REVIEW

Donor specific HLA antibodies & allograft injury: mechanisms, methods of detection, manifestations and management

Jennifer A. McCaughan[1,2] & Kathryn J. Tinckam[1,3]

1 HLA Laboratory, Laboratory Medicine Program, University Health Network, Toronto, ON, Canada
2 Department of Nephrology and Transplantation, Belfast City Hospital, Belfast, UK
3 Division of Nephrology, Department of Medicine, University Health Network, Toronto, ON, Canada

Correspondence
Dr. Kathryn J. Tinckam, Toronto General Hospital, University Health Network, 585 University Avenue, 11PMB-187, Toronto, ON M5G 2N2, Canada.
Tel.: 416 340 4800 ext 8225;
fax: +1 647 689 3070;
e-mail: kathryn.tinckam@uhn.ca

SUMMARY

The impact of donor specific HLA antibodies (DSA) on solid organ transplant outcomes has been recognised for over half a century. This article reviews the mechanisms of DSA formation, details the laboratory methods for detecting DSA, discusses the clinical and histological manifestations of DSA in the allograft and explores the options for management of DSA. The challenges posed by pre-existing and *de novo* DSA are explored with current therapeutic strategies described. A method for stratifying the risk associated with pre-existing DSA is explained and the importance of understanding immunological risk associated with transplantation to facilitate optimal personalised decision making for transplant recipients is highlighted. Future directions for further managing the risk associated with DSA are proposed.

Transplant International 2018; 31: 1059–1070

Key words
donor specific antibodies, rejection, risk

Received: 3 May 2018; Revision requested: 11 June 2018; Accepted: 27 July 2018

Solid organ transplantation is the definitive treatment for end-stage cardiac, hepatic, nutritional, pulmonary and renal failure. There are myriad issues which must be faced to achieve successful transplantation: those of performing major anaesthesia and surgery on a physiologically fragile recipient, those of optimising donor quality, those related to organ preservation and the immunological hurdles which must be surmounted when a genetically non-identical organ is transplanted into a recipient. The latter is a particular challenge. The majority of the earliest solid organ transplants failed as a consequence of either rapid aggressive alloimmune injury or because the recipient succumbed to infection following attempts to attenuate the alloimmune response [1,2]. It is now understood that this early injury to the allograft was driven by pre-existing donor specific antibodies [3]. In this review, we discuss the mechanisms by which donor specific antibodies are produced, their histopathological and clinical manifestations and options for managing the associated risk.

Donor specific antibodies

Donor specific antibodies are defined as antibodies which are complementary to a region of a peptide in the donor which is not present in the recipient i.e. the peptide region is considered *non-self* by the recipient's immune system. Donor specific antibodies (DSA) can develop to any polymorphic protein which differs between donor and recipient. In transplantation, the term DSA is commonly used to refer to antibodies specific for donor HLA which are foreign to the recipient, but DSA can also exist to foreign donor ABO

doi:10.1111/tri.13324

antigens and to other non-HLA antigens. The focus of this manuscript will be on HLA DSA.

The importance of HLA DSA in transplantation has been recognised for over 50 years [3]. The function of HLA is to present peptides from the intracellular and extracellular compartments to T lymphocytes, facilitating recognition of foreign proteins and the initiation of an immune response against pathogens and dysplastic cells [4]. The CD4 T lymphocytes activated by this process play a key role in providing help to B cells during the process of antibody generation [5]. Non-self HLA is an extremely potent stimulus of the humoral response; this is likely to be attributable to the high level of expression of HLA by donor cells generating multiple opportunities for allorecognition to occur [6].

Mechanisms of DSA production

The initial step in HLA antibody generation is exposure to a non-self HLA peptide which is recognised by a B lymphocyte; this process is known as sensitisation. Sensitisation to non-self HLA can occur via pregnancy (sensitisation to paternal HLA), transplantation, homografts or blood transfusion (sensitisation to donor HLA). HLA antibodies have also been detected in some individuals who have not had sensitising events; this may reflect cross reactivity between antibodies which are formed to pathogens and HLA [7,8]. Sensitisation alone, however, does not always result in the generation of antibodies [9,10]. Two theories of immune activation exist: the non-self theory and the danger theory [11]. It is plausible that alloimmune activation requires a "double hit" and that HLA DSA are generated when a threshold is exceeded (Fig. 1). In recipients who have identical HLA to their donors, no amount of danger signal is going to result in the generation of HLA DSA because there is no non-self stimulus and it is recognised that there may be hyporesponsiveness of the immune system to non-self HLA in the absence of a danger signal [12]. The early period after solid organ transplantation provides a perfect storm for the initiation of antibody development; the danger signal is stimulated by surgery and the implantation of an organ injured by the process of donation and preservation and there is a strong non-self signal from highly expressed foreign donor HLA. The variation in danger signal between transplants is a simple concept to grasp; a critically ill, ventilated patient who receives lungs donated after circulatory death is likely to mount a greater inflammatory response than a renal transplant recipient receiving a pre-emptive transplant from a young living donor with a short ischaemic time. There is also variability in the non-self signal; an understanding of antigen-antibody interaction is necessary to appreciate this.

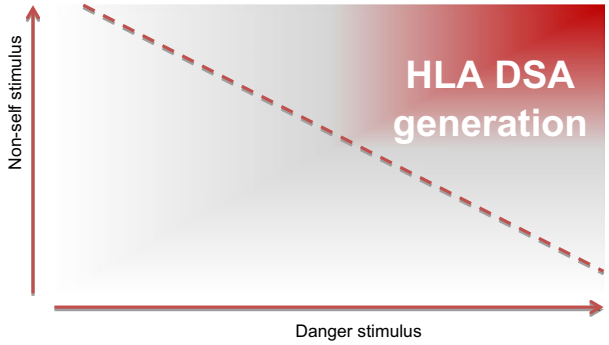

Figure 1 A threshold model for generation of HLA donor specific antibodies by the recipient immune system.

An antibody is specific for a non-self eplet. Eplets are polymorphic 3 Å regions on the exposed surface of an antigen which are comprised of amino acids that are in close proximity on the antigen surface (Fig. 2) [13]. An eplet consists of approximately three amino acids so an HLA class I molecule has a large number of distinct eplets. Each individual is tolerant to their self eplets. Antigenicity is the likelihood that an antigen will be recognised as foreign by the immune system and the antigenicity of a single HLA antigen mismatch in transplantation varies according to the recipient's self HLA and the mismatched donor HLA [14]. The number of foreign eplets in the donor HLA partly explains this. An association has been demonstrated between the number of donor HLA eplet mismatches and the risk of DSA development after transplantation [15,16].

A greater number of non-self eplet mismatches increases the potential antigenicity of an allograft, but the concept of immunogenicity must also be considered. Immunogenicity is the likelihood that a foreign eplet or antigen will be recognised by the immune system and induce a destructive immune response. The electophysical properties of an amino acid polymorphism within an eplet influence the affinity of the antigen-antibody complex [17,18] by facilitating the initial interaction between eplet and antibody and allowing non-covalent bonds to form [19]. Differences in electrostatic potential between polymorphic amino acids in donor-recipient pairs have been shown to correlate with both the development and amount of HLA DSA [20,21].

