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Review

The role of B cell immunity in VCA graft rejection and acceptance

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ABSTRACT

Vascularized composite allotransplantation (VCA) has emerged as the most recent field of transplantation to offer an alternative treatment for those patients that have failed or are not suitable candidates for conventional therapy. Most of the current clinical experience in this field is with recipients of skin containing grafts such as the face, upper extremity and abdominal wall transplants. Like solid organ recipients, VCA recipients require lifelong systematic immunosuppression to maintain their grafts. To date, the most successful immunosuppressant regimens are calcineurin inhibitor based and have been targeted to the control of T cells. While these regimens have resulted in excellent short term graft survival in solid organ transplantation, achieving significant improvements in long term survival has been more challenging. The reasons are multi-factorial, but a role for B cells and humoral immunity has been proposed. Antibody mediated rejection leading to chronic rejection has been cited as the leading cause of renal graft loss. While the number of VCA transplants performed is still small, evidence to date suggests that antibody mediated rejection may occur less frequently than seen in solid organ transplants. Here we will discuss the role of B cell immunity in solid organ transplantation as it pertains and contrasts to the field of VCA and present some examples of possible sequela of B cell immunity in a series of hand transplant recipients.

1. B cells in solid organ transplantation

The field of clinical organ transplantation is now more than sixty years old. Organ transplants currently provide the preferred treatment for end stage failure of the kidneys, heart, liver and lungs. The main biological limitations to organ transplantation are widely known and understood. These include progressive loss of function associated with chronic pathologic changes often termed “chronic rejection.” Although the causes of chronic rejection remain a matter of discussion, most evidence suggests chronic rejection of transplanted kidneys, heart and lungs reflects, at least in part, antibody-mediated processes [1–4]. For this reason, much attention has been given to understanding the B cell biology of organ transplantation and to the possibility of developing therapeutics and regimens for more effective suppression of B cell functions or for induction of B cell tolerance. The role of B cells in solid organ transplantation has been elegantly reviewed elsewhere [5–9]. The field of vascularized composite allotransplantation (VCA) has emerged more recently. VCA potentially provides an alternative to use of prosthetics or reconstructive surgery for treatment of catastrophic tissue loss, as might occur from trauma, surgical removal of tumors, or repair of congenital anomalies. Understanding about whether and how B cells may govern the fate of VCA is far less advanced than understanding for organ transplantation and observations made to date by us and others suggest the impact of B cells and antibodies in the outcome of VCA may depart significantly from the commonly understood impact in organ transplantation [10]. Here we will discuss the role of B cell immunity in VCA as it may contrast to the role in organ transplants and present some examples of possible sequelae of B cell immunity in a series of hand transplant recipients.

Traditionally, interest in B cell biology in organ transplantation has

Abbreviations: ABMR, Antibody mediated rejection; Breg, B regulatory cell; BCR, B cell receptor; DSA, Donor specific antibodies; dnDSA, de novo Donor specific antibodies; IRI, Ischemia reperfusion injury; MZL, marginal zone lymphoma; VCA, Vascularized Composite Allotransplantation

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mainly reflected involvement of B cells as the source of donor-specific antibodies (DSA) that serve as markers of risk and evidence of antibody-mediated injury. Consistent with this concept were findings linking pre-existing donor specific antibodies to hyperacute rejection. While hyperacute rejection was a major stumbling block early in the field, it is largely avoidable today by pre-transplant testing [11]. However, donor-specific B cells often develop *de novo* even in recipients with adequate immunosuppression [8,9]. How and to what extent donor-specific B cells develop into cells secreting pathogenic antibodies and to what extent some donor-specific B cells may have graft protective functions is currently a matter of debate.

B cells develop in the bone marrow where the B cell receptor (BCR) assembles by recombination of variable (V), diversity (D) and joining (J) gene segments of the heavy and light chains [12]. During development in the bone marrow, the B cells with receptors that bind to self molecules (usually expressed at the surface of cells) are deleted while non-self binding B cells are allowed to exit the bone marrow. Once B cells encounter antigens they differentiate into antibody producing plasma cells or memory B cells [13]. The decision of which path B cells will take (either plasma cells or memory B cells) takes place in the secondary lymphoid organs, at the borders of the T and B cell zones, and appears to be highly dependent on T cell help and antigen presentation by dendritic cells [13].

In transplantation, donor specific antibodies (soluble and as a receptor on the surface of B cells) recognize sugars (such as the blood types) and proteins including HLA and non-HLA molecules. Antibodies bind to conformational epitopes sometimes referred to as “eplets”. The mismatch of HLA loci, as well as the ability of the recipient’s immune system to respond to donor HLA determines how strongly and of what type that immune response will be [14]. Besides matching HLA alleles, there is substantial evidence that matching based on conformational HLA epitopes may provide better long term outcomes [15,16].

After organ transplantation, the de novo development of DSA is widely taken as a harbinger of acute cellular rejection as well as acute antibody mediated rejection (ABMR). Presently, the diagnosis of ABMR in kidney transplants requires i) histologic evidence of acute tissue injury ii) histologic evidence of current/recent antibody interaction with vascular endothelium, as defined by complement component deposition, or expression of transcripts indicative of endothelial cell injury, and iii) detection of DSA in the serum [17]. Among the factors determining outcome, the immunoglobulin subclass of DSA has been thought to determine whether subclinical or acute or chronic ABMR ensues [6]. IgM DSA appears to have little or no clinical effect, unless IgG DSA is also present. IgG3 DSA is most often associated with ABMR [18]. The association of DSA isotype and subclass with clinical manifestations, however, can be confounded by selective binding of DSA to the graft [19–21]. In principle, DSA of highest affinity and/or directed against the most abundant targets are selectively depleted from blood, while DSA of lower affinity or directed against scarce targets are left disproportionately represented in blood. For this reason, we have proposed that donor-specific B cell responses might provide valuable insight into risk and graft biology [7].

Although IgM DSA in the absence of IgG DSA is reported to have little impact on kidney transplants, we have suggested that some IgM DSA could exert biological properties not typical of IgG DSA [22]. Natural IgM, encoded by germ line V region sequences, often exhibiting polyreactivity, has been postulated to subserve these unusual functions [23], mediating ischemia-reperfusion injury in some systems [13,14] and healing of damaged cells and tissues, including nerve tissue, in others [15,16]. Conversion of natural antibodies from an IgM to IgG subclass has been associated with AMBR [24].

The majority of immunosuppressive protocols are targeted to controlling T cell mediated cellular rejection. While calcineurin inhibitors such as tacrolimus and cyclosporine have little effect on B cells, sirolimus, mycophenolate mofetil, and alemtuzumab (also known as Campath) all affect B cells, especially the latter, which can suppress circulating B cells for a year post transplant [5]. For the treatment of antibody mediated rejection, many centers rely on depleting DSA through plasmapheresis and use of anti-B cell antibodies such as rituximab or alemtuzumab, as well as high dose Immunoglobulin [25]. However, rituximab or alemtuzumab do not recognize plasma cells, and therefore DSA production and untoward biological impact may continue despite these measures. Bortezomib, a proteasome inhibitor, is active against plasma cells has been used with some success [26,27]; however DSA is not always cleared by this treatment [27]. A recent randomized trial in the treatment of late ABMR in renal transplants failed to show a difference between the experimental and placebo groups [28], suggesting depletion or reduction of plasma cells did not prevent DSA production.

