TRANSPLANT INTERNATIONAL

Transplant International ISSN 0934-0874

CASE REPORT

Antibody-mediated rejection in hand transplantation

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Keywords
antibodies, outcome, rejection treatment, rituximab, vascularized composite allotransplantation.

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Conflict of interest
There is no conflict of interest to disclose.

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Received: 23 August 2013
Revision requested: 23 September 2013
Accepted: 28 October 2013
Published online: 25 November 2013

doi:10.1111/tri.12233

Summary

Clinical relevance of antibody-mediated rejection (ABMR) in vascularized composite allotransplantation (VCA) has not been defined. We herein describe a novel type of donor-specific antibody (DSA) and B-cell-associated rejection in hand transplantation. In 2003, a bilateral forearm transplantation was performed on a 42-year-old male patient. In 2012, the patient presented with edematous hands and forearms without skin lesions. Punch skin biopsies revealed rejection grade Banff II. Immunohistochemical analysis identified large aggregates of CD20 + lymphocytes with an architecture resembling lymph nodes. De novo DSA was found at a high level. Steroid treatment was ineffective, but administration of rituximab resulted in complete remission of clinical symptoms, evaporation of B-cell aggregates, and disappearance of DSA. We herein report the first case of what we suggest is an ABMR in VCA occurring at 9 years after forearm transplantation. Rituximab therapy successfully reversed the event.

Introduction

The impact of anti-donor antibodies directed against HLA, ABO blood group and a variety of endothelial cell antigens on rejection in renal transplantation was established in the 1970s [1,2]. Antibody-mediated rejection (ABMR) is often refractory to conventional immunosuppressive therapy and the single most important predictor of early and late allograft loss [3]. In vascularized composite allotransplantation (VCA), many aspects of cell-mediated acute skin rejection have been elucidated [4,5]. The role of B cells and anti-donor antibodies as well as the clinical relevance of ABMR, however, remains unclear. C4d depositions have been detected in some cases, but not shown any correlation with skin lesions, cellular infiltrates, or functional impairment in hand or face transplantation [6,7]. Early reports have all demonstrated the absence of circulating donor-specific antibodies (DSA) [5,6]. More recently, DSA was identified in some VCA patients, coinciding with either cellular rejection or non-adherence [8].

Patient and methods

A 42-year-old man received the world's first bilateral forearm transplantation at the Innsbruck Medical University on February 17, 2003. A total of six rejection episodes occurred in the first 3 years after transplantation, requiring treatment with steroids, basiliximab, ATG, alemtuzumab,

or tacrolimus dose augmentation [9]. The patient received erythrocytes, platelets, and fresh frozen plasma. The last erythrocyte concentrate was administered on February 26, 2003. DSA remained undetectable during the entire course. Radio morphological studies revealed normal blood flow through radial and ulnar arteries in both allografts at all time points. No luminal narrowing as an indirect sign of myointimal proliferation was observed, and all arteries were preserved and unchanged in caliber. Total active range of motion and hand function continued to improve with time after transplant.

In February 2012, the patient presented with a significant edema over left and right hands and forearms without any exanthema or rash typical for acute rejection. He reported significant pain in both arms with deterioration of sensitivity, but no other clinical symptoms. The immunosuppressive therapy had been continuously reduced over the past 6 years in a stepwise fashion and consisted of tacrolimus (trough level 3.1 ng/ml) and everolimus (trough level 4 ng/ml). A clinical, radiological, and laboratory workup was initiated immediately. Punch skin biopsies of both arms were performed for histological evaluation and immunohistochemical (IHC) analysis.

Results

Clinical examination, Doppler and contrast-enhanced ultrasound, CT angiography, conventional angiography, and X-rays showed patent vessels with no luminal narrowing/occlusion. Blood workup revealed no metabolic alterations but a mild leukocytosis (11.2 G/l, upper normal 10.0 G/l). Assessment for de novo DSA by LUMINEX (Luminex® 200™) and ELISA was positive for HLA class I and HLA class II DSA (Table 1) with mean MFI values of 825 (anti-HLA I) and 967 (anti-HLA II).

