TRANSPLANT INTERNATIONAL

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Clinical relevance of the *de novo* production of anti-HLA antibodies following intestinal and multivisceral transplantation

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Keywords
Antibody-mediated rejection, donor-specific anti-HLA antibodies, intestine, multivisceral clinical.

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Conflict of interest
We declare that there are no conflict of interest. In particular, there are no conflicts related to financial funding that are potentially relevant to the content of this manuscript.

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Received: 18 September 2013
Revision requested: 20 October 2013
Accepted: 23 November 2013
Published online: 16 January 2014

doi:10.1111/tri.12250

Summary

Despite a negative pretransplant cross-match, intestinal transplant recipients can mount humoral immune responses soon after transplantation. Moreover, the development of donor-specific anti-HLA antibodies (DSAs) is associated with severe graft injury. Between June 2000 and August 2011, 30 patients (median age 37.6 ± 9.8 years) received isolated intestinal transplantations (ITX, $n = 18$) or multivisceral transplantations (MVTXs, $n = 12$) at our center. We screened for human leukocyte antigen (HLA) antibodies pre- and post-transplant. If patients produced DSAs, treatment with plasmapheresis and intravenous immunoglobulin (IVIG) was initiated. In the event of DSA persistence and/or treatment-refractory rejection, rituximab and/or bortezomib were added. Ten patients developed DSAs and simultaneously showed significant signs of rejection. These patients received plasmapheresis and IVIG. Eight patients additionally received rituximab, and two patients were treated with bortezomib. DSA values decreased upon antirejection therapy in 8 of the 10 patients. The development of DSAs following ITX is often associated with acute rejection. We observed that the number of mismatched antigens and epitopes correlates with the probability of developing *de novo* DSAs. Early diagnosis and therapy, including B-cell depletion and plasma cell inhibition, are crucial to preventing further graft injury.

Introduction

Short-term survival after intestinal transplantation (ITX) has significantly improved over the last several years, driven by a better understanding of the management of immunosuppression and acute allograft rejection [1]. Interestingly, the management focus was previously on controlling T-cell-mediated immunity [2]. Recently, with the increasing awareness of consistently inferior long-term survival, the dynamic interaction of T and B cells and the subsequent development of anti-HLA antibodies (HLAabs) have gained increased attention [2,3]. Whereas the challenge of controlling antibody-mediated rejection (AMR) has long been recognized in heart and kidney transplantation [4–12], this

issue only recently gained attention in the context of ITX. Importantly, compared with other solid organ transplants, the intestine represents a highly vascularized and thus very immunogenic allograft. Although data are still scarce, an increasing number of authors have reported the challenges associated with diagnosing and treating AMR following ITX [2,3,13–15]. In most of these studies, the diagnosis of AMR was based on the coincidence of DSAs and acute biopsy-proven rejection [2]. However, the detection of distinct histological signs of vascular graft injury may be hampered, especially in the early phase in which AMR is first suspected [3,16–18]. Staining for C4d of the mesenterial vasculature would be more informative but requires full-thickness graft biopsies, which are associated with an increased risk of graft perforation [2]. In addition, the C4d staining of mucosal biopsies has been shown to be rather unspecific due to the high degree of complement activation in the intestinal wall [19]. Thus, the diagnosis of AMR of an intestinal graft can often only be confirmed after explantation of the injured graft. From heart and kidney transplantation studies, it is well known that AMR can follow a rapid and severe course and that subclinical acute rejection and DSA persistence may progress to chronic rejection and late graft loss [20–25]. Recent reports from the field of ITX have attributed the current high rates of long-term graft attrition to antibody-mediated rejection [3]. The existence of AMR after ITX is no longer questionable, and studies should now focus on the identification, treatment, and prevention of this unique form of rejection. Here, we retrospectively studied 30 patients after ITX or multivisceral transplantation (MVTX) and identified HLA epitope mismatches as potential risk factors for the *de novo* production of HLAabs, leading to rejection. The role of preformed HLAabs could not be elucidated in this study and remains to be clarified. MVTX recipients seemed to have a reduced risk of AMR, most likely due to the immunoprotective effect of the liver, which was recently reported in larger studies [3]. Similar to reports of kidney transplantation [26–29], we successfully implemented treatment strategies, including proteasome inhibitors, for refractory antibody-mediated rejection [30] to overcome graft injury at an early stage and to prevent chronic rejection and graft loss.

Subjects and methods

Between June 2000 and August 2011, 30 patients (9 females, 21 males, median age 37.6 ± 9.8 years) received an isolated intestinal (ITX, $n = 18$), modified (mMVTX, $n = 2$), or typical multivisceral (MVTX, $n = 10$) transplant. Four MVTX cases included a kidney graft (Table 1). Two time periods were analyzed: era I, from June 2000 to May 2005 (13 ITX, 2 MVTX) and era II, from June 2007 to August 2011 (5 ITX, 2 mMVTX, 8 MVTX).

