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Detection of HLA antibodies prior to renal transplantation: prospects and limitations of new assays

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Summary

Donor-specific HLA antibodies are responsible for most early renal allograft rejections and allograft losses. Therefore, detection of HLA antibodies prior to transplantation is an important step in the evaluation of renal allograft recipients. In this mini-review we will describe recent advances in the techniques used to detect donor-specific HLA antibodies with emphasis on their potential application for patient management and their current limitations.

Key words: HLA antibodies; transplantation; flow cytometric crossmatch; virtual crossmatch

Introduction

The Human Leukocyte Antigens (HLA) are highly polymorphic molecules that can present "self" and "non-self" peptides to the immune system and thus are critical for induction of an immune response. Class I HLA (i.e., HLA-A, HLA-B, HLA-C) are constitutively expressed on all nucleated cells, while class II HLA (i.e., HLA-DR, HLA-DQ, HLA-DP) are primarily expressed on antigen-presenting cells, but also on other cell types (e.g., vascular endothelial cells, tubular epithelial cells in the kidney) [1].

Due to their high polymorphism and high expression rate, "non-self" or mismatched HLA are major targets for the immune system. Indeed, after blood transfusions, pregnancies or transplants the immune system may produce antibodies against the mismatched HLA. If these circulating HLA antibodies are directed against HLA of a subsequently transplanted organ, they immediately bind to their targets on the vascular endothelial cells of the allograft. The bound HLA antibodies will then activate the complement system as well as macrophages and neutrophils leading to severe endothelial cell damage and allograft dysfunction. This clinico-pathological entity induced by preformed donor-specific HLA antibodies (HLA-DSA) is called antibody-mediated or humoral rejection and is responsible for most early allograft losses. Therefore, detection of HLA-DSA prior to transplantation is an important step in the assessment of the patient's immunological risk and for exclusion of incompatible donors (2). In this mini-review we will describe recent advances in the techniques used to detect HLA-DSA with emphasis on their prospects and limitations for patient management.

Cell-based assays to detect HLA-DSA

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In 1969 the clinical importance of preformed HLA-DSA was demonstrated by Patel and Terasaki [3]. They introduced the complement-dependent cytotoxicity crossmatch (CDC crossmatch) as a simple test to assess the presence of HLA-DSA (figure 1). In a retrospective study involving 225 renal allograft recipients they observed that 80% of the recipients with a positive CDC crossmatch lost their allograft presumably due to acute humoral rejection, whereas less than 5% of the patients with a negative CDC crossmatch experienced this bad outcome. Since this

Table 1

Antibodies detectable on T-/B-lymphocytes and their clinical relevance.

Antibody	Detectable on T-cells	Detectable on B-cells	Associated with rejection
Anti-HLA A, B, C	+++	++++	yes
Anti-HLA DR, DQ, DP	–	++++	yes
Anti-endothelial cell*	–	–	yes
Auto-antibodies	+	++	no
Immune complexes, FcR-binding	–	++	no

FcR = Fc-receptor

*) MHC class I related chain A (MICA) [18], AT1-receptor [19], and other – currently unknown – antigens

landmark study, the CDC crossmatch – with some modifications – became the standard assay for detection of HLA-DSA for many years. While a positive CDC crossmatch was considered an absolute contraindication for transplantation, a negative CDC crossmatch was regarded to indicate the absence of HLA-DSA. However, in Patel's study 20% of patients with a positive CDC crossmatch had an uneventful course and 5% of patients with a negative CDC crossmatch experienced early rejection/allograft loss, suggesting a limited sensitivity and specificity of the CDC crossmatch assay.

In the 1980s the flow cytometric crossmatch (flow crossmatch) was developed (figure 1). Due to its higher sensitivity to detect clinically relevant HLA-DSA, the flow crossmatch was introduced as the standard assay in many renal transplant centres [2]. Unfortunately, it became evident that the flow crossmatch did not resolve, but rather enhanced the specificity problem using cell-based assays for detection of HLA-DSA [2, 4, 5]. In fact, T- and B-lymphocytes carry many different molecules on their surface, which can bind clinically irrelevant antibodies leading to false positive results (table 1). Although much effort has been invested to optimise the specificity, the interpretation of a flow crossmatch remains challenging [5, 6]. Currently, it is widely accepted that antibodies against the HLA are most likely the only clinically relevant antibodies that are detectable on T- and B-lymphocytes [2]. Notably, the main advantage of both cell-based assays (CDC crossmatch and flow crossmatch) is the ability to detect antibodies against the "real" donor HLA, which is of major importance.