Deep tissue punch skin biopsies revealed a prominent and intense perivascular cellular lymphocytic infiltrate in the dermis with only mild epidermal involvement. IHC examination identified an overall T-cell (80% $CD4^+$, 20% $CD8^+$)- dominated infiltrate. Large cell aggregates resembling nodular lymphoid aggregates (lymph follicle-like features) were observed within the deep dermal layer consisting mainly of B cells. High endothelial venule (HEV)-like vessels within the structure stained positive for peripheral node addressin (PNAd). These findings strongly indicate the presence of tertiary lymphoid organs (TLOs) and lymphoid neogenesis (Fig. 1a–g). The majority of the cell infiltrate was positive for BAFF (Fig. 1h), and a potent activator of B cells suggested to play a role during ABMR and the development of DSA [10]. Capillaries and vessels were strongly positive for C4d (Fig. 1i). The high number of infiltrating B cells contrasts previous findings in hand transplantation, where B cells were routinely representing 0.5–5% of the infiltrate. Epstein–Barr virus (EBV) was negative, indicating no hint for a B-cell lymphoma. Serum electrophoresis was performed to rule out a B-cell neoplasm.

Table 1. Pretransplant HLA characteristics and donor-specific HLA antibodies at the time point of rejection.

HLA characteristics	
Recipient	HLA-A1, 24; B8, 35; Cw3, 7; DRB1*03, *12; DQB1*02, *03
Donor	HLA-A1, 3; B55, 58; Cw3, 7; DRB1*12, *13; DQB1*03, *06
DSA	A3, DQ6, DR13
PRA pre-Tx	0%

First-line treatment consisted of steroids and an increase in maintenance immunosuppression, including the initiation of mycophenolate mofetil (MMF, 1 g/day). Clinical symptoms and histology remained unaffected by this treatment. Based on the presence of large B-cell aggregates, de novo antibodies, and lack of response to conventional therapy, rituximab (Mabthera®, Roche, Basel, Switzerland), a chimeric monoclonal antibody against CD20 on B cells, was given once at a dose of 375 mg/m^2 body surface area. Subsequently, the edema decreased and ultimately diminished within 1 month after rituximab administration. DSA continued to decrease and were negative at 3 months. Complete blood count revealed minimal lymphocytopenia (18.8%, 0.70 G/l). FACS analysis of peripheral blood demonstrated $<2/\mu l$ $CD19^+$ B cells and $<2/\mu l$ $CD19^+/CD20^+$ cells. A repeat biopsy performed at 2.5 weeks after rituximab administration showed a mild perivascular infiltrate in the dermis consisting of $CD3^+$ T cells and the complete absence of B cells (Fig. 2a–f). Only isolated vessels within the perivascular infiltrate were positive for PNAd (Fig. 2g); however, most vessels were still positive for C4d (Fig. 2i). In addition, lower levels of BAFF expression were found (Fig. 2h). The treatment was well tolerated, no side effects were noticed, and graft function returned to the high level present prior to this rejection episode.

Discussion

Since the inception of human hand transplantation in 1998, much progress has been made in this field. Specifically, the mechanism, dynamic, and treatment of skin rejection have received much attention. Unlike in solid organ transplantation, DSA and ABMR did not seem to play a major role in VCA. An experimental study carried out in a rat limb transplantation model revealed a possible effect of preexisting DSA and accelerated rejection [11]. No such effect has been observed in human VCA prior to the case described here. De novo DSA in solid organ transplantation

