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Review

Sensitization and desensitization of burn patients as potential candidates for vascularized composite allotransplantation

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ARTICLE INFO

Article history:
Accepted 25 May 2015

Keywords:
Composite tissue allotransplantation
Face transplantation
Hand transplantation
HLA
IVIG
Plasmapheresis
Rejection

ABSTRACT

Sensitization describes the acquired ability of the immune system to react to foreign human leukocyte antigens (HLA) by producing antibodies and developing memory cells. In the field of transplantation, recipient preformed HLA antibodies due to previous sensitization have been identified – beneath ABO incompatibility – as a major factor for acute graft rejection. Several reasons for sensitization have largely been studied, such as previous blood transfusions, pregnancies or former transplants. Recent studies indicate that the use of assist devices (e.g. ECMO) or cadaveric skin allotransplantation providing temporary coverage in burn patients may lead to additional sensitization. As vascularized composite allotransplantation (VCA) has become a rapidly advancing therapeutic option for reconstruction of complex tissue defects in burns, it seems even more important to become familiar with immunological principles and to be cautiously aware of both sources of sensitization and therapeutic concepts in burns avoiding sensitization. This may also include emergency VCAs in burn patients as potential strategy for early definitive reconstruction avoiding procedures triggering HLA antibody formation.

We hereby provide an overview on current evidence in the field of pre- and peritransplant sensitization, followed by posttransplant strategies of desensitization and their potential impact on future treatments of burn patients.

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http://dx.doi.org/10.1016/j.burns.2015.05.019
0305-4179/

Contents

1. Introduction

Vascularized composite allotransplantation (VCA) is a rapidly advancing therapeutic option for reconstruction of complex tissue defects [1–4]. Major trauma to the face and extremities frequently leaves massive soft and bony tissue defects that are not amenable to conventional reconstruction. Functional or esthetic outcomes may be suboptimal and may be associated with substantial morbidity. VCA facilitates the ideal goals of reconstructive surgery – ''to replace like with like''. However, advancement of VCA as a routine reconstruction option is hampered by the burden of immunosuppression for long term graft acceptance [5].

Since the inception of clinical VCA over a decade ago, burn victims have been identified as immunologically complex patients for these procedures [6,7]. This population may be prone to sensitization for various reasons, which has been an exclusion criterion for many VCA programs around the world. Different concepts of both desensitization as well as induction and maintenance immunosuppression have been investigated and implemented in solid organ transplants [8–13], while similar protocols or experiences in VCA are limited [13]. The principles of sensitization and desensitization in burns and VCA appear largely uncharted [14,15].

Sensitization describes the acquired ability of the immune system to react to foreign human leukocyte antigens (HLA) by producing anti-HLA antibodies and developing memory cells. In the field of transplantation, donor-specific, preformed recipient HLA antibodies (DSA) have been identified – beneath ABO incompatibility – as a major risk factor for hyperacute and acute allograft rejection [14,16–20]. Several reasons for sensitization have been identified, such as previous blood transfusions, pregnancies or former transplants. Recent studies indicate also that the use of cardiac assist devices (e.g. extracorporeal membrane oxygenation (ECMO), ventricular assist device (VAD)) or even cadaveric skin allotransplantation providing temporary coverage in burn patients may lead to a primary or additional sensitization [15,21,22].

About 35% of all patients on a renal transplant waiting list in the US are HLA-sensitized due to previous transplantation, blood transfusions or pregnancies [23]. Historically this led to the introduction of anti-HLA antibody screening and pre-transplant complement-dependent cytotoxcitiy (CDC) crossmatch testing to avoid antibody-mediated rejection [24,25]. Successful solid organ transplantation across HLA and/or ABO barriers – emerging in the 1980s – using now refined desensitization protocols aiming at reduction of pre-existing antibodies to a level that qualifies for successful engraftment, have stimulated the interest in grafts from HLA-incompatible or immunologically less favorable donors [26,27]. Different strategies to remove pre-existing antibodies have been tested by using techniques like plasmapheresis and immunoabsorption, while other protocols using splenectomy or the application of antibodies (e.g. rituximab) or intravenous immunoglobulins (IVIG) target anti-HLA antibodies indirectly [20]. The trend of immunologically incompatible organ replacement with good short-term results is meanwhile widespread for kidney transplantations based on a broad understanding of the principles of sensitization and desensitization [28–32].