Solid-phase assays to detect HLA-DSA and the concept of the virtual crossmatch

Given the fact that preformed HLA-DSA are responsible for most acute humoral allograft rejections, assays that measure exclusively antibodies against HLA were developed. These assays consist of HLA that are coupled to a solid phase (e.g., plastic plate or latex beads). Anti-HLA antibodies that bind to the solid phase are then detected either by flow cytometry (i.e., flow beads) or spectrometry (i.e., ELISA). The flow beads are more sensitive than the ELISA and are currently the preferred solid phase assay in many transplant centres [7]. The most advanced flow beads carry

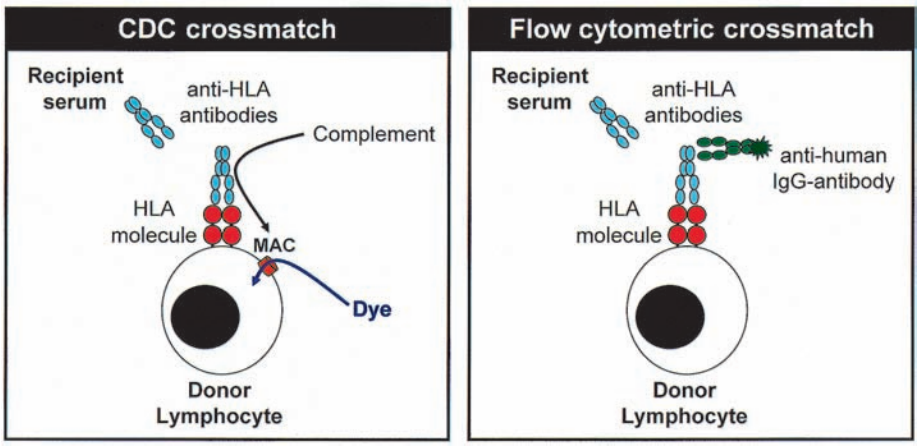


Figure 1

Cell-based methods for detecting HLA-DSA. The complement-dependent cytotoxicity crossmatch (CDC crossmatch) is performed by incubating T- and B-lymphocytes of the donor with serum from the recipient with subsequent addition of complement. If recipient antibodies bind to the donor cells, complement is activated, which creates "holes" in the cell membrane through the membrane-attack complex (MAC). A dye will then stain all cells with permeable membranes indicating antibody binding. The result (percentage of stained cells) is determined by eye using fluorescence microscopy. The flow cytometric crossmatch is performed by incubating T- and B-lymphocytes of the donor with serum from the recipient with subsequent addition of a fluorescent secondary antibody binding to human IgG-antibodies. The fluorescent intensity correlating with the amount of antibody binding to the cells is measured by a flow cytometer.

only one HLA (ie, single HLA flow beads) (figure 2). These beads allow assigning the specificity of the HLA antibodies [8]. Therefore, the anti-HLA antibody repertoire of an allograft recipient can be defined and it becomes possible to determine the presence of HLA-DSA without performing a cell-based crossmatch by comparing the HLA-typing of the donor with the HLA-antibody specificities of the recipient (i.e., virtual crossmatch) (figure 2). As the HLA-antibody specificities can be identified when a patient is listed for kidney transplantation, the result of the virtual crossmatch is immediately available once the HLA-typing of the donor has been performed. Obviously, the accuracy of virtual crossmatch depends on the precision of the HLA-typing of the donor and a detailed HLA-antibody analysis of the recipient.

Prospects of solid phase assays

There is currently only one small prospective single centre study involving 65 patients that evaluated the accuracy of virtual crossmatch for pre-transplant risk assessment in renal transplantation [4]. The study demonstrated that virtual crossmatch is more accurate than CDC crossmatch and flow crossmatch at predicting the presence or absence of clinically relevant HLA-DSA. Impor-

Figure 2

Single HLA flow bead analysis and the concept of virtual crossmatch. Single HLA flow beads consist of solid phase particles that carry only one specific HLA molecule on the surface. After incubation with the recipient's serum a fluorescent secondary antibody binding to human IgG antibodies is added. All individual beads (currently covering around 150 different HLA molecules) are then analysed by flow cytometry. This allows assigning the specificity of HLA antibodies. By comparison with the HLA-typing of the donor, the presence of HLA-DSA can be determined (i.e., virtual crossmatch).

