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Abstracts
Oral Communications

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Impressum

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

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 amenorrhoea, hirsutism and weight gain. On clinical examination she had acanthosis nigricans, clitoromegaly and virilisation. LH-RH stimulation test showed LH levels not to be reduced significantly, while LH-RH did not inhibit hormonal responsiveness. 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 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 the ovarian tumor occurs through the backdoor pathway such as RoDH (retinol dehydrogenase), CYP17A1 (17α-hydroxylase) and AKR1C3 (aldo-keto-reductase family 1 C3). Postoperatively, androgen levels normalized and menstrual bleedings became regular. But 12 months later, severe acne and amenorrhoea 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. Therefore, LH-RH could be used to treat our patient’s tumor. However, these drugs are not in routine use in pediatrics.

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 523 (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: Of 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.
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 symptoms 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 weekly 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.

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

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Background: Newborn screening (NBS) for cystic fibrosis (CF) is based on immuno-reactive trypsinogen (IRT)-DNA-IRT algorithm, was introduced in Switzerland in 2011. The program aims to detect all children with CF, to avoid detecting children with “CF screen positive, inconclusive diagnosis” (CFSPIID). 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, 356 (66.8%) children were CF negative, and two have yet unknown diagnosis. Ten (1.9%) children died of severe diseases (e.g. 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 CFSPIID cases were included. The specificity (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 for CF, pancreatitis-associated protein (PAP) measurement, as a further technique or a more efficient safety-loop, is under evaluation.

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


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Background: Asthma is more frequent in children with atopic dermatitis (AD) compared to children without AD. The timing of onset and progression in childhood AD may impact the development of asthma in the long run. The presence of other atopic diseases might be associated with specific AD phenotypes.

Methods: We performed a secondary analysis on 344 asthmatic children, who stayed in the rehabilitation program for at least 14 days. We compared the baseline, the discharge, and the follow-up spirometry, nitric oxide measurements (FeNO). Associations with allergic sensitization (skin prick testing and/or specific IgE), level of asthma control and level of inflammatory cytokines (IL-5 and IL-13) were assessed. Children were classified into three main phenotypes: type I patients had high levels of IL-5 and IL-13 and were sensitized to house dust mites and birch pollen. Type II patients had low levels of IL-5 and IL-13 and were sensitized to house dust mites, birch pollen, or cat. Type III patients had low levels of IL-5 and IL-13 and were sensitized to birch pollen only.

Results: Pulmonary conditions improved significantly on average during the sojourn. Patients with uncontrolled asthma benefited most with an increase of FEV1, MEF25 and MEF75 by 7.7, 9.9 respectively. A decrease of 9.9, 7.7 pp was found in children with asthma not meeting control criteria. Children with FeNO had a significantly lower increase of IL-5 and IL-13 compared to children without FeNO. Children with FeNO had a significantly lower increase of IL-5 and IL-13 compared to children without FeNO. Children with FeNO had a significantly lower increase of IL-5 and IL-13 compared to children without FeNO. Children with FeNO had a significantly lower increase of IL-5 and IL-13 compared to children without FeNO. Children with FeNO had a significantly lower increase of IL-5 and IL-13 compared to children without FeNO.
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%).

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 associated with having an early-onset 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. 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.

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 sorosis. 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 sorosis (5/39). Among the 61 patients, 39 received a treatment with NSAID (64%), 26 systemic corticosteroids (43%), 5 intra-articular steroids (8%), 36 Methotrexxate (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% Tolctizumab (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-IL-1 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 decision-making process. Some elements favored good communication between parents and the healthcare 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 depreciated 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 clinical management in VLBW infants. Besides, CVC are the major risk for catheter-associated bloodstream infections (CABSI) which per se are related to significant morbidity and mortality. Therefore, invasive central venous catheters (CVC) are essential for clinical management in VLBW infants. Besides, CVC are the major risk for catheter-associated bloodstream 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 automated 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 in the laboratory software to a central database. All VLBW infants aged ≤90 days with placement of a CVC between January 2011 and December 2016 were included. CABSI was defined as presence of any laboratory results were directly entered by the correspondent clinician in the laboratory software to a central database. All VLBW infants aged ≤90 days with placement of a CVC between January 2011 and December 2016 were included. CABSI was defined as presence of any laboratory test result consistent with the clinical syndrome of bloodstream infection.

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 results 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 ≤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 (CrVG) 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 192 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 involvement. Given the different neurological findings, 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 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 transitory 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 transitory psychotic disorder treated with risperidone. 48h later she presented general tonico-clonic seizure. CSF showed no abnormalities including lymphocytosis and oligoclonal bands. Diagnosis shows non-specific T2 hyperintensity in hippocampus, frontal and parietal lobes. Group A includes healthy hips that need no further intervention and can be discharged. Group B hips are dysplastic with trapped cartilage need, after an initial try with an orthosis, surgical management. Group C includes dysplastic hips that need immediate treatment to perform “hip-friendly” swaddling with abduction and flexion of both hips. Group D includes dysplastic hips that need immediate treatment. Group E constitutes to Graf, to only 4 types. Group A hips are healthy hips that need no further intervention and can be discharged.

Methods: A simplification of its necessary for those children with DDH, a simplification of its diagnostic criteria was also justified.

Conclusion: Early diagnosis and non-surgical treatment of DDH has corresponded to an incidence of 17 per 1000 children with DDH, which means that more than 3.500 children with DDH could be successfully treated in Switzerland.

Results: In the period between 1/4/2014–31/12/2016, 1045 voluntary cases 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/m2 (N = 274, 26%); Abdominal circumference >94.0 cm (N = 177, 11%); Arterial blood pressure ≥140/90 mmHg (N = 83, 8%); 25-OH-vitamin D3 D3 rate ≤50 mmol/L (N = 201, 19%); total cholesterol ≥5.2 mmol/L (N = 54, 5%); uricemia ≥500 mmol/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 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 cases 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/m2 (N = 274, 26%); Abdominal circumference >94.0 cm (N = 177, 11%); Arterial blood pressure ≥140/90 mmHg (N = 83, 8%); 25-OH-vitamin D3 D3 rate ≤50 mmol/L (N = 201, 19%); total cholesterol ≥5.2 mmol/L (N = 54, 5%); uricemia ≥500 mmol/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.

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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 launched 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 ultrasound 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.
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 patient 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 their 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, 31 (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 enhanced care offer, a 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.
(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 cardiac education 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 recommendations. However, the clinical significance and the appropriate management of elevated BP at a single visit remain to be established.

**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 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 non-drug 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 visceral pain load in the gut. Studies in adults 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 in the initial phase and one patient was still in the testing phase. After reintroducing carbohydrates during the daily food plan, three patients stayed asymptomatic and tolerated the diet well, one with no food restrictions, one with lactose free and one with wheat, lactose and low fructose diet. The average follow up time was 23 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.

**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-to-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 minute, 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 during CC, parental PA specific, staff support (inverse), and mobile equipment. Significant correlates for SB were written PA convention (inverse), socio-cultural 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 to promote 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 correlates.
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 effectiveness.

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.
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: Neonates 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 (65%) 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% (0.0–0.3). 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% (16/72), 12% (33/278) and 0% (79/79) 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% (279) 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%, 45/278) in HA-LOS, and Staphylococcus epidermidis (22%, 75/339) and Group B Streptococcus (11%, 37/319) 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, pathogenesis and outcomes.

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.
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 = 50], biological DMARDs [n = 59], 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 increased by 8% during the observation period. Compared with age adapted healthy Swiss population vaccine coverage was 14% lower in PedRD patients at reference date.

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.

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 outcome 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 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.

Primary results: SPAC 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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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 any 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.

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 (SaO2).

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 compared to clinical signs or a combinations of sign. Cyanosis had a good diagnostic 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 unnecessarily.

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 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.

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.
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 and are necessary for treatment. 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 allows 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 prepared hotkeys for side of hip (left and 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 uploaded daily by the screeners 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 which are 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.

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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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 axillary fold to 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. It 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.

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 dysgenesys (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.
Variations of Currarino triad
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Background: Currarino triad is a rare congenital disease with a prevalence of 1-8/100,000 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 either 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.

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 from a consanguineous family presented with bleeding of the trocar wounds. On admission, she underwent diagnostic laparoscopy with resection of a presacral mass. A neurogenic bladder must not be overseen.

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.

Correlates of preschool children’s 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 are 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 study aimed to compare the answers from the parental questionnaire with a standardised motor test.

Methods: The data were collected within the frame of the Swiss Preschooler’s Health Study (SPLASHY). 449 children typically developing between ages 2 and 6 years of age (213 female, M age = 3.9 years, SD = 0.7, range 2.5–6.6 years) were examined. Motor skills were measured 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 <6, n = 22) differed from children with normal motor development (score ≥6) 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.
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.

