related genes and promoted B cell recruitment from the circulation; ATLO B-2 B cells included naïve, transitional, follicular, germinal center, switched IgG1+, IgA+, and IgE+ memory cells, plasmablasts, and long-lived plasma cells (PCs); ATLOs recruited

large numbers of B-1 cells whose subtypes were skewed towards IL-10+ B-1b cells vs. IL-10- B-1a cells. Moreover, ATLO B-1 cells and PCs constitutively produced IgM and IgG and a fraction of PCs expressed IL-10. These data show that innate and adaptive atherosclerosis B cell responses are organized within the arterial wall adventitia and raise the possibility that atherosclerotic autoimmune B cells are generated in ATLO germinal centers.

LTMP 50

Fibroblastic reticular cells facilitate myeloid cellular crosstalk via the provision of FRC derived growth and chemotactic factors

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Lymph nodes play a pivotal role in the maintenance and initiation of the host's immune response through their ability to facilitate dynamic interactions between various cells, resulting in immune activation or tolerance. Non-hematopoietic cells have recently been discovered to be important in these cellular interactions. In particular, fibroblastic reticular cells (FRCs) are crucial to lymph node homeostasis, playing unique immunoregulatory roles within the lymph node. FRCs have previously been shown to secrete chemokines, cytokines and growth factors to facilitate leukocyte migration and priming within the periphery, to achieve functions encompassing lymphocyte survival, deletional tolerance, antigen presentation and T cell suppression. Here, we focus on the immunological interactions that occur between FRCs and myeloid cell types in mouse and human model systems. Transcriptomic analysis of human and mouse FRCs suggest that FRCs constitutively produce factors associated with the innate immune response. Both human and mouse FRCs express CCL2 and CXCL12, providing stimuli for the chemoattraction of macrophages and monocytes. Accordingly, lymph node imaging showed that a significantly higher frequency of medullary myeloid cells was attached to FRCs than not attached, and that attached cells had a significantly higher ratio cell perimeter to area, showing elongation associated with attachment. As lymph nodes are sites of inflammation, we stimulated FRCs with LPS or Poly I:C and found p38/p65 phosphorylation, indicating active toll like receptor signaling pathways. LPS stimulation of human FRCs were also shown to increase the production of CCL2, IL-6 and IL-8 after 24 hours. Strikingly, we report that in vitro interactions between FRCs and monocytes were sufficient to induce monocytes to adopt a macrophage phenotype. In mouse and human cells, this occurs through the provision of FRC-derived growth factors that signal through CSF1R. Notably, TLR4 stimulation of mouse FRCs dramatically enhances monocyte differentiation. In vivo studies showed that FRC surface phenotype was altered following macrophage depletion, indicating likely two-way crosstalk between FRCs and myeloid cells within secondary lymphoid organs. Our data reveals a novel role for FRCs in the regulation of myeloid cell differentiation within secondary lymphoid organs.

LTMP 51

The role of tissue-specific mesenchymal stromal cells in initiating early inflammatory processes and tertiary lymphoid structures formation in systemic lupus erythematosus patients

