ORIGINAL ARTICLE

Preformed donor-reactive T cells that persist after ABO desensitization predict severe T cell-mediated rejection after living donor kidney transplantation – a retrospective study

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SUMMARY

Preformed donor-reactive T cells are relatively resistant to standard immunosuppression and account for an increased incidence of T cell-mediated rejection (TCMR) and inferior kidney allograft outcomes. We analyzed 150 living donor kidney transplant recipients (KTRs) of a first kidney allograft. Ninety-eight ABO-compatible (ABOc) and 52 ABO-incompatible (ABOi) KTRs were included. Samples were collected at 6 time points, before rituximab, before immunoadsorption and pretransplantation, at +1, +2, and +3 months posttransplantation, and donor-reactive T cells were measured by interferon-γ ELISPOT assay. Twenty of 98 ABOc (20%) and 12 of 52 ABOi KTRs (23%) showed positive pretransplant ELISPOT. Eight of 20 ABOc-KTRs (40%) with positive pretransplant ELISPOT showed TCMR, whereas 17 of 78 ABOc-KTRs (22%) with negative pretransplant ELISPOT did ($P = 0.148$). Seven of 12 ABOi KTRs (57%) with positive pretransplant ELISPOT showed TCMR, whereas only 3 of 40 ABOi KTRs (8%) with negative pretransplant ELISPOT did ($P < 0.001$). Interestingly, 6 of 7 ABOi KTRs with positive pretransplant ELISPOT that persists after ABO desensitization developed TCMR. Among 118 KTRs with negative pretransplant ELISPOT, 10 of 72 ABOc-KTRs (14%), but 0 of 46 ABOi KTRs, developed positive posttransplant ELISPOT ($P = 0.006$). Preformed donor-reactive T cells that persist despite ABO desensitization identify KTRs at highest risk of TCMR. Less *de-novo* donor-reactive T cells after ABO desensitization may account for less TCMR. Both, the use of rituximab and early initiation of calcineurin inhibitor-based maintenance immunosuppression may contribute to these findings.

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Key words
ABO desensitization, donor-reactive T cells, immunosuppression, kidney transplantation, rituximab, T cell-mediated rejection

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Introduction

Previous studies on donor-reactive T cells identified almost one third of all first kidney transplant recipients (KTRs) with detectable donor-reactive T cells pretransplantation [1,2]. In addition, the majority of KTRs undergoing renal retransplantation who show a sensitization with preformed human leukocyte antigen (HLA) antibodies, in addition show preformed donor-reactive T cells [3]. If not adequately suppressed, the presence of such donor-reactive T cells is likely to result in T cell-mediated rejection (TCMR) early after transplantation [1–5]. Here, a very recent and first meta-analysis about the association between pretransplant donor-reactive T cells and kidney allograft outcomes suggest and confirm a higher risk of TCMR among KTRs with preformed donor-reactive T cells particularly in those not receiving T-cell depleting induction therapy [6].

The combination of intravenous immunoglobulins (IVIG) and rituximab has been supposed to be a safe and effective approach to reduce anti-HLA antibodies among highly sensitized KTRs [7,8]. However, the impact of this desensitization strategy on preformed donor-reactive T cells has not been addressed so far.

ABO desensitization represents a quite similar approach to achieve temporary elimination of anti-AB IgM and prevention of IgG switch. The administration of a single dose of rituximab, antigen-specific immunoadsorption, IVIG, and early initiation of triple-drug maintenance immunosuppression led to excellent long-term results after ABO-incompatible (ABOi) transplantation. Reports on ABO desensitization also suggest direct and indirect effects on T-cell immunity in several ways: Rituximab reduces T-cell activation due to down-regulation of co-stimulatory molecules, tacrolimus suppresses T-cell-derived growth factors, and IVIG reduces antigen presentation due to decreased internalization of immune complexes [9–12].

To provide an estimate of donor-reactive T-cell sensitization, an ELISPOT assay detecting interferon-γ (IFNg) secreting recipient T cells after in vitro stimulation with donor antigens has been established and has been proven to be a reliable and feasible approach to monitor donor-reactive T-cell sensitization. These donor-reactive T cells have been associated with an increased risk of TCMR early after transplantation [13–16].

Therefore, we hypothesized that ABO desensitization may impact preformed donor-reactive T cells and tried to address the following questions: (i) What impact does ABO desensitization have on the presence of preformed donor-reactive T cells? (ii) What impact does ABO desensitization have on outcomes after kidney transplantation? (iii) What impact does ABO desensitization have on the development of *de-novo* donor-reactive T cells?

Materials and methods

Patients

This study was approved by our local ethical review committee in compliance with the Declaration of Helsinki (Ethic Committee Charité University Medicine Berlin, Germany, 126/2001, 07/30/2001). Informed consent was obtained from all patients. In total, we examined 202 living donor KTRs of a first kidney allograft transplanted at our single transplant center at Charité Campus Virchow Clinic between 2008 and 2015. We included 98 consecutive ABO-compatible (ABOc) KTRs and 52 consecutive ABOi KTRs for analysis. In total, 52 KTRs were excluded from the analysis, 8 KTRs with a previous nonrenal solid organ transplantation, 23 KTRs who did not give their consent or withdrew their consent posttransplantation, 17 KTRs with insufficient monitoring of preformed donor-reactive T cells due to technical issues during weekends, and 4 KTRs who were lost to follow-up within the first posttransplant year. We compared 1-year outcomes of patient survival, death-censored allograft survival, allograft function, delayed graft function, and T cell-mediated rejection. Recipient and donor clinical characteristics and 1-year outcomes are shown in Table 1.