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

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 severe 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 heart. 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.

Behavioural problems, eating disorder and short stature: 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 Methyphenidate (Ritaline®) at 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.
Pedia tic 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 dermatological problems encountered in Southern Switzerland.

Methods: Retrospective data collection from September 2015 (begin of the Pediatric Dermatology 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). In total, five to six patients were seen each session. 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 pediatric subspecialists, dermatologists and parents.

The most frequent diagnosis were: inflammatory diseases (like atopic dermatitis) followed by intestinal hemorrhagic and benign cutaneous tumors, viral infections (like molluscum contagiosum and warts), angiomata 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. Pediatrician 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.
**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 unnessecary procedures in unclear cases.

**Take home message:** All suspicion 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.

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

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Glycogen storage disease 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-hereditary red blood cell 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 a 6-month-old boy of Syrian descent diagnosed with GSD-I at age 8 months by liver biopsy and treated with allopurinol, fenofibrate and frequent corticosteroid administrations. Upon arrival in Switzerland he presented with dehydration, electrolytic imbalances, recurrent fasting hypoglycemia, severe lactic acidosis, growth failure, developmental delay, hypertigrycideremia, hyperuricemia, hepatospplenomegaly, anemia, mild neutropenia and proteinuria. He was hospitalized for IV rehydration. Continuous nocturnal lube 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 SLC37A4 data revealed a second, non-identifiable but highly suggestive 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 case of a 6-month-old boy 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.

**Glycogen storage disease Ib**

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**Background:** Glycogen storage disease type I (GA-I) is a rare autosomal-recessive disorder of the degradation of the amino acids lysine and tryptophan carried out by the enzyme GA-1. The encoded enzyme permits the glycogen metabolism to be cleaned from the glycogen that has been degraded by glycogen phosphorylase. Deficiency of glycogen resistance (here GA-1) is part of the NBS scheme and respectively defect (here GA-1) is part of the NBS scheme and metabolism a thorough workup has to be performed, even if the 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 false negative newborn screening results had been negative. Screening tests can neither be performed nor performed.

**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 inborn metabolic phenotype can easily be missed by NBS as this example shows.

**Parechovirus is not rare and no more unknown; do not forget it!**

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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 severe illness in neonatal and young infants and in older children, and we present 3 cases of neonatal CNS HPeV infection. 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 hemimascara of unclear origin. In all three cases CSF HPeV RT-PCR led to diagnosis. In one case, it was an incidental finding thanks 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 metabolite, 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 scientific culture and clinical experience can also help 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!
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 dominant or recessive 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 macromacrosomic male infant was born at 33 1/7 weeks of gestational age at 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 μU/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 (c.1801G>A, p.Val601Ile) 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 persistent 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.
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 metatal stenosis. No biopsy 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 metatal 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. We report two publications describing patients, which are under preexisting oral anticoagulation to prevent thromboembolism or intoxications with superwarfarins (rodoxidines). 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 the 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 beneficial coincidence of doses because of the long half-life. In contrast, 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. Limiting 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 a one-year’s child 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, loded 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 NASPAN recommend an emergent 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, ear stifness, tracheoesophageal fistula, mediastinitis 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. Esophagogram helps to diagnose stenosis and to exclusion of the extension of lesion.

Conclusion: Life threatening complication can follow BB ingestion. It is important to know them in order to avoid severe outcome. Parents should also be aware of the danger associated with ingesting BB.
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 BHN, 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 BHN 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 general or tumor involvement. Due to low free T4 and free T3 (8.0 and 4.5 pmol/L) levels and increased TSH (65.6 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 hemangiomatosis in infants, there is no evidence, whether thyroid tests are necessary in all children concerned. Therefore, further research is needed, all the more as BHN 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].


SGP 27

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 concomitant 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 likely pathogenic variants in ATM. Further investigations confirmed a combined severe immunodeficiency. He was 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, a low level of immunoglobulins 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 mosaicism name as the patient 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 maligny associated with this disorder.

SGP 29

SGP 28

Acute abdominal pain in an adolescent girl with Mayer-Rokitansky-Küster-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 15½ year-old girl with previously known Mayer-Rokitansky-Küster-Hauser-syndrome was admitted for acute abdominal pain in the right lower quadrant. The clinical examination, laboratory results (CRP 1.9 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 performed and...
showed a ruptured follicle 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 possibility of appendicitis, perforation of tubo-ovarian infectious disease, cystitis, psychosomatic conditions nd many more. In this case the diagnosis was complicated by the fact, that the intraabdominal anatomy was different because of the Mayers-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 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 redness, pain or palpable bony step-off around the orbital rim. There was no proptosis and the remainder of his optical exam was normal including extracocular 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 subcutaneous 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 incidence 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’, can occur when pressure 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. In many cases there is a history of trauma and fracture of an orbital bone.

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 visible. 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 penicillin and observed for 3 months. At present, the patient is doing well.

Discussion: Children affected by Lyme neuroborreliosis typically show cranial nerve palsy 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, CSP 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.

References
HSV type 2 conjunctival 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 conjunctivitis 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 no fever. On the 8 day postpartum and based on clinical diagnosis of bilateral conjunctival conjunctivitis, the newborn was treated empirically with Polymyxin B suflas and Neomycin drops. At 10 days postpartum, a conjunctival swab was obtained due to persistence of symptoms and treatment was switched to Ofloxacin 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 in a pannular form with Chlamydia involvement in up to 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 1% 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 Acriflavin 40 mg/kg/day, Truffinidine ophthalmitic 1 drops and an topical neomycin/erythromycin/fluoromethasone with no ophthalmologic sequelae up to most recent follow up. The reported case presents a rare case of conjunctival co-infection 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 conjunctival 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 antibacterial therapy.

HSV type 2 conjunctival infection with bacterial coinfection

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 hip. She was on daily treatment with Hydroxycarbamid (20 mg/kg/d), Amoxicillin (20 mg/kg/d, prophylaxis) and folic acid. On the 4 day postpartum, the newborn presented with bilateral conjunctival erythema and purulent secretions no fever. On the 8 day postpartum and based on clinical diagnosis of bilateral conjunctival conjunctivitis, the newborn was treated empirically with Polymyxin B suflas and Neomycin drops. At 10 days postpartum, a conjunctival swab was obtained due to persistence of symptoms and treatment was switched to Ofloxacin 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 in a pannular form with Chlamydia involvement in up to 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 1% 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 Acriflavin 40 mg/kg/day, Truffinidine ophthalmitic 1 drops and an topical neomycin/erythromycin/fluoromethasone with no ophthalmologic sequelae up to most recent follow up. The reported case presents a rare case of conjunctival co-infection 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 conjunctival 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 antibacterial therapy.

Extremity pain in a child with sickle cell disease

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.

Case report: A 14-year-old boy who presented with TBE despite vaccination.

Tick-borne encephalitis despite immunisation

Tick-borne encephalitis despite immunisation

Tick-borne encephalitis despite immunisation

Tick-borne encephalitis despite immunisation

Tick-borne encephalitis despite immunisation

Tick-borne encephalitis despite immunisation
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 ticks (for eg. forest rangers) and living in endemic areas may benefit from a shorter booster interval (as recommended in Austria and Germany).

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.

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. Superseding tick-borne encephalitis as 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 tick-biting Amoq -Clav since 8 days for a presumed bacterial superinfection with cellitidis 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 around the tick burris and crusts were 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 antibiotic. No other diagnostic measures were initiated. 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 rocky streams. Francisella tularensis serology was positive in arrtG. Oral treatment with Ciprofloxacin was started. Follow-up 2 weeks later showed resolution of the ulcer and retroauricular lymphadenitis.

Case presentation: 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.

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. Going in certain cases – the most common being ulceroglandular disease.

Case presentation: Summary: A 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, he 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 no abnormalities would be ruled out. The final diagnosis was a septic encephalitis. In the clinical diagnosis of a meningitis, we excluded all other pathogens, in particular viral, by the CSF analysis. Furthermore, the absence of CSF 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 the 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 encephalitis had a positive PCR in CSF. The cerebral MRI images showed basal ganglia involvement, which we also 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.

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, rhinorhea, cough and weight loss. Six days earlier, he started amoxicillin for bilateral 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 pterygoid plates, of the left ethmoidal cells, maxillary and sphenoidal sinuses bilaterally. The patient underwent endoscopic drainage of the abscessed associated with anterior ethmoidectomy and middle meatoctomy in the left side. Corticotherapy was given for 5 days, and then successfully tapered off.