In the history of VCA, transplantations have been performed with HLA mismatch between donor and recipient, whereas negative CDC crossmatch and ABO compatibility remain a prerequisite [33]. The prevalence of sensitization in patients awaiting VCA is thought to be essentially lower than in patients being scheduled for solid organ transplantation for various reasons. Devastating trauma to the hand or face qualifying for VCA mostly happen to previously healthy, young, often male patients with a low risk of previous sensitization. According to the literature, 80% of the patients who have received reconstructive VCAs are male with an average age of 34 years for face transplantation and 84% of the patients with hand transplantation are male with a median age of 32 years [34]. The average age for renal transplantation

is 47.5 years in comparison, with 61% males in the population. These patients have previously received numerous transfusions because of renal anemia and/or former kidney transplants [35] as potential sensitizing triggers.

As VCA is about to become a valid and accepted alternative for reconstruction of complex tissue defects, it seems even more important to familiarize with immunological principles and to be cautiously aware of both sources of sensitization and therapeutic concepts avoiding sensitization during primary trauma care and burn treatment. The current article aims at providing an interdisciplinary overview of sensitization and desensitization for the understanding of reconstructive surgeons based on current evidence in the field of pre- and peritransplant sensitization, followed by posttransplant immunosuppressive strategies and their potential impact on future treatments of massive tissue defects in patients qualifying for VCA.

2. Pretransplant assessment of the HLA system

The HLA system as a part of the major histocompatibility complex (MHC) was first described in the 1950s [36]. HLAs are cell surface glycoproteins that serve as recognition molecules in the initiation of an adaptive immune response. They present self and foreign peptides to effector T-cells of the immune system [16]. Pretransplant assessment of the HLA system consists of different testing methods. HLA typing is performed with serological methods based on complement-dependent cytotoxicity (CDC) [37]. The CDC technique is widely used in patients when listed for transplantation. Since the 1980s, the introduction of molecular techniques and polymerase chain reaction (PCR) enabled scientists to type for HLA on a genetic level guaranteeing higher accuracy and resolution of HLA typing [38].

2.1. *HLA matching*

HLA matching tests the immunologic compatibility of two human organisms. Whereas some data suggests that the degree of HLA matching between donor and recipient greatly influences transplant outcomes [39] and twin studies emphasize the role of HLA matching by demonstrating that grafts from monozygotic twins are tolerated without immunosuppression, mostly VCA has been performed with HLA mismatches [40]. Although transplants with none or few mismatches show favorable outcomes compared to transplants with several HLA mismatches, the restricted donor pool for VCA – due to difficulties in adequate matching for skin color, gender, size, age, AB0 blood group and sometimes the urgency of the procedure – requires more liberal acceptance of HLA mismatched donors [41,42].

2.2. *HLA crossmatching*

In contrast to HLA matching, crossmatching identifies preexistent donor-specific anti-HLA antibodies potentially increasing the risk of hyperacute and acute antibody-mediated rejection. Crossmatching is therefore mandatory prior to any allotransplantation. For solid organ transplantation, a positive CDC crossmatch test is considered a contraindication [43,44]. The same principles apply for VCA [33]. Since 1969, the CDC crossmatch (CDCXm) has routinely been established in transplant centers all over the world to detect preformed donor-specific antibodies [26,45] and led to a significant reduction in hyperacute and acute organ rejection [46]. Lately, bead-based solid-phase crossmatching has been introduced as another more sensitive technique for the detection of pretransplant anti-HLA antibodies [47,48]. This approach incubates patients' sera with microspheres coated with single HLA proteins, identifying both complement and non-complement activating anti-HLA antibodies [49]. With this novel technique, the specificity of the anti-HLA antibodies can be accurately defined [49,50]. Furthermore, if the donor HLA type is known, a crossmatch test can be performed in silico, a procedure called virtual crossmatching [50,51]. Virtual crossmatching allows testing over larger distances and can therefore increase the chance to find a suitable organ. Whereas virtual crossmatching is increasingly used in solid organ transplantation, its introduction for VCA is currently under discussion. Possibly, with UNOS incorporation of VCA in the US, there will be a renewed review of the utility of the virtual crossmatch to enable donor sharing across geographically separated regions.