ABO desensitization protocol

Kidney transplant recipients considered for iABO transplantation received a single dose (375 mg/m^2) of anti-CD20 antibody rituximab 4 weeks prior to transplantation, and maintenance immunosuppression was initiated at that time. Maintenance immunosuppression was a triple-drug regimen with tacrolimus, MMF, and methylprednisolone. Target tacrolimus trough levels were 5–7 ng/ml pretransplantation, MMF dose was 500 mg twice daily pretransplantation, and methylprednisolone dose was 24 mg pretransplantation. Six days before scheduled transplantation, iABO-KTRs underwent antigen-specific immunoadsorption (Glycosorb A/B columns) to remove isoagglutinin antibodies, until antidonor isoagglutinin (anti-A and/or anti-B) IgG titer decreased to a level of 1:8. Postoperative antigen-specific immunoadsorption was performed on days +3, +6, and +9. Immunoadsorption was only continued if there was

Table 1. Clinical characteristics and outcomes of all living donor kidney transplant recipients (KTRs) divided into ABO-compatible and ABO-incompatible KTRs.

	Total (n = 150)	ABO-compatible KTRs (n = 98)	ABO-incompatible KTRs (n = 52)	P value
Characteristics				
Age, years*	49 (18–77)	50 (21–77)	48 (18–74)	0.617
Male sex, n (%)	99 (66)	60 (61)	39 (75)	0.105
Donor age, years*	53 (25–78)	54 (28–78)	49 (25–70)	0.187
Living donation, n (%)				
Related donors, n (%)	73 (49)	52 (53)	21 (40)	0.127
Time on dialysis, months*	10 (0–116)	9 (0–75)	13 (0–116)	0.189
Induction immunosuppression, n (%)				
IL2-receptor blockade	150 (100)	98 (100)	52 (100)	1
Maintenance immunosuppression, n (%)				
Tacrolimus, MMF, steroids	120 (80)	72 (73)	52 (100)	<0.001
Cyclosporine, MMF, steroids	30 (20)	26 (27)	0 (0)	
Causes of ESRD, n (%)				
Glomerulonephritis/vasculitis	50 (33)	29 (30)	21 (40)	0.314
Diabetes/nephroangiosclersosis	36 (24)	27 (28)	9 (17)	
Polycystic kidney disease	13 (9)	7 (7)	6 (12)	
Uropathy	11 (7)	9 (9)	2 (4)	
Other or undetermined	40 (27)	26 (27)	14 (27)	
Total HLA mismatches, n (%)	3 (0–6)	3 (0–6)	4 (0–6)	0.021
4–6 HLA mismatches	58 (39)	27 (28)	31 (60)	<0.001
Panel-reactive antibodies (PRA), n (%)				
<10%	150 (100)	98 (100)	52 (100)	1
>10%	0 (0)	0 (0)	0 (0)	
Outcomes				
Delayed graf function (DGF), n (%)	8 (5)	4 (4)	4 (8)	0.449
T cell-mediated rejection (TCMR) within the first posttransplant year, n (%)	33 (22)	23 (23)	10 (19)	0.680
IA/IB	24 (16)	18 (18)	6 (12)	0.353
IIA/IIB/III	9 (6)	5 (5)	4 (8)	0.720
De-novo donor-specific antibodies (DSA) within the first posttransplant year, n (%)	5 (3)	4 (4)	1 (2)	0.659
Antibody-mediated rejection (ABMR) within the first posttransplant year, n (%)	3 (2)	2 (2)	1 (2)	1

*Median (range).

a rise in isoagglutinin antibody titer or histological evidence of antibody-mediated rejection. IVIG (0.5 g/kg body weight) was administered 1 day ahead of the scheduled transplantation and at day +9 posttransplantation.

Immunosuppressive therapy

All patients received induction with IL-2R antagonist (basiliximab), which was given before transplantation and +4 days posttransplantation. Maintenance immunosuppression was a triple-drug regimen with a calcineurin inhibitor (tacrolimus or cyclosporine), MMF, and methylprednisolone. Target tacrolimus trough levels were 8–10 ng/ml for the first 6 months, 5–7 ng/ml from 7 to 12 months, and 4–6 ng/ml thereafter. All KTRs received an initial MMF dose of 1000 mg twice daily as tolerated. Methylprednisone was tapered to 4 mg daily over 4 weeks posttransplantation.

Collection of samples for monitoring of donor-reactive T cells

One hundred and fifty KTRs were successfully enrolled in our monitoring of donor-reactive T cells. Among ABOc-KTRs, 30–40 ml of citrate blood was collected at the following four time points: pretransplantation, at +1

+2, and +3 months posttransplantation. Among ABOi KTRs, 30–40 ml of citrate blood was collected at the following six time points: before administration of rituximab, before immunoadsorption, before transplantation, at +1, +2, and + 3 months posttransplantation. We collected 10–20 ml of citrate blood from donors pretransplantation. Living donor peripheral blood mononuclear cells (PBMC) were CD3-depleted to avoid donor PBMC IFNg release and isolated using standard Ficoll–Hypaque density gradient technique. PBMC were T-cell depleted using a human CD3 Cell Depletion Cocktail (RosetteSep Human CD3 Depletion Cocktail (Catalog No 15661), STEMCELL Technologies, Vancouver, BC, Canada). In addition, a 40 Gy irradiation of all T-cell depleted donor PBMC was added to achieve an almost total inhibition of IFNg release by donor PBMC. Recipient PBMC were isolated using standard Ficoll–Hypaque density gradient technique. Donor PBMC and recipient PBMC were cryopreserved in liquid nitrogen until their use.

