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AB0 blood group incompatible haematopoietic stem cell transplantation and xenograft rejection

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Summary

The current organ shortage in transplantation medicine stimulates the exploration of new strategies to expand the donor pool including the utilisation of living donors, AB0-incompatible grafts, and xenotransplantation. Preformed natural antibodies (Ab) such as anti-Gal or anti-A/B Ab mediate hyperacute graft rejection and thus represent a major hurdle to the employment of such strategies. In contrast to solid organ transplantation (SOT), AB0 blood group incompatibilities are of minor importance in haematopoietic stem cell transplantation (HSCT). Thus, AB0 incompatible HSCT may serve as an *in vivo* model to study carbohydrate antigen (Ag)-mismatched transplantations such as AB0-incompatible SOT or the effect of preformed Ab against Gal in xenotransplantation. This mini-review summarises our clinical and experimental studies performed with the support of the Swiss National Science Foundation program on Implants and Transplants (NFP-46). Part 1 describes data on the clinical outcome of AB0-incompatible HSCT, in particular the incidence of several immunohaematological complications, acute graft-versus-host-disease (GvHD), and the overall survival. Part 2 summarises the measurements of anti-A/B Ab in healthy blood donors and AB0-incompatible HSCT using a novel flow cytometry based method and the potential mechanisms responsible for the loss of anti-A/B Ab observed following minor AB0-incompatible HSCT, ie the occurrence of humoral tolerance. Part 3 analyses the potential of eliminating Gal expression as well as specific complement inhibitors such as dextran sulfate and synthetic tyrosine analogues to protect porcine endothelial cells from xenoreactive Ab-mediated damage *in vitro* and in a hamster-to-rat heart transplantation model. In conclusion, due to similarities of the immunological hurdles of AB0 incompatible transplantations and xenotransplantation, the knowledge obtained from both fields might lead to new strategies to overcome humoral rejection in transplantation.

Key words: AB0-incompatible grafts; haematopoietic stem cells; stem cell transplantation; xenotransplantation

Introduction

This minireview summarises the results of several studies – both clinical and experimental – performed in our laboratories and clinics during the last few years with the support of the Swiss National Science Foundation program on Implants and Transplants (NFP-46). The project has the long-term objective to explore new strategies in order to enlarge the pool of organ donors in transplantation medicine. Using AB0 incompatible haematopoietic stem cell transplantation (HSCT) as an *in vivo* model the specific aims are to expand both the clinical and basic knowledge regarding AB0-incompatibility, hyperacute Ab-mediated allo- and xenograft rejection, and the induction of tolerance and accommodation to carbohydrate Ag.

Over the past three decades HSCT has gained major importance in the treatment of various haematological diseases and approximately one third of all patients are transplanted across the AB0-blood group barrier [1]. Studies on AB0-incompatible HSCT started in the 1970s and with some exceptions found no association between ABO-match and overall survival or graft rejection [2–4]. In contrast, matching of AB0 blood groups is of outmost importance for solid organ transplantation (SOT) [5]. Yet, AB0-incompatible SOT has

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become a clinical reality for a small number of patients over the last two decades with the use of specific immunomodulatory protocols and various procedures to eliminate anti-donor Ab prior to transplantation. In fact, AB0-incompatible adult kidney and infant heart transplants are nowadays performed with similar patient and graft survival as AB0-compatible SOT [6–8].

Hyperacute rejection in pig-to-primate xenotransplantation shares several similarities with acute rejection in AB0-incompatible SOT including the important role of carbohydrate Ag, namely the Galα1,3Gal (Gal) oligosaccharide, and preformed xenoreactive Ab [9]. Carbohydrate Ag are expressed on red blood cells (RBC), endothelial cells (EC) and other tissues. Although the Ag involved in hyperacute xenograft rejection share several similarities with the AB0 Ag, it remains unknown how rejection, tolerance and accommodation observed in AB0 incompatible HSCT and SOT are applicable to xenotransplantation.