2.3. *The acceptable mismatch*

During the last two decades, a new paradigm in terms of "acceptable HLA mismatches" in solid organ transplantation has gradually replaced the efforts for a perfect HLA match as the driving force in organ allocation [16,52,53]. More effective regimens of immunosuppression and significant advances in anti-HLA antibody detection techniques have been enabling factors. The acceptable mismatch program was established for the purpose of finding crossmatch-negative donors for highly sensitized patients [54,55]. The program includes a systematic analysis of those HLA antigens toward which the recipient-to-come has not produced antibodies so far. These antigens are transferred to an international database as acceptable mismatches. Organ donor selection is based on complete compatibility with both the patient's own HLA antigens and the acceptable mismatches [54]. This strategy has led to successful transplantation of several hundred renal allografts within the group of the highly sensitized patients [56,57]. Acceptable mismatches in sensitized patients have yet not been considered in VCA, but it is currently debated to make use of this strategy as an efficient method of broadening the transplant offer in VCA, too.

3. Origin of HLA sensitization

Anti-HLA antibodies do not occur naturally. They are produced after contact with foreign HLA antigens. Antibody formation is initiated by the uptake and processing of foreign antigens by antigen-presenting cells that present it to $CD4^+$ T-cells. At the same time, antigen-specific B2-cells recognize, bind, internalize and process these antigens through their B-cell-receptor. Clonal expansion of B-cells is induced, when a

co-stimulatory signal is present. As a result B-cells will differentiate and develop into either IgG-secreting plasma cells or into memory B-cells [58]. The main well-known antigenic exposures leading to HLA antibody formation are pregnancy, blood transfusion and previous transplants [59,60]. Recently, further triggers for the development of HLA alloantibodies have been suggested, such as the use of assist devices in heart failure or cadaveric skin transplantation in burns [12,15]. The incidence, degree and duration of the anti-HLA antibody response – and therefore its clinical significance – are the result of complex interactions largely determined by the nature of the alloantigenic event and the immunologic history of the patient [61].

3.1. *Pregnancy*

Pregnancy represents the only natural source of HLA sensitization. Reported incidences vary throughout the relevant literature from approximately 25% to 55% of pregnancies, in which women develop antibodies against paternal antigens present on the fetal cells that enter the maternal circulation [61–64]. HLA alloantibodies were not detected until about the 28th week of gestation [65], and the probability of pregnancy-induced sensitization is obviously increased with the number of pregnancies, reaching up to 74% in women with more than two deliveries [62,64]. The level of immunization may decrease over time, but can also increase and may acquire a wider spectrum after a new antigenic challenge such as blood transfusion or transplantation [61,66]. The latter ''multiplier'' effect is not to be underestimated, as multiparous women with little – or even no – HLA antibodies at presentation can become highly sensitized by blood transfusion or transplantation [61].

3.2. *Blood transfusions*

Blood transfusion is a further well-known cause for sensitization and potentially preventable in contrast to pregnancy and transplantation [10]. The incidence of HLA antibodies in renal transplant candidates, who had no previous pregnancies or transplants, was 10–12% in patients who received up to 20 transfusions (measured by CDC testing or enzyme-linked immunosorbent assay (ELISA)) [67,68]. In general, blood transfusions in naïve recipients are a relatively poor immunogenic stimulus [61]. This low immunogenicity of blood transfusions is supported by recent findings in male and nulliparous female blood donors where sensitization was detected in 1.7% of donors with transfusions in the past versus 1.0% of donors without previous transfusions [69].