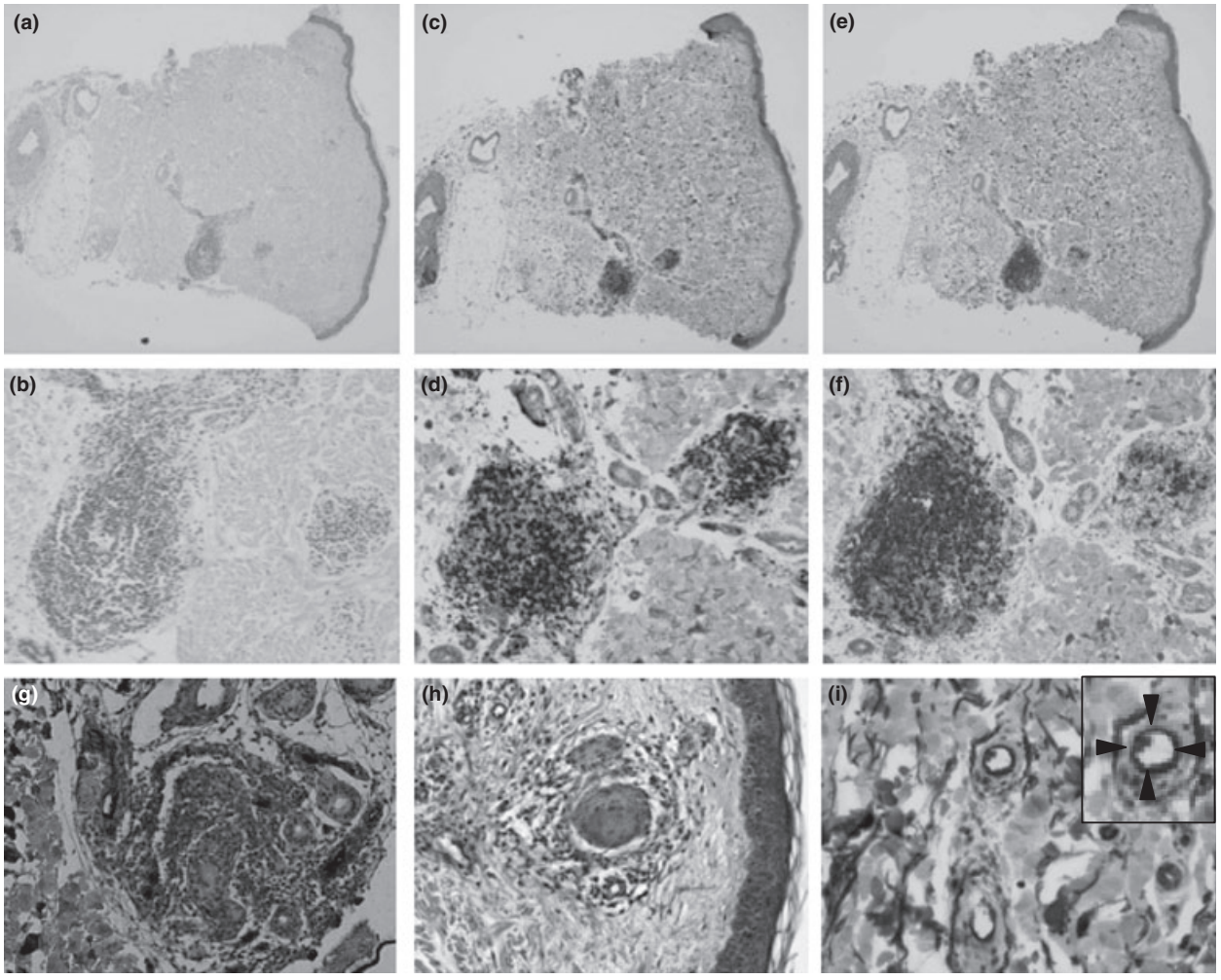

Figure 1 (a–i) Histology and immunohistochemistry of an allograft skin biopsy at the time of diagnosis: H&E histology revealed Banff grade II rejection with prominent perivascular cell infiltrates and mild epidermal involvement (a) together with a cellular accumulation resembling a nodular lymphoid aggregate (lymph follicle-like feature) in the deep dermis (b). The overall cell infiltrate was dominated by CD3 + T cells (c + d), whereas the cell accumulation mainly consisted of B cells, CD20 + (e + f). PNAd+ HEV-like vessels were found within the cell accumulation (g). The majority of infiltrating cells were positive for BAFF (h). Vessels were highly positive for C4d (i); arrows in the high power inset mark specific C4d staining of vascular endothelium. C4d staining for elastic fibers was considered unspecific.