Another factor which may influence immunogenicity is the epitope that is associated with a particular eplet. While antibody specificity is determined by the eplet, there is a larger region around the eplet which also

Transplant International 2018; 31: 1059–1070

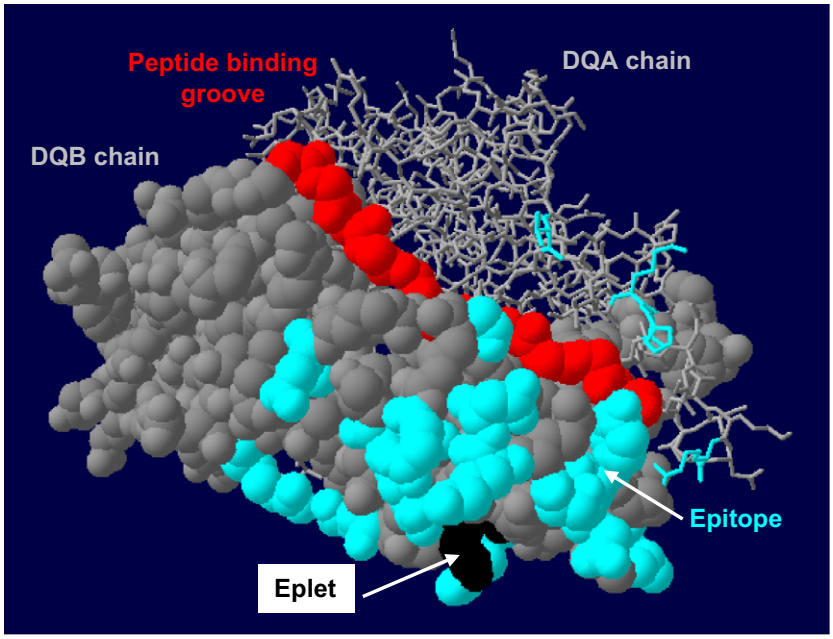

Figure 2 Diagram to illustrate the concept of eplets and epitopes. This model of the DQ7 HLA (peptide chains encoded by DQA1*05 and DQB1*03:01) shows the 45EV eplet in black. This has a corresponding Terasaki eplet (TerEp 2001) confirming its immunogenicity *in vivo*. The blue area on both the DQA and DQB chains surrounding the eplet represents the epitope. An antibody specific for the 45EV eplet will make contact with the amino acids contained in the larger blue region when it binds to the HLA at 45EV. This homology model was generated within Swissmodeller and considers the area for a structural epitope to be within 15 Å of the eplet. Resolved X-ray crystallography structures (2NNA, 1S9V, 402F, 5KSV, 4DBP) were used as templates (all templates had a resolution of 2.7 Å or less) for alignment of HLA allele sequence data (obtained from https://www.ebi.ac.uk/ipd/imgt/hla/) in Swissmodeller (https://www.swissmodel.expasy.org/); mean sequence homology was >95%, quality of the generated homology model was assessed by QMEAN and Ramachandran plots.

makes contact with the antibody footprint; this is called the epitope (Fig. 2). There are some key amino acid positions within the epitope where interaction with the antibody footprint influences the stability of the antigen-antibody complex [21,22]. It has been shown that, while amino acid polymorphisms in these regions do not affect the antibody specificity, these variations can influence the capacity of an antibody to bind and initiate a destructive immune response [22–24].

A description of the cellular processes involved in DSA generation is beyond the scope of this manuscript but these have been described eloquently elsewhere [25].

Methods of DSA detection

There are a number of methods for detecting HLA antibodies in the laboratory. These differ according to the source of the HLA antigens, the type of antibody detected (immunoglobulin class, complement fixing) and the implications of a positive result for transplant outcomes (Table 1). A positive complement dependent cytotoxic (CDC) crossmatch in the presence of HLA DSA is specific for a high risk of early allograft loss but lacks sensitivity for detecting low titre or non-complement fixing DSA [3] while Luminex single antigen bead (SAB) testing is extremely sensitive for DSA detection but has a less rigorous association with alloimmune injury and allograft survival [26]. Despite this reduced specificity, detectable DSA by Luminex alone prior to transplantation does confer an increased risk of antibody mediated injury and recipients receiving transplants in this context should be monitored closely. Luminex SAB testing has the advantages of not requiring donor cells, determining HLA antibody specificity and facilitating eplet analysis of an antibody profile [27]. Most HLA laboratories employ a combination of Luminex (bead based) and cross match (cell based) methods when assigning immunological risk.

There are a number of adaptations which can be made to the Luminex assay to provide further information about the antibodies that are present. Although Luminex is normally used to test for IgG antibodies in the serum, the assay can be modified to allow detection of other immunoglobulin classes. In addition, it may also be used to determine IgG subclass. There are four recognised subclasses which vary in their capacity to fix

Table 1. Comparison of laboratory methods for HLA antibody detection.

Method	HLA source	Antibodies detected	Pre-transplant screening	Donor assessment	Post-transplant screening	Specificity	Sensitivity
CDC cross match	Cells	IgG & IgM, complement fixing only	Yes – using cell panels	Yes	No	++	+
Flow cross match	Cells	IgG routinely, other Ig according to secondary antibody	No	Yes	No	++	++
Luminex single antigen beads	Recombinant HLA on beads	IgG routinely, other Ig according to secondary antibody, capacity for investigating antibody properties e.g. C1q fixing	Yes	Yes – virtual	Yes	+	+++

complement and interact with Fc receptors on immune cells; IgG1 and IgG3 are considered to be potent complement activators while IgG2 and IgG4 are less effective at initiating the complement cascade [28]. A number of groups have identified an association between the IgG subclass of DSA and specific phenotypes of allograft injury [29–31]. A further characteristic of antibodies which can be assessed by Luminex technology is the ability of antibodies to fix complement; the methods most commonly employed detect the C1q, C3d or C4d products of activation of the classical complement pathway following the addition of complement to the Luminex assay. In heart and lung transplantation, there has been an association reported between pre-transplant complement fixing DSA and antibody mediated rejection and early allograft loss respectively [32,33] while in kidney transplantation, the development of complement fixing DSA after transplantation has been associated with an increased risk of antibody mediated rejection [34]. It is important to acknowledge that there are a number of factors which influence the interpretation of complement-fixing assays including antibody titre, complement inhibitors and antibodies to denatured epitopes [35].

Another adaptation to the Luminex assay involves the antibody source. Conventionally, serum is used as the source for HLA antibodies but it is also possible to test eluates from allograft biopsies for the presence of DSA. A small number of studies have suggested that intragraft DSA may be more specific for allograft injury than DSA detected in the serum [36,37].

Non-HLA antibodies can also be detected by a number of methods. There are well established methods for identifying blood group antibodies in blood transfusion laboratories. There are also platforms available to detect antibodies to other non-HLA antigens, but the association between the presence of such antibodies and transplant outcomes has not been conclusively demonstrated [27]. It may be that with the development and standardisation of testing for non-HLA antibodies that their role in transplant outcomes, and subsequently the place of testing for these antibodies in solid organ transplantation, will be clarified.