Because most DSA are products of T cell-dependent B cell responses, agents that hinder interaction of B cells with T cells (i.e. provision of T cell help) or activation of T cells might prevent or suppress initiation of these responses. Consistent with that concept, belatacept, a fusion protein consisting of IgG1 constant domain and CTLA-4, blocks the B7-1 and B7-2 ligands on antigen presenting cells [29] and in so doing limits or prevents provision of T cell help. The BENEFIT and BENEFIT-Ext cohorts recently showed renal transplant recipients treated with Belatacept had lower rates of de novo IgG DSA compared to renal transplant recipients treated with cyclosporine A, suggesting that the conversion of IgM to IgG DSA was inhibited [30], and DSA in general was reduced in patients receiving Belatacept vs. Cyclosporine [31].

Recently, B cell functions besides production of DSA functions have been explored in organ transplantation [8,9,32]. These other functions include antigen presentation and immune regulation. In some settings, the regulation of the alloresponse may be as important as antibody production. For example, in some trials of B cell depletion in kidney transplantation, recipients reveal paradoxically higher rather than lower rates of rejection [9,32–35]. As one example, five of six (84%) non-sensitized renal transplant recipients given rituximab (anti-CD20) at the time of transplantation experienced acute rejection within three months of transplantation while only one of seven (14%) control subjects who received daclizumab (anti-CD25) induction [34] experienced rejection in this time period. The number of subjects in this study was small, and a cytokine release syndrome seen in some of the rituximab group subjects may have contributed to setting the stage for rejection. However, the results are consistent with the possibility that immune regulation by B cells exerts a powerful impact on alloimmune responses. Marino et al. [33] extended this idea in mice, reporting that whereas depletion of B cells using anti CD20 monoclonal antibody in wild type vs. anti-OVA transgenic mice did not affect the direct alloresponse, it heightened the indirect responses to skin transplantation. Anti-CD20 treatment also enhanced activation of memory T cells and accelerated second set rejection of skin allografts in this system [33].

In the late 1980s ABO-incompatible kidney transplants were sometimes observed to continue to function normally despite the presence of high levels of anti-donor blood group antibodies in the blood of the recipients [8,32,36–39]. This apparent resistance of the graft to injury was termed ‘accommodation’. So effective is accommodation that the outcome ABO incompatible renal and cardiac transplants is generally comparable to that of ABO compatible transplants [40]. A registry study of ABO incompatible heart transplants performed after 2005 and series of ABO-incompatible kidney transplants [41–43] demonstrated heightened susceptibility to graft loss during the first weeks after transplantation but once this period has passed ABO-incompatible and ABO-compatible transplants exhibit indistinguishable outcomes [44]. Several explanations have been proposed for the sensitivity of some and resistance of other ABO-incompatible organ transplants to antibody-mediated injury [39]. The explanations include differences among individuals in the intrinsic threshold and/or ability to repair complement mediated injury and/or in the inducible resistance to cytotoxicity. Regardless, the observations also tend to confirm the concept that accommodation requires but is not mediated by expression of anti-

apoptotic genes but rather reflects changes that take days or weeks to enact [39,45]. Consistent with these concepts, no relationship exists between the level of antibody against foreign ABO antigens and the function of ABO incompatible transplants after the first several weeks [36,37,42,46].

2. Differences in VCA containing skin and solid organ transplant

Although the experience with VCA is quite limited (less than 200 hand and face transplant recipients world-wide) compared to the experience with organ transplants, the experience to date suggests the impact and implications of B cell responses and DSA in VCA recipients differ profoundly from the impact and implications of DSA in organ transplant recipients [10]. Because VCA include anastomoses between donor and recipient blood vessels, the management of clinical VCA has been modeled on the management of solid organ transplants. VCA grafts undergo acute cellular rejection at least as often and probably more often than solid organ grafts [47]. However, despite the robust antibody responses observed in recipients of skin grafts, recipients of clinical VCA, which include skin grafts, appear less apt to suffer acute or chronic antibody-mediated rejection, especially in compliant recipients. Although recipients of VCA are sometimes found to have DSA in their blood, VCA in adequately immunosuppressed patients only occasionally exhibit significant vascular disease and are rarely destroyed as a consequence [47]. Antibody mediated rejection in VCA recipients is relatively rare in adequately immunosuppressed patients [47,48] with only 2 reported cases, excluding crossmatch positive recipients [49]. Weissenbacher et al. [49] describe a bilateral hand recipient who presented at nine years post transplant with edematous hands and forearms, and without the skin lesions normally seen in acute cellular rejection. These clinical symptoms appeared in conjunction with de novo DSA against class I and class II, albeit at low levels (< 1000 MFI). Capillaries and vessels of deep tissue punch biopsies were strongly positive for C4d and tertiary lymphoid organs had large numbers of B cells. The recipient did not respond to steroid treatment and an increase in maintenance immunosuppression but did respond to one dose of rituximab, with edema resolving in 1 month and DSA becoming negative at three months (although blood vessels continued to be positive for C4d) [49]. Besides this episode, the authors suggest appearance of de novo DSA may have been related to the six episodes of acute cellular rejection the subject underwent.

The Lyon group reported the second case of ABMR in a face transplant recipient in the tenth year post transplant, which resulted in partial graft loss [50]. DSA specific for HLA class II (DR and DQ) were first detected in the recipient 7.5 years after transplantation without clinical or histologic evidence of rejection. Four months later grade III cell-mediated rejection in both the face, as well as the sentinel graft, with no complement deposition, capillary thrombosis, or changes in vessels, was diagnosed which responded steroid boluses. Nine months later, another episode of rejection occurred, with grade III rejection in the face and necrosis of the sentinel graft. Examination of the sentinel graft showed superficial necrosis, C4d deposition, and thrombosis of vessels of various sizes. The MFI of the DQ6 DSA gradually increased over a nearly two year period from 3800 at 7.5 years to 14,000 at year 9. The patient was treated with plasmapheresis, bortezumab, and eculizumab, unfortunately without success. The patient lost the lower portion of the graft, which was covered with an autologous skin graft. The case was further complicated by the finding of a small cell lung carcinoma which did not respond to treatment. Of note the recipient was a smoker prior to and after transplantation [50]. The authors concluded that in VCA, the skin is the primary target of cell mediated rejection as opposed to the vasculature, which seems to be more susceptible to ABMR. One could argue that perivascular infiltrates are one of the first signs of problematic cellular rejection and the finding of thrombotic or ischemic vasculopathy with complement deposition and DSA is an end stage combination of cellular and humoral immune damage.