Induction therapy

As shown in Table 1, we initially used daclizumab (Zenapax®, Hoffmann-La Roche, Basel, Switzerland; 20 mg iv) and one dose of ATG-Fresenius [ATG-Fresenius S®, Fresenius-Biotech, Munich, Germany; 8 mg/kg body weight (BW)] to mitigate IRI. Based on upcoming reports on depletion strategies, we then utilized alemtuzumab instead (Campath®, Genzyme, Cambridge, Mass., USA; 30 mg iv on postoperative day (POD) 1 + 4). Because of frequent late-onset rejection following alemtuzumab, which was attributed to lymphopenia-induced proliferation, the protocol was again modified to include thymoglobulin (Thymoglobulin®, Genzyme, Cambridge, Mass., USA; 7.5 mg/kg BW total dose) and one dose of infliximab (Remicade®, Centocor Inc., Essex Pharma GmbH; 5 mg/kg BW). Infliximab was used to mitigate IRI and to deplete effector memory $CD8^+$ T cells [31,32].

Baseline and maintenance immunosuppression

The initial immunosuppression consisted of tacrolimus (Prograf® Astellas, Japan; initial trough levels of 15–20 ng/ml in era I were decreased to 10–15 ng/ml in era II) and steroids (40 mg/day), which were tapered off by POD 80. Maintenance immunosuppression consisted of tacrolimus (trough levels of 5–6 ng/ml) and either mycophenolate mofetil (MMF, Cellcept®, Hoffmann-La Roche, Switzerland; 500 mg or 1000 mg q12) or sirolimus (Rapamune®, Wyeth Ayerst Pharmaceuticals, USA; trough levels of 2–3 ng/ml) depending on various determinants such as the presence of proteinuria, disturbed wound healing, diarrhea, and myelotoxicity. Two patients were given triple combination therapy (tacrolimus, MMF, and sirolimus) because of recurrent rejection during the first 2 years post-transplant (Table 1).

Pre- and post-transplant HLAab monitoring

Pretransplant HLAab monitoring was performed in both eras. Regular, frequent post-transplant HLAab screening was, however, only initiated in the late phase of era I, after the graft loss and death of one patient (no. 14) due to severe treatment-refractory rejection, which was associated with DSA development. The importance of DSAs was indicated by the histopathological results of the explanted graft, which showed severe cellular and humoral rejection. Following that event, post-transplant HLAab screening was performed weekly, or whenever necessary for diagnosis, until discharge from the hospital. Outpatients were screened for alloantibodies every 6 months.

Donor and recipient HLA typing was performed by serological and molecular methods using lymphocyte-typing trays (Bio-Rad, Hercules, CA, USA) and Dynal RELI SSO

Table 1. Characteristics of all ITX and MVTX recipients at our center, including patients who received maintenance immunosuppression and induction therapy according to era I (patients 1–15) or era II (patients 16–30).