Manifestations of DSA in the allograft

There are a number of possible manifestations of the interaction of DSA with the endothelium of the transplanted organ.

Complement activation

When DSA bind to the graft endothelium, activation of the classical complement pathway results in the formation of anaphylatoxins which attract leucocytes into the graft, the production of complement proteins which opsonise donor cells, the stimulation of cytokine production and ultimately in the generation of the membrane attack complex (MAC) [38]. The MAC causes direct injury to the transplant by inducing cell lysis. Historically, the classical feature of antibody mediated rejection has been positive staining for C4d (a product of the classical complement pathway) on allograft biopsies [39–42] (Fig. 3a). The capacity of DSA to activate complement depends upon their class, with IgM and IgG being the most effective complement activators, and their IgG subclass, with IgG1 and IgG3 being the most potent initiators of the classical complement cascade [29,43].

Antibody-dependent cell mediated cytotoxicity

The recognition of C4d-negative antibody mediated rejection (Fig. 3b) highlighted the existence of a complement-independent mechanism of DSA mediated injury to the transplant [40,44]. The majority of work in C4d negative antibody mediated rejection is in kidney transplants where C4d positivity is not an essential criteria for the diagnosis of antibody mediated rejection [39], but C4d negative antibody mediated rejection has also been reported in other solid organ allografts [45,46]. Antibody dependent cell mediated cytotoxicity (ADCC) occurs when the Fc portion of a DSA bound to the

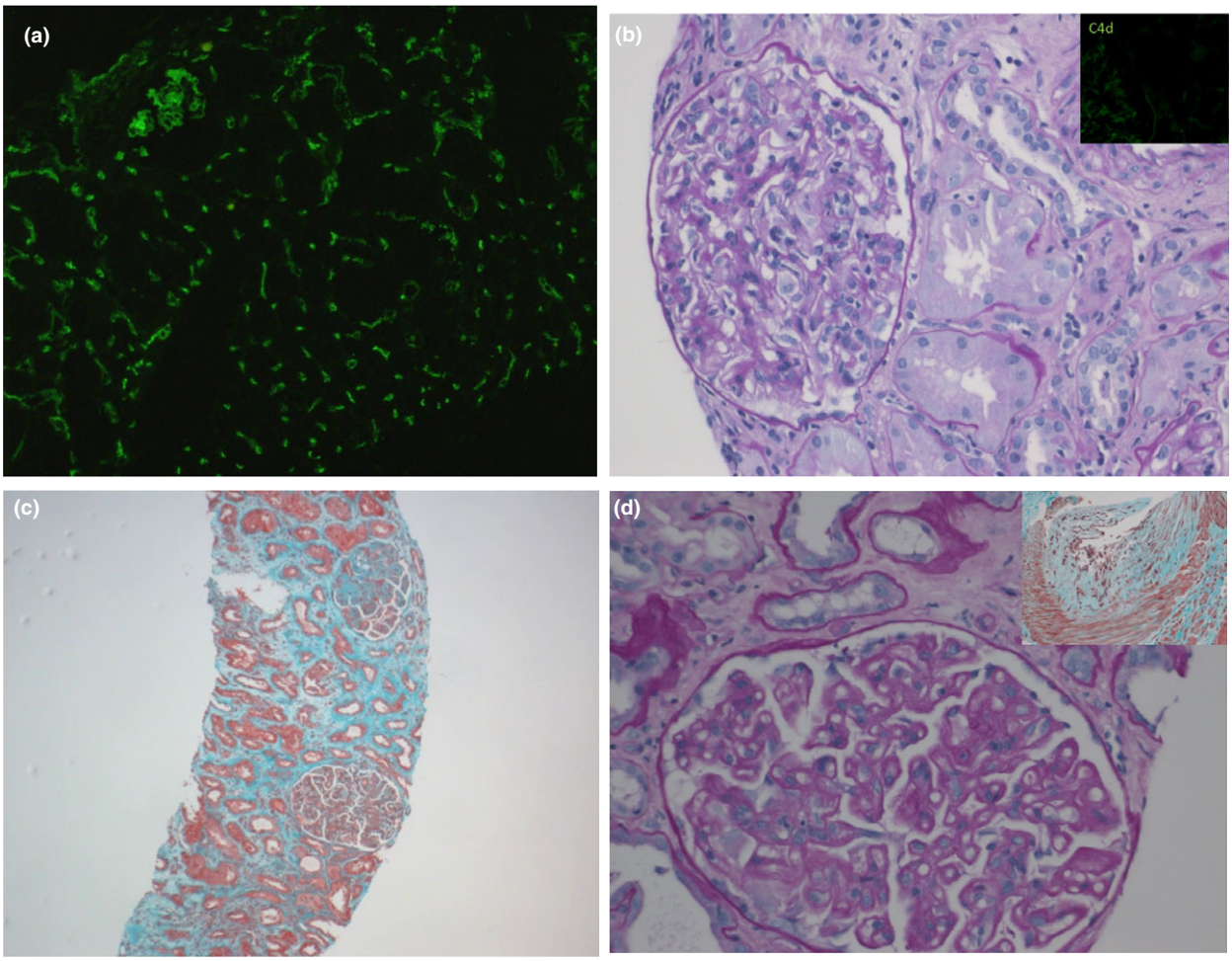

Figure 3 Histological manifestations of donor specific antibodies in the renal allograft. (a) Diffuse peritubular capillary staining for C4d (IF 5×). (b) Glomerulitis and peritubular capillaritis in a renal allograft (PAS 20×); inset showing C4d immunofluorescence with no peritubular capillary staining (IF 10×). (c) Chronic antibody mediated rejection in a renal allograft: interstitial fibrosis and transplant glomerulopathy (Trichrome 5×). (d) Chronic antibody mediated rejection in a renal allograft: glomerular capillary wall double contouring and segmental glomerulitis (PAS 20×); inset showing artery with intimal proliferation (Trichrome 20×).

transplant endothelium interacts with the Fc receptor on a leucocyte, usually a natural killer cell. This results in the formation of an intercellular synapse that allows the natural killer cell to induce apoptosis of the associated graft endothelium and stimulate the secretion of cytokines which recruit other inflammatory cells to the site [47]. Microvascular inflammation which occurs in the presence of DSA, but in the absence of C4d positivity, may be driven by this manifestation of DSA [47,48].

Endothelial cell dysfunction

The binding of DSA to the graft endothelium activates signalling cascades which modify endothelial cell function by changing the cytoskeleton, promoting cell proliferation and enhancing the binding of monocytes to the endothelium [49,50]. These manifestations contribute to the intimal proliferation and fibrosis which characterise chronic antibody mediated injury in solid organ transplantation (Fig. 3c,d) [44,51].

Accommodation

There are occasions when there is evidence of DSA binding to the graft endothelium without signs of allograft injury or inflammation; this is known as accommodation. This phenomenon is most commonly reported in ABO incompatible transplantation when there is return of the donor-specific AB antibodies after transplantation, there is evidence of C4d positivity on graft biopsy, but there are no clinical or histological manifestations of allograft injury [52]. The process behind accommodation is not well understood but is clearly of great interest. In cases where there are detectable DSA but no evidence of histological damage, it is also possible that there is early allograft injury present but that this falls below the threshold of detection of current technologies.