The男孩在14个月大的时候从肯尼亚收养了。肯尼亚的筛查显示，病毒载量为53,000拷贝/毫升。

He had fever for a week (39 °C), otalgia, rhinorhea, cough and weight loss. Six days earlier, he started amoxicillin for bilateral 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 pterygoid plates, of the left ethmoidal cells, maxillary and sphenoidal sinuses bilaterally. The patient underwent endoscopic drainage of the abscessed associated with anterior ethmoidectomy and middle meatoctomy in the left side. Corticotherapy was given for 5 days, and then successfully tapered off.
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 oculomotoric irritability. 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.

SGP 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 which 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 migrating 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 coinfected 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.

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

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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 urinanalysis showed leucocytes and blood. Pyleonphritis 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-iliiitis. Parenteral antibiotic (amoxicillin, clavulanate) was given. Blood cultures grew Staphylococcus aureus, and therapy was changed to fluocxacine. 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 sacroliliitis is particularly rare in paediatrics (~1–2% of osteoarticular infections). Staphylococcus aureus is the most frequent pathogen. Clinical presentation of septic sacro-iliiitis is variable and may include nonspecific signs such as febrile back pain and fever. 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-iliiitis 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.
A rare manifestation of Lyme disease

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Clinical case: A 14-year-old boy was treated quickly and without complications by endovascular surgery, because of reduced level of consciousness. Incidental finding on brain CT scan led to the diagnosis of coarctation of the aorta, which could be confirmed with Borrelia serology. 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.

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 back pain on chest. Cardiac ultrasound 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-notchting 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.

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 symptoms during childhood can be epistaxis, followed by symptomatic pulmonary arteriovenous malformations (PAVMs) in 70% of all patients by the age of 16. Case report: We report a case of an infant with proven HHT and his three children (a boy of 16 A, a girl of 13 B, and girl of 11 C years) at the age of 46. The father became symptomatic with an ischemic stroke most likely due to paradoxical thrombosis 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 echocardiography 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 clincially 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 children B and C is pended.

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.

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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 cardiac 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 period of 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 detected. 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.

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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.

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

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Introduction: Trimethylaminuria (TMU) 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. TMU is therefore also known as “fish odor syndrome” (FOS). We report two pediatric patients (aged 5 and 10 years, respectively) with TMU.

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 unrevealed 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.

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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
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 to estimate percentage of Fat Mass (FM%) in pediatric patients with IBD, in comparison with dual energy X ray absorptiometry (DEXA).

Results: Correlation between skinfold and DEXA values ranged to estimate percentage of Fat Mass (FM%) in pediatric patients with IBD, in comparison with dual energy X ray absorptiometry (DEXA).

Discussion: 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 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 met 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 jejunoileal atresia. For a pelvic cystic hey of both kidneys was not associated malformations were detected. As feeding problem persisted, a laparoscopic hiatal hernia repair and fundoplication 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 reflex. 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 an important diagnostic tool is the radiological work-up of the GI-tract. In the case of microgastria, surgical intervention can lead to complete resolution of symptoms.
disorders, cystic fibrosis, ileal disease, congenital biliary or hepatic disease, drugs, prolongation of parental nutrition, amniolympathy, hypercholesterolemia, obesity. Cases of ABC4 gene mutations have also been reported. Therapeutic options are cholecyctectomy or ERCP (Endoscopic Retrograde Cholangiopancreatography).

Conclusion: Cholelithiasis 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.

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 or permanent. 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 then again changed 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, ABC2 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.

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.

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 36 weeks of gestation. There was no consanguinity. Family histories were 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 twice. 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 neonuromal diabetic diabetes there was 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, neonuromal 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.

Conclusions: 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.

“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, 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.

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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 taxe 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.

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**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 tooth is a tooth which appears in the first month of life whose root growth is prevented. A neonatal tooth is present less common than a natal tooth. 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.

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**Unexpected genetic findings in a female newborn with severe IUGR and epidermolysis 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, somonomorphic screenings and genetic evaluations are performed, respectively. Some times 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 somonomorphic abnormalities (bulky stomach, conspicuous legs with discrepancy between thigh and lower leg). The TORCH panel showed normal results. An amnioncentesis 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 α6 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 uniparental disomy of the ITGA6 gene as cause of the lethal hyaline degeneration 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.

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**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, smilling, caring, and protecting can 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.

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**Distal humeral epiphysyal separation**

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Distal humeral epiphysyal 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 neonatal hypernatremic dehydration. **Conclusion:** Our study illustrates that parents of extremely preterm 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 infants, 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 lowered in the study group (18.7%, 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.

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**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 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 (DWI) 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 C2 tetraparesis with neurogenic bladder and bowel dysfunction, up-beat nystagmus, and still no sensory loss. Our patient underwent an intensive rehabilitation program and slowly 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.
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. At birth, fetal ultrasound 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 anti-fungal treatment was added due to muco-cutaneous candidiasis. She was discharged home at 2 months of life, and topical emollients were continued. 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.

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, eclairum (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 possible 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 a heterozygous variant ALOX12B, in favour of autosomal recessive congenital ichthyosis (ARCI) type II.
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 presence with hyperreflexia and hyperreactivity. At H1
 psychologist 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 paroxysmal 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.

SGP77

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 proteins, 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 mls, fat 4.5 ± 0.7 g/100 mls. Atbirth
 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 intakes.
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 caesarean 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.

Severe endocarditis presenting with acute renal failure
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A 13-year-old female patient with pyonematoma, 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 coartation 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. Septic 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 slow regular 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).

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 fulfill 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 TREC’s (T-cell receptor excision circles) from dried blood spots (DBS) is a sensitive and specific screening test for SCID and severe T cell deficiency. TREC’s 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, TREC’s 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.
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 their 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 workflow. 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 central nervous system diseases. Somatosensory 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 peripheral 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.

Retrospective analysis of treatment strategy and outcome in infantile spasms in the neonopediatric 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 neonopediatric 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 Babi-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 after 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 no mutations associated with infantile spasms: three infants with Trisomie 21, one with Neurofibrromatosis type 1 and one with STXB1-1 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.

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 frequency (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 ± .08) and spindle density over parietal brain regions correlated with the volume of the thalamic cluster projecting to parietal brain regions (mean r = .44 ± .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 ± .06 and .46 ± .09 in the very preterm and term-born group, respectively).

Conclusion: Region-specific associations between structural and functional measures of thalamocortical connectivity 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.
Sudden postnatal collapse – a case too early to be screened

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Introduction: Sudden unexpected postnatal collapse (SUPC) in an apparently healthy newborn is a rare event carrying a high risk of mortality or persistent 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 neonatal screening program aiming to prevent adverse events. According to the national program, screening is performed at the age of 72 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 36 + 7/7 weeks of gestation following an uneventful pregnancy. Initial clinical examination revealed a small, somnolent 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 cardiopulmonary 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 present case, 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.

Ponine 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 unclear. As described earlier, clinical manifestations vary from severe cognitive impairment and 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 scaling from hot liquids. In addition, physiotherapy, ophthalmological and otorhinolaryngological 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.
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). TIA can 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 a right sided hemiparesis and motor aphasia. She reported rhinitis, fever and reduced appetite during the previous three days. Suspicions of stroke prompted immediate cerebral Anglo-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; otherwise, her left carotid angiography was 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 E-c 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.

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 manoeuvres.

Case report: During a trip on a merry-go-round, a 10 y.o. girl experiences a sudden malaise with ocular revision 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
**Case-report:** A 3 y.o. boy presented important asthenia for more than a week with diffuse paresis 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 lumbar 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 fluid showed fluid, pleocytosis (29 µL) and oligoclonal bands. ADEM was diagnosed and methyl-prednisolone (500 mg/m²) given. We excluded an optic nevrits 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 were 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 pleocytosis. New seric antibodies, anti-MOG, can predict a relapsing course. Treatment of ADEM is methyl-prednisolone iv for 2–5 days and then continuing 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.
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 infectious neurological system infection a lumbar puncture (LP) was performed, showing hyperproteinorrachia (1435 mg/l) and a mild hypoglycorrhachia (3.21 mmol/l) without pleocytosis. She then experienced an acute neurological deterioration with increased irritability, upper limb paresis and dysaesthesia. An MRI disclosed a CT scan showing a widespread CVST (inferior sagittal sinus, straight sinus and left sigmoid sinus) and acute hydrocephalus. MRL 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 not her 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 hypercoagulability 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 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 infectious neurological system infection a lumbar puncture (LP) was performed, showing hyperproteinorrachia (1435 mg/l) and a mild hypoglycorrhachia (3.21 mmol/l) without pleocytosis. She then experienced an acute neurological deterioration with increased irritability, upper limb paresis and dysaesthesia. An MRI disclosed a CT scan showing a widespread CVST (inferior sagittal sinus, straight sinus and left sigmoid sinus) and acute hydrocephalus. MRL 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 not her hemiparesis. One year after, she has mild motor deficit of her right upper limb.