ELISPOT assay for IFNg detection of donor-reactive T cells

Donor-reactive T cells were determined by measuring IFNg upon stimulation of PBMC as described previously [16–18]. For ELISPOT assay, 96-well multiscreen filter plates (MAIPS4510; EMD Millipore, Billerica, MA, USA) were coated with 100 µl of primary IFNG monoclonal antibody (mAb) at a concentration of 3 µg/ml (anti-human IFN-c; M700A; Endogen, Woburn, MA, USA) and incubated overnight at 4 °C. A standardized responder T-cell number of 3.0×10^5 isolated PBMC per well were added in duplicate wells with 3.0×10^5 T-cell-depleted donor PBMC and with Staphylococcus enterotoxin B (SEB; Sigma, 1 µg/ml; Sigma-Alldrich, St. Louis, MO, USA) as positive control and incubated for 24 h at 37 °C. Negative controls were always run in parallel using 3.0×10^5 recipient PBMC plus medium and DMSO. Plates were incubated overnight at 4 °C with 100 µl (1 µl/ml) biotinylated detection IFNG antibody (ahu-IFNG biotin-Endogen M701). After adding streptavidine (1 µg/ml) for 2 h at room temperature, spots were developed by adding 200 µl visualization solution, AEC (3-amino-9-ethylcarbazole; Sigma-Aldrich) in acetate buffer supplemented with H_2O_2 30% for 3–5 min. Resulting spots were counted using a computer-assisted ELISPOT reader (Immunospot; Cellular Technologies, Ltd., Cleveland, OH, USA). Positive ELISPOT signals were predefined as containing at least 25 spot forming units per well after subtraction of negative control. The mean number of spots from duplicate was used for analysis.

Statistical methods

Statistical tests were performed using SPSS Version 23 (SPSS, Chicago, IL, USA). For comparisons of study groups, two-sided Mann–Whitney *U* test for nonparametric independent samples was used. Clinical characteristics were compared across groups using Fisher's exact test categorical variables. Outcomes were measured with reverse Kaplan–Meier models and overall strata comparisons measured by log-rank tests. Two-sided *P*-values <0.05 were considered statistical significant.

Results

Clinical characteristics and overall patient and kidney allograft outcomes

Ninety-eight ABOc-KTRs and 52 ABOi KTRs were analyzed. Median follow-up of ABOc-KTRs was 71 months (range 9–126 months), during which five patients died (5.1%), and three returned to dialysis (3.1%). One ABOc-KTR lost his kidney allograft at +9 months posttransplantation due to severe TCMR, 1 ABOc-KTR lost her kidney allograft at +13 months posttransplantation due to TCMR and severe sepsis, and 1 ABOc-KTR lost his kidney allograft at 49 months posttransplantation due to recurrent TCMR and antibody-mediated rejection (ABMR). Median follow-up of ABOi KTRs was 52 months (range 13–125 months), during which two patients died (3.8%), and two returned to dialysis (3.8%). One ABOi KTR lost his kidney allograft at +24 months posttransplantation due BKV-associated nephropathy, and 1 ABOi KTR lost his kidney allograft at +113 months posttransplantation due to severe TCMR and ABMR. Overall, no differences were observed for patient survival and death-censored kidney allograft survival between ABOc and ABOi KTRs ($P > 0.05$).

No differences were observed for recipient age, recipient sex, and donor age. Unrelated donors were more common among ABOi KTRs that was associated with a higher HLA mismatch in this group. No KTRs with humoral presensitization were included. Besides ABO desensitization, all KTRs received the same induction immunosuppression with IL2-receptor blocker basiliximab and maintenance immunosuppression with

calcineurin inhibitor, mycophenolate mofetil, and steroids. Patient characteristics of ABOc and ABOi KTRs are shown in Table 1.

Impact of ABO desensitization on preformed donor-reactive T cells and TCMR

Among 150 living donor KTRs, we identified 32 KTRs (21%) with positive pretransplant ELISPOT. Twenty of 98 ABOc-KTRs (20%) and 12 of 52 ABOi (23%) KTRs showed positive pretransplant ELISPOT. These KTRs showed a median of 112 SFU/300.000 PBMC (range 29–463). One hundred and eighteen KTRs (79%) showed negative pretransplant ELISPOT.

ABO-compatible KTRs

Eight of 20 ABOc-KTRs (40%) with positive pretransplant ELISPOT developed TCMR within the first posttransplant year, whereas 17 of 78 ABOc-KTRs (22%) with negative pretransplant ELISPOT developed TCMR ($P = 0.148$). This difference did not reach statistical significance (Fig. 1a). Among 12 ABOc-KTRs with positive pretransplant ELISPOT, who did not develop TCMR, 2 ABOc-KTRs (17%) showed persistence of positive posttransplant ELISPOT at 1, 2, and 3 months posttransplantation. Among 8 ABOc-KTRs positive pretransplant ELISPOT, who developed TCMR, 4 ABOc-KTRs (50%) showed persistence of positive posttransplant ELISPOT at 1, 2, and 3 months posttransplantation (1 ABOc-KTR showed positive posttransplant ELISPOT after rejection treatment with steroid pulses only, 1 ABOc-KTR showed positive posttransplant ELISPOT after rejection treatment with steroid pulses plus T-cell depleting therapy, and 2 ABOc-KTRs developed TCMR after 3 months posttransplantation). Three of 4 ABOc-KTRs (75%), who showed negative posttransplant ELISPOT at 1 month despite positive pretransplant ELISPOT, developed TCMR within the first month and negative posttransplant ELISPOT was observed after rejection treatment (1 ABOc-KTR received steroid pulses only and 2 ABOc-KTRs received steroid pulses plus T-cell depleting therapy). One of 4 ABOc-KTRs (25%), who showed negative posttransplant ELISPOT at 1 month despite positive pretransplant ELISPOT, developed TCMR at 2 months posttransplantation.