Part I: AB0 incompatible haematopoietic stem cell transplantation from a clinical point of view

The first aim was to analyse the clinical outcome of AB0 incompatible HSCT. In particular, our studies on immunohaematological complications and strategies to avoid these are presented. Three different groups of AB0-incompatibility are distinguished in allogeneic HSCT: (1) **Major** AB0-incompatible HSCT (eg A into 0) is characterised by the presence of preformed anti-donor A/B Ab directed against donor AB0 Ag expressed on transplanted cells. In this setting host-versus-graft (HvG) reactions may occur such as immediate or delayed haemolysis of donor RBC and destruction of donor erythroid precursor cells causing delayed RBC engraftment and pure red cell aplasia (PRCA). (2) Recipients of **minor** AB0-incompatible HSCT (eg 0 into A) express AB0 Ag not expressed in the donor and are at risk for graft-versus-host (GvH) reactions such as delayed haemolysis of recipient RBC due to donor-derived anti-recipient A/B Ab. (3) **Bidirectional** AB0-incompatibility (eg A into B) represents a combination of major and minor AB0-incompatibility and puts the recipient at risk for both HvG and GvH reactions. The influence of AB0 incompatibilities on the incidence of acute graft-versus-host disease (GvHD) and the overall survival was analysed in three clinical retrospective studies [10–12]. The results of two studies, which analysed all patients receiving allogeneic HSCT since 1980 transplanted at the University Hospitals of Zürich and Basel suggested a higher incidence of acute GvHD in patients with minor AB0 incompatibility and an inferior survival in the group of patients with bidirectional AB0 incompatibility. Similar results have been published by several other groups [13, 14]. To confirm these results, we addressed these questions in collaboration with the Centre for International Blood and Marrow Transplant Research (CIBMTR) in a large homogenous group of over 3000 patients. The latter analysis revealed that the overall survival, transplant-related mortality, and grade II–IV acute GvHD were not significantly different among the four groups of AB0-incompatibility. Bidirectional AB0 incompatibility was associated with a higher risk of grade III–IV acute GvHD, but this did not translate into a higher transplant-related mortality. These results corroborate the generally applied practice of allogeneic HSCT across AB0 barriers and underline that the use of AB0-incompatible transplants in the modern era of allogeneic bone marrow transplantation is safe and not associated with major disadvantages. Nevertheless, several immunohaematological complications may arise. (i) Patients with major AB0 incompatibility (ie A in 0, B in 0, AB in A, AB in B) have a delayed RBC engraftment and are at risk for PRCA. (ii) Delayed neutrophil recovery was observed after major AB0 incompatibility. (iii) AB0 incompatible HSCT increases the risk for transplant-associated microangiopathy (TAM). (iv) Patients with minor or major AB0 incompatible HSCT are at risk for posttransplant haemolysis. All of these issues were addressed as summarised below. The results of these clinical studies are subject of several original articles (published or in preparation) and of a recent detailed review [15].

Delayed red blood cell engraftment and pure red cell aplasia

In general, donor-type erythropoiesis is established in the majority of patients within the first three weeks after allogeneic HSCT. However, delayed RBC engraftment and posttransplant PRCA may occur in patients receiving major AB0 incompatible HSCT. The delay in RBC engraftment is caused by continued anti-donor anti-A/B isoagglutinin production by persisting recipient-type plasma cells. The question whether posttransplant PRCA depends on the level and/or reduction of anti-donor isoagglutinins prior to HSCT was addressed [16]. In the major AB0 incompatible set-

ting immediate haemolysis due to preformed anti-donor isoagglutinins is usually prevented by removal of anti-donor isoagglutinins from the patient's plasma by plasmapheresis or pretransplant infusion of donor-type RBC. Alternatively, the stem cell product can be depleted from RBC leaving the patient free of potentially hazardous manipulations. The incidence of posttransplant PRCA was significantly higher in patients without pretransplant anti-donor isoagglutinin depletion (16% *vs* 3%) and RBC engraftment was delayed to 225 days at which time a simultaneous decrease of anti-donor anti-A/B Ab was noted. In addition, pretransplant anti-A/B Ab reduction, the use of peripheral blood stem cells, acute GvHD, and younger age at HSCT were significantly associated with a faster RBC engraftment. Thus, delayed RBC engraftment and posttransplant PRCA following major AB0 incompatible HSCT was partly prevented by pretransplant reduction of anti-donor isoagglutinins. Our data indicate the potential of long-living recipient-type plasma cells after HSCT, but the reason for the persistence of these cells in some but not all patients remains to be elucidated.