Conclusion: In our treatment group we saw a good response to our double track system of the possibility of multimodal headache treatment individualized to the patient and a low imperative of preventive medication. The behavioral treatment herefor serves also as a connector to a potentially inevitable following psychologic therapy.
Conclusion: CAPS is a rare life-threatening autoimmune disease with disseminated intravascular coagulation and multiple organ failure. Awareness of this condition may enable prompt diagnostic and treatment initiation with anticoagulation and immunotherapy.

Stridor – not always what it’s supposed to be

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Introduction: Stridor is a frequent finding and indicates an extrathoracic 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 subglottal 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.

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 (6) 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.

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

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SGPP 97

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SGPP 100

SGPP 101
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 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 GPIb/IX, which is highly suspicious a Bernard-Soulier Syndrome (BSS). BSS was confirmed by a 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/chiastic 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/chiastic tumors (most commonly low-grade gliomas [LGGs]). DS is characterized by severe emaciation despite average caloric intake, often combined with normal or subnormal growth, loss of subcutaneous fat, anemia, adrenal and gonadal insufficiency, adrenocortical and gonadal hypofunction, proteinuria, hyperkeratosis, uremic mood, hyperthermia, vomiting, and/or nyctagmus. 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, hyperkeratosis, euphoria and relative hyperthermia. Magnetic resonance imaging showed a hypothalamic/chiastic tumor compatible with a LGG. Treatment with carboplatin/vincristine was started. Due to rapid progression, resection of the diencephalon 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 ghrelin 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 hormone ghrelin, leptin, and growth hormone, or FGF21 for the pathogenesis of DS. Chemotherapy is the mainstay of tumor treatment, as complete resection of hypothalamic/chiastic 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 SIOPEN, all patients receive platin-based chemotherapy prior to and after surgery. Diagnostic tumor biopsy is mandatory, the preferred method is a percutaneous ultrasound guided needle biopsy, using a "true cut" needle to avoid seeding tumor cells into uninvolved liver segments, the abdomen or peritoneum.

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 SIOPEN standards, for the biopsy using a "true

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 already identified on the third day of life and progressed to a picture of full-blown radiological BPD on the fifth day of life. Ureaemia, ureaemia ureatycum 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 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.
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 Plain cook biopsies 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 laparoscopy or minilaparatomy, providing sufficient material for histological classification as well as biological research to identify more precise risk profiles and treatment.

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 methemoglobin (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 signs 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 vegetables in Brassica oleracea (n = 3), Cucurbita pepo (n = 2), Fenouiculum vulgare (n = 2) and Spinacia oleracea (n = 1). Other offending agents were: lidocaine/prilocaine (n = 3), dapsone (n = 2), and amyl nitrite (n = 1, poppers abuse by a 15-year-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 (1–15 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.

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 GL) 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 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 μg/mL (range, 0.02 to 6.97; IQR, 0.18 to 1.11). The median APC was 0.55 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 (≤0.5 G/L) in 56% of all cases and in 100% of the patients with very low APC (0 of 5; 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.

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 (SpO₂) 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 SpO₂ 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.

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Discussion: Methemoglobin, an altered state of hemoglobin in which the ferrous (Fe++) ion 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 anabolic blood-transfusion, as methylene blue, the usual treatment, may increase hemolysis since the capacity to reduce the drug to active leucumethylene blue is limited in G6PD. The first negative G6PD test in our patient was thought to be secondary to the important reticuloysis at the time of the illness.

Conclusion: G6PD deficiency is a condition that makes erythrocytes susceptible to hemolysis under conditions of oxidative stress, such as 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.

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

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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, 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 susceptibility to bacterial infection. No major differences between infections caused by the different pathogens or sepsis severity and the susceptibility to bacterial infection. No major differences between infections caused by the different pathogens or sepsis severity and the susceptibility to bacterial infection. No major differences between infections caused by the different pathogens or sepsis severity and the susceptibility to bacterial infection.

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.

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. 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 nature. 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 agreements 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 among those 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 the prevalence of food allergies in Switzerland.

SUGP 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 broad cellular 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 anticoagulant prophylaxis to prevent life-threatening sepsis and fungal and viral infections. However, 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.
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 altered clinical examination revealed reduced normal muscle tone and mild hypotension. Anthropometrics showed weight <50th (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, and elevated thyroid-stimulating hormone with normal T3A. 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-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.

Hematopoietic stem cell transplantation for purine nucleoside phosphorylase deficiency: patients cures combined immunodeficiency and prevents progeroid-like and dermatologic symptoms
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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 XLP-gene compatible with Cernunnos/SLG deficiency. In this worldwide largest cohort of PNP patients, myelocytic 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.

Cernunos disease: a syndromic, radiosensitive primary immunodeficiency syndrome
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In conclusion, patients with a combination of unusual hematopoietic stem cell transplantation (HSCT) but the current knowledge about clinical presentation and outcome is still scarce. Despite HLA-mismatched HSCT to 36 children and 12 unrelated donors transplants led to long-term full or partial myeloid donor chimerism, 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) Busulphan, 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 transfused 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 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, and microcephaly, some with peripheral dysautonomics, 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 infection (n = 9), GVHD (n = 1) or unrelated causes.

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. Despite HLA-mismatched HSCT to 36 children and 12 unrelated donors transplants led to long-term full or partial myeloid donor chimerism, 11 patients from matched unrelated donors, other 12 received transplants from HLA-mismatched 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) Busulphan, 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 transfused 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, and microcephaly, some with peripheral dysautonomics, 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 infection (n = 9), GVHD (n = 1) or unrelated causes.
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, mucusosis/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 her infancy. 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 febrile episodes during periods of low ANCs. By extending the observation period and measuring CRP, a clear cyclic pattern was observed with neutrophilic 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 5. After 19 days 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 weeks and/or combine it with repeated CRP measurements may reveal the diagnosis.

Causes of low neonatal T-cell receptor excision circles (TREC) screening: 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 TREC and the associated diseases, which range from geneticaly caused Severe Combined Immunodeficiency to idiopathic lymphopenia. We aim to provide clinicians with guide on what diseases to assess when evaluating neonates with low TREC 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 1747 studies, the 42 included papers investigated 24 different genes in 60933942 individuals. One additional genetic cause for low TREC were 22q11.2 deletion syndrome followed by Interleukin-2 receptor gamma (IL2RG) and adenosine deaminase (ADA) deficiency. Furthermore 12 syndromes were associated with low TREC at birth as well as 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 further low TREC. 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.

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. Our experience with GATA2 deficiency 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.

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. The largest single study enrolled almost half of all included newborns. The most common genetic cause for low TREC was 22q11.2 deletion syndrome followed by Interleukin-2 receptor gamma (IL2RG) and adenosine deaminase
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. 

**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 immunomodulatory properties, and known to enhance T helper cell skewing towards Th1. Lactobacillus acidophilus (L. acidophilus) is a commonly used probiotic known 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 (Winclowe 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. 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 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. Immuno-deficiency 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 menigitis, 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 H2O [15–20]), while eye fundus examination and brain MRI were normal. Despite switching from canakinumab (interleukin-1 antagonist) to anakinra (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 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-naive patients aged <18 years with SGA or IGHD participating in the ANSWER Program. SGA patients were further stratified by peak GH levels to treatment, ≤0.0132; Y1 to Y3) and SGA patients with GHD at Y1 (<0.05). SGA patients w/o GHD achieved greater bone maturity compared to those with GHD (BA/CA Y1: 0.86 ± 0.98 vs 0.84 ± 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.

**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, HSDS in this group was significantly lower vs IGHD patients (p <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: 0.86 ± 0.98 vs 0.84 ± 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.
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 antitranstglutaminase 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 one year of a gluten-free diet, with height increase persisting over the second year. Age at diagnosis did not influence evolution of growth.

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 nephopathy. 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 us to achieve 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 115/70 mmHg, heart rate 81 bpm. Blood tests revealed severe HK (1.9 mmol/l), hyponatraemia (125 mmol/l), hypochloremia (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: Hyperkalaemia abnormalities 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.
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 therapeutic 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 department (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 MRT to better assess the size of the RA, which showed three lesions, the biggest being >3 cm in the upper renal pole. 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 months showed a significant weekly fever >40 °C for six days and increasing CRP despite of antibiotic treatment. In atypical KD, fever lasting more than five days, then 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.

References

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 individual 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 especially if a mutation has not yet been described yet. To overcome these barriers, we are now setting 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.
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, diphencylenediamide (DPI), DNase, or blocking Fcγ receptor blocking antibody (CD16, CD19, CD20 and CD44). 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 <0.001), 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 and depend on IL-5-activated eosinophils on adhesion and Fcγ receptor activation, requires elevated ROS production and degranulation, and involves EET formation. Thus, targeting eosinophils may be a promising therapeutic approach for BP.