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Formation of tertiary lymphoid structure (TLS) occurs in tissues targeted by chronic inflammatory processes, such as infection and autoimmunity. In systemic lupus erythematosus (SLE), TLSs have been observed in the kidneys of lupus-prone mice and kidney biopsies of SLE patients with Lupus Nephritis (LN). Here we investigated the role of tissue-specific mesenchymal stromal cells (MSCs) as lymphoid tissue organizer cells on the activation of CD4+ T cells from three groups; Healthy, SLE patients and LN patients, five donors in each. Human MSCs were stimulated with the pro-inflammatory cytokines TNF-α and IL-1β to resemble an inflammatory condition. CD4+ T cells isolated from PBMC were co-cultured with stimulated (sMSCs) and non-stimulated MSCs (nsMSCs) at 1:1 and 1:100 ratios (MSCs:CD4+ T cells) or seeded alone as a control. Proliferation assay was performed on CD4+ T cells at day zero, 5, 7 and 10 after co-culture. Flow cytometric analyses were conducted on CD4+ T cells at day zero and day 10 to analyze Th1, Th2, Th9, Th17, Th22, and Th1/17 subsets before and after co-culturing with MSCs. In addition, we made a 3D model of developed TLS using complete serial histopathological images of a kidney from a (NZBxNZW)F1 mouse. We used confocal imaging to detect MSCs within TLS in kidneys of lupus-prone mice. After stimulation, a significant increase in the expression of CCL19, VCAM1, ICAM1, TNF-α, and IL-1β was observed in MSCs. For all groups CD4+ T cells co-cultured with sMSCs and nsMSCs at 1:100 ratio proliferated significantly more at day 10 compared to day zero and CD4+ T cells alone at day 10. CD4+ T cells co-cultured with sMSCs at 1:100 ratio proliferated significantly more than co-cultured with nsMSCs at day 10 in healthy and SLE groups, but not in the LN group. We detected no difference in cell proliferation at 1:1 ratio. A significant increase in Th2 and Th17 subsets were observed in the healthy group at day 10 when co-cultured with sMSCs at 1:100 ratio compared to day zero and CD4+ T cells alone at day 10. We could detect stromal cells within the pelvic wall of the kidneys and within the developed TLS. The 3D structure of the TLS within the kidney revealed a network of areas that seemed to be connected. Our data suggest that tissue-specific MSCs could have pivotal roles in accelerating early inflammatory processes and initiating the formation of TLS in chronic inflammatory condition.

LTMP 52

Deep sequencing of renal specific tertiary lymphoid structure and lymph nodes in lupus-prone mice

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Tertiary lymphoid structures (TLS) are accumulations of well-organized lymphoid cells that resemble secondary lymphoid organs (SLO) in their cellular contents and presence of high endothelial venules (HEV) and lymphatic vessels (LV). Chronic inflammatory processes, such as infection and autoimmunity cause TLS to develop within different organs. Systemic lupus erythematosus (SLE) is a chronic autoimmune syndrome characterized by systemic inflammation and damage, and may lead to the development of lupus nephritis. In a longitudinal study on lupus prone mice (NZBXNZW)F1, we have observed within the kidneys the formation of TLS during the progression of the disease. These structures contained all cells characteristic for SLO and proved to be functional ectopic germinal centres. In the present study, our purpose was to investigate whether the TLS within kidneys of lupus prone mice was similar to SLO with regard to gene expression profile. We performed RNA sequencing of transcriptomes isolated from lymph nodes (LN) and macro-dissected TLS from kidneys of mice at different stages of the disease by using Ion torrent Personal Genome Machine (Ion PGM). Based on annotation and gene ontology analysis we

profiled the expressed mRNA transcripts according to known biological processes. TLS isolated from the kidneys of proteinuric mice expressed the same immunoglobulin, recombinase gene transcripts and T cell receptor transcripts as LN isolated from both antibody positive and proteinuric mice. The expression of Ltα, Ltβ and lymph node homing cytokines were expressed in all tissue. We also found expression of genes related to cell – cell adhesion such as ICAM1, ICAM2 and VCAM1 and other genes important for development of lymphoid tissue. The differentially expressed transcripts in TLS compared to LNs were involved in biological processes like tissue development (nervous), tissue morphogenesis, and angiogenesis among others. We conclude that TLS in kidneys of lupus prone mice have the same gene signature as LNs and thus could have the same function as activated LNs. The induction of anti-dsDNA antibodies, formation and deposition of immune complexes, and the formation and expansion of TLS in lupus nephritis, may promote progression into an end stage kidney disease in SLE. Treatment strategies targeting the development of TLS may be important for preventing the development of lupus nephritis.