No.	Age at TX years	Graft type	Induction therapy	Pre-TX HLAabs	*De novo* HLAabs post-TX DSA in MFI	Time of DSA development post-TX	Grade of rejection at DSA detection	Grade of isolated ACR within the 1st year	Antirejection treatment
1	27	ITX	ATG, Dac	0	DSA DQ6: 8018 DR7: 1587	10 years	III°	III°	ivIG, PP, rituximab
2	39	ITX	ATG, Dac	0	0	/	/	/	
3	27	ITX	ATG, Dac	0	0	/	/	/	
4	49	ITX	ATG, Dac	0	0	/	/	II°	
5	31	ITX	ATG, Dac	0	DSA A3: 1830 A24: 2336 DQ7: 7974	10 years	III°	/	ivIG, PP, rituximab, bortezomib
6	33	ITX	ATG, Dac	0	0	/	/	III°	
7	57	ITX	ATG, Dac	0	0	/	/	III°	
8	59	ITX	ATG, Dac	0	0	/	/	/	
9	28	ITX	ATG, Dac	0	0	/	/	/	
10	35	ITX	ATG, Dac	0	0	/	/	II°	
11	31	ITX	Alemtuzumab	0	0	/	/	III°	
12	36	MVTX + K	Alemtuzumab	0	NDSA	5 years	/	I°	
13	38	ITX	Alemtuzumab	0	0	/	/	III°	
14	39	ITX	Alemtuzumab	0	DSA A2:/DR4:/ Only ELISA	20 days	III°	/	ivIG, PP, rituximab
15	41	MVTX + K	Infliximab, TG	0	0	/	/	/	
16	31	ITX	Infliximab, TG	0	0	/	/	/	
17	24	MVTX + K	Infliximab, TG	0	0	/	/	I°	
18	36	MVTX	Infliximab, TG	0	DSA B8: 997	39 days	I°	/	ivIG, PP, rituximab
19	23	ITX	Infliximab, TG	0	NDSA	27 days	no rej.	/	
20	21	mMVTX	Infliximab, TG	0	DSA DR15: 350	15 days	III°	/	ivIG, PP
21	42	MVTX	Infliximab, TG	0	NDSA	4 days	no rej.	/	
22	38	ITX	Infliximab, TG	0	DSA DQ7: 6060 DQ8: 3938	31 days	II°	/	ivIG, PP, rituximab
23	45	ITX	Infliximab, TG	0	DSA A24: 1186 DQ7: 4278 DQ8: 2457 DR53: 4390	14 days	I°	/	ivIG, PP, rituximab, bortezomib
24	44	ITX	Infliximab, TG	0	0	/	/	I°	
25	38	mMVTX	Infliximab, TG	0	DSA DR4: 943	36 days	II°	/	ivIG, PP, rituximab
26	49	MVTX	Infliximab, TG	Class I NDSA	DSA B60: 2672	36 days	I°	/	ivIG, PP, rituximab
27	37	MVTX	Infliximab, TG	0	NDSA	26 days	no rej.	/	
28	48	MVTX	Infliximab, TG	0	DSA B7: 2810 DQ7: 3337	16 days	I°	/	ivIG, PP
29	29	MVTX + K	Infliximab, TG	Class I NDSA	0	/	/	/	
30	52	MVTX	Infliximab, TG	Class I NDSA	0	/	/	I°	

ITX, isolated intestinal transplantation; mMVTX, modified multivisceral transplantation; MVTX, multivisceral transplantation; ATG, antihuman T-lymphocyte immunglobulin; Dac, Daclizumab; TG, Thymoglobulin; DSA, donor-specific anti-HLA antibodies; DSA+, positive DSA sampling; NDSA, non-donor-specific anti-HLA antibodies; MFI, mean fluorescence intensity; AB, antibodies; CL I, HLA-antibodies Class I; CL II, HLA-antibodies Class II; ivIG, intravenous immunglobulins; PP, plasmapheresis.

typing kits (Invitrogen, San Diego, CA, USA). Waitlisted patients were screened for HLAabs every 3 months using a CDC and solid-phase assays (ELISA and Luminex). Determination of HLAab specificity was performed using LABScreen single antigen beads and a cutoff value of 1000 normalized MFI units. Unacceptable HLA mismatches for transplantation were defined based on the detected cytotoxic and noncytotoxic HLAabs. Transplantation was performed only after receiving a negative pretransplant CDC-based and virtual cross-match. During the post-transplant period, no

fixed MFI-based cutoff value was used, and DSA MFI units were carefully monitored based on the results of previous serum samples. *De novo* DSAs with a significantly increased MFI compared with previous samples (≥+100%) and a level ≥threefold above the respective negative control were indicative of HLA-specific antibodies in a single-antigen assay (N. Lachmann, C. Schoenemann, unpublished data).

Epitope matching

The HLA loci A, B, DR, and DQ were considered for antigen matching on the split level. HLA-DP could not be considered in this analysis due to incomplete donor typing information and a lack of donor DNA/tissue with which to perform retrospective typing. Epitope matching was performed using the HLAMatchmaker algorithm, as described elsewhere [33].

Diagnosis of rejection

Rejection was identified by clinical symptoms and confirmed via graft biopsies, which were assessed according to established histological rejection criteria [34]. Typical clinical signs of rejection were diarrhea; abdominal distension; pain; weight loss; and intestinal wall thickening, inflammation, or hyperperfusion on intestinal power Doppler sonography. In addition, protocol biopsies were performed via graft endoscopy 3 times per week within the first 3 months, twice a week in the second 3 months, and every 6 months thereafter or as clinically indicated. In this way, subclinical rejection was identified.

We hypothesized that the simultaneous appearance of DSAs and cellular graft rejection within a margin of 48 h would be indicative of antibody-mediated rejection. This form of rejection was unresponsive to standard antirejection therapy and triggered graft injury beyond T-cell depletion. Additionally, the published potential histopathological signs of humoral rejection were applied [34]. C4d staining was regularly performed.

Antirejection treatment

Steroid therapy was employed for mild rejection (1000 mg methylprednisolone) for 5 days. For steroid-resistant, moderate, and severe rejection, we applied muromonab in era I (Orthoclone®, OKT3, Janssen-Cilag, Germany; 5 mg/day, 5–10 days) and thymoglobulin in era II (1–1.5 mg/kg BW for 5 days to achieve lymphocyte counts below 500 cells/nl). The absence of rejection was also determined by clinical surveillance.