The importance of blood transfusions in respect of sensitization became especially apparent in patients suffering from anemia while awaiting kidney transplantation [70,71]. Before the introduction of erythropoietin in the 1980s, blood transfusions were frequently given to patients with end-stage renal disease. From nowadays point of view several clinical studies had surprisingly shown that untransfused patients experienced more rejection episodes and presented with worse graft survival than transfused recipients [72–74]. This down-regulatory phenomenon of blood transfusions is commonly called ''transfusion effect'' [75]. Different factors such as anti-idiotypic antibodies, non-specific immunosuppression by plasma factors, macrophages and altered lymphocytes, suppressor cell induction and clonal deletion were hypothesized throughout multiple clinical and experimental studies in order to explain the potential immunomodulating or immunosuppressive effect of blood transfusions [72,76]. Until today, the phenomenon of reduced allo-immune response to the organ allograft has not been clarified, but since the introduction of more potent immunosuppressive regimens on the one hand and erythropoiesis-stimulating agents leading to an abrupt decline of blood transfusions on the other hand, the beneficial effect for graft survival due to pretransplant blood transfusions has become less evident – possibly still being present according to some reports, whereas denied by others [10,77–79]. This phenomenon has not yet been investigated in the field of VCA. Future studies should therefore include careful evaluation of blood transfusion as potential factor of sensitization but also as variable for immunomodulatory effects. Patients after VCA should be monitored for rejection episodes and graft survival with a focus on blood transfusions prior to VCA.

Another interesting conclusion regarding the immunomodulatory effect of transfusions has been drawn from patients with burns or trauma showing an increased rate of infection and mortality [80,81]. This downside effect of transfusion-associated immunosuppression was investigated in a large multi-center study focusing on burn victims with the result that each unit of blood transfused heightened the risk of infection significantly [82]. This led to the conclusion that beneath the systemic immunosuppressive state after burns, the capacity of the immune system seems to be additionally affected by blood transfusions. The administration of blood transfusions in patients with massive tissue defects potentially qualifying for VCA – especially in burns where extensive transfusion of blood products is frequently required [82,83] – should therefore carefully be evaluated. Though, taking the ''transfusion effect'' into account, it remains difficult to delineate between the favorable and disadvantageous effects of blood transfusions in the VCA scenario of burn patients [84]. If blood administration is unavoidable, HLA-matched transfusions may be considered an alternative as they have shown a lower risk for sensitization than ABO-compatible transfusions [61]. On the downside, HLA-matched transfusions are very expensive and resource intensive requiring between 48 and 72 h for their provision. Additionally, the probably beneficial effect of leukoreduced blood transfusions in terms of a diminished rate of infection and mortality should be kept in mind – especially in burn patients, where systemic immunosuppression is generally found as part of the burns [85,86]. Surprisingly, leukocyte reduction of red blood cell transfusions does not decrease allosensitization rates in potential kidney transplant candidates [87]. Above that, platelet transfusions were found to be a major reason for alloimmunization in patients with haemato-oncological disorders. The overall incidence of platelet antibodies was stated with 66% – irrespective of the received number of transfusions [88,89].

3.3. *Previous transplants*

Functioning grafts (e.g. kidney, heart) have been shown to be a risk factor for sensitization and consecutive organ rejection

[61,90]. *De novo* post-transplant anti-HLA antibodies were found to appear to a minor extent early after transplantation, become successively more frequent and can be a major factor in late graft failure [61,91]. The incidence of sensitization in patients after graft loss has been shown being as high as 70%. Furthermore, patients who seemingly remain not sensitized at the time of transplant loss may still be at high risk of developing anti-HLA antibodies in response to transfusion [61,92].