Delayed neutrophil engraftment

Unexpectedly, neutrophil engraftment was also delayed by approximately two days in major AB0-incompatible HSCT [12]. The most likely explanation for this novel finding is the presence or generation of anti-donor Ab against neutrophil-specific Ag. More specifically, it is likely that high pretransplant levels of anti-donor anti-A/B Ab and/or residual host B and plasma cells escaping the conditioning regimen are responsible for the observed delay. Anti-donor isoagglutinins may bind to AB0 Ag absorbed on the surface of neutrophils or their precursors in the bone marrow, thus leading to elimination or suppression [12].

Transplant-associated microangiopathy

Theoretically, donor-derived Ab may bind to the host endothelium causing activation and TAM. Therefore, the occurrence, risk factors, and outcome of patients with a variety of haematological disorders, who developed TAM after allogeneic HSCT, were analysed [17]. The majority received standard myeloablative conditioning, 18% were treated with non-myeloablative conditioning (fludarabine and total body irradiation). The cumulative incidence of TAM was 31% at 100 days. Patients with TAM had higher levels of LDH, bilirubin, and creatinine, and suffered more often from neurological symptoms. TAM was not associated with the source of stem cells (peripheral blood versus bone marrow), and the cyclosporine levels. Risk factors for TAM included donor type, age, gender, major or bidirectional AB0 incompatibility, and acute GvHD. In patients with TAM, one-year survival was lower than in patients without TAM. Poor survival was associated with the number of schistocytes in the peripheral blood. TAM was independently associated with mortality adjusting for donor type, age and acute GvHD occurrence and severity. TAM may therefore represent endothelial damage driven by graft-versus host interactions, mechanisms still largely unknown.

Posttransplant haemolysis

Haemolysis after allogeneic HSCT is mediated by immunological and non-immunological mechanisms. Whereas AB0 incompatibility is a known risk factor for haemolysis, other risk factors, the exact incidence, and the consequences of haemolysis after HSCT are less well studied. Therefore, we performed a retrospective analysis of 860 consecutive patients receiving allogeneic HSCT. The cumulative probability of haemolysis was significantly higher for all groups of AB0 incompatibility (40% for major, 32% for minor, and 27% for bidirectional AB0-incompatibility). However, haemolysis was also observed in 18% of the patients receiving AB0 identical transplants. Consequently, RBC transfusion requirements were increased in patients with haemolysis. Independent risk factors for haemolysis besides AB0-incompatibility were, HSCT from an unrelated donor, and acute GvHD grade II–IV. In contrast, age at HSCT, gender, stem cell source, GvHD prophylaxis, reduced-intensity conditioning, rhesus and HLA mismatches were not associated with the occurrence of haemolysis. The combination of haemolysis and acute GvHD was associated with a higher mortality, suggesting a potentiating effect of these two complications [18].

Part II: AB0 incompatible haematopoietic stem cell transplantation from an immunological point of view

The second aim of this project was to analyse the immunological mechanisms leading to the acceptance of AB0 incompatible HSCT. In particular, we analysed the anti-A/B Ab levels before and after HSCT, anti-A/B Ab and complement deposition onto peripheral EC and a potential EC chimerism. The latter are the first cells encountered by the recipient's immune system after SOT and by the newly transplanted donor's immune system after allogeneic HSCT. Consequently, EC constitute an important target for humoral rejection, accommodation, and tolerance reactions. In general, the immunological reactions following HSCT are reciprocal to SOT insofar as they are directed against the host tissues rather the donor organ. The immunological reactions after allogeneic HSCT are of interest, allowing the analysis of AB0-incompatible transplantations in a large number of patients, and provide an opportunity to study the effects of Ag-mismatched transplantations in the absence of immunosuppression.

After major AB0 incompatible SOT, binding of preformed anti-donor A/B Ab to AB0 Ag abundantly expressed on graft EC leads to complement and endothelial activation and humoral **rejection** of the transplanted organ. In contrast, resistance to organ rejection despite a continued presence of circulating anti-donor A/B Ab and complement is referred to as graft **accommodation**. Acceptance of a donor organ with deletion of donor-specific immune reactions, but normal third party reactivity is referred to as transplant **tolerance**. Since anti-A/B cause rejection after major AB0-incompatible SOT, donor-derived anti-host A/B Ab should induce endothelial activation and damage after minor incompatible HSCT. However, this association has not been convincingly demonstrated due to hitherto unknown mechanisms that prevent either the production of anti-A/B Ab, the binding of anti-A/B Ab to the endothelial AB0 Ag, or the activation of EC.