Upon DSA detection, plasmapheresis was added to antirejection treatment (5 cycles every other day) with alternating IVIG (10 g/day iv) (Fig. 1). Rituximab (MAB THERA®, Hoffmann-La Roche, Switzerland; 375 mg/m^2 body surface iv) was added in the case of DSA persistence (despite repeated plasmapheresis/IVIG) and histological or clinical evidence of ongoing rejection. Bortezomib was added for treatment-refractory AMR (Velcade®, Janssen-Cilag, Germany; 1.6 mg/kg BW) on days 1, 4, 8, and 11 (Fig. 1).

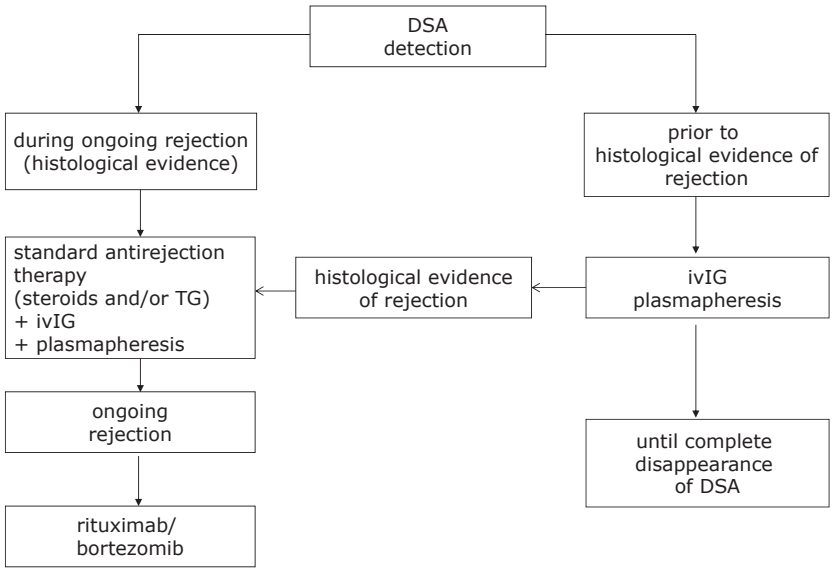

Figure 1 Treatment schedule employed following the appearance of DSAs and associated rejection as currently proposed and as established in our intestinal transplant program. The protocol may need adaptation, particularly when more data on late-onset DSAs and their clinical significance become available.

Data analysis

Data were collected prospectively and obtained by a retrospective review of medical records to assess clinical variables and histopathological results from graft biopsies. Continuous data were analyzed by Student's t-test. Ordinal data were assessed by a Fisher's exact test, where appropriate, or descriptively due to limited patient numbers. The results are provided as the mean ± standard error of the mean (SEM).

Results

De novo HLAabs after ITX

Fifty percentage of patients (15/30) developed *de novo* HLAabs after ITX in this study (Table 1, Fig. 2). In 10 of those patients, *de novo* HLAabs were donor specific and directed against the graft (DSAs), with maximum mean fluorescence intensity (MFI) levels of 997–2810 for HLA class I, and 350–8018 for class II. In 8 of the 10 patients, DSAs primarily developed within the first 6 months, at a mean of 25.9 ± 10.7 days after transplantation, only two patients developed DSAs 10 years after transplantation (Table 1).

Preformed HLAabs

Preformed HLAabs were detected in three patients prior to transplantation (Table 1). These antibodies were non-donor-specific HLAabs (NDSAs); they were not directed against the graft and did not result in rejection. Interestingly, one patient (no. 26) developed an antibody-mediated rejection due to *de novo* class I DSAs (B60), which were cross-reactive with the weak preformed NDSAs (B7, B8, B42, B55, B56, and B67; maximum MFI of 1355) and shared 2 immunogenic eplets.

Simultaneous occurrence of *de novo* DSAs

All patients with *de novo* DSAs exhibited simultaneous cellular rejection at the time of DSA occurrence, 9 of them within 48 h of positive DSA sampling. In one patient (no. 5), the histological evidence of rejection was delayed by 2 months despite positive DSA sampling and was only detected after partial graft resection.

Histological evidence of antibody-mediated rejection

According to standard rejection criteria [34], six patients had moderate (II°) or severe (III°) rejection, whereas four patients showed mild rejection (I°) at the time of positive DSA sampling. (Table 1).