To date, there are no reports about VCA in previously transplanted recipients, but animal studies deliberately use the immunogenicity of HLA-mismatched skin grafts to induce sensitization [14]. Interestingly, presensitization following skin transplantation prior to secondary VCA does not result in hyperacute, but accelerated rejection. This observation is different to findings in the field of renal transplantation, where presensitization immediately induced hyperacute rejection suggesting that the mechanism of rejection for VCA may differ mechanistically from that for organ transplantation [14]. One potential explanation for this phenomenon is that rejection in VCA is considered to be predominantly cell-mediated in contrast to antibody-mediated rejection in organ transplants.

3.4. *Allogeneic skin transplants*

Human skin allografting was first described as allegedly permanent solution for wound covering in 1869 [93]. About 50 years later, incompatibility of cadaver skin was stated by observing complete loss of skin allografts after 35 days leading to the understanding that allogeneic skin transplants only provide temporary coverage.

To date, allogeneic skin transplants are mainly used for transitory coverage of burn patients with limited supply of autologous skin for coverage of open wounds. As the supply of fresh skin from deceased donors is obviously limited, and – although it was the main source in the past – it has led to multiple questions with respect to the transplant law, infectious and immunological complications. For preservation of cadaveric skin there is an ongoing debate on the ideal technique. Two methods have proven useful: cryopreservation and glycerol preservation [94]. Whereas cryopreservation claims not to damage vital structures of the skin imitating the viable properties of fresh cadaver skin, glycerol preservation has turned out to be more cost effective. The latter can easily be stored at +4 °C afterwards. Above that, concentrated glycerol has proven to have antibacterial and antiviral properties [95]. Additionally, the immunogenic reaction to glycerol preserved cadaver skin appears to be reduced as vital structures such as epidermal cells and lymphatic vessels are destroyed in the preservation process [94,95]. Though, any kind of skin allografts underlies a rejection process that simultaneously leads to potential sensitization since MHC molecules are thought to be released from the allogeneic skin cells and presented by antigen-presenting cells of the recipient [96]. Another hypothesized interaction between the skin allograft and the recipient is based on the role of epidermal Langerhans cells migrating from the skin graft to the draining lymph node of the recipient, where they can activate T-cells [97]. The latter mechanism is supposed to explain the lower immunogenicity of glycerol preserved skin, as its nonviable property obstructs the Langerhans cells to migrate out of the allogeneic skin in absence of lymphatic vessels [97]. However, whereas sensitization to skin allografts is a common finding in experimental animal studies and often used for the analysis of solid organ rejection in presensitized animals [98–100], the advantages and disadvantages of different preservation techniques and their impact on sensitization in humans are still a matter of debate.

Preliminary data suggest that cadaveric skin grafting is associated with the emergence of anti-HLA antibodies in severely burned patients [7,15]. This hypothesis was derived from a single patient who was listed for face transplantation after severe electrical burns [101]. The pretransplant screening revealed HLA alloantibodies, but consecutive desensitization treatment was hardly successful resulting in a reduced probability to find a suitable donor. The patient was finally excluded from the waiting list in mutual agreement. Retrospective analysis of the initial burn treatment revealed the temporary use of cadaveric skin coverage as a possible trigger for sensitization [15]. Other risk factors for sensitization however have to be taken into account, such as blood or platelet transfusions and the concomitant multiplier effect. These circumstances should call surgeons' attention to carefully consider pretransplant treatment algorithms if VCA may be a potential future therapeutic option.