ABO-incompatible KTRs

Seven of 12 ABOi KTRs (58%) with positive pretransplant ELISPOT showed TCMR within the first posttransplant year, whereas only 3 of 40 ABOi KTRs (7.5%) with negative pretransplant ELISPOT developed TCMR ($P = 0.001$). This difference reached statistical significance (Fig. 1b).

Among 12 ABOi KTRs with positive pretransplant ELISPOT, 7 ABOi KTRs showed persistence of positive pretransplant ELISPOT through ABO desensitization, and 5 ABOi KTRs showed negative pretransplant ELISPOT mainly after administration of rituximab and initiation of maintenance immunosuppression (Fig. 2).

Six of 7 ABOi KTRs (86%) with positive pretransplant ELISPOT that persists through ABO desensitization developed TCMR, whereas only 1 of 5 ABOi KTRs (20%) with positive pretransplant ELISPOT that turned negative during ABO desensitization and stayed negative after transplantation showed TCMR within the first posttransplant year ($P = 0.072$; Fig. 3). All cases of severe TCMR with Banff category IIA, IIB, and III were observed among those ABOi KTRs that showed positive pretransplant ELISPOT through ABO desensitization. Four of 7 ABOi with positive pretransplant ELISPOT through ABO desensitization stayed ELISPOT positive also at 1, 2, and 3 months after transplantation, all of which developed TCMR. Two of 7 ABOi with positive pretransplant ELISPOT through ABO desensitization turned negative at 1, 2, and 3 months after transplantation, however, were treated for TCMR within the first posttransplant month with steroid pulses. One of 7 ABOi with positive pretransplant ELISPOT through ABO desensitization turned negative at 1, 2, and 3 months and did not develop TCMR.

Predictive value of preformed donor-reactive T cells for TCMR

Receiver operating characteristic curve (ROC) analysis for predicting the risk of TCMR among either ABOc-KTRs, ABOi KTRs before ABO desensitization, or ABOi KTRs after ABO desensitization is depicted in Fig. 4a–c, showing considerably high AUC for ABOi KTRS ranging from 0.777 to 0.790. Sensitivity, specificity, and negative predictive value (NPV) of a positive pretransplant ELISPOT for ABOc-KTRs were 32.0%, 83.3%, and 72.7%, respectively. Sensitivity, specificity, and negative predictive value (NPV) of a positive pretransplant ELISPOT for ABOi KTRs before ABO desensitization were 70.0%, 88.1%, and 55.6%, respectively. Sensitivity, specificity, and negative predictive value (NPV) of a positive pretransplant ELISPOT for ABOi KTRs after ABO desensitization were 60.0%, 97.6%, and 61.9%, respectively.

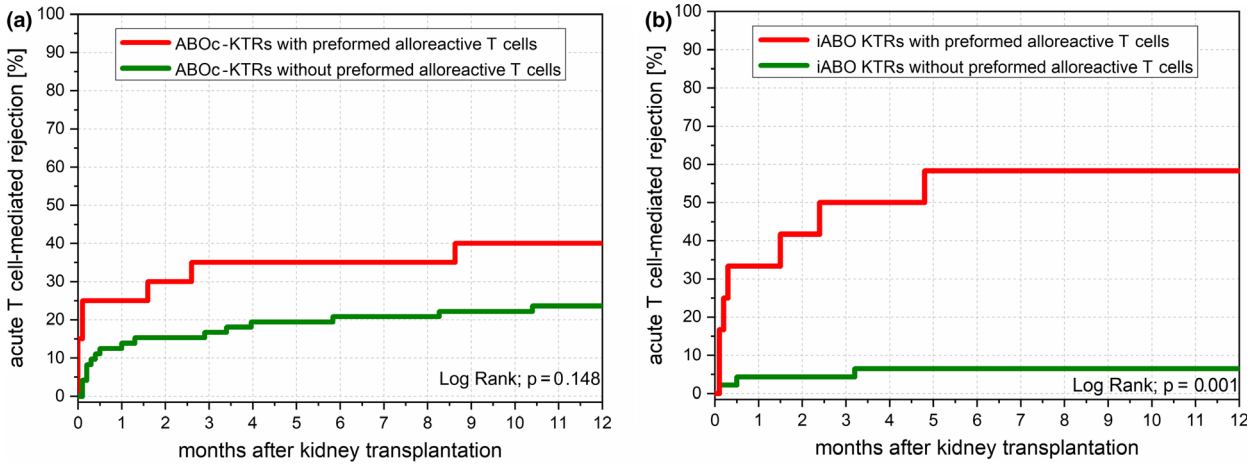

Figure 1 (a) Onset and incidence of T cell-mediated rejection (TCMR) in the first posttransplant year among ABO-compatible kidney transplant recipients (KTRs) with respect to the presence of preformed donor-reactive T cells. Eight of 20 ABOc-KTRs (40%) with preformed donor-reactive T cells showed acute T cell-mediated rejection, whereas 17 of 78 ABOc-KTRs (22%) without preformed donor-reactive T cells developed acute T cell-mediated rejection within the first posttransplant year (P = 0.148). (b) Onset and incidence of TCMR rejection in the first posttransplant year among ABO-incompatible KTRs with respect to the presence of preformed donor-reactive T cells. Seven of 12 ABOi KTRs (57%) with preformed donor-reactive T cells showed acute T cell-mediated rejection, whereas only 3 of 40 ABOi KTRs (8%) without preformed donor-reactive T cells developed T cell-mediated rejection within the first posttransplant year ($P < 0.001$). One ABOi KTR lost the kidney allograft at + 9 months posttransplantation.