Classical histological features of antibody-mediated rejection were only present in two patients, who underwent partial (no. 5) or entire (no. 14) graft resection (Fig. 3a). An examination of the entire intestinal wall,

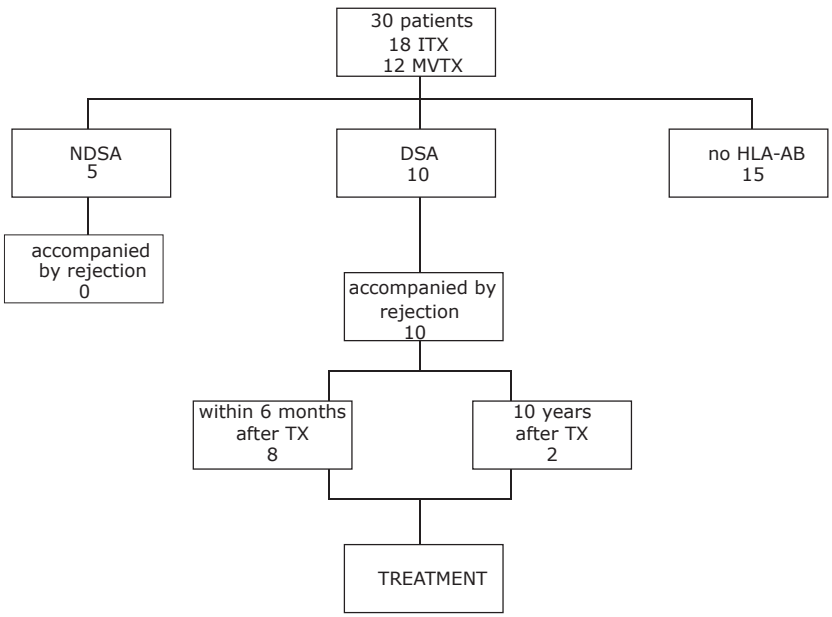

Figure 2 Results of the present study. The patients are divided into those who developed DSAs, NDSAs, or no HLAabs after ITX or MVTX. The 10 patients with DSAs are listed according to the time of DSA development and applied treatment. Five patients developed NDSA without any associated rejection; none of them developed DSA or DSA-associated rejection at any stage after transplantation.