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 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 is a switch in the regulatory role of B cells in 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.

Baseline fatty acids in prevention of the development of allergies in children and mice

Frei R.1,2, Roduit C.2,3, Furstl R.2,3, Loeliger S.2,3, Westermann P.1,2, Ryhner C.1,2, Schiavi E.1,2, Barcik W.1,2, Rodriguez-Perez N.1,2, Wawrzyniak M.2,3, Synihayzus A.2,3, Iwata J.1,2,4, Braun-Fahrländer C.1,2,5, Pekkanen J.1,2, Dalphin J-C.2,3, Robiolo J.1,2, Lauener R.2,11, O'Mahony L.1,12, the team of the PASTURE/EFRAIM study
1Swiss Institute of Allergy and Asthma Research (SIAF), University of Zurich, Switzerland; 2Christine Kühne-Center for Allergy Research and Education, Davos, Switzerland; 3University Children’s Hospital Zurich, Zurich, Switzerland; 4Children’s Hospital St Gallen, Switzerland; 5Children’s Hospital School of Medicine, St. Louis, USA; 6Department of Dermatology, University Medical Center Freiburg, Freiburg, Germany; 7University of Basel, Switzerland; 8Department of Environmental Health, National Institute for Health and Welfare, Kuopio, Finland; 9University of Besançon, Department of Respiratory Disease, UMR CNRS 6249 Chrono-environment, University Hospital of Besançon, France; 10Children’s Hospital Schwarzwald, Austria; 11Children’s Hospital St Gallen, Switzerland; 12Hyvärinen A., Kirjavainen P., Remes S., Roponen M., Dalphin ML., Kaulek V., Ege M., Genuneit J., Illi S., Kabesch M., Loss G., Schaub B., Pfefferle P., Doekes G.

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.

Objective: To test the role of exposure to SCFA by diet in preventing allergic diseases and colitis in children and mice.

Methods: Mice were epidermically sensitized with ovalbumin on an AD-like skin lesion, followed by intra-gastric antigen challenge to induce IgE-mediated bronchial inflammation. The mechanisms by which TSLP-elicited basophils guide the mechanisms by which TSLP-elicited basophils guide the mechanisms by which TSLP-elicited basophils guide the mechanisms by which TSLP-elicited basophils guide the T cell responses. Our preliminary data show that there is a switch in the regulatory role of B cells in 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.

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T cell-induced CSF1 promotes resistance to immunotherapy in melanoma

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Conclusion: SCFA levels were assessed by UPLC in fecal samples of one year old children of the European birth cohort study PASTURE/EFFRAIM 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.

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Results: Child wet weight increased 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 control group. 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 7

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

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SSAIO 8

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

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Conclusion: SCFA are promising anti-inflammatory compounds to prevent the development of diseases such as allergies and colitis.
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 2000 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, indicating that bystander DCs augmented 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 requirements for exponential expansion of responding CD8+ T cells.

**SSAI 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 southern 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 response: 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 these ES products thus renders the parasite susceptible to killing by neutrophils. 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.

**SSAI 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 bacterial infection 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 submamillary 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) sheaths. We dissected in SMG and lacked 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, our results 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.

**SSAI 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 leading to 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 frequencies in TILNs raising the possibility that miR-155 overexpression might be therapeutically useful to 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.

**SSAI 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 Nlrp/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 innate immune axis that shapes the gut microbiota composition. This

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Objective: pro-inflammatory cytokine production in a mouse model of birch pollen allergy. 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 cell (ILC2) responses in a mouse model of birch pollen allergy, and their modulation by rFlaA:Betv1 vaccination.

Background: Previously, we reported that exposure to a farming environment is allergy-protective, while high proportions of neonatal immature/naive CD5+ B cells and putative regulatory T cells (Tregs) are risk factors for, as well as putative regulators of, allergic disease. To explore whether a farming environment is allergy-protective, while high proportions of neonatal immature/naive CD5+ B cells and putative regulatory T cells (Tregs) are risk factors for, as well as putative regulators of, allergic disease, 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 cell (ILC2) responses in a mouse model of birch pollen allergy, and their modulation by rFlaA:Betv1 vaccination.

Results: We observed that ILC2 harvests from mice vaccinated with rFlaA:Bett1, but not with PBS, showed a small reduction of IL-5 and IL-13 positivity. Prophylactically, prophylactic vaccination with rFlaA:Bett1 increased both lymphocyte and ILC2 recruitment to the lungs. In addition, BPE at mice intranasally on 2 consecutive days was not sufficient to recruit additional ILC2. These findings conformed 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:Bett1 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.

Methods: In order to observe early ILC2 recruitment, mice were challenged intranasally with high dose birch pollen extract (BPE) on two consecutive days. Two weeks prior to challenge, mice were prophylactically vaccinated with either PBS or rFlaA:Bett1. 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, TCR-γδ, 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.

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

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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

Method: In order to observe early ILC2 recruitment, mice were challenged intranasally with high dose birch pollen extract (BPE) on two consecutive days. Two weeks prior to challenge, mice were prophylactically vaccinated with either PBS or rFlaA:Bett1. 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, TCR-γδ, 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 harvests from mice vaccinated with rFlaA:Bett1, but not with PBS, showed a small reduction of IL-5 and IL-13 positivity. Prophylactic vaccination with rFlaA:Bett1 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 conformed 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:Bett1 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.

Personalized and rapid food allergy test using natural allergenic extracts

Strategy: At present, 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 a 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 immunoadfinity capillary electrophoresis (IACE) coupled with matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS). Meanwhile, the second method is based on immunomagnetic separation (IMS) with mass spectrometry identification (MALDI MS or peptide mass fingerprinting). In both techniques, magnetic beads coated with anti-human 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 with the observation that 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 species.
mills. 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.

Post organic skin syndrome: is there a place for an immunologist?

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Introduction: Post organic skin 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 after sexual arousal with production of proinflammatory 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 1/4000, but positive at 1/100. 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 Can f 15 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 consistent with an immunological mechanism, which has been proposed by others, with claims of successful desensitization procedures. Further investigations are needed to better evaluate the underlying mechanisms.

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 cindamycin 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 (SDRFE) 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. SDRFE 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.

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. Its clinical use includes disinfection of surgical sites. 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 amino 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 basophil activation assays with CHX, chlorhexidine (CG) and ALX using a commercial IgE assay for CHX (ImmunoCAP).

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 re-sensitized 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 were isolated and donor basophils were stripped of their IgE by lactic acid pre-treatment and re-sensitized 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.
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. CHX showed a strong inhibitory effect (>80%) in 20/22 and CHX in 8/22 tested sera, while ALX (>60%) inhibited CHX positivity in 9/22 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: CHX 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.

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 (transdermal 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 Staphylococcus pneumoniae and S. salivarius 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 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.

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 degradation 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 determining IgE to Asp i 6 (MnSOD from Aspergillus fumigatus, m222), which is strongly crossreacting with Mala s 11.

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 pathogenetic 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, M. sympodialis and M. restricta have long cultivation times and require complex media. Therefore, other species with reduced cultivation times could be used to study these species under culture conditions resembling atopic skin. In addition, we performed basophil activation tests (BAT, CD63 and CD203a as activation markers) with CHX and ALX. Aspergillus fumigatus (m227) and in case of possible auto-reactivity by determining IgE to Asp i 6 (MnSOD from Aspergillus fumigatus, m222), which is strongly crossreacting with Mala s 11.

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
**Unusual presentation of a child with severe combined immunodeficiency**

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**Introduction:** Severe combined immunodeficiency in infancy is characterized by pathological cardiotocography with an excellent newborn adaptation to extraterrain life was admitted to the emergency room for fever (39.8 °C), signs of dehydration, irrtation 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 Amoxicillin and Garamycine was started. During the monitoring 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: xanthochromic cerebrospinal fluid (CSF), proteins 1210 mg/L, leukocytes 3μl, 92.5% mononuclear cells and 75% polynuclear cells. The PCR of the CSF was negative for VPS7A. A skin biopsy was inconclusive, diagnosis of Parechovirus meningencephalitis 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. Only more than 100 cases are reported 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 viral medication is available, but in severe infections mononuclear 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.

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

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**Introduction:** Human immune globulin subcutaneous (HIGS) is a ready-for-use, liquid preparation of highly purified human immune globulin 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 (PIDD) 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 studies (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 causally-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 well-tolerated, irrespective of relatively high infusion volumes and fast infusion rates.

Conclusions: SCIG 20% infusions were well-tolerated, irrespective of relatively high infusion volumes and fast infusion rates.