LTMP 53

IL-33 produced by fibroblastic reticular cells and lymphatic endothelial cells protects from chronic viral infection

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Upon viral infection, virus-specific CD8+ T cells are activated in secondary lymphoid organs (SLOs) becoming cytotoxic T lymphocytes (CTL), capable of producing inflammatory mediators such as IFNγ and TNFα and cytotoxic molecules such as perforin and granzyme B for direct killing of the infected cells. Recent findings showed that Interleukin-33 (IL-33) signaling early during viral infection is required for the efficient generation of CD8+ T cell responses to various viruses. Interestingly, splenic T cell responses to acute LCMV infection depended on IL-33 produced by stromal rather than hematopoietic cells. Interleukin-33 (IL-33) is expressed constitutively by several stromal cell types and most epithelial barrier cells. Stressed or damaged cells can release “alarmins” like IL-33 that act as endogenous danger signals alerting and boosting innate and adaptive immune cells. Although IL-33 plays a key role in mounting antiviral T cell immunity, it is unclear which stromal cell type releases the critical IL-33 and under which conditions. Here, using IL-33 reporter mice we identify fibroblastic reticular cells (FRC) and lymphatic endothelial cells (LEC) as the main IL-33 source in lymph nodes of naïve mice as well as of mice infected with LCMV clone 13. IL-33-producing FRCs were found both in the T zone and medulla, often colocalizing with virus-specific CD8+ T cells. IL-33+ LEC were mainly observed in the medulla, and regularly localized close to virus-specific CD8+ T cells suggesting either LEC, FRC or both are critical IL-33 sources. In the absence of IL-33, these T cell responses were strongly reduced within draining lymph nodes, similar to the published defect in splenic acute responses. Currently we generate mice lacking IL-33 selectively in either FRC or LEC to identify the critical IL-33 source, both in lymph nodes and the spleen. Other efforts aim to improve our understanding of the role of stromal cell infection in IL-33 release, and its precise role for T cells responses. Together, these findings highlight the critical contribution of stroma-derived IL-33 to CD8+ T cell responses during viral infection and should allow to gain insight into an intriguing function of IL-33.

LTMP 54

Multiscale image-based quantitative analysis of bone marrow stromal network topology reveals strict spatial constraints for hematopoietic-stromal cellular interactions

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Adult bone marrow (BM) cavities are vital for the maintenance of a rare population of hematopoietic stem cells (HSCs), which continuously replenishes all mature blood cell types in a demand-adapted and dynamic manner. Besides hematopoietic cells, the BM is populated by a heterogeneous fraction of mesenchymal, endothelial and neural stromal cells, which provide the necessary tissue infrastructure for hematopoiesis to unfold and play essential functional regulatory roles. Recent evidence suggests that tissue regions around BM venous microvessels (termed sinusoids), which are enriched for mesenchymal CXCL12-abundant reticular cells (CARc), serve as the principal regulatory niches for HSCs as well as other hematopoietic progenitor populations . Despite this proposed role as putative cell-specific niche restricted components, comprehensive data on the frequency, global spatial distribution and topology of sinusoidal endothelial and CAR cell networks is largely lacking to date. We have developed (i) advanced microscopy techniques allowing for multiscale 3D visualization of entire bone marrow cavities with cellular and subcellular detail; (ii) customized computational tools enabling the detection and quantification of discrete cell subsets/structures in 3D images of the BM in an unbiased fashion, and a rigorous spatial statistical analysis of cellular interactions. Using 3D-quantitative microscopy (3D-QM) we uncover that BM stromal cells are in fact 10–20 fold more abundant than previously reported. The massive underestimation of these relevant cell subsets results from the highly inefficient isolation of these cellular types with currently employed flow cytometry protocols. Our image-based analyses further reveal that sinusoidal and CAR cell stromal networks occupy a disproportionately large fraction of the BM space, consequently constraining the tissue volume available for hematopoietic cell distribution. In fact, the vast majority of BM resident hematopoietic cells are unavoidably in direct contact with the CAR cellular projections and in close proximity (<25 µm) to the extraluminal surface of sinusoidal endothelium. Collectively, our quantitative description of stromal microarchitecture challenges current models of cell type-specific niche interactions in the BM, which we demonstrate to be based on largely inaccurate estimations of cell frequency and spatial confinement of stromal cells in this organ.