Anti A/B antibody levels after AB0 incompatible HSCT

To quantify the levels of anti-A/B as well as anti-porcine IgM and IgG Ab in the serum of patients after HSCT a flow cytometry based method was established and validated in a population of healthy blood donors [19]. Thereafter, anti-A/B Ab levels were monitored prospectively in 77 patients receiving allogeneic HSCT with a median observation time of more than one year. Some of the results of this study have recently been published [20]. Anti-A/B IgM was found in the majority of patient and donor samples prior to HSCT as predicted by AB0 blood group typing, whereas anti-A/B IgG was almost exclusively present in patients with blood group 0. Donor-directed anti-A/B Ab disappeared rapidly after major AB0 incompatible HSCT in the majority of patients and did not reappear in the further posttransplant course with the exception of the PRCA patients (figure 1). Reciprocally, recipient-directed anti-A/B Ab were not detectable at any time point after minor AB0 incompatible HSCT despite a complete donor-type haematopoietic chimerism. Importantly, anti-A/B Ab not directed against a donor or recipient AB0 Ag as well as anti-porcine Ab levels remained stable after HSCT, excluding unspecific effects on Ab production due to immunosuppression. Moreover, neither Ab nor complement deposition were detectable on recipient EC in skin biopsies of patients after minor AB0-incompatible HSCT excluding the possibility of Ab sequestration to AB0 Ag expressed on EC. Thus, in contrast to AB0 incompatible SOT, where anti-A/B Ab often reappear after a certain time without causing rejection (accommodation), no anti-donor Ab are measurable after minor incompatible HSCT in the serum and skin biopsies (tolerance). To date it is not clear, how B cells are tolerised against host AB0 Ag after minor AB0 incompatible HSCT. We are currently analysing the potential mechanisms of deletion and anergy of anti-host A/B producing B cells by AB0 Ab-specific ELISPOT and FACS assays according to previously published protocols [21].

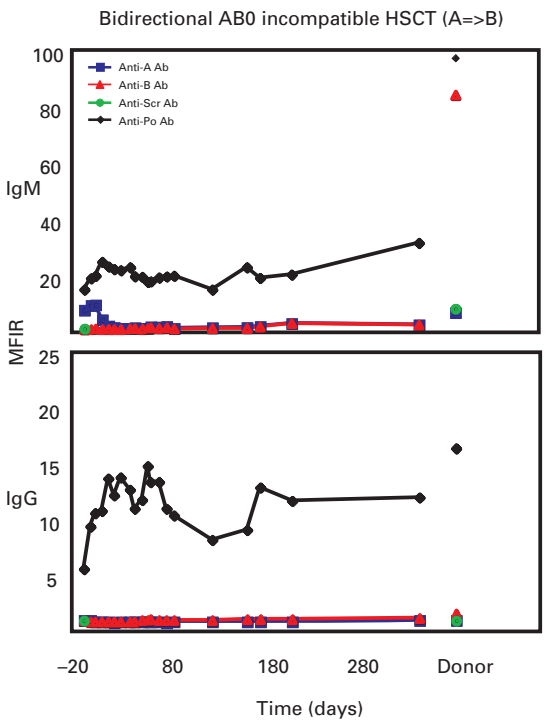

Figure 1

Bidirectional AB0 incompatible HSCT (A in B). The patient received HSCT for advanced acute myelogenous leukaemia after myeloablative conditioning. Engraftment of all haematopoietic cell lines occurred within three weeks. Haemolysis was not observed in the posttransplant course and the patient experienced mild GvHD on day 28. The figure shows the levels of anti-A/B (blue and red line) and anti-porcine antibodies (black line). Anti-A/B and anti-porcine IgM (upper panel) and IgG (lower panel) were measured by indirect flow cytometry using FITC-labelled anti-human IgM and IgG antibodies as secondary antibodies. The mean fluorescence intensity was calculated as ratio between the sample of interest and the negative control (human AB or porcine serum).