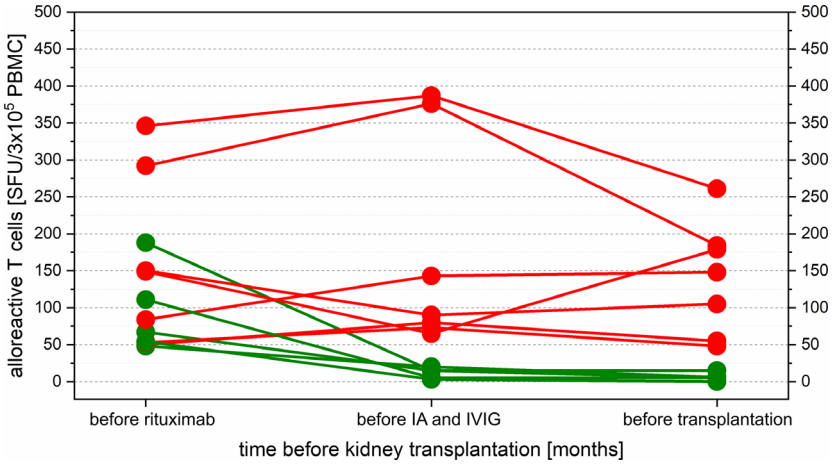

Figure 2 Impact of ABO desensitization on the persistence of preformed donor-reactive T cells. Seven of 12 (58%) ABOi KTRs with preformed donor-reactive T cells show persistence of donor-reactive T cells during ABO desensitization (red lines), whereas 5 of 12 (42%) ABOi KTRs with preformed donor-reactive T cells loose donor-reactive T cells during ABO desensitization (green lines).

Impact of ABO desensitization on de-novo donor-reactive T cells and TCMR

Kidney transplant recipients with negative pretransplant ELISPOT were grouped according to negative or positive posttransplant ELISPOT. Among 118 KTRs with negative pretransplant ELISPOT, 10 of 78 ABOc-KTRs (13%) developed positive posttransplant ELISPOT, whereas 0 of 40 ABOi KTRs (0%) developed positive posttransplant ELISPOT (P = 0.029). Among those 10 ABOc-KTRs with positive posttransplant ELISPOT, 10 ABOc-KTRs showed TCMR within the first posttransplant year.

Discussion

The impact of rituximab and IVIG, which is widely used as a safe and effective desensitization protocol to reduce anti-HLA antibodies among highly sensitized KTRs [7], on preformed donor-reactive T cells remains

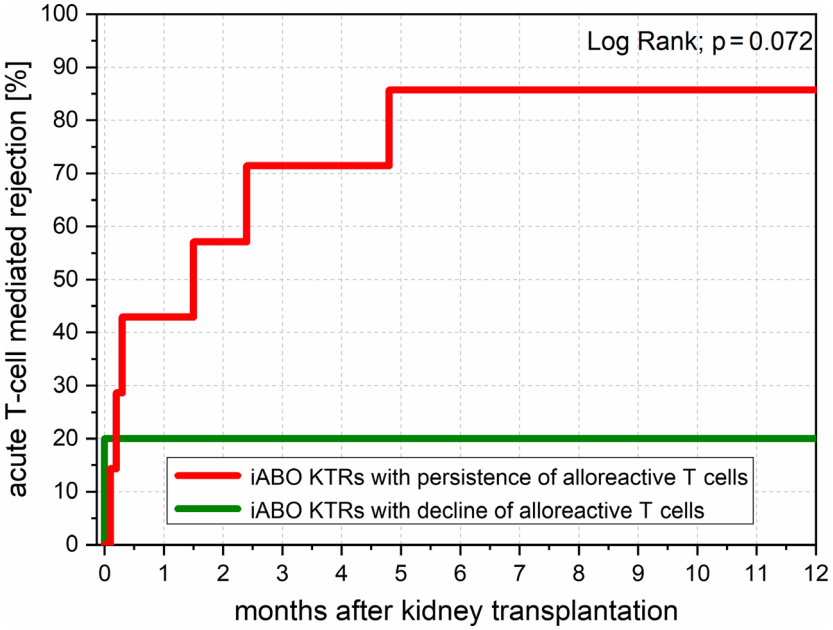

Figure 3 Onset and incidence of T cell-mediated rejection (TCMR) in the first posttransplant year among ABO-incompatible kidney transplant recipients (KTRs) with respect to persistence of preformed donor-reactive T cells during ABO desensitization. Six of 7 (86%) ABOi KTRs with preformed donor-reactive T cells that persist after ABO desensitization developed acute T cell-mediated rejection, whereas only 1 of 5 (20%) ABOi KTRs with preformed donor-reactive T cells that disappeared during ABO desensitization developed acute T cell-mediated rejection within the first posttransplant year (P = 0.072). One ABOi KTR lost the kidney allograft at + 9 months posttransplantation.

unresolved. Since targeted B cells are important contributors to both ABMR and TCMR, knowledge about its impact on preformed donor-reactive T cells is paramount for the design of future desensitization protocols [19].

ABO desensitization allows the investigation of our hypothesis in a low immunological risk cohort without preformed anti-HLA antibodies, excellent donor quality, and short cold ischemia time to minimize other important risk factors for the development of delayed graft function and TCMR.