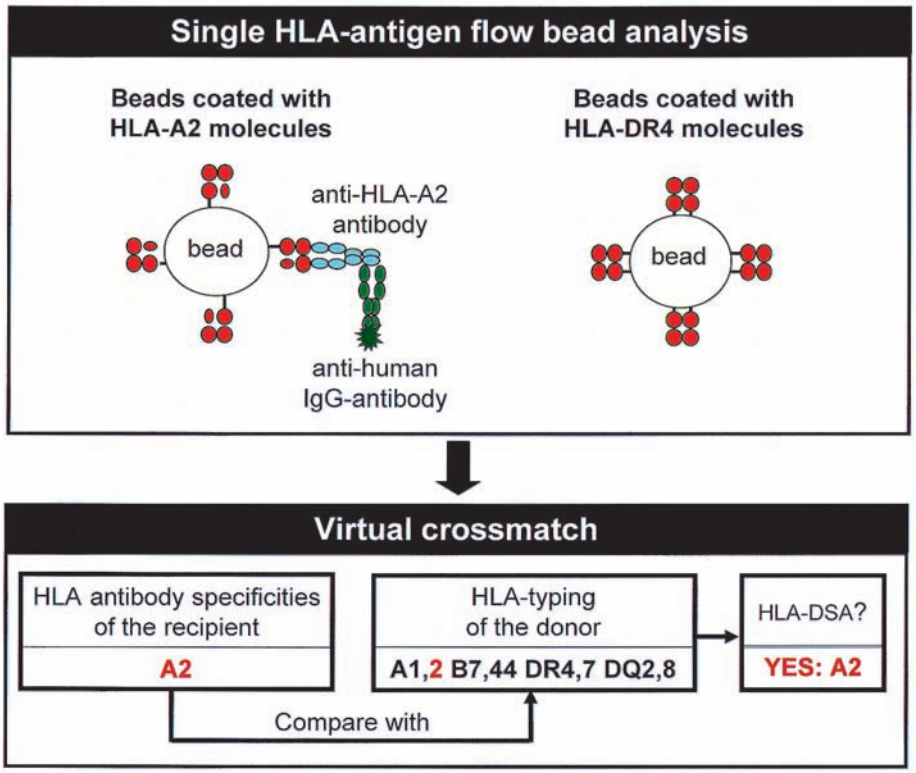
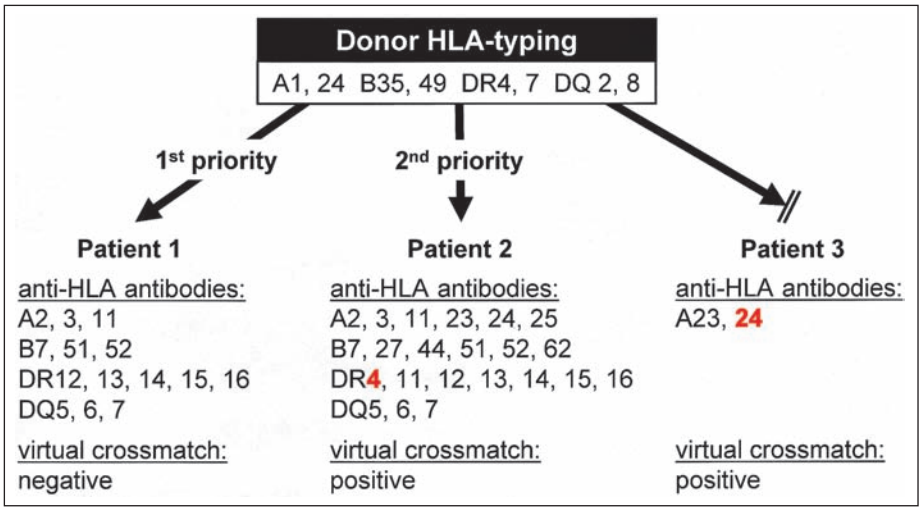


Figure 3

Potential impact of the knowledge of HLA antibody specificities for renal allograft allocation. By knowing the HLA antibody specificities of a recipient, it is possible to estimate the probability of receiving a renal allograft without HLA-DSA. First priority should be given to sensitised patients without a HLA-DSA (patient 1). Second priority might be given to highly sensitised patients with low-level HLA-DSA (i.e., positive virtual or flow crossmatch, but negative CDC crossmatch), but a low probability of receiving a transplant without HLA-DSA (patient 2). Transplantation across HLA-DSA should be avoided in patients with only few HLA antibodies, because there is a high probability of finding a compatible organ (patient 3). HLA-DSA are marked in red.



tantly, a negative virtual crossmatch was associated with a very low risk for early humoral allograft rejection. Taking into account some caveats discussed in the following chapter, patients with a negative virtual crossmatch can be safely transplanted while omitting a pre-transplant CDC or flow crossmatch, which will reduce cold ischaemia time by about 2–4 hours and might reduce the incidence of delayed graft function [9]. This will make virtual crossmatch of particular interest for heart and lung transplantation, where ischaemia time is even more important than in renal transplantation [10, 11].