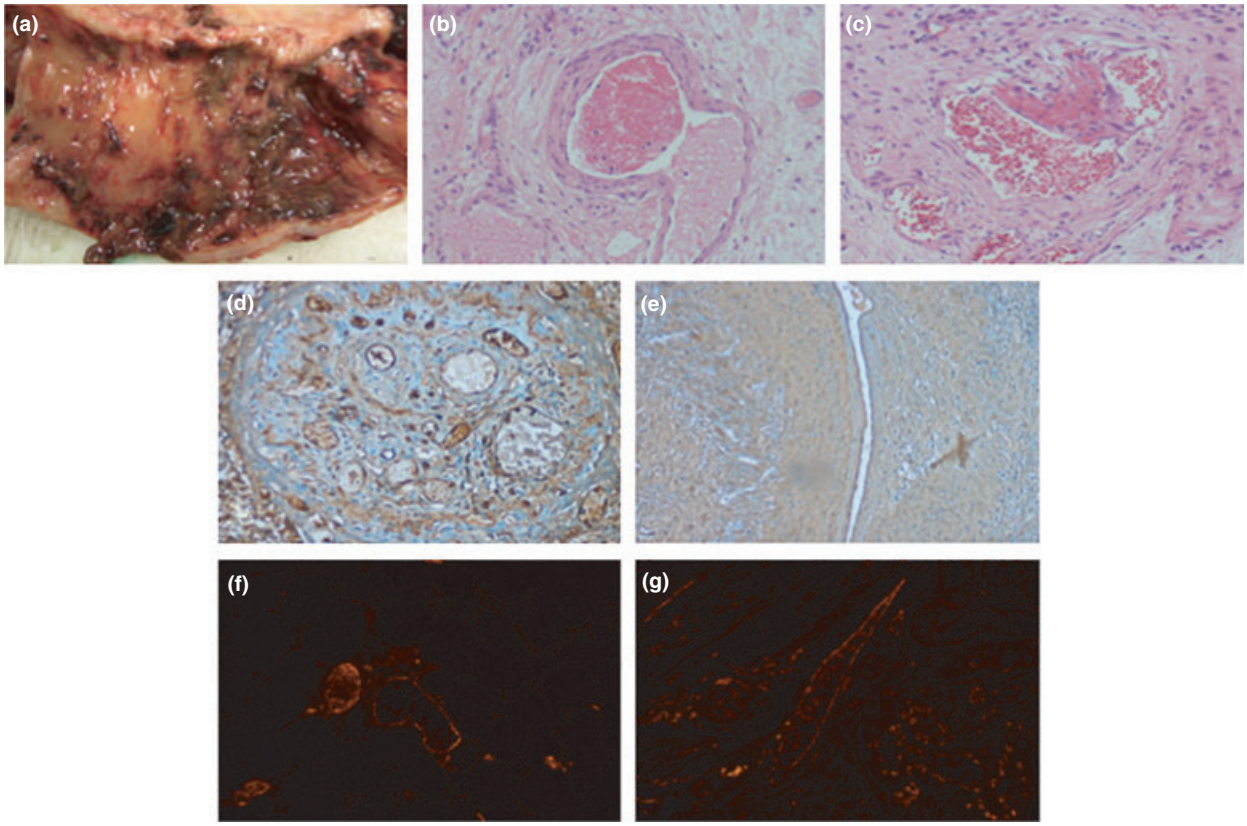

Figure 3 (a–c) Macroscopic (a) image of acute intestinal cellular and DSA-related rejection in patient 14, showing obliterative arteriolopathy and transmural inflammatory infiltration. Microscopic images (b, c) of acute intestinal cellular and DSA-related rejection in patient no. 5, showing middle and small vessels in submucosa and peri-intestinal fat tissue, with fresh thrombosis (b) and signs of recanalization as an indicator of the chronification process (c) (H&E ×300). (d, e) Middle and small vessels in the submucosa and peri-intestinal fat tissue showing the transmural deposition of IgM-positive plasma cells and obliterative arteriolopathy. (f, g) C4d staining of the entire intestinal wall, with partly dilated arterioles in the submucosa and peri-intestinal fett tissue showing the diffuse, linear deposition of C4d (fluorescent microscopy, ×100 magnification).

including the mesenteric vasculature of both grafts, revealed signs of severe cellular and antibody-mediated rejection, including necrotic, ulcerated areas of the mucosa that were characterized by active inflammatory infiltrates, degenerative crypt epithelial damage, villus flattening, and abundant apoptosis. The mesenterial vessels showed recanalization signs, obliterative arteriolopathy, and transmural inflammatory infiltration (Fig. 3b,c). Immunohistostaining showed IgM deposition on the endothelium of the mesenteric arteries and capillaries and within thrombi (Fig. 3d,e). C4d staining was positive on the endothelium of the capillaries and on the medium and large mesenteric arteries (Fig. 3f,g). Although the 1-year rejection rates in the 2 eras were similar (Table 2), we observed an increased incidence of cellular rejection in era I ($P = 0.06$), whereas there was significantly more antibody-mediated rejection in era II ($P = 0.04$). The grade of rejection (either isolated cellular or DSA-associated rejection) was significantly higher in era I than in era II ($P = 0.02$, Table 2).

Table 2. One-year rejection rate and allograft survival rate in eras I and II.

Patients	Era I (15*)	Era II (15)	*P*
General rejection rate	9/14 (64.3%)	10/15 (66.7%)	NS
ACR rate	8/14 (57.1%)	3/15 (20.0%)	0.06
AMR rate	1/14 (7.1%)	7/15 (46.7%)	0.04
Survival rate	9/15 (60.0%)	14/15 (93.3%)	0.08
Mild rejections (I°)	1/9 (11.1%)	7/10 (70%)	0.02
Moderate/severe rejections (II°/III°)	8/9 (88.9%)	3/10 (30%)	

ACR, acute cellular rejection; AMR, antibody mediated rejection; NS, statistically not significant.
*Patient no. 15 died within 24-h post-transplant and was therefore excluded from this analysis.

Significant HLA-epitope mismatch in patients with *de novo* HLAabs

All patients had a negative complement-dependent cytotoxicity test (CDC) result and virtual cross-match on the

day of transplantation. There was no donor typing and thus no calculation of antigen and epitope/eplet mismatches available for patients nos. 2, 3, 4, and 7. The mean numbers of antigen mismatches among patients with *de novo* HLAabs and patients without HLAabs were, respectively, 2.7 ± 1.2 and 2.2 ± 1.2 ($P = 0.2$) for class I (A, B), 3.1 ± 0.9 and 2.4 ± 1.2 ($P = 0.04$) for class II (DR, DQ), and 5.9 ± 1.6 and 4.6 ± 1.7 ($P = 0.04$) for classes I and II combined. Patients with *de novo* DSAs had higher antigen mismatch counts: 2.8 ± 0.9, 3.4 ± 0.8, and 6.2 ± 2.2, respectively. The total number of epitope mismatches between donors and recipients was strongly predictive of postoperative *de novo* HLAab production. Patients with post-transplant HLAabs exhibited a mean eplet mismatch number for class I and II combined of 63.9 ± 19.8, compared with 44.7 ± 20.1 for patients without *de novo* HLAabs ($P = 0.01$). These data suggest a direct correlation between the number of epitope mismatches and the probability of *de novo* HLAab production.