In addition to these reports of antibody mediated rejection associated with de novo production of DSA, two other subjects received face transplants from a donor against whom they had a positive cross match by flow cytometry [51,52]. The first patient received a transplant from a donor with whom the patient had positive T cell and B cell crossmatches. Multiple specificities of DSA were present prior to transplant, and included HLA A2, A32, B57, DQ7, DQ9 and DR7 [51]. Immunosuppression was planned as standard ATG induction with triple drug maintenance, but with plasmapheresis and IVIG treatment on POD 1 and continuing every other day based on DSA levels. The recipient developed rejection by day 5 associated with DSA in blood with C4d deposition in the graft. Therapeutic plasma exchange was stopped, and the patient was treated with eculizumab weekly. At day 19, the patient was treated with another course of plasmapheresis, with the addition of alemtuzumab, bortezomib, and steroids, as well as another dose of ATG. Within six months, the serum DSA were undetectable and the histological changes were resolved [51]. Four years post-transplant the recipient remains negative for DSA, although three episodes of cell-mediated rejection occurred between 12 and 24 months, all of which responded to solumedrol or increases in maintenance immunosuppression [53]. Despite the strength of the treatment of the ABMR, an increased incidence of infection was not reported, suggesting the patient's immune system was competent. Another interesting observation in this case is the good correlation between C4d deposition and histologic grades of rejection. Many groups, including our own, have reported non-specific C4d staining in VCA graft and native tissue [54] or C4d staining in the absence of DSA [54–56]. Kanitakis et al. performed a careful analysis of C4d staining in three VCA recipients and concluded that the lack of staining could truly reflect a lack of complement deposition as the techniques and reagents used showed positive staining in inflammatory dermatoses of non-transplant recipients [57].

A second patient received a face transplant from a donor with whom the patient had a positive B cell crossmatch [52]. The patient received ATG induction with the addition of anti CD20 (rituximab) as an additional induction agent along with standard triple drug immunosuppression based on tacrolimus. This second patient has not experienced any episodes of rejection either antibody or cellular mediated and has not developed DSA at two years post-transplant, despite six revision surgeries performed between day 1 and day 318 [52]. The complete lack of any episodes of rejection in this VCA recipient is notable, in part because of the positive B cell crossmatch (although a false positive BXM cannot be ruled out) to the donor but also because of the repeated surgeries. In the Louisville hand transplant experience, trauma of any sort (thermal, physical, surgical) can be followed by an acute rejection episode.

The Oxford VCA group recently reported on the incidence of de novo development of DSA after combined intestinal and abdominal wall transplantation [58]. Between 2008 and 2015, 18 recipients received an abdominal wall transplant in addition to an intestinal or multivisceral transplant. These patients were compared to 14 who received an intestinal or multi-viseral transplant only. All subjects received alemtuzumab induction and maintenance triple drug therapy based on tacrolimus. The incidence of development of de novo DSA was 48% overall. Fewer recipients of VCA (37%, 6 of 16) developed de novo DSA than recipients of intestinal or multi-visceral transplants (61%, 8 of 13). Despite the relatively high incidence of de novo DSA, no episodes of ABMR occurred [58]. The authors concluded that VCA do not increase the incidence of development of de novo DSA and speculated that use of abdominal wall biopsy as a sentinel could have enabled earlier treatment of rejection.

Studies conducted in kidney transplant recipients [4–6] and work on cellular injury in non-transplant settings [59,60] suggests B cell responses to VCA might also confer a benefit on the graft in some cases [10]. Some B cells produce cytokines, such as IL-10, that suppress T cell

responses to the donor or to other antigens, and could benefit the graft [17–19]. These B cells are called B regulatory cells (Breg) [61]. The extent to which Breg specifically recognize the donor and regulate cellular immune responses to VCA is unknown. In studies of kidney transplant recipients with operational tolerance (did not take immunosuppression for more than one year) genes associated with B cells pathways were upregulated in tolerant recipients [62]. These findings were recently confirmed in additional subjects as well as patients rendered tolerant via induction of transient mixed chimerism, or successfully weaned to minimal immunosuppression. Both groups also showed similar increases in IGKV1D-13 in the peripheral blood [63]. These data not only underscore the regulatory role of B cells in graft rejection, but may offer a marker of operational tolerance in both solid organ and VCA recipients.

Why is ABMR rare in VCA? VCA are certainly immunogenic as the incidence of cellular-mediated rejection is higher for VCA than for solid organ allografts. Could ABMR be more difficult to diagnose in VCA recipients? Of note while antibody mediated rejection is well described in renal transplantation, criteria are less well defined in heart and lung transplantation. In VCA transplantation ABMR is generally defined as clinical rejection in the absence of cellular infiltrate, and the diagnosis is supported by the presence of DSA. We recently discussed various mechanisms that might explain the scarcity of ABMR in VCA [64]. One difference between VCA and organ transplantation is the duration, frequency and character of ischemia associated with the surgical procedure. Warm ischemia time in reconstructive surgery and transplantation of the hand is notably greater than in solid organ transplantation. In conventional solid organ transplantation, blood flow is restored by reperfusion through the vascular anastomosis, and generally not interrupted unless there is a vascular kink or blockage of flow. The surgical technique for VCA often requires intermittent application of a tourniquet to stop blood flow and clear the field for microsurgery, as well as for vascular kinks or blockages. Thus in VCA, normothermic ischemia time and the number of damaging reperfusions that occur are increased relative to organ transplantation. Both mechanical perfusion [65] and pre-conditioning [66] have been proposed as modalities to reduce ischemic reperfusion injury (IRI) associated with VCA. Although one might expect that increased exposure to ischemia would increase the incidence of development of DSA and ABMR, it is possible that the nature of ischemia in VCA procedures limits susceptibility of VCA to injury. Thus, the intermittent periods of ischemia and reperfusion and the up/down application of the tourniquet could provide ischemic preconditioning [67]. Repeated application of the tourniquet is thought to recruit several protective pathways including upregulation of nitric oxide synthase, which renders the graft insensitive to IRI [68]. In animal models of VCA, preconditioning has been found to reduce the number of acute rejection episodes [69]. Whether the type of ischemia VCA grafts undergo versus conventional solid organ transplantation plays a role in lower rates of ABMR remains to be determined.

Of the ten recipients who received a hand transplant in the Louisville VCA Program, only one developed significant de novo DSA after transplantation. A summary of our results is shown in Table 1. Our first patient, who is now nearly 20 years post-transplant has not developed antibody despite weaning and restarting of tacrolimus at year 16. Neither patient 2 nor patient 3 who both experienced repeated episodes of acute cellular rejection developed DSA. Likewise, patient 3 who has significant chronic rejection has not developed DSA. Patient 4 who lost his graft due to ischemic vasculopathy nine months after transplantation [54] did not develop DSA during the time the graft was in place, however 3 days after amputation and cessation of immunosuppression high levels of DSA were detectable in blood. Patient 5 developed de novo DSA (dnDSA) 6 years post-transplant, coincidental with reductions in tacrolimus as a result of changes in kidney function. Although tacrolimus dosage was restored, DSA levels continued to increase. In year 8 post-transplant, the patient had rotator cuff surgery on the non-transplant side, and developed pain in the transplant that was treated with carpal tunnel surgery. Multiple episodes of cell mediated rejection occurred during this period. Infection also developed, and was followed by aggressive rejection and accelerated vasculopathy in the graft leading to ischemic damage and a decision was made by the patient and the surgical team to amputate the graft. Histologic analysis of a biopsy of the median nerve at the time of carpal tunnel surgery in patient 5 revealed an inflammatory process as did the ulnar nerve in analysis of the tissues after amputation. At the time of graft loss the patient had high levels of DSA (A24–3000 MFI which increased to 14,000 MFI after amputation) and high levels of Class II DSA (DR53, 18,000 MFI and DQ2, 5000 MFI – no change in either after amputation). While no co-incident changes were seen when DSA first appeared in this patient, we cannot rule out that three years later this initial antibody production contributed to the demise of the graft. In that the ischemia resulting from obliterative vasculopathy resulted in the patient and team's decision to amputate, this graft loss could be attributed to ABMR. However, graft loss occurred in the presence of clear cellular mediated rejection, infection and high DSA. Whether that graft loss would have occurred if each of these processes were occurring alone is not clear. Of note, in patient 4 the nerves were relatively spared from obvious immune responses.