Endothelial cell chimerism after allogeneic haematopoietic stem cell transplantation

Several studies in AB0 incompatible SOT have suggested a donor-specific endothelial chimerism by replacement of recipient EC with bone marrow-derived EC precursor cells (EPC), thereby providing a potential mechanistic basis of tolerance induction as reviewed recently [22]. In contrast, this mechanism has not been thoroughly analysed in allogeneic HSCT. To address this question an immunohistochemical staining protocol for AB0 Ag on EC of skin, heart, and bone-marrow tissue samples was established. Using this technique we found no evidence of donor-type EC chimerism in 22 skin biopsies up to ten years after HSCT. Similarly, postmortem samples from HSCT patients who died from acute GvHD did not provide evidence for the expression of donor AB0 Ag on EC (figure 2). To confirm these results, EC chimerism after gender-mismatched HSCT was analysed for the presence of donor-derived EC by X/Y-specific chromogen in situ hybridisation and by short tandem repeat analyses (ongoing study) in skin biopsies from seven patients. Again, none of the analysed samples suggested a donor-type endothelial chimerism. The pooled data are still unpublished, but a case study after major AB0-incompatible and gender-mismatched HSCT has recently been published [23].

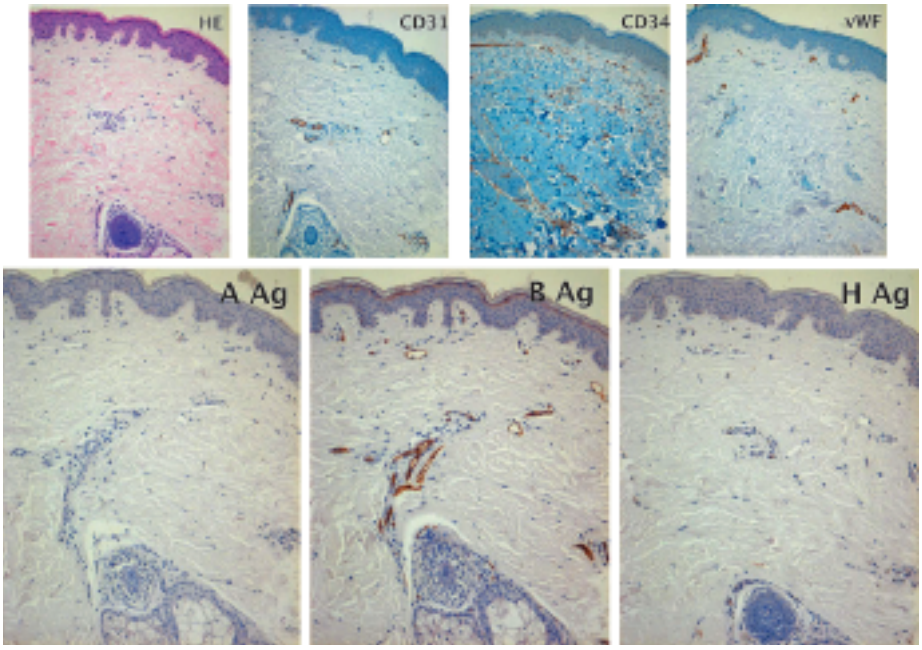

Figure 2

Skin biopsy specimen derived from a patient of blood group B, who underwent minor AB0-incompatible (0 → B) HSCT. HE represents haematoxylin/eosin staining; CD31, CD34, and von Willebrand factor staining were performed to determine EC (top row). Immunohistochemical staining for ABH histo-blood group antigen 4 years after transplantation. Endothelial cells exclusively expressed recipient type B antigen and no donor antigen H was detected on EC 1412 days after transplantation (bottom row).