3.5. *Assist devices*

There is profound evidence showing that the use of ventricular assist devices (VAD) as bridge-to-transplant techniques in terminal heart failure is associated with the emergence of anti-HLA antibodies [22]. One hypothesized reason for this kind of sensitization is seen in the presence of antigens on the biomechanical surface of the assist device [102]. Recent investigations in a group of children with circulatory support of the extracorporeal Berlin Heart EXCOR VAD showed *de novo* occurrence of anti-HLA antibodies in 69% [103]. Some reports propagate an unfavorable effect on the cardiac allograft survival in sensitized left VAD recipients [104], others do not [12] – attributing this effect to the increased and ameliorated use of potent immunomodulatory therapies [22]. Likewise, venovenous extracorporal membrane oxygenation (vvECMO) as bridge-to-transplant strategy in lung transplant candidates showed augmented levels of HLA alloantibodies without evidence of hyperacute organ rejection after transplantation [21,105]. However, vvECMO is not only used in pretransplant patients, but also as lifesaving modality in patients with inhalative trauma and/or ARDS in severely burned patients [106–109]. This fact should be taken into account when listing a burn victim for VCA. The clinical relevance of presensitization related to the exposure to inert biomaterials should probably not be overestimated, but interventions to support the device, such as blood transfusions for replacement after device-associated hemolysis or adverse events, may contribute significantly to the development of anti-HLA antibodies [110]. Additionally there is controversial evidence concerning dialysis being a risk factor for HLA sensitization: Some reports found hemodialysis to increase the anti-HLA antibody level in a single treatment session [111,112], while others did not find any difference in

the anti-HLA antibody levels of end stage renal disease patients before and after dialysis [113]. Still, literature provides no information if temporary dialysis is a potential trigger of *de novo* synthesis of HLA antibodies in patients with acute renal failure as a complication of major burn trauma.

3.6. *Further stimuli*

Recently, it has become clear that HLA alloantibodies inconsistently arise in response to HLA-unrelated immune stimulation [114,115] and that their response is increased by proinflammatory events such as infections and sepsis [116,117]. The latter findings were drawn from a retrospective analysis of renal transplant patients who had at least one culture-proven infection leading to a significant increase in their anti-HLA antibody level. As infection is a frequently occurring complication in burned patients, one should be aware of this unfavorable trigger for anti-HLA antibody stimulation, despite its inevitability.

4. Quantitative determination of sensitization

The degree of sensitization is classically reported as the percentage of CDC panel reactive antibody (PRA). The PRA represents an estimate of the probability of a positive CDC crossmatch to a pool of potential donors. Higher values indicate an increased chance for a positive crossmatch and therefore a decreased chance for a suitable donor. Although PRA cut-off values vary throughout the relevant literature, the most common threshold to consider a patient as sensitized is a PRA value $\geq$10% [118,119], whereas highly sensitized patients are defined at a PRA cut-off value $\geq$85% [120]. It is to be noted, that the value of PRA depends both on the panel composition and the technique used for antibody detection; PRA is therefore said to be highly variable and inconsistent [120,121]. Nevertheless, to date, PRA is the most established quantitative indicator of pretransplant immunologic responsiveness [119].

Several approaches have been established over the last 50 years to quantitatively assess HLA sensitization [121–124]. Depending on the different transplant centers' guidelines, at least one screening test for the determination of the degree of sensitization is required at the time of the first transplant evaluation. The common assays to determine the PRA are similar to the methods of crossmatching as described before. The only – but important – difference is that tests for sensitization detect antibodies against a broad population of donors, whereas crossmatching identifies antibodies against one specific donor. The complement-dependent cytotoxicity (CDC) test combines the patient's serum with a pool of lymphocytes from different donors with defined HLA antigens [125]. With the introduction of more sensitive and more specific methods, such as the Luminex bead-based assay, the CDC has gradually become less important in respect of PRA determination [125,126]. Enzyme-linked immunoabsorption (ELISA) assays combine known HLA antigens with the potential recipient's serum using microtest trays. Compared with the CDC, ELISA can be performed more rapidly. For determination of PRA, the HLA antigens used for screening can be adjusted to the known donor pool. This method has proven to be superior regarding sensitivity and specificity and has thus been integrated in many transplant laboratories [126]. Analogously to the virtual crossmatch, the term of a virtual or calculated PRA (cPRA) has risen from the combination of the exact antibody specificities detected in the recipient's serum by Luminex and the frequency of those antigens present in the donor population [127]. The cPRA established a uniform and more reliable definition of (highly) sensitized patients [120].