is mainly class II and associated with a worse prognosis when compared to HLA class I DSA [12]. Capillary C4d deposition is an established Banff criterion for rejection in kidney transplantation but also a somewhat problematic and inconsistent marker for ABMR. According to the findings in hand and face transplantation, C4d alone may be of limited diagnostic value for ABMR in VCA. Our group has previously shown that more than 60% of all biopsies showing grade I rejection stained positive for C4d [5]. C4d-deposits, however, were also found in samples not showing other evidence of rejection and even in corresponding samples from recipient skin. Four rejection episodes after hand transplantation and coinciding with C4d depositions were described by Landin *et al.* [13], but DSA was negative and no clinical symptoms or histologic features of rejection were present in these cases.

Rituximab, a chimeric monoclonal antibody composed of human immunoglobulin G1 kappa antibody, was introduced for the treatment of B-cell non-Hodgkin lymphomas. A single course of rituximab successfully depletes peripheral human B lymphocytes for 3 months to more than 1 year through mechanisms involving Fc- and complement-dependent killing. Rituximab has been successfully used in combination with intravenous immune globulin (IVIg) for desensitization in renal transplantation [14]. Use of rituximab has no immediate effect

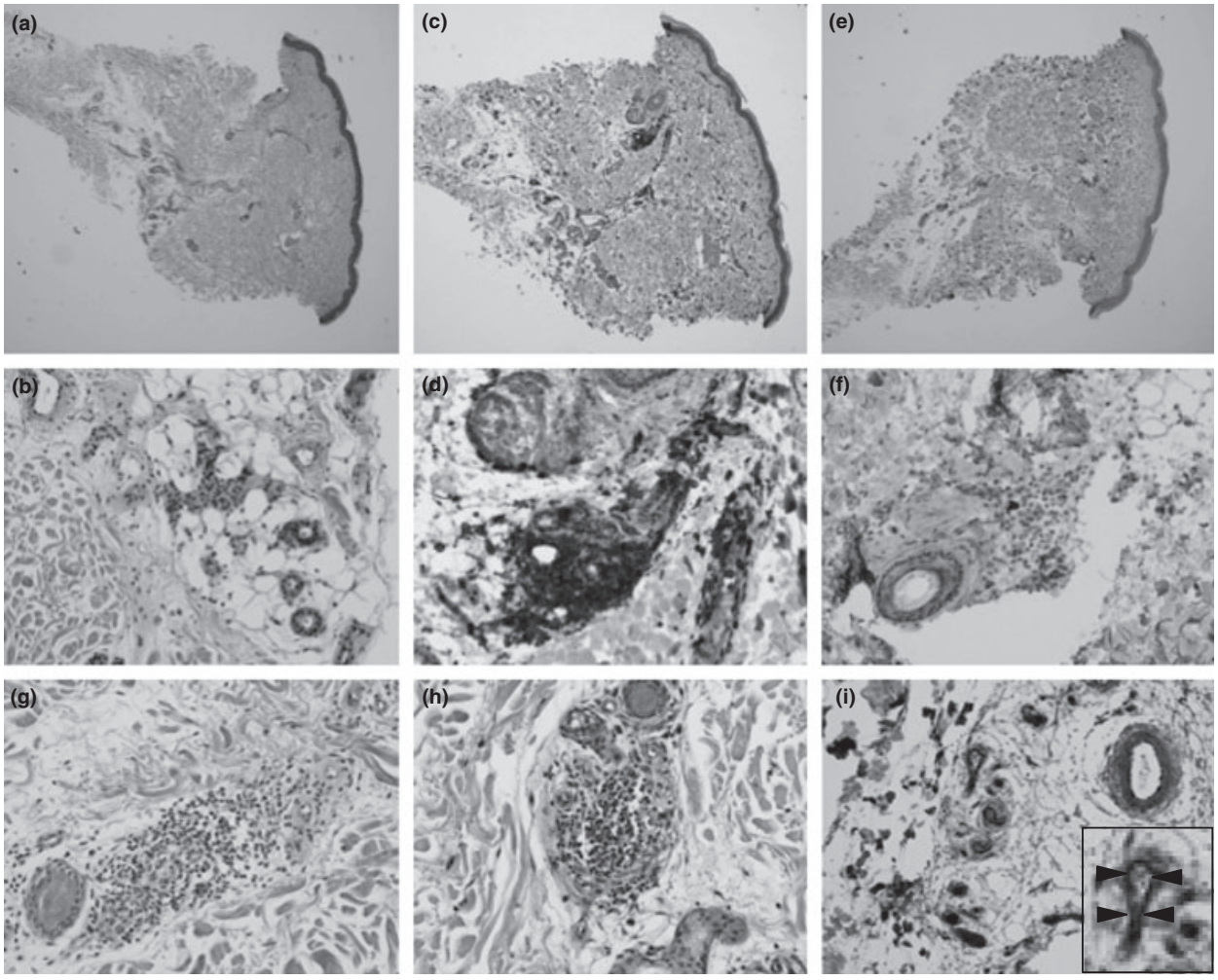

Figure 2 (a–i) Histology and immunohistochemistry of a repeat skin biopsy 2.5 weeks post-rituximab treatment: Histopathologic evaluation displayed normal skin with only mild perivascular cell infiltration in the superficial and deep dermis (a + b), mainly CD3+ (T cells; c + d). Cell accumulations were absent and no B cells, CD20+, were detected (e+f). Only two vessels positive for PNAd were found within the perivascular infiltrates (g) and also BAFF expression was diminished (h). C4d+ deposits were still observed in most vessels (i); arrows in the high power inset mark specific C4d staining of vascular endothelium.

on circulating antibody levels [15], but eventually results in reduction in elimination of DSA through its effect on B cells [16].