Part III: Xenotransplantation

Similar to the AB0 system in allogeneic human SOT, carbohydrate epitopes, ie Gal, represent a major hurdle for successful xenotransplantation using pigs as donors [22]. Gal is expressed in all vertebrates except for humans and higher apes. Consequently, xenoreactive human-anti-pig Ab are predominantly, but not exclusively directed against the Gal epitope. Binding of IgG and IgM Ab activates porcine EC and triggers complement lysis responsible for hyperacute xenograft rejection. The recent generation of Gal knock-out pigs represented a major break-through for the applicability of such organ transplants. However, other naturally occurring or elicited Ab directed against non-Gal Ag on porcine tissues might still cause hyperacute or acute vascular rejection. We therefore examined the levels and functional properties of Gal- and non-Gal Ab in healthy individuals. Human IgM binding to porcine RBC was found in 93% and IgG binding in 86% of all samples. Non-Gal Ab comprised 13% of total IgM and 36% of total IgG binding to pEC and the majority of anti-Gal and non-Gal IgG Ab were of the IgG_2 subclass. Antibody and complement-induced lysis and ADCC of Gal-deficient ($Gal^{-/-}$) compared to wild-type ($Gal^{+/+}$) porcine EC were 21% and 29%, respectively. Thus, non-Gal anti-porcine Ab represent a potentially relevant immunological hurdle in a subgroup of individuals by inducing endothelial damage in xenografts [24].

Adhesion and cytotoxicity of human leukocytes interacting with porcine endothelial cells

Interactions between different human leukocyte subsets and porcine EC contribute substantially to rejection of xenogeneic tissues. Here we analysed whether the carbohydrate porcine Ag Gal plays a role in cellular responses to xenogeneic EC. Therefore, adhesion of human leukocyte subsets to porcine EC was tested by rolling-adhesion assays simulating *in vivo* conditions. Adhesion of human peripheral blood mononuclear cells (PBMC), granulocytes, or purified natural killer (NK) cells to porcine EC did not depend on the Gal expression of porcine EC. NK and B cells preferentially adhered to porcine EC. Tumour-necrosis factor stimulation of $Gal^{-/-}$ and $Gal^{+/+}$ porcine EC induced an upregulation of CD62E and CD106 expression and increased cellular adhesion, in particular of granulocytes. The lack of Gal expression did not prevent xenogeneic human NK cytotoxicity mediated by freshly isolated or interleukin-2-activated NK cells. In summary, neither human leukocyte adhesion nor xenogeneic NK cytotoxicity against porcine EC are impaired by the lack of Gal indicating that Gal is not a dominant target of cellular rejection [25, 26].

Complement in xenogeneic transplantations

The innate immune system, including the coagulation and the complement system, and NK cells, plays a critical role in activation and damage of EC during xenograft rejection [27]. Antibody and complement mediated activation of porcine EC induces shedding of heparan sulfate proteoglycans (HSPG) from the cell surface layer and exhibit a procoagulant and pro-inflammatory cell surface. The semi-synthetic proteoglycan analogue dextran sulfate (DXS) is known to inhibit the complement and coagulation cascade by replacement of HSPG on EC [28, 29]. DXS inhibits all three pathways of complement activation. Binding of DXS to porcine EC increased upon treatment with human serum or heparinase I and correlated positively with the inhibition of human complement deposition. This cytoprotective effect of DXS was still present when the challenge with normal human serum was performed 48 hr after DXS treatment. DXS incubation of porcine EC with and without prior TNFα stimulation reduced xenogeneic cytotoxicity mediated by human NK cells by 47% and 25%, respectively. Thus, DXS binds to porcine cells and protects them from complement- and NK cell-mediated injury *in vitro*. It might therefore be used as a novel therapeutic strategy to prevent xenograft rejection, however, the major disadvantage of DXS is a strong anticoagulant effect and bleeding might therefore compromise systemic use of this substance in a clinical setting.

By screening a variety of other carbohydrate compounds with standard complement and coagulation assays, a novel, fully synthetic tyrosine analogue with reduced inhibition of the coagulation system was identified as a candidate for EC protection (sTyr-PAA). Of all tested compounds, sTyr-PAA was the most effective substance in inhibiting all three pathways of complement activation, whereas the inhibitory effect on the coagulation cascade was significantly lower as compared to DXS. sTyr-PAA inhibited deposition of human complement on porcine EC and this inhibition correlated with the binding of sTyr-PAA to EC. Moreover, sTyr-PAA preferentially bound to damaged EC protecting them from complement-mediated damage. Since sTyr-PAA is less effective on the coagulation system than DXS it may have a potential for *in vivo* application.