In VCA, determination of sensitization is as mandatory as in solid organ transplantation. To the best of our knowledge, only one presensitized patient scheduled for VCA has been reported thus far in the literature [15,101,128].

5. ABO compatibility

The ABO blood group system was discovered by Landsteiner in 1900 and consists of four well-known categories: O, A, B and AB, with types O and A most frequently found in common populations [129]. ABO antigens are glycopeptides and expressed on most human tissues as on red blood cells, lymphocytes, platelets, endothelial and epithelial cells. In contrast to anti-HLA antibodies, ABO antibodies do occur naturally following exposure to bacterial antigens of the gut after birth [130]. They are directed against those antigens that are not natively existent in the individual ABO organism. The ABO antibodies are known as isohaemagglutinins and consist predominantly of the IgM subtype [131]. As well as HLA sensitization, ABO incompatibility plays an important role in organ transplantation. Due to the risk of hyperacute rejection, major ABO incompatibility has commonly been considered a contraindication for transplantation [19,132]. However, there is upcoming evidence that ABO-incompatible transplantation is gradually becoming an acceptable alternative to ABO-matched transplants, for some organs under certain circumstances, mostly living donations [19].

The first ABO-incompatible kidney transplantation was performed in 1955 resulting in poor graft survival [25], which underlined the overall agreement that ABO-matching is indispensable for renal transplantation. In 1987, hyperacute graft rejection in kidney transplants was effectively prevented by pretransplant plasmapheresis re-igniting the idea of ABO-incompatible organ transplantation [27]. Expansion of desensitization protocols and improvement of immunosuppression has successively made ABO-incompatible living-donor kidney transplantation to a valuable option with outcomes comparable to ABO-matched transplants [133]. The liver is commonly considered an immunologically "permissive" organ with respect to incompatible transplantations. While ABO-incompatible liver transplantation was limited to emergency cases in the 1970s [134], advances in immunosuppression and improved transplantation techniques have led to improved outcomes for ABO-incompatible liver transplantation [19,135]. ABO-incompatible lung transplants have only been reported to be performed in clinical emergency situations with limited success, whereas ABO-incompatible heart transplantation has become a promising issue in infants and neonates [19], but not in adults. This trend is related to the fact that the humoral response to ABO-incompatible cardiac transplants is remarkably diminished in infants, as the ABO antibodies develop only

6–8 months after birth in the course of gut colonization with *Escherichia coli* [130,136,137]. This unique window in the absence of acute humoral response is liable for excellent long-term outcomes in cardiac transplantation across the ABO barrier [19].

In VCA, the ABO barrier is universally respected. However, the trends mentioned for ABO-incompatible solid organ transplantation may offer translatable insights in the field of VCA to help enlarging the donor pool.

6. Desensitization

Desensitization protocols have been established to remove both anti-HLA and anti-A/B blood group antibodies with the aim to increase the probability to find a suitable donor. These protocols consist of different combined techniques to either eliminate circulating antibodies and/or prevent their synthesis and secretion. The first broad-based studies evaluating the long-term outcome of transplantations after desensitization trace back to incompatible kidney transplants in the 1990s [138,139]. Since then, various combinations of instrumental, surgical and pharmacological desensitization techniques have significantly increased the chances for sensitized patients to receive an organ. The treatment options for presensitized patient are listed in the following paragraphs:

- Immunoadsorption and plasma exchanges are extracorporeal techniques to eliminate anti-HLA and anti-A/B antibodies in the patients' plasma. While plasma exchange unspecifically removes immunoglobulines (Ig) with consecutive need of plasma products, immunoadsorption selectively binds anti-A or -B antibodies by a sepharose column coated with A or B antigen [140,141]. However, antibody titers rebound some weeks after performing immunoadsorption or plasmapheresis if not combined with the measures described below [142].
- The anti-B cell agent rituximab depletes B-cells by binding to the CD20 receptor. In contrast, plasma cells are not targeted as they lack CD20 receptors [143]. This results in a constrained effectiveness of rituximab on the antibody production making a combination with other antibody depleting measures unavoidable. The effect of rituximab can be observed for up to one year [11].
- The proteasomal inhibitor bortezomib indirectly reduces the alloantibody production by triggering the apoptosis of plasma cells. The peak concentration of bortezomib is reached within 30 min and cleared out of the blood after 60 min, while the effect duration can last for a long time [11].
- Intravenous Immunoglobulins (IVIG) affect the immune system on multiple pathways [144]. These include neutralization of anti-idiotypic antibodies, inhibition of the complement cascade and reduction of antibody formation by down-regulating mechanisms or eliciting apoptosis of B cells. Optimal dosing and frequency of administration of IVIG is discussed controversially. Two major therapeutic principles have risen in the course of the last two decades: the administration of high dose IVIG has proven to be a cost and resource effective method to desensitize patients with low antibody levels. In comparison, low dose IVIG combined with plasma exchange results in better outcomes in (highly) sensitized patients than the sole application of high dose IVIG [145]. On the downside, the low dose IVIG plus plasma exchanges protocol is a cost, time and resource intensive desensitization strategy, as numerous pretransplant sessions may be required to obtain adequately low titers.
- Surgical removal of the spleen has been used in desensitization protocols for ABO-incompatible transplant recipients with the intention to eliminate a major source of B-cells and plasma cells. Its outcome and the lifelong consequences have since been debated controversially [27,146,147]. Newer pretransplant immunodulatory concepts – like the combination of immunoadsorption or plasma exchange and rituximab – have led to replacement of the surgical removal of the spleen in favor of partial pharmacologic splenectomy [146].

Desensitization comes with its price as the agents used in the protocols undoubtedly have side effects. Desensitized patients are at higher risk of severe – predominantly viral – infections and malignancy [11]. Additionally, IVIG has shown to cause mild side effects like headache, nausea, myalgia and arthralgia, back pain, increased blood pressure or less commonly renal failure, hemolysis and thrombotic events like myocardial infarction, stroke or deep vein thrombosis [11].

Whether desensitization strategies will increase the recipient pool of VCA patients remains to be seen. The strategies may also be evaluated for applicability in burn patients to avoid presensitization in the future.

7. Summary

Sensitization and desensitization represent two basic terms in the field of transplant immunology that have successively gained importance for reasons of organ shortage, understanding of rejection mechanisms, effective concepts of immunomodulation and better diagnostic and monitoring strategies. Transplantation across previously incompatible barriers – especially for ABO incompatibility – has become more and more a reality with good long-term graft and patient outcomes.

In contrast to patients awaiting solid organ transplantation, patients qualifying for VCA – especially after burn trauma – are primarily at risk of sensitization during the acute treatment, when blood transfusions are administered, temporary skin coverage is performed or even assist devices such as ECMO are utilized. One should also be aware of the multiplier effect in multiparous women. Thus, prevention of sensitization should cautiously be taken into consideration from the date of the trauma if a patient may qualify for VCA. Sensitization might not be prevented in all cases, but due efforts must be made to minimize patient exposure to predisposing factors that may cause sensitization to worsen: patients, referring physicians and transplant team members should be instructed to think critically about the administration of blood transfusions and the use of cadaveric skin or assist devices. Under certain circumstances, VCA performed as a primary reconstruction as a secondary procedure could indeed possibly obviate the issues of pre-sensitization due to

transfusions, temporary coverage or other triggers. The face transplant recently performed in Poland is the first such example of a primary VCA [148]. In summary, future strategies to avoid sensitization will be of utmost interest non only in burn patients, but also in parous female patients, and most importantly VCA patients requiring retransplantation for primary graft failure.

Conflict of interest

All named authors hereby declare that they have no conflicts of interest to disclose.

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