Patient 7 is notable for a quiet post-transplant course, and has had the highest Carroll score function at 86, in our series of transplants. The patient was initially thought to have developed dnDSA against donor DQ8 approximately 3 years post-transplant. This reactivity was not associated with any significant clinical or histologic change. This was reported based on patient serum reactivity to 1 of 3 beads carrying the DQ8 antigen in the Luminex LabScreen Single Antigen Bead assay. His samples were never positive on all three DQ8 beads and subsequent experience suggests this was likely a false positive, possibly due to denatured antigen on the beads. In addition, this patient's sera reacted to one bead (the DQ8 (DQB1*03:02) with the DQA1*03:02 that was positive. However, two other DQB1*03:02 beads were negative, as was one other DQA1*03:02 (which is paired with a DQB1*03:03), further supporting the conclusion of non-specificity. While it is possible that there was a combinatorial specific antibody (only recognizing a very specific Alpha, Beta combination), we have not found this routinely. Patient 8 in this series is also doing very well, with few episodes of CMR, and also had a de novo, but non-specific HLA antibody response, similar to patient 7. Patient 10 is now twenty-seven months post-transplant and has had no episodes of cell-mediated rejection and has not developed DSA. She is remarkable as she received a bilateral hand transplant at 69 years of age and she was also enrolled in a clinical trial of induction with recipient adipose derived stromal vascular fraction cells.

Patient 3 in our series received a transplant in 2006 with alemtuzumab induction and a steroid sparing maintenance regimen. The patient experienced multiple early rejection episodes and was subsequently converted to standard triple drug tacrolimus based immunosuppression. Eighteen months after transplantation a routine CBC revealed an increased number of circulating lymphocytes. Flow cytometry revealed many of the lymphocytes were CD19/lambda + consistent with a B cell neoplasm. To address this possibility, immunosuppression was reduced. The reduction in immunosuppression was followed by an episode of severe acute rejection which responded to steroids and increased maintenance immunosuppression. Further investigation revealed the patient to have a marginal zone lymphoma (MZL). The MZL was detectable in specimens obtained at the time of transplantation and hence preceded the hand transplant. Because MZL often responds to agents used for immunosuppression, with the patient's consent standard immunosuppression was re-started. A leukemia/lymphoma flow cytometry panel is used to monitor this patient, as well as annual PET scans. Flow cytometry analysis revealed that all circulating B cells are of the same clone, CD20/CD19/lambda light chain positive cells, which do not express CD5, CD10, or CD11c. At diagnosis the clone comprised the majority of the lymphoid gate, and

Table 1
De Novo DSA in Louisville VCA Program Hand Transplant Recipients.

Patient	dnDSA	#ACR episodes (grade 2 or higher)	Follow up	Comments
HTx-01	No	4	19 years	Despite tacrolimus weaning and restart
HTx-02	No	> 18	17 years	Non-specific reactivity observed on LabScreen SAB Class I*
HTx-03	No	> 15	11 years	Severe chronic rejection
HTx-04	No	2	9 months	Severe ischemic vasculopathy, graft loss, DSA detected in serum 3 days post amputation
HTx-05	Yes	6	~9 years	Class II C1q + DSA at ~6 years post-transplant, Graft loss at year ~9 years.
HTx-06	No	2	1 year	Transferred care, lost to follow up
HTx-07	No	4	7 years	Non-specific reactivity observed on LabScreen SAB Class II*
HTx-08	No	3	6 years	Non-specific reactivity observed on LabScreen SAB Class II*
HTx-09	N/A	N/A	TF	Graft loss at day 5
HTx-10	No	0	1.5 years	Age 69 at transplant, treated with autologous adipose derived stromal vascular fraction cells

* Non-specific reactivity is identified based on previously published patterns of beads known to carry denatured antigen or on patterns of reactivity that do not demonstrate a common epitope.

there was no change in the population before or after immunosuppression was reduced, nor did it change significantly once immunosuppression was restarted. We have continued to monitor this population and it decreased to 32% of the lymphoid gate and 15% of all cells at year 4 post-transplant, and at his latest annual visit at year 11 the B cell clone is now 25% of all lymphocytes and 9% of all analyzed white blood cells. Of note, the patient has also maintained an inverted CD4/CD8 ratio of 0.3.

Although patient 3 mounted a substantial cellular immune response leading to multiple (> 15) episodes of cellular rejection, he has never had DSA detected in blood. Nevertheless, he is one of the few VCA recipients to develop "classic" chronic rejection (resembling chronic GVHD of the skin) while adequately immunosuppressed. As shown in Fig. 1, the adnexal structures such as the fingernails started disappearing at year 4, and other chronic rejection hallmarks such as atrophy and thinning of the skin as well as development of fibrosis has occurred and continues to slowly progress over time. High resolution ultrasound and MRA of the vessels reveals thickening over time, but all vessels, including digital arteries, are patent and the patient has always had excellent digital temperatures. The skin biopsies reveal thickening of vessels consistent with chronic rejection. Capillary thrombosis was present as early as month 3 in a histologic grade 3 skin biopsy (Fig. 2). Because of declining kidney and hand function, a decision was made to convert the patient from tacrolimus/sirolimus/prednisone maintenance immunosuppression to sirolimus/MMF/prednisone triple therapy 8 years post-transplant. Serum creatinine and BUN improved significantly (year 11; Cr 1.2, GFR 63) and hand function was not affected by the change. Presently, 11 years after transplantation, hand function is limited by stiffness as a result of fibrosis; however, the patient currently considers the hand to be an assist functionally. This subject presents an unusual case of VCA in the presence of aberrant B cell function. The case is notable for the presence of severe chronic rejection in the face of good medication compliance, an absence of DSA. Vasculopathy is present in this patient, although at a mild to moderate level. The high number of cellular mediated rejection episodes may also suggest that the dysfunctional B cell population resulted in reduced regulation of T cell alloimmunity.