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 patients were isolated by MACS and stimulated with LPS. Levels of α and β secretion of IL-1β, IL-6, IL-18 and IL-6 were measured by ELISA.

Results: We report 13 patients with the Q703K NLRP3 variant: 10 Q703K+ PFAPA patients were compared by ELISA. Q703K+ PFAPA patients were similar to Q703K-PFAPA patients. The cytokine profile of Q703K+ versus Q703K- asymptomatic adults.

Conclusions: SCIG 20% infusions were well-tolerated, irrespective of relatively high infusion volumes and fast infusion rates.

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, diarrhea and vomiting) along with recurrent ear nose and throat (ENT) infections. He was multi-investigated for his abdominal symptoms by echography and CT-scan 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 PET-scan. Colonooscopy confirmed several nodules disseminating along the colon with masses in the caecum. Lymphoma was first suspected. However the non conclusive 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 eosinophils; 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+ and increased CD8+ T cells (included recent thymic emigrants) and B cells with normal distribution. T lymphocytes proliferated well after stimulation with mitogens. After lymphoma and PIDa classically prone to lymphoproliferation were ruled out we undertook to explore further our patient with a whole exome sequencing scan. 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 disorder recently described. This PID displays variable phenotypes 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 bronchiectasis. He started on intravenous polyvalent immunoglobulin substitution with the plan to assess the effect after 6 months.
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)-treated patients had a higher rate (50.2% vs 34.4% respectively; P = 0.006); conversely, late (versus early) treaters had shorter time to resolution ([3hrs (0.8, 9.3) versus 7hrs (3, 19.3)] and attack duration [4hrs (1, 10.3) versus 12.5hrs (6, 20.0)]) 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 access to treatment 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.

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 check-point 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, FoxP3, CD8, and PD-1. A total of 107 patients were included in this retrospective study. Median patient age was 60 years (range 26-88) and median follow-up was 43 months (range 1-130). The overall response rate was 32% (complete response 17%, partial response 15%). Median progression-free survival was 11 months (range 1-38) and overall survival was 65 months (range 10-116). Thirty-six patients had lymph node metastases of stage III melanoma. The proportion of TILs in primary melanomas and lymph node metastases was determined. The presence of TILs was higher in lymph node metastases compared to the primary melanoma (20% vs 10%, respectively; P = 0.04). The presence of TILs in the lymph node metastases correlated with a longer progression-free survival (P = 0.04). These findings may help in selecting patients with completely resected high-risk stage III melanoma for adjuvant treatment with checkpoint inhibitors.

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 immunological 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 prepared. The factors produced by keratinocytes were identified by a proteomic approach. 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-6, IL-1α, MCP-1 and MMP-1, but not of type I collagen. However, IL-17A significantly decreased type I collagen production induced by TNFα. Pretreatment of keratinocytes with TGF-β1 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-β1 surprisingly resulted in a synergistic positive effect on fibroblast production of these mediators. By proteomic approach, we identified GM-CSF and TGF-αα as mediators produced by keratinocytes under the synergistic effect of IL-17A and TGF-β1. In full human skin, IL-17A promoted pro-inflammatory responses by inducing IL-1α, IL-6, IL-8, MCP-1 and MMP-1 levels, while showing direct anti-fibrotic effects and decreasing by 2-fold collagen production triggered by TGF-β1 (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-αα and other factors released by keratinocytes that activate fibroblasts.

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 burden 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 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 JIRcohort 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 JIRcohort from 3800 to 2188 items with 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: JIRcohort 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.

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 γ-subunit of the high affinity IgE receptor (anti-FcεRI) and/or against mast cell and basophil surface bound IgE (anti-IgE). These presumably autoimmune forms of CU can be identified by functional tests such as 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-FcεRI 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-FcεRI 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-FcεRI as well as anti-IgE autoAbs, which are found in CU-patient sera, differ greatly in their quantity and affinity. Hence, based on autoAbs quantity and affinity, CU-patients can be divided in different subgroups: anti-FcεRI 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 determine the possibility of therapeutic interventions such as anti-IgE therapy.

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. In the treatment of other 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.

Treatment preference on the new Subcutaneous Immunoglobulin 20% (SCIG 20%) treatment in patients with Primary Immunodeficiency Diseases (PIDD) 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 patient 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 PIDD treated with IVIG 10% for 3 months followed by SCIG 20% for ≥12 months. Questionnaires were administered by 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, 86% 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, 89% 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.

Cellulitis after two-step excision of a basal cell carcinoma favored by rituximab induced hypogammaglobulinemia
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A 78-year-old multimorbidity patient 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 a 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
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 increased 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. Mutant vs unmutated STAT3 was used to define differences in multilineage complex 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 bone marrow cultures and in bone marrow cultures we could show an arrest of cells in the pre-erythroblastic state of hematopoiesis (CD235a intermediate stages).

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, capsular or eyelid edema. These signs form the clinical activity score (CAS), which allows for disease progression. Increase methylprednisolone pulses (MP) are the mainstay of therapy. Patients unresponsive to glucocorticosteroids (GC) or with contraindications may benefit from immunobiologics.

Conclusion:

Toxiciluzum 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, capsular or eyelid edema. These signs form the clinical activity score (CAS), which allows for disease progression. Increase methylprednisolone pulses (MP) are the mainstay of therapy. Patients unresponsive to glucocorticosteroids (GC) or with contraindications may benefit from immunobiologics. Toxiciluzum (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 rifampicin. 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, toxiciluzum improved symptoms within 3 months. Treatment was well tolerated. Controlled, prospective studies are urgently needed to assess whether IL-6 receptor blockade could benefit patients with early severe ophthalmopathy.

Reference:

Generation of a human p47phox-deficient chronic granulomatosus 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 microbicial activity of phagocytes leading to recurrent life-threatening infections. Mutations in gp91phox, p47phox, p40phox, p40phox or p22phox subunits of the phagocytic nicotinamide adenine dinucleotide phosphate (NADPH) oxidase may result in CGD. The p47phox subunit is the most frequently mutated in CGD (65%), and the mutations are scattered throughout the whole cytobrheim b-246 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 (∆GT) in exon 2 of neutrophil cytosolic factor 1 (NCF1) gene. The ∆GT 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 two copies of the NCF1 pseudogenes results in the predominance of the ∆GT 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 ∆GT 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 new model for ∆GT-p47phox-CGD p50 cells on a human acute myeloid leukemia PLB-985 cell line. We utilized the CRISPR/Cas9 technology to introduce the ∆GT mutation in p47phox encoding NCF1 gene. The established PLB-985 NCF1 ∆GT 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 ∆GT cell line creates an attractive alternative to currently used iPSC models for rapid testing of novel gene therapy approaches.

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 4 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 TGFS-α and IL-15 synergistically induce elevated Rgs1 expression, which was further enhanced by concomitant anti-CD3/CD28 mediated activation of CD8βT cells. Intriguingly, we observed in vivo a rapid down-regulation of Rgs1 expression in unconventional CD8αx TCRαβ intraepithelial lymphocytes (IEL) during onset of experimental colitis concomitant with the appearance of this animal T cell 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 microenvironmental 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 congeneric Rgs1+/-, and Rgs1+/-, mixed bone marrow chimera suggest that absence of Rgs1 may indeed directly affect the distribution pattern of various T cell subsets in vivo.

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 β1i, β2i, and β5i subunits of the constitutive proteasome are replaced by β1j, β2j, and β5j (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.

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, steiodogenesis, 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 choriomeningitis 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 LHR-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 LRH-1 for the cellular response to LCMV in the control of T cell-mediated anti-viral immune responses.
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 binds to a dominant-negative p97-repressor and controls mutant we show that MHC class I restricted presentation of virus-induced and endogenous epitopes as well as bulk MHC class I surface degradation of proteins into small peptides. One source of antigenic class I molecules allows immunosurveillance of proteins synthesized in the intestinal mucosa after azoxyethane (AOM) and dextran sodium sulphate (DSS) treatment (mouse model of colon carcinogenesis) compared to wildtype mice. Small heterodimer partner (SHP/NRIB2) is a nuclear receptor with no DNA binding domain. SHP is a downregulation-negative 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 escapes 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.

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.

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 ΔδDgbata-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 ΔδDgbata-1 mice exhibit impaired fat absorption. In all we show a novel role for eosinophils in intestinal maintenance and function.
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 glandular tissues such as the intestinal skin, and lung. The development of spontaneous dermatitis-like skin inflammation in lymphoid-deficient mice was recently described. Similar responses were also observed in mice lacking the enzyme 11β-hydroxysteroid dehydrogenase-1 (11β-HSD-1), which is responsible for the inactivation of GC in the skin. These findings suggest that GC can be produced de novo in the skin and may offer novel understandings and therapeutic opportunities in inflammatory skin diseases.