Knowledge of the HLA antibodies specificities of renal allograft recipients on the waiting list allows allocating deceased donor organs to those patients without HLA-DSA. Bray et al investigated this approach in 492 renal transplants, and found that allograft survival was similar in patients without HLA antibodies and highly sensitised patients [12]. Furthermore, more kidneys could be allocated to highly sensitised patients promoting access of this disadvantaged patient group to suitable deceased donor kidneys. Clearly, the large donor pool in the United States (250 million persons) offers the potential for highly sensitised patients to receive a kidney without HLA-DSA, but this chance is significantly lower in Switzerland with a population of 7 million persons. Thus, for patients with a very low probability of receiving a kidney without HLA-DSA within a reasonable time frame, transplantation across low-level HLA-DSA (i.e., positive by virtual or flow-crossmatch, but negative by the less sensitive CDC-crossmatch) can be an acceptable solution adopting an enhanced immunosuppression [4, 13, 14] (figure 3). Although many centres have performed transplantations in the presence of such low-level HLA-DSA with good short-term outcomes, long-term allograft survival might be lower than in patients without HLA-DSA [15].

Limitations of solid phase assays

Although the current panel of single HLA flow beads can detect antibodies against the most prevalent 150 HLA, they do not cover the whole diversity of all HLA alleles (i.e., >1000). Therefore, antibodies against rare HLA alleles can be missed. However, since implementation of virtual crossmatch for pre-transplant risk assessment at our centre we have not observed an early humoral allograft rejection due to HLA-DSA in 154 patients [16]. The risk of missing clinically relevant HLA antibodies might be higher in populations with different ethnicities and consequentially a higher diversity of HLA than in our predominantly Caucasian population. Nevertheless, antibodies against all mismatched HLA of the donor have to be actively excluded before virtual crossmatch can be assigned negative. Only when this is assured, a negative virtual crossmatch will be associated with a very low risk for early humoral allograft rejection.

On the other hand, it is not clear yet whether all HLA antibodies detected by single HLA flow beads are in fact clinically relevant. First, the assay may give a technically-related false positive result. During coupling of the HLA molecules to the solid phase particles, they may be denatured and disclose a new epitope normally not existing on properly configured HLA molecules. In addition, the antibodies may bind to the peptide presented by the HLA and not to the HLA itself. Second, the assay may give a true positive result, but the detected HLA antibodies are only of limited clinical relevance. The incidence of clinically irrelevant HLA antibodies detected by single HLA flow beads is as yet unknown and should be further investigated. However, limited data from two studies suggest that most HLA-DSA detected by single HLA flow beads are clinically relevant, even if flow crossmatch is negative [4, 17]. This supports the concept that virtual crossmatch is more sensitive than flow crossmatch and may therefore represent the currently best pre-transplant risk assessment. The consequence of a false assignment of HLA-DSA by virtual crossmatch is that an allograft may be detained from a patient or that the patient receives an unnecessary high load of immunosuppression. This has to be balanced against the risk of early humoral allograft rejection by missing a clinically relevant HLA-DSA.

Another limitation of solid phase assays is their restriction to detect HLA antibodies, while all other potentially relevant antibodies will be missed by definition. These so called non-HLA antibodies are directed against molecules expressed only on endothelial cells in the transplanted kidney and can induce acute humoral allograft rejection indistinguishable from HLA-DSA. Some targets of non-HLA antibodies were recently identified (i.e., MHC class I related chain A (MICA) [18], AT1-receptor [19]). Indeed, both MICA-antibodies and AT1-receptor antibodies were reported as being associated with acute humoral allograft rejection and allograft loss [19, 20]. However, a recent study found that the incidence of early humoral allograft rejection due to non-HLA antibodies is low (10/433 patients; 2%) [16]. Furthermore, AT1-receptor antibodies were not predictive and donor-specific MICA antibodies were found in at most 3/10 patients, while the antibodies remained unexplained in 7/10 cases. This suggests that there remains a small gap in our ability to detect all clinically relevant donor-directed antibodies. Unfortunately, no assay is currently available that reliably helps to close this gap.

Conclusion

Solid phase assays have largely expanded the ability to detect HLA-DSA. Implementation of these assays for pre-transplant risk assessment and organ allocation will be expected to improve short and long-term renal allograft survival. However, knowledge of the current limitations of solid phase assays is important for accurate interpretation of the results and optimal patient management.

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