In our series of ten hand transplants, no recipient with de novo DSA had a coincident change in graft function or graft pathology. Conversely, neither of two recipients who developed severe vasculopathy [54] nor one who developed severe chronic rejection of the skin had detectable circulating DSA. It is possible that non HLA antibodies may have played a role, but commercial standardized reagents to most non HLA antibodies are not available. Additionally, while the absence of detectable DSA may have been due to tissue absorption, we do not believe false low reactivity was present as we do not report results based on a c MFI, but rather on patterns of reactivity. Based on these patterns we would have detected low reactivity to public antigens such as Bw6 or Bw4. We did not. Two other hand transplant recipients followed at the Louisville VCA Program (now 11 years and 17 years post-transplant respectively) have had more than fifteen episodes of cellular rejection without evidence of de novo DSA, while one recipient who had only three episodes of ACR developed very high levels of de novo DSA six years post-transplant (Table 1). Of note, review of our DSA monitoring revealed that identifying DSA based only on Luminex testing is problematic. We did find that false positives, likely due to denatured antigen, occurred in three of our recipients. The reactivity was determined to be false positive as the bead pattern is frequently observed in patients with no history of sensitizing events and these patients were negative by FlowPRA. While this does not completely rule out the possibility of real antibody, when all of the data are considered in the clinical context that is, in fact, the most congruous interpretation. To truly understand the nature of DSA in transplantation it is imperative to not over-estimate the incidence.

Our experience and our perspective on the experience of others would suggest that VCA differ markedly from other transplants in the manifestations and impact of B cell responses. Tissue and organ transplants generally elicit powerful B cell responses leading to production of DSA in many immunosuppressed recipients and to DSA-mediated acute and chronic graft changes. In contrast, clinical VCA infrequently induce DSA and when present DSA usually appear to have little or no impact on the graft. Indeed, an episode of classical ABMR in a VCA with DSA in blood and C4d in the graft would merit a report in the literature. We recently discussed in some detail the various mechanisms that might account for the limited impact of DSA on VCA [10]. Rather than reiterate these yet unproven explanations we will discuss, in closing, some questions, the answers to which would advance this field.

The foremost question from the perspective of transplantation immunology must be whether and in which ways B cells of the clinical VCA recipient respond specifically to the VCA donor. Put in another way – does the infrequency of DSA in VCA recipients reflect infrequency of B cell responses or are B cell responses cloaked in some way. In fundamental systems, T cell-dependent B cell responses can be limited by MHC (i.e. T cell help) but clinical VCA clearly elicit T cell responses. Another potential mechanism is the follicular helper T (Tfh) cells that interact with B cells and may be providing some regulation of the response [70]. In some fundamental and clinical systems, B cell responses can be suppressed by presence of soluble antigen in blood. It is possible that extensive tissue disruption in the course of VCA procedures liberates "soluble" or suspended antigen that suppresses B cell responses but the presence of such antigen remains to be tested. We think a more direct approach, such as the assay of donor specific B cell responses [7] might address this question.

If B cell responses to VCA do occur more often than the low prevalence of DSA would suggest, then a key question is whether DSA produced by VCA recipients is bound to the graft or blocked by circulating antigen. We previously discussed the pertinence of this question [19,20] and will not do so again here. We would point out however that

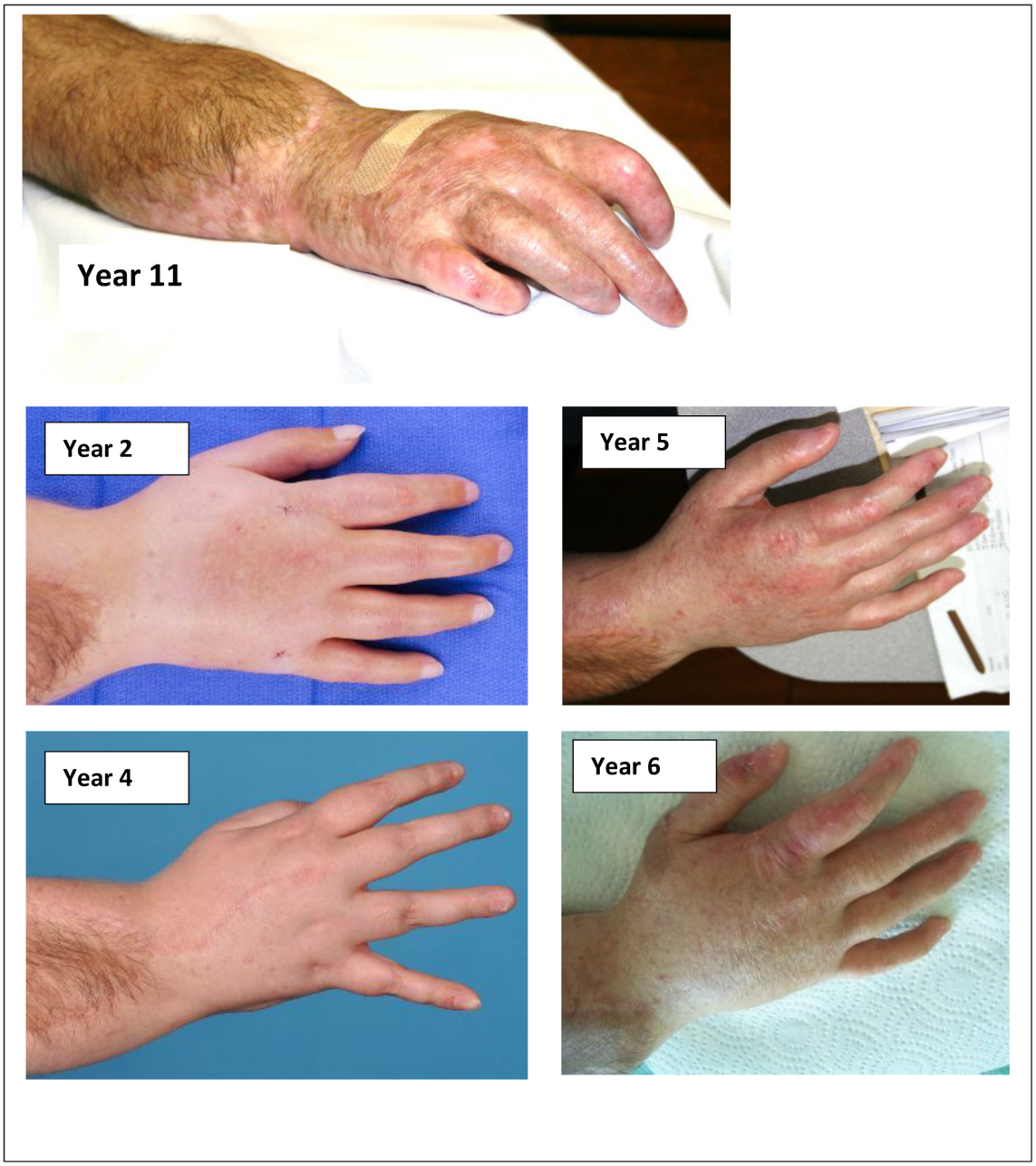

Fig. 1. Changes in hand allograft over time for Patient 3. Four years after transplantation, the patient started losing his fingernails, and this continued until all fingernails were lost. Thinning and atrophy of the skin was also progressive with loss of hair, and evidence of hard fibrotic areas in the forearm. Over time there has been increased brown mottling of the skin, which is very apparent in the year 11 photo. Progressive stiffing and loss of the ability to extend or flex the fingers also occurred, the hand is functional as an assist only.