Clinical manifestations of DSA

The clinical syndromes associated with DSA-mediated injury to the allograft can be classified according to timing.

Hyperacute rejection

This is irreversible immunological injury which occurs within minutes to hours of organ perfusion. Hyperacute rejection is usually attributable to the presence of very high titre donor specific AB or HLA class I antibodies which bind to highly expressed targets on the graft endothelium [3,53]. This manifests as massive complement activation and endothelial injury followed rapidly by graft thrombosis. Hyperacute rejection due to high titre HLA and ABO DSA was a feature of the earliest kidney transplants [54] but should never occur in the modern era with appropriate pre-transplant blood group and HLA testing. Hyperacute rejection due to non-HLA and non-ABO donor specific antibodies has been reported; these rare instances occur when there are high titre, complement fixing donor specific antibodies to targets that have a high degree of expression on the graft endothelium [55].

Accelerated antibody mediated rejection

This occurs within the first 10 days after transplantation and is attributable to the reactivation of a memory response in recipients who have been previously sensitised to the donor HLA mismatches. It is a common complication of HLA incompatible transplantation [56]. Accelerated antibody mediated rejection (ABMR) is characterised by rapidly deteriorating graft function, a significant increase in DSA and the characteristic histological findings of antibody interaction with the endothelium and tissue injury (microvascular inflammation, arteritis, thrombotic microangiopathy or allograft injury without an alternative explanation) [39]. Accelerated ABMR is usually detected early in the absence of any chronicity to the allograft injury and can often be reversed with rapid intervention [39,57].

Active ABMR

This can present at any stage after transplantation and may be characterised by a detectable deterioration in allograft function or identified incidentally on protocol biopsy. Three criteria must be met for a diagnosis of active ABMR: firstly, histological evidence of tissue injury; secondly, evidence of current or recent antibody interaction with the endothelium and thirdly, evidence of DSA or C4d positivity or increased expression of validated transcripts which suggest antibody mediated injury [39]. There is no chronic allograft injury in active ABMR.

Chronic active ABMR

This is characterised by evidence of remodelling of the allograft in the context of antibody-endothelium interaction and DSA. In kidney transplantation, the histological features of chronic ABMR are transplant glomerulopathy, capillary basement membrane multilayering and arterial intimal fibrosis. Chronic active ABMR

Transplant International 2018; 31: 1059–1070

often coexists with chronic active T cell mediated rejection so features such as interstitial inflammation, interstitial fibrosis and tubular atrophy are also commonly observed (Fig. 3c,d) [39] and chronic ABMR commonly manifests clinically as a slowly rising creatinine and increasing proteinuria. There is no consistently effective intervention for chronic ABMR [58,59].

Management of pre-existing DSA

Potential transplant recipients with pre-existing DSA are at higher risk of antibody mediated rejection and premature allograft loss because of antibody mediated injury [3,26,33,60,61]. There are two options for managing pre-existing DSA: avoidance of the DSA or acceptance of the DSA and adaptation of therapy.

Avoidance of pre-existing DSA

Avoidance of pre-existing DSA has been the mainstay of managing the risk associated with pre-existing DSA for five decades. Donated organs are allocated after testing for blood group and HLA compatibility to mitigate the risk of hyperacute and accelerated ABMR. Potential transplant recipients now have regular testing for HLA antibodies during their transplant waiting time. HLA antigens to which antibodies are detected in the recipient serum at a level deemed unacceptable by the local centre or national transplant programme are listed as "unacceptable mismatches" with the relevant transplant allocation body [27]. When an organ becomes available, a virtual crossmatch is performed using this information to ensure that a donor organ is not allocated to a recipient who has pre-existing HLA DSA [27,62]. The development of kidney sharing schemes where kidney transplant recipients with incompatible donors exchange their donors so that both recipients receive compatible kidney transplants has provided another option which allows avoidance of pre-existing DSA. EuroTransplant have developed a unique and successful allocation scheme for the most highly sensitised renal patients where the acceptable HLA mismatches for a potential recipient are identified and these patients receive priority for donor organs with an acceptable HLA type [63].

Acceptance of pre-existing DSA and adaptation of therapy

Complete avoidance of DSA is undoubtedly associated with the lowest immunological risk in solid organ transplantation. However, transplantation is a process in which multiple competing risks must be considered for each individual patient. Any recipient considering transplantation faces anaesthetic risk, surgical risk, immunological risk, medical risk (with the potential for immunosuppression to exacerbate pre-existing conditions or contribute to the development of new comorbidity) and economic risk (with the cost of potential lost earnings or relocation for transplant). All of these facets must be considered. The greater consideration, however, is that the overall risk of transplanting must be balanced against the risk of *not* transplanting for each patient, with the prognosis from their end-stage organ failure being the main driver of the latter. This may vary according to the organ involved, the patient's clinical status or the quality of care available [64,65]. In this context, it may be appropriate to accept increased immunological risk in transplantation because of the magnitude of the risk of harm faced by an individual if they are not transplanted [27,66]. The aim in this personalised decision making should be to identify the "sweet spot" where there is the lowest global risk of harm for the patient (Fig. 4).

To achieve optimal outcomes, it is essential that the risk associated with crossing pre-existing DSA is stratified so that those DSA that are crossed in transplantation are associated with the lowest risk of rejection but the greatest likelihood of receiving an offer. The risk associated with crossing HLA DSA can be stratified according to the method of detection of DSA in the HLA laboratory (with HLA DSA associated with a positive CDC crossmatch being the highest risk and those identified by Luminex SAB alone being the lowest) [64,67], the timeframe of the HLA DSA detection (greater risk with those that are currently detectable versus those that have been detected historically) [68] and the mechanism of sensitisation (with greater risk likely to be attributable to sensitisation via pregnancy) [56] (Fig. 5). Further information may be provided by eplet analysis and IgG subclass [29,69]. Employment of these steps allows the lowest risk HLA DSA to be identified. The biggest difference in access to transplantation is achieved when it is deemed acceptable to cross HLA DSA where the associated HLA antigen has a high population frequency in the donor pool. This strategy facilitates the identification of a group of HLA antigens to which DSA have been detected above the usual threshold of acceptability but which a transplant programme is *willing to cross* for a particular recipient; excellent outcomes have been reported [70,71].