LTMP 55

CCL19-producing fibroblastic stromal cells control CD8+ T cell memory inflation following adenoviral immunization

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CD8+ T cell memory inflation is defined as an accumulation of large numbers of antigen-specific effector memory CD8+ T cells residing mainly within non-lymphoid organs. We have shown previously that CD8+ T cells reacting against a particular set of antigenic peptides show a high propensity to memory inflation making such epitopes excellent constituents of vaccines such as recombinant adenoviruses. While persistent antigen presentation by non-hematopoietic cells has been shown to be instrumental for the maintenance of inflating CD8+ memory T cells, the identity and localization of these stromal cells remains elusive. We have developed a Cre recombinase-dependent, b-galactosidase (bgal)-recombinant adenovirus vector that allows for cell-specific expression of two H2-Kb-restricted bgal epitopes. We found that expression of the inflating (bgal96) and classical-memory forming (bgal497) epitopes exclusively in CCL19-producing fibroblastic stromal cells resulted in induction of robust CD8+ T cells responses. In contrast, targeting the expression of both epitopes to CD11c-expressing dendritic cells, LysM-expressing myeloid cells, or VE-cadherin-expressing endothelial cells failed to elicit significant bgal-specific CD8+ T cell activation. Moreover, generation of bone marrow chimeric mice to abolish major histocompatibility complex I (MHC-I) expression by hematopoietic cells revealed that presentation of bgal epitopes by CC19-producing cells alone was sufficient to induce CD8+ T cell memory inflation. Overall, our data reveal a hitherto unknown role for CCL19-expressiong fibroblastic stromal cells in the induction and maintenance of inflating CD8+ memory T cells.

LTMP 56

CD44 regulates nitric oxide production by fibroblastic reticular cells to promote chronic viral infection

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The role of adhesion molecules in the regulation of immunity to chronic viral infections has been poorly investigated. Using the chronic Lymphocytic Choriomeningitis Virus Clone 13 model (LCMV-Cl13), we found that expression of CD44, a cell surface glycoprotein, plays a critical role in suppressing virus-specific T cell responses to enable the establishment of chronic viral infection. In Cd44–/– hosts, we observed a striking increase in multifunctional effector CD4 and CD8 T cells that was linked to downregulation of multiple inhibitory receptors, including PD-1, Lag-3, CD160 and Tim-3, as well as to viral clearance at 15 days post infection (dpi). Underscoring this dramatic release of the anti-viral response from immune checkpoint inhibition, viral clearance occurred at the expense of increased morbidity, with 30% of the Cd44–/– mice succumbing to the infection. Using a bone marrow (BM) chimera approach in which Cd44–/– hosts were reconstituted with WT BM to restrict CD44-deficiency to non-hematopoietic cells, we observed comparable changes in CD4 and CD8 T cells together with viral clearance. In contrast, restriction of CD44-deficiency to T cells was not sufficient to promote better immunity to LCMV-Cl13 but instead decreased antigen specific T cell accumulation. Fibroblastic reticular cells (FRCs) are non-hematopoietic cells found in the T cell zone of secondary lymphoid organs, express CD44 and are essential immune-regulators. Importantly, they can block T cell proliferation and survival via nitric oxide (NO) production. RNA-seq analysis revealed that nitric oxide synthase 2 (Nos2) transcripts were lower in Cd44–/– compared to WT FRCs at 3 dpi, which was confirmed at the protein level in vivo. Furthermore, in vitro stimulation of FRCs with IFN-gamma and TNF-alpha induced significantly less Nos2 expression and NO production by Cd44–/– FRCs and treatment with the CD44-ligand, osteopontin increased NO production by WT FRCs. Importantly, treatment of WT mice with a CD44-blocking antibody increased the recovery of virus-specific CD4 and CD8 T cell with improved T cell function as early as 9 dpi, along with a significant reduction in viremia, suggesting that CD44 can be targeted to reinvigorate the immune system during chronic viral infection. From these results we propose that CD44 is a novel immune checkpoint regulator that promotes NO production by FRCs to support virus persistence and dampen the immune response to prevent immunopathology.