VCA pose an interesting challenge in that various tissues, e.g. skin, muscle, bone, could have differential impact on antibody adsorption or antigen release and because biopsy limited to skin (while serving as a useful sentinel for CMR) might not faithfully represent the extent of Ab binding.

B cells are found in both normal and inflamed human skin [71]. In an ovine model, Geherin et al. demonstrated that B cells not only traffic through, but are present in both normal and inflamed skin. In addition, B cells within the skin expressed markers suggesting both antigen presenting function as well as antibody secreting cells [72]. In VCA, donor B cells come with the graft as skin resident immune cells, and may recirculate back to the skin in the same manner. Weissenbacher et al. have observed B cells and TLO like structures in the skin of a patient with steroid resistant rejection, although whether they were of host or donor origin was not determined [49]. In a study of 113 skin biopsies from face transplant recipients, Lian et al. reported that immune cells spatially associated with target cell injury were often predominantly of donor, not recipient origin [73]. A role for donor as well as host B cells re-circulating in the skin may occur and should be examined.

If VCA do elicit B cell responses and Ab to bind to the graft, the question of whether VCA have greater baseline or induced resistance to injury, i.e. accommodation, comes to the fore. We will not review the putative mechanisms of accommodation here as we have done so for organ transplants of various types [8,20,39,74] and VCA [10]. What we should add here is that aspects of the procedure and the biology of VCA that distinguish those grafts from organ grafts could be pertinent to accommodation. Above we discussed the possibility that the surgical

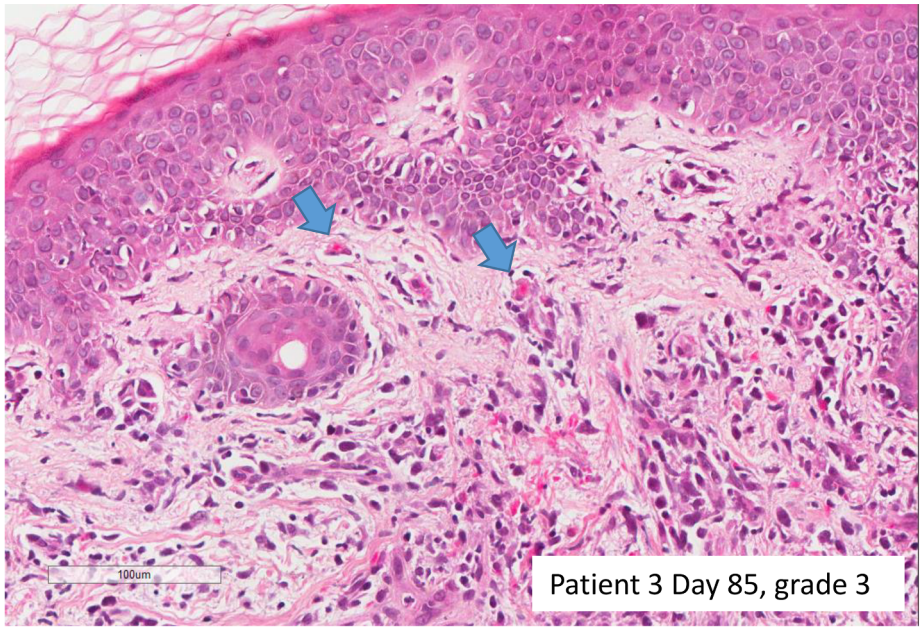

Fig. 2. Evidence of capillary thrombosis at month 3 in skin biopsy of Patient 3. H&E staining of skin biopsies revealed capillary thrombosis early post transplant. This recipient had grade III histologic rejection at the same time as a CMV infection. Both processes responded to treatment, however this patient went on to have more than 15 episodes of grade II or higher histologic rejection, and has what appears to be severe chronic rejection targeted primarily to the skin.

procedure for VCA transplants probably induces ischemic-preconditioning. Whether and in what ways preconditioning impacts induction of accommodation by DSA and complement is not known and we think a subject meriting investigation. As another factor, antigen liberated from VCA might well modify interaction of DSA with the graft, displacing complement activation from the surface of the graft or limiting the availability of sites for covalent reaction of graft cells with C4 and C3, conditions potentially favoring accommodation [10]. On the other hand, the greater extent of tissue injury incurred by VCA might hinder or delay induction of accommodation in VCA.

In summary, the role of B cells with respect to development of pathologic humoral immunity, regulation of T cell responses, and as an indicator of accommodation or operational tolerance is an exciting area of research in the field of VCA. While numbers of VCA recipients are still small, it appears that there is less production of dnDSA than seen in solid organ transplantation, and clear cases of pathology due to ABMR with or without concomitant acute cellular rejection also seem to be lower. Whether this is due to mechanisms unique to VCA that more easily induce graft accommodation, increased activity of B cell regulatory cells or if VCA grafts may be less susceptible to complement mediated damage than solid organ grafts should be investigated.

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References

[1] C. Wehmeier, G. Honger, H. Cun, et al., Donor specificity but not broadness of sensitization is associated with antibody-mediated rejection and graft loss in renal allograft recipients, Am. J. Transplant. 17 (2017) 2092–2102.
[2] J. Sellares, D.G. de Freitas, M. Mengel, et al., Understanding the causes of kidney transplant failure: the dominant role of antibody-mediated rejection and non-adherence, Am. J. Transplant. 12 (2012) 388–399.
[3] S.A. Lodhi, K.E. Lamb, H.U. Meier-Kriesche, Solid organ allograft survival improvement in the United States: the long-term does not mirror the dramatic short-term success, Am. J. Transplant. 11 (2011) 1226–1235.
[4] G. Guidicelli, F. Guerville, S. Lepreux, et al., Non-complement-binding de novo donor-specific anti-HLA antibodies and kidney allograft survival, J. Am. Soc. Nephrol. 27 (2016) 615–625.
[5] J. Kwun, P. Bulut, E. Kim, et al., The role of B cells in solid organ transplantation, Semin. Immunol. 24 (2012) 96–108.
[6] G.E. Karahan, F.H. Claas, S. Heidt, B cell immunity in solid organ transplantation, Front. Immunol. 7 (2016) 686.
[7] R.J. Lynch, I. Silva, B.J. Chen, J.D. Punch, M. Cascalho, J.L. Platt, Cryptic B cell response to renal transplantation, Am. J. Transplant. 13 (2013) 1713–1723.
[8] M.I. Cascalho, B.J. Chen, M. Kain, J.L. Platt, The paradoxical functions of B cells and antibodies in transplantation, J. Immunol. 190 (2013) 875–879.
[9] E.I. Dijke, J.L. Platt, P. Blair, et al., B cells in transplantation, J. Heart Lung Transplant. 35 (2016) 704–710.
[10] J.L. Platt, C.L. Kaufman, B.M. Garcia de Mattos, M. Cascalho, Accommodation and related conditions in vascularized composite allografts, Curr. Opin. Organ Transplant. 22 (2017) 470–476.
[11] R. Patel, P.I. Terasaki, Significance of the positive crossmatch test in kidney transplantation, N. Engl. J. Med. 280 (1969) 735–739.
[12] A. Granato, Y. Chen, D.R. Wesemann, Primary immunoglobulin repertoire development: time and space matter, Curr. Opin. Immunol. 33 (2015) 126–131.
[13] R. Shinnakasu, T. Kurosaki, Regulation of memory B and plasma cell differentiation, Curr. Opin. Immunol. 45 (2017) 126–131.
[14] S. Takemoto, P.I. Terasaki, J.M. Cecka, Y.W. Cho, Gjertson DW. Survival of nationally shared, HLA-matched kidney transplants from cadaveric donors. The UNOS Scientific Renal Transplant Registry, N. Engl. J. Med. 327 (1992) 834–839.
[15] R.J. Duquesnoy, Are we ready for epitope-based HLA matching in clinical organ transplantation? Transplantation 101 (2017) 1755–1765.
[16] C. Wiebe, P. Nickerson, Strategic use of epitope matching to improve outcomes, Transplantation 100 (2016) 2048–2052.
[17] M. Haas, A. Loupy, C. Lefaucheur, et al., The Banff 2017 kidney meeting report: revised diagnostic criteria for chronic active T cell-mediated rejection, antibody-mediated rejection, and prospects for integrative endpoints for next-generation clinical trials, Am. J. Transplant. 18 (2018) 293–307.
[18] C. Lefaucheur, D. Viglietti, C. Bentlejewski, et al., IgG donor-specific anti-human HLA antibody subclasses and kidney allograft antibody-mediated injury, J. Am. Soc. Nephrol. 27 (2016) 293–304.
[19] J.L. Platt, M. Cascalho, Donor specific antibodies after transplantation, Pediatr. Transplant. 15 (2011) 686–690.
[20] J.L. Platt, Accommodation: how you see it, how you don't, Am. J. Transplant. 11 (2011) 2007–2008.
[21] B.D. Tait, C. Susal, H.M. Gebel, et al., Consensus guidelines on the testing and clinical management issues associated with HLA and non-HLA antibodies in