Adaptation of therapy may be necessary when a willing to cross strategy is implemented. There are three

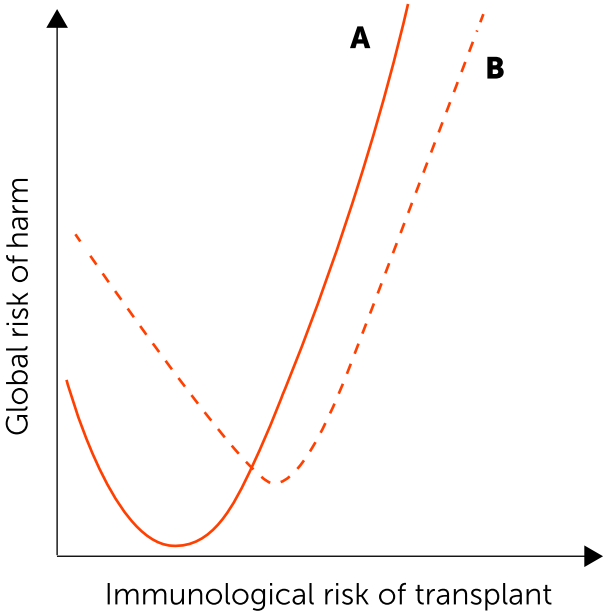

Figure 4 The "sweet spot" for transplantation. (A) The lowest immunological risk in transplantation occurs when recipients receive an organ which is perfectly matched. The likelihood of such an organ being available, however, is very small and to wait for such an offer would increase a potential recipient's time on the waiting list with the associated risk of accruing additional comorbidity or succumbing to end stage organ failure. For this reason, most centres will accept organs to which a recipient has no HLA antibodies without waiting for a perfectly matched organ. (B) For some recipients, the global risk of harm without transplantation is higher due to the severity of their end-stage organ failure so it is appropriate to consider higher immunological risk transplantation because of the magnitude of the overall benefit conferred by transplant. This may involve crossing low risk donor specific antibodies.

potential targets: the circulating DSA, the cells implicated in antibody production and the mediators involved in ABMR. In HLA incompatible transplantation where there is a current positive crossmatch, antibody removal is performed with the aim of rendering the crossmatch negative at the time of transplant [57]. This minimises the risk of hyperacute rejection. If, however, the DSA which is being crossed was only present historically, or is not at an adequate titre to cause a positive cross match, this may not be necessary [70]. In targeting the cells implicated in DSA production, many centres employ lymphocyte depleting induction therapy using polyclonal and monoclonal antibodies such as anti-thymocyte globulin, alemtuzumab and rituximab. The aim of this is to reduce the likelihood of reactivating a memory response which could cause accelerated ABMR. The evidence for the effectiveness of these agents in this context, however, is inconclusive [72]. The final possibility is to target the mediators which are responsible for at least part of the pathophysiology of ABMR. Eculizumab is a monoclonal antibody which targets the final step of complement activation to prevent the formation of the MAC. While it can be effective in preventing the acute injury associated with complement activation by high titre DSA, active ABMR has been reported in the context of complete suppression of complement activation by eculizumab and it has no effect on the longer term injury in which other pathophysiological processes are implicated [73,74]. This adaptation of immunosuppression has allowed successful transplantation to occur despite the presence of HLA DSA, with outcomes that are superior to potential recipients who continued to wait for a compatible transplant in some programmes [64,75]. However, in others, the benefit seems less clear [65]. This further highlights the importance of balancing the risk of transplanting against the risk of not transplanting when making personalised decisions for each recipient.

Management of *de novo* DSA

The development of HLA DSA for the first time after solid organ transplantation occurs in 10–40% of solid

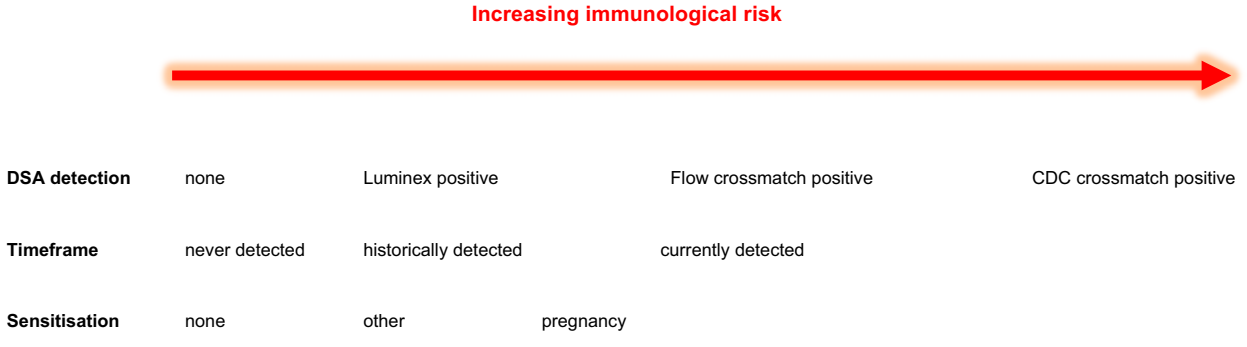

Figure 5 Stratifying the risk associated with HLA donor specific antibodies.

Transplant International 2018; 31: 1059–1070

organ transplant recipients [76–78]. The variability in the reported frequency of *de novo* HLA DSA (dnDSA) may be explained by differences in the frequency of HLA antibody monitoring after transplantation, laboratory methods of antibody detection, immunosuppression protocols or organ immunogenicity. The development of dnDSA has consistently been associated with allograft injury and reduced graft survival [76,78–82].

It has recently been reported that there may be a greater risk of allograft loss associated with dnDSA than with pre-existing DSA [83]. In a large study of renal transplant recipients, the development of dnDSA was associated with more chronic allograft injury on biopsy than pre-existing DSA. When analysis of gene expression was performed, there was also greater expression of IFNγ, natural killer cell and T cell transcripts [83]. This provides an important insight into the differences in the processes which drive the alloimmune injury associated with pre-existing and dnDSA. Pre-existing DSA instigate endothelial injury early after implantation due to the introduction of their complementary target eplet. Initially, this injury is driven solely by the effect of the bound antibody at the endothelial surface and is mainly comprised of complement activation and ADCC. This results in an early deterioration in graft function at a time when the recipient is being closely monitored which facilitates early recognition [67,83]. Rapid and aggressive intervention at this early stage can prevent chronic injury and the involvement of other components of the immune system in the alloimmune response [57,84]. The development of dnDSA, however, involves the presence of an inflammatory stimulus in the allograft which upregulates the expression of HLA and recruits macrophages and natural killer cells, requires the activation of CD4 T helper cells which are required for the *de novo* activation of B cells and formation of plasma cells and involves the refined maturation of the antibody response which is driven directly by the donor antigen [25,85,86]. The diversity of potentially injurious immune cells which are activated by this process combined with the timeframe for development, which is often much later after transplantation at a time when clinical monitoring is less frequent, means that there is more established injury by the time of biopsy and that treatments which target the antibody component of the process are much less effective in preventing immunological injury and allograft loss [58].

The principles of managing dnDSA are identical to those for managing pre-existing DSA: targeting the circulating DSA, the cells implicated in antibody production and the mediators involved in ABMR. Many excellent reviews of the treatments available have been written [87–89] but there is a huge diversity in practice across centres managing ABMR due to dnDSA. This reflects the lack of high quality evidence for effectiveness of any specific intervention [89,90].