Specific treatment can resolve antibody-mediated rejection

Therapy for AMR consisted of a combination of the following T-cell- and B-cell-directed treatments: steroids, increased tacrolimus trough levels, and plasmapheresis with alternating IVIG. The six patients with moderate or severe rejection additionally received thymoglobulin. Rituximab was added in eight patients (1.8 ± 0.9 applications/patient), and bortezomib was applied in two patients with treatment-refractory AMR (Table 1).

A sustained response to antirejection therapy was defined by the total absence of any histological or clinical signs of rejection or graft injury. Patient 14 was excluded from this analysis due to graft loss following AMR 4 weeks after ITX. The mean time to resolution of graft injury was 17.3 ± 18.5 days. The mean time period between the appearance of DSAs and DSA withdrawal below the detection level was 36.6 ± 30.1 days.

Outcome after antibody-mediated rejection

Nine of the 10 patients (90%) recovered from antibody-mediated rejection under the specific treatment and remain alive with a functioning graft. This group also includes patient no. 5, who lost 50 cm of proximal jejunum on account of a segmental ischemia due to AMR and still has detectable DSA levels despite bortezomib treatment. DSA levels have decreased after the first cycle of bortezomib, but have not completely disappeared. This patient is clinically well and his graft is histologically in a stable condition. He is under regular observation in our outpatient clinic and currently undergoing a second cycle of bortezomib. The other eight patients have not experienced any further DSA development or associated rejection.

Patient no. 14 died due to multi-organ failure following treatment-refractory AMR and graft loss. His death resulted in a mortality rate of 10% for antibody-mediated rejection in this cohort.

Discussion

The results of this study suggest that DSA development after ITX is associated with rejection and that high numbers of antigen end epitope mismatches between donors and recipients represent significant risk factors for DSA development. These findings are in accordance with the definition of AMR in other solid-organ transplantations so that AMR may be accepted as an entity of vascular rejection in the field of ITX.

Antibody-mediated rejection (AMR) constitutes a condition in which not only humoral but also cellular immune responses are activated [2,3,13,19]. The T-cell response is a prerequisite for B-cell activation and DSA development, promoting more severe graft injury than acute cellular rejection alone [35]. Thus, the appearance of HLAabs may be considered to be a biomarker for T-cell activation and ACR [36].

However, there are obvious limitations to the study. This study was a retrospective analysis of a relatively small number of patients over a time period of more than 10 years. Therefore, the higher rate of AMR in era II may be due to the learning curve of the center and an increased awareness toward DSA development. Yet, it may also be discussed, whether the use of infliximab may have changed the phenotype of rejection. Infliximab was initially used to mitigate ischemia-reperfusion injury (IRI) and to prevent severe cellular rejection by depleting $CD8^+$ T cells expressing membrane-bound tumor necrosis factor-alpha [37]. The depletion of $CD8^+$ T cells may have evoked an imbalance of T cells, with a preference for $CD4^+$ T cells, and this setting may have favored B-cell activation and AMR [38]. However, larger patient cohorts would be needed to further investigate the impact of, for example, infliximab on the development of AMR.

The majority of patients in this study showed *de novo* DSA development within 6 months after transplantation, accompanied by an early onset of severe AMR. In contrast, two patients (nos. 1 and 5) showed *de novo* DSA occurrence at 10 years after transplantation. A recent study revealed that the late onset of DSAs frequently occurs in the context of relative under immunosuppression and may significantly contribute to chronic AMR with allograft failure [24]. Other observational studies showed that *de novo* DSA formation was associated with HLA-DR matching, early ACR, nonadherence, and pretransplant immunization. Whether

heterologous immunity or cross-reactivity plays a role has not been elucidated to date, but a relationship between the intensity of immunosuppression and sensitization has been suggested [5,10,11,20,21,39]. The two patients of the presented study had no immunizing events that could explain the late DSA development in terms of a heterologous immunity- or cross-reactivity-associated event. However, tacrolimus trough levels of patient no. 5 showed strong deviations over time, which may be attributed to nonadherence. Unfortunately, DSA detection in this patient was initially not accompanied by any histological or clinical rejection signs and was misleading to the point that adequate antirejection therapy did not seem necessary.

Persistingly high DSA levels may continuously injure the graft, yielding the risk of treatment-refractory allograft rejection. Reasons for that may be the generation of short-lived plasma cells by reactivation and recall stimulation of memory B cells, which have a high DSA-production, but do not express CD20 and are therefore not responsive to rituximab [27,40]. Plasma cells are however susceptible to proteasome inhibitors like bortezomib, which was successfully used in early acute AMR in patient no. 23 [30].