transplantation, Transplantation 95 (2013) 19–47.
[22] J.L. Platt, M. Cascalho, IgM in the kidney: a multiple personality disorder, Kidney Int. 88 (2015) 439–441.
[23] P.I. Lobo, Role of natural autoantibodies and natural IgM anti-leucocyte autoantibodies in health and disease, Front. Immunol. 7 (2016) 198.
[24] E. Zorn, S.B. See, Polyreactive natural antibodies in transplantation, Curr. Opin. Organ Transplant 22 (2017) 8–13.
[25] G.E. Karahan, F.H. Claas, S. Heidt, B cell immunity in solid organ transplantation, Front. Immunol. 7 (2017) 686, https://doi.org/10.3389/fimmu.2016.00686. eCollection;%2016.: 686.
[26] W.R. Morrow, E.A. Frazier, W.T. Mahle, et al., Rapid reduction in donor-specific anti-human leukocyte antigen antibodies and reversal of antibody-mediated rejection with bortezomib in pediatric heart transplant patients, Transplantation 93 (2012) 319–324.
[27] Y. Ding, J. Francis, A. Gautam, L. Pelletier, V. Sanchorawala, K. Quillen, Durable renal response after combination of bortezomib, corticosteroids, rituximab, and plasmapheresis for late antibody-mediated renal transplant rejection, Clin. Nephrol. 89 (2018) 252–259.
[28] F. Eskandary, H. Regele, L. Baumann, et al., A randomized trial of bortezomib in late antibody-mediated kidney transplant rejection, J. Am. Soc. Nephrol. 29 (2018) 591–605.
[29] P. Masson, L. Henderson, J.R. Chapman, J.C. Craig, A.C. Webster, Belatacept for kidney transplant recipients, Cochrane Database Syst. Rev. 11 (2014) CD010699.
[30] M.J. Everly, M. Roberts, R. Townsend, R.A. Bray, H.M. Gebel, Comparison of de novo IgM and IgG anti-HLA DSAs between belatacept- and calcineurin-treated patients: an analysis of the BENEFIT and BENEFIT-EXT trial cohorts, Am. J. Transplant. 18 (2018) 2305–2313.
[31] F. Vincenti, Belatacept and long-term outcomes in kidney transplantation, N. Engl. J. Med. 374 (2016) 2600–2601.
[32] J.L. Platt, S. Tsuji, M. Cascalho, Novel functions of B cells in transplantation, Curr. Opin. Organ Transplant 16 (2011) 61–68.
[33] J. Marino, J.T. Paster, A. Trowell, et al., B cell depletion with an anti-CD20 antibody enhances alloreactive memory T cell responses after transplantation, Am. J. Transplant. 16 (2016) 672–678.
[34] M.R. Clatworthy, C.J. Watson, G. Plotnek, et al., B-cell-depleting induction therapy and acute cellular rejection, N. Engl. J. Med. 360 (2009) 2683–2685.
[35] D.J. Firl, G. Benichou, J.I. Kim, H. Yeh, A paradigm shift on the question of B cells in transplantation? Recent insights on regulating the alloresponse, Front. Immunol. 8 (2017) 80, https://doi.org/10.3389/fimmu.2017.00080. eCollection;%2017.: 80.
[36] M.W. Chopek, R.L. Simmons, J.L. Platt, ABO-incompatible kidney transplantation: initial immunopathologic evaluation, Transplant Proc 19 (1987) 4553–4557.
[37] A.D. Bannett, R.F. McAlack, M. Morris, M.W. Chopek, J.L. Platt, ABO incompatible renal transplantation: a qualitative analysis of native endothelial tissue ABO antigens after transplantation, Transplant Proc. 21 (1989) 783–785.
[38] M. Cascalho, J.L. Platt, Harnessing B cells in immunotherapy, Immunotherapy 8 (2016) 237–239.
[39] B.M. Garcia de Mattos, M. Cascalho, J.L. Platt, Accommodation in ABO-incompatible organ transplants, Xenotransplantation 25 (2018) e12418.
[40] S. Zschiedrich, A. Kramer-Zucker, B. Janigen, et al., An update on ABO-incompatible kidney transplantation, Transpl. Int. 28 (2015) 387–397.
[41] J.M. Gloor, D.J. Lager, M.E. Fidler, et al., A Comparison of splenectomy versus intensive posttransplant antidonor blood group antibody monitoring without splenectomy in ABO-incompatible kidney transplantation, Transplantation 80 (2005) 1572–1577.
[42] J.R. Montgomery, J.C. Berger, D.S. Warren, N.T. James, R.A. Montgomery, D.L. Segev, Outcomes of ABO-incompatible kidney transplantation in the United States, Transplantation 93 (2012) 603–609.
[43] C. Morath, M. Zeier, B. Dohler, G. Opelz, C. Susal, ABO-incompatible kidney transplantation, Front. Immunol. 8 (2017) 234, https://doi.org/10.3389/fimmu.2017.00234. eCollection;%2017.: 234.
[44] H. Bergenfeldt, B. Andersson, D. Bucin, et al., Outcomes after ABO-incompatible heart transplantation in adults: a registry study, J. Heart Lung Transplant. 34 (2015) 892–898.
[45] Z.E. Holzknecht, K.L. Kuypers, T.B. Plummer, et al., Apoptosis and cellular activation in the pathogenesis of acute vascular rejection, Circ. Res. 91 (2002) 1135–1141.
[46] G.P. Alexandre, B.M. De, J.P. Squifflet, M. Moriau, D. Latinne, Y. Pirson, Human ABO-incompatible living donor renal homografts, Neth. J. Med. 28 (1985) 231–234.
[47] E. Morelon, J. Kanitakis, P. Petruzzo, Immunological issues in clinical composite tissue allotransplantation: where do we stand today? Transplantation 93 (2012) 855–859.
[48] A. Weissenbacher, A. Loupy, A. Chandraker, S. Schneeberger, Donor-specific antibodies and antibody-mediated rejection in vascularized composite allotransplantation, Curr. Opin. Organ Transplant 21 (2016) 510–515.
[49] A. Weissenbacher, T. Hautz, B. Zelger, et al., Antibody-mediated rejection in hand transplantation, Transpl. Int. 27 (2014) e13–e17.