Management of the potential for *de novo* DSA

Given the ineffectiveness of therapy for dnDSA and the associated ABMR, it may be appropriate to consider managing the risk of dnDSA development at the time of organ allocation. This involves managing the degree of the non-self stimulus provided by a donor organ and may be of particular relevance when there are a number of potential living donors for a recipient. There are a number of ways to approach this. Traditionally, the number of HLA antigen mismatches has been considered in some allocation algorithms, but this fails to address the fact that HLA antigen mismatches vary in their immunogenicity and the likelihood that they will induce dnDSA development [14]. Alternatively, it may be worth considering the number of HLA eplet mismatches which has been associated with the risk of dnDSA in lung and kidney transplantation [15,16]. However, eplets also differ in their immunogenicity according to their position *in vivo* and their electrophysicochemical properties [20] and it may be that specific eplets are particularly immunogenic [21,91]. It is likely that these are the eplets which defined the cross-reactive groups (CREGs) of HLA antigens which were identifiable serologically [14]. The most pragmatic approach may be to identify these highly immunogenic eplets and to aim to avoid these in allocation.

The potential impact of considering this in organ allocation must be placed in the context of the other competing risks for each patient as avoiding even a single immunogenic eplet will reduce an individual's access to potential donors. The impact of the risk associated with a longer period prior to transplantation which is a consequence of restricting the donor pool must be balanced against the potential for avoiding dnDSA after transplantation with the associated improvement in graft survival.

Recent years have seen progress in the understanding of mechanisms of DSA development, methods of DSA detection, appreciation of the variety of histological and clinical manifestations of DSA and the options for managing the risk associated with DSA. Challenges remain. Some of these centre on the complexity of understanding immunological risk in the context of the patient as a whole, others are focussed on assigning the correct immunological risk to an individual transplant while still others relate to the most appropriate way to manage that risk. It is likely that a combination of

personalised preventative strategies along with individualised interventions for those with DSA will be required to optimally manage the risk associated with DSA in solid organ transplantation.

Funding

JAM receives support from the Northern Ireland Kidney Research Fund.

Conflict of interest

The authors have no conflicts of interest to declare.

Acknowledgements

We wish to acknowledge the contribution of Dr Richard Battle and Dr Rohan John in the provision of images for this article.

REFERENCES

1. Kuss R, Teinturier J, Milliez P. Some attempts at kidney transplantation in man. *Mem Acad Chir (Paris)* 1951; **77**: 755.
2. Murray JE, Merrill JP, Dammin GJ, *et al.* Study on transplantation immunity after total body irradiation: clinical and experimental investigation. *Surgery* 1960; **48**: 272.
3. Patel R, Terasaki PI. Significance of the positive crossmatch test in kidney transplantation. *N Engl J Med* 1969; **280**: 735.
4. Ali JM, Bolton EM, Bradley JA, Pettigrew GJ. Allorecognition pathways in transplant rejection and tolerance. *Transplantation* 2013; **96**: 681.
5. Noelle RJ, Snow EC. Cognate interactions between helper T cells and B cells. *Immunol Today* 1990; **11**: 361.
6. Bharat A, Mohanakumar T. Allopeptides and the alloimmune response. *Cell Immunol* 2007; **248**: 31.
7. Alberu J, Morales-Buenrostro LE, de Leo C, Vargas-Rojas MI, Marino-Vazquez LA, Crispin JC. A non-allogeneic stimulus triggers the production of de novo HLA antibodies in healthy adults. *Transpl Immunol* 2007; **18**: 166.
8. Gombos P, Opelz G, Scherer S, *et al.* Influence of test technique on sensitization status of patients on the kidney transplant waiting list. *Am J Transplant* 2013; **13**: 2075.
9. Magee BA, Martin J, Cole MP, Morris KG, Courtney AE. Effects of HLA-matched blood transfusion for patients awaiting renal transplantation. *Transplantation* 2012; **94**: 1111.
10. Picascia A, Grimaldi V, Sabia C, Napoli C. Comprehensive assessment of sensitizing events and anti-HLA antibody development in women awaiting kidney transplantation. *Transpl Immunol* 2016; **36**: 14.
11. Kohl J. Self, non-self, and danger: a complementary view. *Adv Exp Med Biol* 2006; **586**: 71.
12. Safinia N, Afzali B, Atalar K, Lombardi G, Lechler RI. T-cell alloimmunity and chronic allograft dysfunction. *Kidney Int Suppl* 2010; **78**: S2.
13. Duquesnoy RJ. A structurally based approach to determine HLA compatibility at the humoral immune level. *Hum Immunol* 2006; **67**: 847.
14. Lucas DP, Leffell MS, Zachary AA. Differences in immunogenicity of HLA antigens and the impact of cross-reactivity on the humoral response. *Transplantation* 2015; **99**: 77.
15. Wiebe C, Pochinco D, Blydt-Hansen TD, *et al.* Class II HLA epitope matching-A strategy to minimize de novo donor-specific antibody development and improve outcomes. *Am J Transplant* 2013; **13**: 3114.
16. Walton DC, Hiho SJ, Cantwell LS, *et al.* HLA matching at the eplet level protects against chronic lung allograft dysfunction. *Am J Transplant* 2016; **16**: 2695.
17. Sinha N, Mohan S, Lipschultz CA, Smith-Gill SJ. Differences in electrostatic properties at antibody-antigen binding sites: implications for specificity and cross-reactivity. *Biophys J* 2002; **83**: 2946.
18. Chong LT, Duan Y, Wang L, Massova I, Kollman PA. Molecular dynamics and free-energy calculations applied to affinity maturation in antibody 48G7. *Proc Natl Acad Sci USA* 1999; **96**: 14330.
19. Fiorucci S, Zacharias M. Prediction of protein-protein interaction sites using electrostatic desolvation profiles. *Biophys J* 2010; **98**: 1921.
20. Kosmoliaptsis V, Chaudhry AN, Sharples LD, *et al.* Predicting HLA class I alloantigen immunogenicity from the number and physiochemical properties of amino acid polymorphisms. *Transplantation* 2009; **88**: 791.
21. McCaughan JA, Battle RK, Singh SKS, *et al.* Identification of risk epitope mismatches associated with de novo donor-specific HLA antibody development in cardiothoracic transplantation. *Am J Transplant* 2018; https://doi.org/10.1111/ajt.14951. [Epub ahead of print]
22. Chothia C, Lesk AM. Canonical structures for the hypervariable regions of immunoglobulins. *J Mol Biol* 1987; **196**: 901.
23. Duquesnoy RJ. The antibody response to an HLA mismatch: a model for nonself-self discrimination in relation to HLA epitope immunogenicity. *Int J Immunogenet* 2012; **39**: 1.
24. Marrari M, Mostecki J, Mulder A, Claas F, Balazs I, Duquesnoy RJ. Human monoclonal antibody reactivity with human leukocyte antigen class I epitopes defined by pairs of mismatched eplets and self-eplets. *Transplantation* 2010; **90**: 1468.
25. Clatworthy MR, Espeli M, Torpey N, Smith KG. The generation and maintenance of serum alloantibody. *Curr Opin Immunol* 2010; **22**: 669.
26. Mohan S, Palanisamy A, Tsapepas D, *et al.* Donor-specific antibodies adversely affect kidney allograft outcomes. *J Am Soc Nephrol* 2012; **23**: 2061.
27. Tait BD, Susal C, Gebel HM, *et al.* Consensus guidelines on the testing and clinical management issues associated with HLA and non-HLA antibodies in transplantation. *Transplantation* 2013; **95**: 19.
28. Lowe D, Higgins R, Zehnder D, Briggs DC. Significant IgG subclass heterogeneity in HLA-specific antibodies: implications for pathogenicity, prognosis, and the rejection response. *Hum Immunol* 2013; **74**: 666.
29. Lefaucheur C, Viglietti D, Bentlejewski C, *et al.* IgG donor-specific anti-human HLA antibody subclasses and kidney allograft antibody-mediated injury. *J Am Soc Nephrol* 2016; **27**: 293.
30. Kaneku H, O'Leary JG, Taniguchi M, Susskind BM, Terasaki PI, Klintmalm GB. Donor-specific human leukocyte antigen antibodies of the immunoglobulin G3 subclass are associated with chronic rejection and