Firstly, ABO desensitization reduces frequencies of preformed donor-reactive T cells in a subgroup of ABOi KTRs. Mechanisms that may account for this observation may include direct effects of rituximab, tacrolimus, and IVIG on T-cell activation, proliferation, and B cell-mediated antigen presentation and co-stimulation [19–21]. Very recently, van den Hoogen et al. [22] showed in a placebo-controlled trial using rituximab for induction that rituximab may reduce the incidence of TCMR among immunological high-risk KTRs, whereas no benefit was observed among immunological low-risk KTRs. Here, the impact of rituximab on TCMR may be attributed to the effects on preformed donor-reactive T cells and the development of *de-novo* donor-reactive T cells that are more likely among immunological high-risk KTRs. However, the administration of rituximab at the time of transplantation may account for a less pronounced impact on TCMR compared with the earlier administration in our population. Similarly, Tyden et al. [23] observed a tendency toward fewer and milder TCMR in KTRs who received rituximab at the time of transplantation.

Secondly, our data suggest the highest incidence of TCMR among ABOi KTRs with positive pretransplant ELISPOT that stayed positive after ABO desensitization. This observation supports previous reports that donor-reactive T cells are hard to target using standard induction and maintenance immunosuppression, due to a memory phenotype. Those T cells do not require co-stimulation compared with naïve T cells and therefore may exert a fast response with more severe consequences [23]. Activated donor-reactive memory T cells harm the kidney allograft through activation of naïve donor-reactive T cells and alloantibody production [2,24–27]]. In previous studies, TCMR has been associated with the presence of preformed donor-reactive T cells, whereas the strength of association highly differed between different studies [1–5]. This difference is most likely attributed to our finding that some preformed donor-reactive T cells are targeted by widely used desensitization protocols.

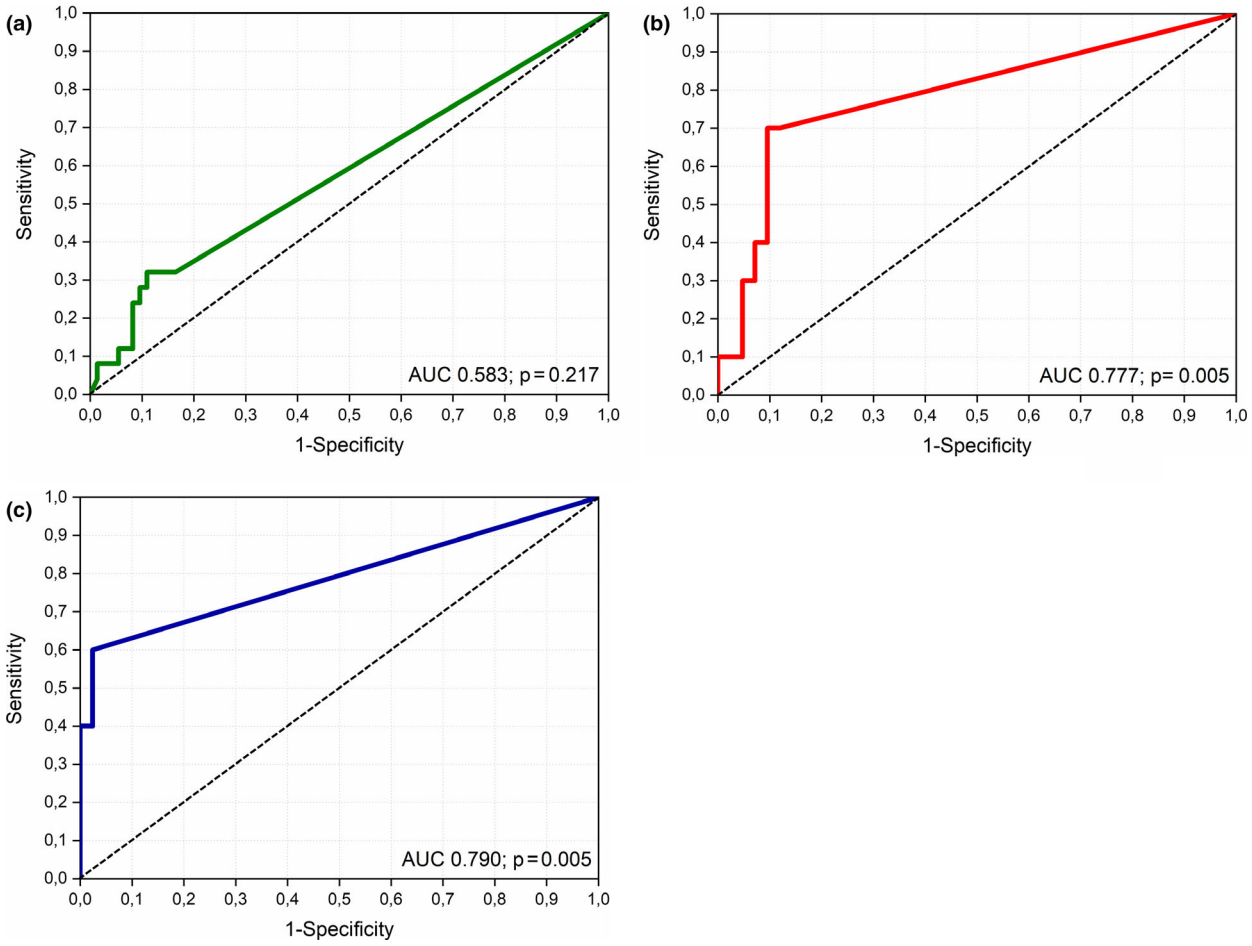

Figure 4 (a) ROC curve analysis estimates sensitivity and specificity of pretransplant ELISPOT for predicting the risk of T cell-mediated rejection (TCMR) among ABOc- kidney transplant recipients (KTRs). (b) ROC curve analysis estimates sensitivity and specificity of pretransplant ELISPOT (before ABO desensitization) for predicting the risk of TCMR among ABOi KTRs. (c) ROC curve analysis estimates sensitivity and specificity of pretransplant ELISPOT (after ABO desensitization) for predicting the risk of TCMR among ABOc-KTRs.