LTMP 58

The lymphatic system: a gatekeeper for migration of pathogenic T-cells towards synovial joints and entheses in psoriasis

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Background: Psoriasis (PsO) is characterized by acanthosis, impaired immune cell migration, and remodeling of the vascular and lymphatic system. Up to ~30% of PsO patients develop psoriatic arthritis (PsA). The lymphatic system may control the migration of pathogenic T-cells to either skin or synovial joints and entheses.
Methods: Human dermal lymphatic endothelial cells (LEC; 0.5×10^4), and fibroblast- like synoviocytes of a patient with PsA (PsA-FLS; 1.0×10^4) were pre-incubated for 3 days with media or PsA synovial fluid (PsA-SF; 10/20% v/v). Then, LEC or PsA-FLS were co-cultured with 2.5×10^4 CD4+CD45RO+CD25– T-cells that were sorted from healthy donors with or without stimulation with αCD3/αCD28. After 72 h, T-cells were immunophenotyped by flow cytometry. Relevant T-helper (Th) subsets were characterized, including the CCR6+ subsets Th17.1 (CCR4–/CXCR3+), Th17/Th22 (CCR4+/CXCR3–), Th17 (CCR4+/CXCR3–/CCR10–) and Th22 (CCR4+/CXCR3–/CCR10+). We also looked at cutaneous lymphocyte- associated antigen (CLA), a skin homing receptor. IL-17A, IL-22, and TNF protein levels in the co-cultures were determined by ELISA.
Results: Stimulation of CD4+CD45RO+ T-cells in co-culture with PsA-FLS skewed towards the CCR6+ subset Th17/Th22, which were predominantly Th17 cells. Th17 differentiation was suppressed in co-culture with LEC even when the LECs were pre- incubated with PsA-SF. Stimulation of CD4+CD45RO+ T-cells in co-culture with LEC, as compared to PsA-FLS, promoted the generation of the Th22 subset. Upon co- culture, activated LEC conserved CLA expression on stimulated CD4+CD45RO+ T- cells at a higher level than PsA-FLS, particularly in the CCR6+ T-cell subset. In line with FACS results, a trend towards lower IL-17A and higher IL-22 levels were observed in the co-cultures with LEC that were pretreated with PsA-SF 20%, as compared to the co-culture with PsA-FLS. Blockade of the lymphotoxin beta receptor (LTbR) pathway during co-culture of the CD4+CD45RO+ T-cells and LECs, but not NO pathway, resulted in higher IL-17A levels, and higher proportion of the Th17/22 subset.
Conclusion: LECs are directly involved in T-cell differentiation, and homing capabilities, as shown by suppression of Th17 differentiation in co-culture experiments, as compared to PsA-FLS. Also, LEC promoted Th22 generation, and conserved CLA expression in CCR6+ T-cells. The LTbR pathway may be involved in LEC mediated modulation of T-cell homing and deserves further exploration.

LTMP 59

Neuropilin-1 is expressed on lymphoid tissue residing LTi-like ILC3s and associated with ectopic pulmonary lymphoid aggregates

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The study shows that Neuropilin-1 (NRP1) is a functional marker for human and mouse LTi-like ILC3s, a finding that provides an important tool to study these cells in health and disease. We demonstrate that NRP1+ ILC3 are present in lymphoid tissues but not in the peripheral blood or skin, and these cells display in vitro Lymphoid tissue inducer activity. We demonstrate that the NRP1+ ILC are primed cells as, like memory T cells, they express CD45RO and produce higher amounts of cytokines than NRP1- ILC3, which express CD45RA. We observe distinct functional features between lymphoid and peripheral blood ILC3, as IL-1β induced NRP1 expression on NRP1 negative ILC3 from lymphoid tissue but not on peripheral blood ILC3. In addition, ILC3 in secondary lymph nodes either do express NRP1 or have the capacity to upregulate NRP1, providing for the first time a clear distinction between ILC3 and ILC3/LTi cells in humans, despite their overlapping phenotypes and cytokine production profiles. Finally, we show that NRP1+ LTi cells are present in inflammatory aggregates in lungs of smokers and COPD patients providing insight into the initiation of smoke-induced ectopic pulmonary lymphoid aggregates in the lungs.

The numbers refer to the pages of this supplement.