We herein report the first case of a B-cell driven rejection episode with the presence of DSA, indicating ABMR in VCA at 9 years after forearm transplantation. The reason for this B-cell-mediated rejection at 9 years after transplantation remains incompletely understood. A viral infection, a flu, about a month prior to rejection, could be considered a possible trigger. Also, the patient had started to regularly visit a thermal bath. Any causal relation between these events and the rejection, however, remains highly speculative. One of the major findings in this case was the evidence of lymphoid neogenesis in the dermis of both hands. B cells have been shown to play a critical role in lymphoid neogenesis, a process by which ectopic lymphoid structures appear de novo during chronic inflammation [17]. TLOs, characterized by discrete T- and B-cell zones and HEV expressing PNAd (Fig. 1g) were found in our patient. Baddoura *et al.* [18] described such a phenomenon in murine cardiac allografts undergoing chronic rejection. Thaunat *et al.* [19] have discovered clusters of $CD20^+$ B cells in chronically rejected human kidney allografts. However, as stated by Thaunat [20], a quantitative assessment of the size and number of all TLOs and B-cell clusters may not be possible with punch biopsies as the sample size is limited and may or may not be representative for the entire graft. Moreover, the functionality of the TLOs and their contribution to the development of DSA in our case remain hypothetical at this point.

Overall, the clinical picture, the B-cell aggregates, and the fact that DSA were positive for the very first time in ELISA and Luminex® indicated the relevance of B cells and antibodies for this rejection. Rituximab has emerged as a rational choice for therapy in our patient with the aim to interrupt the B-cell-mediated event. It resulted in depletion of B cells, abrogation of DSA, and disappearance of symptoms. However, a more systematic investigation of this phenomenon in VCA is warranted.

Authorship

A.W. and T.H. analyzed the data and wrote the manuscript; B.Z. and B.G.Z. did the histological and immunohistochemical analysis; V.M. did the Luminex® and ELISA analysis; G.B. contributed to analysis of data; J.P. and S.S. critically revised and finally approved the article.

Funding

No funding sources were given to the authors.

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