Thirdly, ABO desensitization impacts the development of *de-novo* donor-reactive T cells and accounts for a lower incidence of TCMR. Particularly rituximab mediates down-regulation of co-stimulatory molecules on B cells thereby affecting activation of naïve T cells. The development of *de-novo* donor-reactive T cells may also explain, why differences in the incidence of TCMR did not reach statistical significance among ABOc-KTRs with positive and negative pretransplant ELISPOT.

This is the first study addressing the impact of desensitization with rituximab, IVIG, and calcineurin inhibitor (CNI)-based maintenance immunosuppression on preformed donor-reactive T cells. On the basis of our results, we would like to introduce a proposal for donor-reactive T-cell monitoring to be implemented among different subgroups of KTRs. Monitoring of donor-reactive T-cell dynamics through desensitization may provide a sensitive marker to identify a subgroup of KTRs at increased risk of TCMR:

Firstly, KTRs with positive pretransplant ELISPOT that turns negative through desensitization are at low risk of early TCMR. These KTRs may qualify for induction therapy with IL-2 receptor blockade. Performing posttransplant ELISPOT may be valuable in cases of immunosuppression reduction, as BKV-associated nephropathy, severe infection, drug-induced adverse events that require switch of medication, or any kind of minimization protocols. Secondly, KTRs with positive pretransplant ELISPOT that stays positive through desensitization are at highly increased risk of early severe TCMR. These KTRs may qualify for T-cell depleting induction therapy, although previous studies suggest that T-cell depleting induction may fail to target donor-reactive T cells of an effector memory phenotype [28–30]. Recent work by Ayasoufi et al. [31] demonstrated

that administration of anti-thymocyte globulin in a mouse cardiac allograft model one week prior to transplantation was superior in targeting preexisting donor-reactive T-cell responses compared with administration of anti-thymocyte globulin at the time of transplantation. Thirdly, KTRs with negative pretransplant ELISPOT are at low risk of TCMR. These KTRs may qualify for induction therapy with IL-2 receptor blockade. Performing posttransplant ELISPOT may be valuable in cases of immunosuppression reduction as mentioned above. Our approach might be of special value in those KTRs undergoing HLA or ABO desensitization for a living donor kidney transplantation. Although the use of rituximab as induction therapy at the time of transplantation did not show a benefit in unselected deceased-donor KTRs [21,22], a potential improvement may be supposed in a specific subgroup, possibly characterized by the presence of preformed donor-reactive T cells.

Limitations of our study include the retrospective nature, the single-center approach, and particularly the lack of protocol biopsies. In addition, the simultaneous administration of rituximab and initiation of CNI-based maintenance immunosuppression does not allow to attribute the effects on preformed donor-reactive T cells to a single agent. Furthermore, using the ELISPOT assay to estimate T-cell alloreactivity in fact measures all IFNg-producing cells and may among others also include natural killer cells. Due to the limited number of patients with 12 ABOi KTRs with positive pretransplant ELISPOT only, the hypothesis raised here needs to be validated in a much larger cohort. If the effects on preformed donor-reactive T cells differ in KTRs with higher immunological risk (i.e., preformed HLA antibodies, retransplantation) compared to this low immunological risk group needs to be addressed in upcoming studies.

In summary, preformed donor-reactive T cells that are relatively resistant to standard immunosuppression are targeted using desensitization with rituximab, CNI-based maintenance immunosuppression, and IVIG in a subgroup of KTRs.

This finding allows a risk stratification, identifying those KTRs that show persistence of preformed donor-reactive T cells through ABO desensitization at the greatest risk of severe TCMR. In addition, less *de-novo* donor-reactive T cells after desensitization with rituximab, CNI-based maintenance immunosuppression, and IVIG may account for less TCMR in this group.

Authorship

TS: contributed to research design, writing of the paper, performance of the research, and data analysis. MS: contributed to performance of the research. NMO: contributed to writing of the paper. PR: contributed to research design and writing of the paper.

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Conflicts of interest

The authors have declared no conflicts of interest.

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REFERENCES

1. Andree H, Nickel P, Nasiadko C, *et al.* Identification of dialysis patients with panel-reactive memory T cells before kidney transplantation using an allogeneic cell bank. *J Am Soc Nephrol* 2006; **17**: 573.
2. Bestard O, Cruzado JM, Lucia M, *et al.* Prospective assessment of anti-donor cellular alloreactivity is a tool for guidance of immunosuppression in kidney transplantation. *Kidney Int* 2013; **84**: 1226.
3. Schachtner T, Otto NM, Stein M, Reinke P. Transplantectomy is associated with presensitization with donor-reactive T cells and graft failure after kidney retransplantation: a cohort study. *Nephrol Dial Transplant* 2018; **33**: 889.
4. Najafian N, Salama AD, Fedoseyeva EV, Benichou G, Sayegh MH. Enzyme-linked immunosorbent spot assay analysis of peripheral blood lymphocyte reactivity to donor HLA-DR peptides: potential novel assay for prediction of outcomes for renal transplant recipients. *J Am Soc Nephrol* 2002; **13**: 252.
5. Heeger PS, Greenspan NS, Kuhlenschmidt S, *et al.* Pretransplant frequency of donor-specific, IFN-gamma-producing lymphocytes is a