[50] E. Morelon, P. Petruzzo, J. Kanitakis, et al., Face transplantation: partial graft loss of the first case 10 years later, Am. J. Transplant. 17 (2017) 1935–1940.
[51] A. Chandraker, R. Arscott, G.F. Murphy, et al., The management of antibody-mediated rejection in the first presensitized recipient of a full-face allotransplant, Am. J. Transplant. 14 (2014) 1446–1452.
[52] Bruce E. Gelb, J. Rodrigo Diaz-Siso, Natalie M. Plana, Adam Jacoby, William J. Rifkin, Kimberly S. Khouri, Daniel J. Ceradini, Eduardo D. Rodriguez, Absence of rejection in a facial allograft recipient with a positive flow crossmatch 24 months after induction with rabbit anti-thymocyte globulin and anti-CD20 monoclonal antibody, Case Rep. Transplant. 2018 (2018) 1–9, https://doi.org/10.1155/2018/7691072.
[53] T.S. Win, N. Murakami, T.J. Borges, et al., Longitudinal immunological characterization of the first presensitized recipient of a face transplant, JCI Insight 2 (2017) 93894.
[54] C.L. Kaufman, R. Ouseph, B.W. Blair, et al., Graft vasculopathy in clinical hand transplantation, Am. J. Transplant. 12 (2012) 1004–1016.
[55] L. Landin, P.C. Cavadas, J. Ibanez, I. Roger, F. Vera-Sempere, CD3+-mediated rejection and C4d deposition in two composite tissue (bilateral hand) allograft recipients after induction with alemtuzumab, Transplantation 87 (2009) 776–781.
[56] P. Petruzzo, J. Kanitakis, L. Badet, et al., Long-term follow-up in composite tissue allotransplantation: in-depth study of five (hand and face) recipients, Am. J. Transplant. 11 (2011) 808–816.
[57] J. Kanitakis, B. McGregor, L. Badet, et al., Absence of c4d deposition in human composite tissue (hands and face) allograft biopsies: an immunoperoxidase study, Transplantation 84 (2007) 265–267.
[58] A. Weissenbacher, G. Vrakas, M. Chen, et al., De novo donor-specific HLA antibodies after combined intestinal and vascularized composite allotransplantation - a retrospective study, Transpl. Int. 31 (2018) 398–407.
[59] P. Ferdinandy, D.J. Hausenloy, G. Heusch, G.F. Baxter, R. Schulz, Interaction of risk factors, comorbidities, and comedications with ischemia/reperfusion injury and cardioprotection by preconditioning, postconditioning, and remote conditioning, Pharmacol. Rev. 66 (2014) 1142–1174.
[60] S.N. Heyman, D. Leibowitz, L.I. Mor-Yosef, et al., Adaptive response to hypoxia and remote ischaemia pre-conditioning: a new hypoxia-inducible factors era in clinical medicine, Acta Physiol. (Oxf.) 216 (2016) 395–406.
[61] C. Mauri, M. Menon, Human regulatory B cells in health and disease: therapeutic potential, J. Clin. Invest. 127 (2017) 772–779.
[62] K.A. Newell, A. Asare, A.D. Kirk, et al., Identification of a B cell signature associated with renal transplant tolerance in humans, J. Clin. Invest. 120 (2010) 1836–1847.
[63] K.A. Newell, A. Asare, I. Sanz, et al., Longitudinal studies of a B cell-derived signature of tolerance in renal transplant recipients, Am. J. Transplant. 15 (2015) 2908–2920.
[64] C.L. Kaufman, R. Ouseph, A. Ramirez, et al., Role of donor specific antibody in chronic rejection and graft accomodation in hand transplant recipients, Military Health System Research Symposium (MHSRS), (2017) 8–27-0017.
[65] K.R. Amin, J.K.F. Wong, J.E. Fildes, Strategies to reduce ischemia reperfusion injury in vascularized composite allotransplantation of the limb, J. Hand Surg. Am. 42 (2017) 1019–1024.
[66] S. Khalifian, D.S. Cooney, W.P.A. Lee, G. Brandacher, The role of ischemia-reperfusion injury in reconstructive transplantation, Transplant. Technol. Res. S3 (2013) 1–6.
[67] T.M. Zahir, K.S. Zahir, S.A. Syed, R.J. Restifo, J.G. Thomson, Ischemic preconditioning of musculocutaneous flaps: effects of ischemia cycle length and number of cycles, Ann. Plast. Surg. 40 (1998) 430–435.
[68] S.S. Huang, F.C. Wei, L.M. Hung, Ischemic preconditioning attenuates postischemic leukocyte–endothelial cell interactions: role of nitric oxide and protein kinase C, Circ. J. 70 (2006) 1070–1075.
[69] Y.H. Hsieh, S.S. Huang, F.C. Wei, L.M. Hung, Resveratrol attenuates ischemia - reperfusion-induced leukocyte – endothelial cell adhesive interactions and prolongs allograft survival across the MHC barrier, Circ. J. 71 (2007) 423–428.
[70] Carola G. Vinuesa, Michelle A. Linterman, Di Yu, Ian C.M. MacLennan, Follicular helper T cells, Annu. Rev. Immunol. 34 (1) (2016) 335–368, https://doi.org/10.1146/annurev-immunol-041015-055605.
[71] I.U. Egbuniwe, S.N. Karagiannis, F.O. Nestle, K.E. Lacy, Revisiting the role of B cells in skin immune surveillance, Trends Immunol. 36 (2015) 102–111.
[72] S.A. Geherin, S.R. Fintushel, M.H. Lee, et al., The skin, a novel niche for recirculating B cells, J. Immunol. 188 (2012) 6027–6035.
[73] C.G. Lian, E.M. Bueno, S.R. Granter, et al., Biomarker evaluation of face transplant rejection: association of donor T cells with target cell injury, Mod. Pathol. 27 (2014) 788–799.
[74] R.J. Lynch, J.L. Platt, Accommodation in organ transplantation, Curr. Opin. Organ Transplant. 13 (2008) 165–170.