Unfortunately, bortezomib was shown to be ineffective in resolving late AMR after KTX, dominated by antibody-secreting, long-lived $CD138^{+}CD20^{-}$ plasma cells from the bone marrow compartment [41], which is in accordance with what we witnessed in patient no. 5.

Obviously, the diagnosis of AMR in an intestinal allograft is a result of distinct findings using a variety of techniques, the cornerstone of which is noninvasive HLAab screening. In fact, C4d staining remains inconclusive as a histological surrogate marker for AMR due to the high level of complement fixation in the intestinal wall and the patchy distribution of C4d, which is also traceable in native organs and allografts without rejection [19]. Thus, C4d staining of the vasculature within mucosal biopsies of intestinal grafts is not specific for rejection [42]. In our study, such typical characteristics of AMR as C4d deposition could only be revealed in cases of partial or complete graft removal, in which the entire intestinal wall, including the mesentery, was examined.

According to our study and the current literature [2,3,13], the mere presence of DSAs is a biomarker for impending graft injury. Persistent high DSA levels continuously injure the graft, yielding a risk of treatment-refractory allograft rejection.

Therefore, two important arguments can be made for early DSA elimination in addition to the use of standard antirejection therapy. First, AMR can be subclinical and may result in severe chronic graft injury, causing high morbidity, if it is not assessed properly. Second, the delayed initiation of adequate treatment can be fatal, as the depletion of DSA-secreting, long-lived plasma cells is not feasible.

The identification of potential risk factors promoting AMR is essential for an early diagnosis and thus for the prevention of severe graft injury. We found a significant correlation between the number of antigen/epitope mismatches between a donor and a recipient and the risk of DSA development with subsequent AMR. Due to the increasing organ shortage, however, it is questionable whether intestinal allografts can be selected according to a complete antigen matching and, thus, whether these findings are applicable to clinical practice. It may be more promising to create a risk profile and thereby increase the efficacy of diagnosing AMR or to initiate preventive therapy in eligible patients. Another risk factor is most likely the transplantation of a high load of immunogenic tissue (isolated ITX or mMVTX) without an additional liver graft. Abu-Elmagd *et al.* recently reported the immunoprotective effect of a concomitantly transplanted liver with respect to the development of AMR [3] and long-term survival [43,44]. This hypothesis is supported by data from the Intestinal Transplant Registry [45] that showed improved conditional 1-year allotransplant survival in patients who received a liver as part of the graft.

Patients who developed non-donor-specific HLAabs (NDSAs) in this study did not show any clinical or histological signs of graft injury and were not treated. However, it is important to emphasize that the appearance of NDSAs, which share epitopes and are thus cross-reactive with the donor specificity, may be indicative of the presence of DSAs bound to the graft. This phenomenon was observed in patient no. 26 and was previously reported in a KTX-study in which DSAs, although absent in the periphery, could be eluted from renal transplant biopsies [46]. This possibility needs to be considered, particularly if DSA levels decrease spontaneously after a previous high DSA level and ongoing rejection that is unresponsive to standard antirejection therapy.

Conclusion

Antibody-mediated rejection (AMR) is a severe form of rejection and an unavoidable outcome of T- and B-cell immune activation. Adequate treatment should address cellular and humoral immune responses, which is the current standard in KTX. However, C4d staining is not reliable for the diagnosis of AMR in ITX recipients. Therefore, DSA detection is the most important indicator of ongoing B-cell activation and antibody-mediated graft injury. Furthermore, the analysis of preformed HLAabs and antigen/epitope mismatches may help to identify patients with an elevated risk of AMR. Presumably, MVTX recipients have a reduced risk of DSA development and AMR because these individuals benefit from the immunoprotective effect of a concomitantly transplanted liver or kidney graft.

Authorship

UA: Gerlach provided clinical treatment, contributed to the acquisition and analysis of data, and was involved in the preparation and writing of the manuscript. NL: performed the analysis of HLAabs and of antigen and epitope mismatches and was involved in the writing and review/proofreading of the manuscript. BS: contributed to the data analysis and manuscript review/proofreading. RA: performed the histological analysis of graft biopsies, the analysis of the histological data, and manuscript review/proofreading. PN: head of the Department of General, Visceral and Transplantation Surgery and was involved in the manuscript review/proofreading. CS: performed and supervised the analysis of HLAabs and of antigen and epitope mismatches and was involved in the manuscript review/proofreading. AP: director of the Organ Transplant Program and is responsible for the Program of Intestinal and Multivisceral Transplantation and the Clinic for Intestinal Failure Management. He provided clinical treatment, contributed to the acquisition and analysis of data, and was involved in the manuscript writing and review/proofreading.

Funding

The authors have declared no funding.

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