manifestation of immunologic memory and correlates with the risk of posttransplant rejection episodes. *J Immunol* 2004; **163**: 2267.
6. Montero N, Farouk S, Gandolfini I, *et al*. Pretransplant donor-specific IFNγ ELISPOT as a predictor of graft rejection: a diagnostic test accuracy meta-analysis. *Transplant Direct* 2019; **5**: e451.
7. Vo AA, Lukovsky M, Toyoda M, *et al*. Rituximab and intravenous immune globulin for desensitization during renal transplantation. *N Engl J Med* 2008; **359**: 242.
8. Vo AA, Peng A, Toyoda M, *et al*. Use of intravenous immune globulin and rituximab for desensitization of highly HLA-sensitized patients awaiting kidney transplantation. *Transplantation* 2010; **89**: 1095.
9. Leandro MJ, Cambridge G, Ehrenstein MR, Edwards JC. Reconstitution of peripheral blood B cells after depletion with rituximab in patients with rheumatoid arthritis. *Arthritis Rheum* 2006; **54**: 613.
10. Tokunaga M, Fujii K, Saito K, *et al*. Down-regulation of CD40 and CD80 on B cells in patients with life-threatening systemic lupus erythematosus after successful with rituximab. *Rheumatology* 2005; **44**: 176.
11. Sfikakis PP, Boletis JN, Lionaki S, *et al*. Remission of proliferative lupus nephritis following B cell depletion therapy is preceded by down-regulation of the T cell co-stimulatory molecule CD40 ligand: an open-label trial. *Arthritis Rheum* 2005; **52**: 501.
12. Aubin E, Lemieux R, Bazin R. Indirect inhibition of in vivo and in vitro T-cell responses by intravenous immunoglobulins due to impaired antigen presentation. *Blood* 2010; **115**: 1727.
13. Bestard O, Crespo E, Stein M, *et al*. Cross-validation of IFN-γ Elispot assay for measuring memory/effector T cell responses in renal transplant recipients. *Am J Transplant* 2013; **13**: 1880.
14. Poggio ED, Augustine JJ, Clemente M, *et al*. Pretransplant cellular alloimmunity as assessed by a panel of reactive T cells assay correlates with acute renal graft rejection. *Transplantation* 2007; **83**: 847.
15. Crespo E, Lucia M, Cruzado JM, *et al*. Pre-transplant donor-specific T-cell alloreactivity is strongly associated with early acute cellular rejection in kidney transplant recipients not receiving T-cell depleting induction therapy. *PLoS ONE* 2015; **10**: e0117618.
16. Nickel P, Presber F, Bold G, *et al*. Enzyme-linked immunosorbent spot assay for donor-reactive interferon-gamma-producing cells identifies T-cell presensitization and correlates with graft function at 6 and 12 months in renal-transplant recipients. *Transplantation* 2004; **78**: 1640.
17. Schachtner T, Stein M, Reinke P. ABO desensitization affects cellular immunity and infection control after renal transplantation. *Transplant Int* 2015; **28**: 1179.
18. Schachtner T, Stein M, Reinke P. Kidney transplant recipients after nonrenal solid organ transplantation show low alloreactivity but an increased risk of infections. *Transplant Int* 2016; **29**: 1296.
19. Jordan SC, Kahwaji J, Toyoda M, *et al*. B-cell immunotherapeutics: emerging roles in solid organ transplantation. *Curr Opin Organ Transplant* 2011; **16**: 416.
20. Vallerskog T, Gunnarsson I, Widhe M, *et al*. Treatment with rituximab affects both the cellular and the humoral arm of the immune system in patients with SLE. *Clin Immunol* 2007; **122**: 62.
21. Trepanier P, Bazin R. Intravenous immunoglobulin (IVIg) inhibits CD8 cytotoxic T-cell activation. *Blood* 2012; **120**: 2769.
22. van den Hoogen MW, Kamburova EG, Baas MC, *et al*. Rituximab as induction therapy after renal transplantation: a randomized, double-blind, placebo-controlled study of efficacy and safety. *Am J Transplant* 2015; **15**: 407.
23. Tydén G, Genberg H, Tollemar J, *et al*. A randomized, doubleblind, placebo-controlled, study of single-dose rituximab as induction in renal transplantation. *Transplantation* 2009; **15**: 1325.
24. Issa F, Schiopu A, Wood KJ. Role of T cells in graft rejection and transplantation tolerance. *Expert Rev Clin Immunol* 2010; **6**: 155.
25. Pearl JP, Parris J, Hale DA, *et al*. Immunocompetent T-cells with a memorylike phenotype are the dominant cell type following antibody-mediated T cell depletion. *Am J Transplant* 2005; **5**: 465.
26. Zhang QW, Rabant M, Schenk A, Valujskikh A. ICOS-Dependent and -independent functions of memory CD4 T cells in allograft rejection. *Am J Transplant* 2008; **8**: 497.
27. Koyama I, Nadazdin O, Boskovic S, *et al*. Depletion of CD8 memory T cells for induction of tolerance of a previously transplanted kidney allograft. *Am J Transplant* 2007; **7**: 1055.
28. Golshayan D, Pascual M. Tolerance-inducing immunosuppressive strategies in clinical transplantation: an overview. *Drugs* 2008; **68**: 2113.
29. Haudebourg T, Poirier N, Vanhove B. Depleting T-cell subpopulations in organ transplantation. *Transpl Int* 2009; **22**: 509.
30. Mohty M. Mechanisms of action of antithymocyte globulin: T-cell depletion and beyond. *Leukemia* 2007; **21**: 1387.
31. Ayasoufi K, Yu H, Fan R, Wang X, Williams J, Valujskikh A. Pre-transplant antithymocyte globulin has increased efficacy in controlling donor-reactive memory T cells in mice. *Am J Transplant* 2013; **13**: 589.