After the finding that low molecular weight DXS acts as an EC protectant and prevents human complement- and NK cell-mediated cytotoxicity towards porcine cells *in vitro* it was hypothesised that DXS, combined with cyclosporine A (CyA), could prevent acute vascular rejection in a hamster-to-rat cardiac xenotransplantation model [30]. Untreated, CyA-only, and DXS-only treated rats rejected their grafts within 4–5 days. Of the hearts grafted into rats receiving DXS in combination with CyA, 28% survived more than 30 days. Deposition of anti-hamster Ab and complement was detected in long-term surviving grafts. Combined with the expression of haemoxygenase 1 (HO-1) on graft EC, these results indicate that accommodation had occurred. In conclusion, it was shown that DXS + CyA induce long-term xenograft survival and we provide evidence that DXS might act as a local EC protectant also *in vivo*.

Discussion and conclusions

Our clinical studies indicate that HSCT can safely be performed across the AB0-blood group barrier without affecting overall survival and rejection. However, the presence and/or continued generation of anti-donor and/or anti-recipient Ab may lead to several immunohaematological com-

plications due to humoral responses. Although the risk for PRCA, TAM and haemolysis increases after AB0-incompatible HSCT, it does not translate into a higher transplant-related mortality. These results confirm earlier studies and reinforce the current practice to allow AB0-incompatible donors in HSCT. On the other hand, such transplants are performed since more than 30 years and the knowledge of the mechanistic background of AB0-incompatible HSCT is still scarce. Whereas the potential role of accommodation and B cell tolerance for AB0 incompatible SOT and xenotransplantation has been studied profoundly, it has not been appropriately studied in HSCT. We therefore analysed the mechanisms of tolerance or accommodation in AB0 incompatible HSCT.

Several authors demonstrated a recipient-type EC chimerism after AB0 or gender mismatched SOT, which is a potential mechanism for accommodation [31]. Likewise, replacing the recipient-type EC by donor-type EC might inhibit immune stimulation following HSCT and induce a donor-specific tolerance or accommodation. However, in the presented studies we found no evidence for EC chimerism in skin, bone marrow and heart tissue samples even years after HSCT. Similar to the results observed by Fan and colleagues [21], tolerance might be a predominant mechanism responsible for successful AB0-incompatible HSCT. This hypothesis is supported by our finding that anti-recipient Ab were not present in the serum of patients after minor AB0-incompatible HSCT although the incompatible AB0 Ag is almost universally present on a variety of recipient cells. Likewise, there was no indication of anti-recipient Ab or complement deposition on EC in skin biopsies. However, the final proof for B-cell tolerance will be to show the lack of B cells capable of producing anti-donor Ab in the presence of a normal B cell response to third party Ag. This has been elegantly shown for neonatal heart transplantation, but not for HSCT yet. In summary, we were able to exclude several possible immunological mechanisms leading to the acceptance of AB0 incompatible HSCT. Our current experiments focus on the role of anti-A/B Ab producing B and plasma cells for the induction of humoral tolerance after AB0-incompatible HSCT.

With regards to xenotransplantation our projects analysing interactions between human leukocytes and porcine EC have shown so far that neither human leukocyte adhesion nor xenogeneic NK cytotoxicity against porcine EC are impaired by the lack of Gal. Thus, Gal is not a dominant target of cellular rejection and non-Gal targets need to be identified to avoid xenograft rejection. Testing approaches to protect porcine EC from cellular and humoral immune responses, it has been shown that DXS and sTyr-PAA inhibit complement activation on PAEC *in vitro*, that DXS inhibits xenogeneic NK cytotoxicity, and that DXS in combination with CyA induces long-term xenograft survival in a cardiac hamster-to-rat model. Thereby we provided novel evidence that DXS might act as a local EC protectant *in vivo*.

Consequently, AB0-incompatible HSCT provides an excellent model to study the immunological mechanisms of accommodation and/or tolerance in a large number of patients *in vivo*. With respect to the ratio of Ab and Ag, the maturity of the immune system, and the level and duration of immunosuppression, AB0-incompatible SOT, HSCT and xenotransplantation differ considerably as discussed elsewhere in detail [22]. The relative contribution of each factor might be important for the induction of either accommodation or tolerance. However, due to the similarities of the immunological hurdles, the knowledge obtained from AB0-incompatible transplants might promote further advances in the field of xenotransplantation and vice versa.

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