graft loss after liver transplantation. *Liver Transpl* 2012; **18**: 984.
31. Khovanova N, Daga S, Shaikhina T, *et al.* Subclass analysis of donor HLA-specific IgG in antibody-incompatible renal transplantation reveals a significant association of IgG4 with rejection and graft failure. *Transpl Int* 2015; **28**: 1405.
32. Chin C, Chen G, Sequeria F, *et al.* Clinical usefulness of a novel C1q assay to detect immunoglobulin G antibodies capable of fixing complement in sensitized pediatric heart transplant patients. *J Heart Lung Transplant* 2011; **30**: 158.
33. Smith JD, Ibrahim MW, Newell H, *et al.* Pre-transplant donor HLA-specific antibodies: characteristics causing detrimental effects on survival after lung transplantation. *J Heart Lung Transplant* 2014; **33**: 1074.
34. Loupy A, Lefaucheur C, Vernerey D, *et al.* Complement-binding anti-HLA antibodies and kidney-allograft survival. *N Engl J Med* 2013; **369**: 1215.
35. Taylor CJ, Kosmoliaptsis V, Martin J, *et al.* Technical limitations of the C1q single-antigen bead assay to detect complement binding HLA-specific antibodies. *Transplantation* 2017; **101**: 1206.
36. Bachelet T, Couzi L, Lepreux S, *et al.* Kidney intragraft donor-specific antibodies as determinant of antibody-mediated lesions and poor graft outcome. *Am J Transplant* 2013; **13**: 2855.
37. Visentin J, Chartier A, Massara L, *et al.* Lung intragraft donor-specific antibodies as a risk factor for graft loss. *J Heart Lung Transplant* 2016; **35**: 1418.
38. Ehrnthaller C, Ignatius A, Gebhard F, Huber-Lang M. New insights of an old defense system: structure, function, and clinical relevance of the complement system. *Mol Med* 2011; **17**: 317.
39. Haas M, Loupy A, Lefaucheur C, *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* 2018; **18**: 293.
40. Levine DJ, Glanville AR, Aboyoun C, *et al.* Antibody-mediated rejection of the lung: a consensus report of the International Society for Heart and Lung Transplantation. *J Heart Lung Transplant* 2016; **35**: 397.
41. Kobashigawa J, Colvin M, Potena L, *et al.* The management of antibodies in heart transplantation: an ISHLT consensus document. *J Heart Lung Transplant* 2018; **37**: 537.
42. Collins AB, Schneeberger EE, Pascual MA, *et al.* Complement activation in acute humoral renal allograft rejection: diagnostic significance of C4d deposits in peritubular capillaries. *J Am Soc Nephrol* 1999; **10**: 2208.
43. Schaub S, Honger G, Koller MT, Liwski R, Amico P. Determinants of C1q binding in the single antigen bead assay. *Transplantation* 2014; **98**: 387.
44. Haas M, Sis B, Racusen LC, *et al.* Banff 2013 meeting report: inclusion of c4d-negative antibody-mediated rejection and antibody-associated arterial lesions. *Am J Transplant* 2014; **14**: 272.
45. Tible M, Loupy A, Vernerey D, *et al.* Pathologic classification of antibody-mediated rejection correlates with donor-specific antibodies and endothelial cell activation. *J Heart Lung Transplant* 2013; **32**: 769.
46. Roux A, Bendib Le Lan I, Holifanjaniaina S, *et al.* Antibody-mediated rejection in lung transplantation: clinical outcomes and donor-specific antibody characteristics. *Am J Transplant* 2016; **16**: 1216.
47. Resch T, Fabritius C, Ebner S, Ritschl P, Kotsch K. The role of natural killer cells in humoral rejection. *Transplantation* 2015; **99**: 1335.
48. Lee CY, Lotfi-Emran S, Erdinc M, *et al.* The involvement of FcR mechanisms in antibody-mediated rejection. *Transplantation* 2007; **84**: 1324.
49. Valenzuela NM, Reed EF. Antibodies to HLA molecules mimic agonistic stimulation to trigger vascular cell changes and induce allograft injury. *Curr Transplant Rep* 2015; **2**: 222.
50. Jin YP, Valenzuela NM, Zhang X, Rozengurt E, Reed EF. HLA class II-triggered signaling cascades cause endothelial cell proliferation and migration: relevance to antibody-mediated transplant rejection. *J Immunol* 2018; **200**: 2372.
51. Mehra MR, Crespo-Leiro MG, Dipchand A, *et al.* International Society for Heart and Lung Transplantation working formulation of a standardized nomenclature for cardiac allograft vasculopathy-2010. *J Heart Lung Transplant* 2010; **29**: 717.
52. Tang AH, Platt JL. Accommodation of grafts: implications for health and disease. *Hum Immunol* 2007; **68**: 645.
53. Brynger H, Rydberg L, Samuelsson B, Sandberg L. Experience with 14 renal transplants with kidneys from blood group A (subgroup A2) to O recipients. *Transplant Proc* 1984; **16**: 1175.
54. Kissmeyer-Nielsen F, Olsen S, Petersen VP, Fjeldborg O. Hyperacute rejection of kidney allografts, associated with pre-existing humoral antibodies against donor cells. *Lancet* 1966; **2**: 662.
55. Sumitran-Karuppan S, Tyden G, Reinholt F, Berg U, Moller E. Hyperacute rejections of two consecutive renal allografts and early loss of the third transplant caused by non-HLA antibodies specific for endothelial cells. *Transpl Immunol* 1997; **5**: 321.
56. Higgins R, Lowe D, Daga S, *et al.* Pregnancy-induced HLA antibodies respond more vigorously after renal transplantation than antibodies induced by prior transplantation. *Hum Immunol* 2015; **76**: 546.
57. Montgomery RA, Zachary AA, Racusen LC, *et al.* Plasmapheresis and intravenous immune globulin provides effective rescue therapy for refractory humoral rejection and allows kidneys to be successfully transplanted into cross-match-positive recipients. *Transplantation* 2000; **70**: 887.
58. Roberts DM, Jiang SH, Chadban SJ. The treatment of acute antibody-mediated rejection in kidney transplant recipients-a systematic review. *Transplantation* 2012; **94**: 775.
59. Moreso F, Crespo M, Ruiz JC, *et al.* Treatment of chronic antibody mediated rejection with intravenous immunoglobulins and rituximab: a multicenter, prospective, randomized, double-blind clinical trial. *Am J Transplant* 2018; **18**: 927.
60. Kim M, Townsend KR, Wood IG, *et al.* Impact of pretransplant anti-HLA antibodies on outcomes in lung transplant candidates. *Am J Respir Crit Care Med* 2014; **189**: 1234.
61. Opelz G, Dohler B, Susal C. Analysis of positive kidney, heart, and liver transplant crossmatches reported to the Collaborative Transplant Study. *Hum Immunol* 2009; **70**: 627.
62. Claas FH, Doxiadis II. Human leukocyte antigen antibody detection and kidney allocation within Eurotransplant. *Hum Immunol* 2009; **70**: 636.
63. Heidt S, Witvliet MD, Haasnoot GW, Claas FH. The 25th anniversary of the Eurotransplant Acceptable Mismatch program for highly sensitized patients. *Transpl Immunol* 2015; **33**: 51.
64. Orandi BJ, Garonzik-Wang JM, Massie AB, *et al.* Quantifying the risk of incompatible kidney transplantation: a multicenter study. *Am J Transplant* 2014; **14**: 1573.
65. Manook M, Koeser L, Ahmed Z, *et al.* Post-listing survival for highly sensitised patients on the UK kidney transplant waiting list: a matched cohort analysis. *Lancet* 2017; **389**: 727.
66. Tambur AR, Campbell P, Claas FH, *et al.* Sensitization in transplantation:

assessment of risk (STAR) 2017 working group meeting report. *Am J Transplant* 2018; **18**: 1604.
67. Higgins R, Lowe D, Hathaway M, *et al.* Human leukocyte antigen antibody-incompatible renal transplantation: excellent medium-term outcomes with negative cytotoxic crossmatch. *Transplantation* 2011; **92**: 900.
68. Couzi L, Araujo C, Guidicelli G, *et al.* Interpretation of positive flow cytometric crossmatch in the era of the single-antigen bead assay. *Transplantation* 2011; **91**: 527.
69. Duquesnoy RJ, Awadalla Y, Lomago J, *et al.* Retransplant candidates have donor-specific antibodies that react with structurally defined HLA-DR, DQ, DP epitopes. *Transpl Immunol* 2008; **18**: 352.
70. McCaughan JA, Courtney AE. Successful kidney transplantation in highly sensitized, ultra-long-term dialysis patients. *Transpl Int* 2017; **30**: 844.
71. Schinstock CA, Gandhi M, Cheungpasitporn W, *et al.* Kidney transplant with low levels of DSA or low positive B-flow crossmatch: an underappreciated option for highly sensitized transplant candidates. *Transplantation* 2017; **101**: 2429.
72. Higgins RM, Daga S, Mitchell DA. Antibody-incompatible kidney transplantation in 2015 and beyond. *Nephrol Dial Transplant* 2015; **30**: 1972.
73. Stegall MD, Diwan T, Raghavaiah S, *et al.* Terminal complement inhibition decreases antibody-mediated rejection in sensitized renal transplant recipients. *Am J Transplant* 2011; **11**: 2405.
74. Burbach M, Suberbielle C, Brocheriou I, *et al.* Report of the inefficacy of eculizumab in two cases of severe antibody-mediated rejection of renal grafts. *Transplantation* 2014; **98**: 1056.
75. Tinckam KJ, Keshavjee S, Chaparro C, *et al.* Survival in sensitized lung transplant recipients with perioperative desensitization. *Am J Transplant* 2015; **15**: 417.
76. Lachmann N, Terasaki PI, Budde K, *et al.* Anti-human leukocyte antigen and donor-specific antibodies detected by luminex posttransplant serve as biomarkers for chronic rejection of renal allografts. *Transplantation* 2009; **87**: 1505.
77. Tikkanen JM, Singer LG, Kim SJ, *et al.* De novo DQ donor-specific antibodies are associated with chronic lung allograft dysfunction after lung transplantation. *Am J Respir Crit Care Med* 2016; **194**: 596.
78. Smith JD, Banner NR, Hamour IM, *et al.* De novo donor HLA-specific antibodies after heart transplantation are an independent predictor of poor patient survival. *Am J Transplant* 2011; **11**: 312.
79. O'Leary JG, Kaneku H, Jennings L, Susskind BM, Terasaki PI, Klintmalm GB. Donor-specific alloantibodies are associated with fibrosis progression after liver transplantation in hepatitis C virus-infected patients. *Liver Transpl* 2014; **20**: 655.
80. Morrell MR, Pilewski JM, Gries CJ, *et al.* De novo donor-specific HLA antibodies are associated with early and high-grade bronchiolitis obliterans syndrome and death after lung transplantation. *J Heart Lung Transplant* 2014; **33**: 1288.
81. Hodges AM, Lyster H, McDermott A, *et al.* Late antibody-mediated rejection after heart transplantation following the development of de novo donor-specific human leukocyte antigen antibody. *Transplantation* 2012; **93**: 650.
82. Le Pavec J, Suberbielle C, Lamrani L, *et al.* De-novo donor-specific anti-HLA antibodies 30 days after lung transplantation are associated with a worse outcome. *J Heart Lung Transplant* 2016; **35**: 1067.
83. Aubert O, Loupy A, Hidalgo L, *et al.* Antibody-mediated rejection due to preexisting versus de novo donor-specific antibodies in kidney allograft recipients. *J Am Soc Nephrol* 2017; **28**: 1912.
84. Orandi BJ, Zachary AA, Dagher NN, *et al.* Eculizumab and splenectomy as salvage therapy for severe antibody-mediated rejection after HLA-incompatible kidney transplantation. *Transplantation* 2014; **98**: 857.
85. Taylor AL, Negus SL, Negus M, Bolton EM, Bradley JA, Pettigrew GJ. Pathways of helper CD4 T cell allorecognition in generating alloantibody and CD8 T cell alloimmunity. *Transplantation* 2007; **83**: 931.
86. Wedgwood JF, Hatam L, Bonagura VR. Effect of interferon-gamma and tumor necrosis factor on the expression of class I and class II major histocompatibility molecules by cultured human umbilical vein endothelial cells. *Cell Immunol* 1988; **111**: 1.
87. Valenzuela NM, Reed EF. Antibody-mediated rejection across solid organ transplants: manifestations, mechanisms, and therapies. *J Clin Invest* 2017; **127**: 2492.
88. Lee M. Antibody-mediated rejection after liver transplant. *Gastroenterol Clin North Am* 2017; **46**: 297.
89. Kim M, Martin ST, Townsend KR, Gabardi S. Antibody-mediated rejection in kidney transplantation: a review of pathophysiology, diagnosis, and treatment options. *Pharmacotherapy* 2014; **34**: 733.
90. Burton SA, Amir N, Asbury A, Lange A, Hardinger KL. Treatment of antibody-mediated rejection in renal transplant patients: a clinical practice survey. *Clin Transplant* 2015; **29**: 118.
91. Duquesnoy RJ. Reflections on HLA epitope-based matching for transplantation. *Front Immunol* 2016; **7**: 469.