Immunoregulatory glucocorticoids result 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 glandular tissues such as the intestinal skin, and lung. The development of spontaneous dermatitis-like skin inflammation in lymphoid-deficient mice was recently described. Similar responses were also observed in mice lacking the enzyme 11β-hydroxysteroid dehydrogenase-1 (11β-HSD-1), which is responsible for the inactivation of GC in the skin. These findings suggest that GC can be produced de novo in the skin and may offer novel understandings and therapeutic opportunities in inflammatory skin diseases.

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 under the 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.

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The Lin28β/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 (ETPs), 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 responsible 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 functions for miRNAs in the thymus. The most abundant family of miRNAs in mammals is lethal-7 (let-7). Differences in the let-7 family members are bound to the RNA binding protein Lin28. Lin28 is found in thymus progenitors and thymocytes, and Lin28 expression results in thymus hypercellularity and promotes an increase in cTEC numbers. Lin28β 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 Lin28β expression. The TEC compartment is to our knowledge the first example where Lin28A and B exert qualitatively and temporally distinct host responses determining the fine balance between commensalism and pathogenicity.

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 immune responses are emerging 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 fungal overgrowth at the epithelial barriers, thereby limiting fungal overgrowth at the epithelial barriers.

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, sharing many similar features with mast cells. Even though mast cells are tissue resident cells, basophils were long thought to be too early in their development, not performing related functions. 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 functionality of the newly developed BH3 mimetic compounds to regulate their fate decision of cell death. This reveals 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 Bcl3-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 suggesting 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 in 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-lymphoid tissue resident 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 naive 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 naive 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, naive CD4+ T cells become tissue resident (non-labeled) in the intestine and express CD69 and CD103 (signature markers of tissue resident memory CD4+ T cells) within the cryptic marker Bcl-2. These resident CD4+ T cells are not depleted during remission induction by 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 function 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 hypothesis 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.
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 KLF2, 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 suggest 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 Galα 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 PIDs; 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. Highly expressed in the gut, ROR-γt+ Treg cells have an extensive carbohydrate recognition capacity 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 (IkappaBα, IL-10) and for cellular carbohydrate recognition, is also upregulated in some Treg cells, particularly in ROR-γt+ Treg cells. To study the role of MAF in this population of T cells, we generated mice deficient for MAF in Foxp3+ cells. These mice 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, and its endogenous 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 (ESRPI) is an epithelial-specific regulator of mRNA splicing. The Triaka mouse model has a point mutation in Esrp1 leading to the rapid secretion of Th1 and Th2 cytokines. 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 significantly increased CD8+ T cells, which mainly load on the APC cell surface, are able to maintain iNKT cell 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 endogenous 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.
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 mouse (designated Atg5SE?). Because eosinophils in mice comprise only 1–3% of circulating leukocytes, we provoke eosinophilia by crossbreeding our genetically modified mice with chemokine receptor driving eosinophils

Results: Atg5 is efficiently deleted within eosinophil lineage and deletion is eosinophil specific. We measured eosinophil numbers and determined decreased absolute (p = 0.0085) and relative (p = 0.0271) numbers in the peripheral blood of Atg5SE? IL-5 transgenic mice as compared with control IL-5 transgenic mice. We found that Atg5SE? 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 biologic stimulation with CsA 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 eosinophiliogenesis 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.

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 mice, 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 Atg5-IL-5 transgenic mouse as compared with control IL-5 transgenic mice. We found that Atg5SE? 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 biologic stimulation with CsA following GM-CSF priming, eosinophils deficient for Atg5 exhibit an impaired ability to de novo synthesise lipid bodies.

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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-tumoural 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 play a pivotal role most prominently downstream of PD-1. It was shown that Shp-2 is recruited to the phosphorylated inhibitory receptor tyrosine-based switch motif (ITSM) 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, Ptn11fl/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 animals bearing Ptn11fl/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 signalling, advancing our understanding of signaling downstream of inhibitory receptors and potentially opening novel opportunities for improved combined therapies concomitantly targeting PD1 and Shp-2.

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 homeing 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 and its ligands act in concert with other chemokine receptors important for 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 required for the design of therapeutic strategies to intervene in this process in Th cell-driven pathologies.
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 or foreign antigens encoded by LCMV, 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.

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

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Germinal center 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 TLSs 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 chemotherapies inhibit the development of TLSs and GCs, suggesting that these steroids impair the immune control of cancer. Based on our findings, we propose that deliberate induction of TLSs may be a novel immunotherapeutic approach for LSCC.

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

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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 IL-7--/- 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 numbers and in 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 node nodes relies upon IL-7 dependent maintenance ILC3 cells.

LTM0 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 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 phenotypic. Competitive transplantation assay 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 fluorescence 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 bothColVI-Cre+ 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 venous (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 S1pr1 in HEVs using the Lyve1 promoter, and found that both HEVs and complete knockout 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 bothColVI-Cre+ 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 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 recombinease under control of the ColVI-Cre promoter, and found that both HEVs 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 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 effrent lymphatic vessels. Unlike lymph node, PPs lack a conventional source ofafferent 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 luminal fluids to the flow of PP conduits. Surprisingly, this alteration to conduit flow did not result in significant functional changes to PP conduit flow (as assessed by RNAseq). However, disruption of fluid absorption over the course of at least 2 days had profound effects on the structural integrity of the high endothelial venule and surrounding peripheral FRCs. These alterations correlate with a striking defect in the recruitment of naive 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 involves B cell receptors, which 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.

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 thymopoietin 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+ precursor 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+ precursor 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 state along a developmental trajectory without previous knowledge of the exact variance of markers that identity sequential maturation states. 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 transcriptionic 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 components. Two cell clusters positioned at either extremity of the trajectory displayed multiple transcriptionic 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 demonstrated the existence of active β5t+ cortical progenitors to the maintenance of the thymic medulla.

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 response. Tertiary lymphoid structures (TLS) are assembly 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 RORγt−/−, lymphotixin (LT)R−/−, LTα−/−, Rag2−/−, IgMnull (B cell deficient) and CD40−/− T cell deficient mice to investigate the signals that determine the generation of TLS. Both stromal cell lines responded to LTβR stimulation with systemic manifestations of disease and worse clinical outcome. These data suggest that stromal cells respond significantly to LTβR and RORγt−/−, LTα−/−, Rag2−/−, IgMnull (B cell deficient) and CD40−/− T cell deficient mice induced stromal cell activation and chemokine production thereby preventing TLS organization. Lymphocytes (in particular T cells) emerged as major sources of LTα in TLS, with a strong contribution from B cells. As such, mice displayed a milder phenotype compared to LTβR−/− 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 CD3+ Tg cells 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 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 chemokine expression. This study was partly regulated by LT, however, the specific signals regulating CCL19 versus CXCL13 production are still under investigation.
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 implicit 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 sustained impairment of immune responses.

Intranaodal T cell migration, and dendritic cell-mediated activation of 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.

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 organs (SLOs). 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 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 lymphotaxis, which are molecules implicated in secondary lymphoid organ formation, to investigate if they are sufficient to promote TLS formation by this tumour in vivo.

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 mouse 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 reaveal a novel role for CD112 in stabilizing LEC-LEC interactions and in supporting lymphatic function.

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 various tissues and control immune responsiveness through regulation of T cell egress. However, during infection with an enteropathogenic virus, cell-specific formation, structure and composition under homeostatic conditions.

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, the significance of S1PR1 signaling in the stromal cells remains unclear. To address this issue, we developed conditional knockout mice (Lyve1-CRE/ S1pr1f/f) 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 medullus failed to undergo thymus egress. Using a Lyve1 reporter strain in which Lyve1 lineage cells expressed tdTomato fluorescence 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.
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 and 12 with clinical disease only (no 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, αβCD3/CD28 induced T cell proliferation was measured using CFSE dilution.

Results: Fibroblast-like cells grown out of the biopsy consisted of fibroblastic reticular cells (FRCs: Podoplanin+CD31-), and double negative cells (DN; CD45+CD3-CD19-). Cultured LNSCs expressed characteristic adhesion molecules and cytokines (e.g., TNFα) 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 lymphotxin α1[2], 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.

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 their outcome. In this work we develop 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 reticulocyte 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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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-known lymph node and skin 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.
the different cell populations, with highest reads (FPKM values) of vWF and VEGFR1 in BcEcs; Prox1, PDNP and VEGFR3 in coloECs and capECs, and highest reads of Lyve-1 in capECs. Interestingly, we found that capECs and coloECs differentially express several genes reportedly or presumably involved in leukocyte migration, and in many cases these gene levels are modulated by inflammation. Overall, our results shed light on the gene expression differences between capECs, coloECs and BcEcs and will serve as the starting point for functional studies investigating the molecular control of leukocyte migration through LVs.

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 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 regulate damage-driven epithelial stem cell renewal 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 epithelial crypt regeneration.

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 a key role in maintaining homeostatic 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 (fRRC), which localize next to IgA+ cells. Given the role fibroblasts play in PC survival within the bone marrow we asked whether fRRC have an important function in PC survival within the lamina propria. To this end we have developed an isolation protocol for fRRC allowing to look for expression of PC survival genes. Indeed, fRRC were found to be a major source of baf, april and also ccl12 transcripts. In coculture experiments we observed that purified fRRC 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.

Depletion of a subset of activated stromal cells impairs ectopic lymphnode genesis in inflammation
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Background: Crucial structural and immunological functions of stromal cells have been recently shown by stromal cell depletion both in the context of cancer and [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 maturation of lymphocytes in inflammation [3].

Here 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 lymphnodegenesis in murine salivary glands.

Methods: FAP-DTR mice were treated with Diptheria 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-duodenally 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 and platelet populations 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 organise 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, producing nitric oxide (NO) in a dose-dependent manner.

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 (PDLPN) 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 PDLPN/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 PDLPN up-expression on a pro-inflammatory SF subset with concurrent accumulation of PDLPN+ anti-inflammatory microphages. 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.: Rasa2+/Er2Cre x Clec1bFloxFlox mice) and in the absence of platelet-derived CLEC-2 (i.e.: Pia4Cre x Clec1bFloxFlox mice). Wild-type mice treated with an agonist anti-PDLPN 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 PDLPN/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 PDLPN/CLEC-2 interactions with an agonist anti-PDLPN 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.

References

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. Antigen-presenting cells (APCs) in secondary lymphoid organs include dendritic cells (DCs) and B cells. APCs function to communicate with T cells and dendritic cells in order to maintain immune homeostasis but also to modulate immune responses. We show 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 COX2 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 COX2 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.
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 infections 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 may drive a common mechanism of trafficking of B cells into infiltrated tissues and the initiation of TLO formation. Thus, these early events offer a therapeutic target to limit TLO formation in infection and/or autoimmunity.

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 Has 1, 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 homoeostasis 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 periportal immune tone**

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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 immune-stimulating FSC using the novel Ccl19-1TAeyfp inducible Cre mouse model. Using the novel Ccl19-1TAeyfp inducible Cre mouse model revealed that splenic fibroblastic stromal cells descend from a common perivascular progenitor cell. Further, 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 LTo-dependent manner.

**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) represent 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, these findings indicate that a novel subset of fibroblastic stromal cells plays an important role in tumor growth and metastasis.

**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 LTIR 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 Ccl19-1TAeyfp 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, the analysis 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 Ccl19-1TAeyfp 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 pathway. 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 an LTIR-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-domain in a complement receptor 2 (CD21)-mediated fashion, leading to enlarged and more numerous cervical 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 promoter 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. Importantly, the effects on GC functions were observed 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 defects and TH impairment found in infants. Based on 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 organ responses**

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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 lymphoid nodules and that lymphocytes are recruited to these nodules via LTIR. Recently, the LTIR-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 these cells are not only present in lymphoid nodules but also in skin, we have crossed Ltof/f mice with Cxcl13-Cre/tdTomato; R26R-EGFP dual reporter mice. While the absence of LTIR 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 Ltb-competent littermate 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 (LN) and back to the blood vascular system (BVS). In 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 forhead 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 cilia/lophorax and rapid 100% lethality, as a result of degeneration of intraluminal vessels, 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 Foxc2−/− Proxl-CreERT2 T 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) play a major role in 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 Cxcl3-Cre tdtTomato; R26R-EYFP reporter mouse that permits the cell-specific tracing of CXCL13-expressing fibroblasts. The dual reporter mouse expresses tdtTomato and Cre recombine directly under the control of the Cxcl3 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 Cxcl3-Cre tdtTomato; 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 lymphoxygenis-l receptor (LTD4) signals. Ablation of Ccl19 on Cxcl13-expressing fibroblasts demonstrated 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 proportion of CCL19-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 function 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-17 and TNF-α. 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 report a number of chromatin regions of chromatin near 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 biosyntheses 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. Moreover, 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.

**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 spatial and temporal dynamics by a combinatorial imaging approach to disrupt tumor-promoting effects of CAFs on cancer 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. To better understand the role of cancer-associated fibroblasts (CAFs) in CRC, we have investigated their network properties. The localization of CXCL13 expression in CRC has been shown to be associated with poor-prognosis colorectal cancer (CRC).

**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) constitute the lymphoid organ microenvironment and control the migration and localization of lymphocytes in the draining lymph node (dLN). 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.

**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 cancer, tumors require the interaction with other cells, 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. To better understand the role of cancer-associated fibroblasts (CAFs) in CRC, we have investigated their network properties. The localization of CXCL13 expression in CRC has been shown to be associated with poor-prognosis colorectal cancer (CRC).
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 hematopoietic cells. LTi precursor and LTi cells contribute to the complete formation of complex lymph node structure. However, precise characterization of LTi cell for investigation for molecular mechanism underlying LTi-LTo cell interplay were not completely performed. RANKL, a TNF family cytokine indispensable for the lymph node development. In this study, we found that RANKL is a TNF family cytokine indispensable for the lymph node development. In this study, we found that RANKL expression on LTi cell was indispensable for the lymph node development and that RANK, a receptor of RANKL was expressed on local mesenchymal stromal cell termed Lymphoid 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 LTi cell was indispensable for the lymph node development and that RANK, a receptor of RANKL was expressed on LTi precursor. Transcriprome 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 in its near surroundings.

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 autoimmunedeficiencies due to an intrinsic function of NDFIP1 in dAMPing 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-4KO 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.

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 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 modulated 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 on cells in 12 hours before RNA isolation and qPCR for IF 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 = 6) by IL-1β but not by TNF or 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 non-stimulated. A synergistic effect was seen using IL-1β + TNF (66 ± 33 fold, p < 0.0001). 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.
significant immigration of lymphocytes. At the same time, we observed immigrated lymphocytes only in the outer part of the fibrin-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 scaffold-implant mixture did not affect either vessel ingrowth or immigration of the cells to the inner part of the implants. In summary, we found that fibrin scaffolds affected fibroblasts in vitro by upregulation of adhesion molecules ICAM-1 and VCAM-1 and induced immigration of lymphocytes and B cells 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.

**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 the immune system 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 a 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.

**Robo4 controls B cell migration – a new regulator**

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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 theafferent 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 lymph node 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 the deficient mice, and compared the data. As a result, Robo4 deficient mice had a slightly diminished amount of transferred B cells in their secondary lymphoid organs. This suggests that early IFNγ 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, CD31, in the interaction, 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.

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

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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.

**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.
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 to promote 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 anti-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 monocyteic (CD14+) or erythroid (CD38+) 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 IL-10-rich elements in the IFNγ-3 untranslated region. BM lymphocytes in ARE-Del mice produced more IFNγ, which remodelled 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-phenotypic progenitors. Thus, we demonstrate that IFNγ has a negative impact on expansion and hematopoietic support of BM MSCs in vitro and in vivo, and 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.
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, deleterional 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. Transcriptional 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 chemotaxis 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 frequency of medullary myeloid cell attachment. As lymph nodes are sites of inflammation, we stimulated FRCs and myeloid cells in initiating early inflammatory processes and initiating the formation of TLS in chronic inflammatory condition.
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 IFN-γ 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 depend on IL-33 produced by stromal rather than hematopoietic cells. Interleukin-33 (IL-33) signaling by stromal cells is constitutively expressed 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 identified 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+ LECs were mainly observed in the medulla, and regularly localized close to virus-specific CD8+ T cells suggesting either local IL-33 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 end stage kidney disease in SLE. Treatment strategies targeting the development of TLS may be important for preventing the development of lupus nephritis.

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

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Bone marrow stromal cells and niche cells are key regulators of 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 hematopoietic 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 volume, 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 extramural surfaces of sinusoidal endothelial cells. 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.

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-C13), 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). Undergoing 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-C13 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.

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; 0.5×10⁴), and fibroblast- like synoviocytes of a patient with PsA (PsA-FLS; 1.0×10⁴) 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 2.5×10⁴ 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 (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+ 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 lymphoxin 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.

Neuropilin-1 is expressed on lymphoid tissue residing 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 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+ ILC3 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-17I1 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/LTis in humans, despite their overlapping phenotypes and cytokine production profiles. Finally, we show that NRP1+ LTis are present in inflammatory aggregates in lungs of smokers and COPD patients providing insight into the initiation of smoke-induced ectopic lymphoid pulmonary aggregates in the lungs.