Role and Value of Luminex®-Detected HLA Antibodies before and after Kidney Transplantation

Caner Süsal^a Gerhard Opelz^a Christian Morath^b

^a Department of Transplant Immunology,

^b Department of Nephrology, University of Heidelberg, Heidelberg, Germany

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Summary

The complement-dependent lymphocytotoxicity (CDC) method has been the classical technique to detect human leukocyte antigen (HLA) antibodies in sera of patients who are listed for kidney transplantation. Because of the drawbacks of CDC, such as low sensitivity and low resolution in characterizing antibody specificities, the more specific ELISA technology was introduced in the 1990s which utilizes solubilized HLA molecules instead of lymphocytes. During the last 10 years, the introduction of the Luminex-based single antigen bead (L-SAB) technology, which uses recombinant single HLA molecules, allows detection and characterization of HLA antibodies at greater sensitivity than CDC and ELISA. A drawback associated with this technique is that the interpretation of results is demanding and requires comprehensive experience in HLA antibody diagnostics. Herein we discuss the current role and value of L-SAB technology in the clinical management of sensitized kidney transplant recipients.

pathological investigations indicate that more than 60% of late kidney graft losses nowadays are due to antibody-mediated humoral rejection [1, 2]. Because of increasing evidence that HLA antibodies are responsible for graft losses not only in kidney but also in other organ transplantation, HLA antibodies have become the main focus of research in organ transplantation.

Tissue Damage Caused by Donor-Specific HLA Antibodies

Unrecognized donor-specific HLA antibodies (DSA), if strongly reactive and complement-activating, can cause hyperacute or accelerated humoral rejections in the early phase after kidney transplantation. Weak DSA have been associated with rather subtle types of graft damage, often leading to delayed graft function [3]. It is well known that early damage can later on translate to chronic rejection, most probably because the structure of the endothelium is not anymore intact and new antigenic epitopes, including autoantigens, are expressed on the surface of transplanted tissue. During later phases after transplantation, non-sufficient immunosuppression can support the development of de novo DSA and autoantibodies against these antigenic structures and result in failure of the transplanted organ.

Introduction

In organ transplantation, the development of effective immunosuppressive agents in the 1980s and 1990s and their effective use to control T-cell alloimmunity led to a striking decrease in the occurrence of severe T-cell-mediated acute rejections. Simultaneously, our shortcomings in controlling antibody-mediated rejection processes were revealed. Recent

Lymphocyte Cross-Match, Antibody Screening and Determination of Unacceptable HLA Antigen Mismatches as Preventive Measures to Avoid Donor-Specific HLA Antibody-Mediated Damage

Since the early 1970s, prospective lymphocyte cross-matches are established as a routine procedure in kidney transplantation for the prevention of DSA-mediated damage

due to preformed HLA antibodies. Furthermore, while listed on the transplant waiting list, the patient's HLA antibodies are characterized in order to predict the result of a lymphocyte cross-match in advance. HLA specificities against which antibodies are detected are registered as unacceptable HLA antigen mismatches (UAMs), and potential kidney donors are excluded during the organ allocation process when they possess an HLA antigen mismatch against which the potential recipient is sensitized.

Since the 1960s, the complement-dependent lymphocytotoxicity (CDC) method has been the classical technique to detect HLA antibodies in sera of patients who are listed for organ transplantation. This technique has been used for antibody screening as well as cross-matching, and after its introduction hyperacute rejections have become a rare event. However, because accelerated forms of antibody-mediated acute rejections have still been occurring in sensitized recipients, CDC was criticized for not being able to detect all clinically relevant antibodies.

To overcome the sensitivity problems associated with the CDC methodology, solid-phase immunoassays, such as ELISA and Luminex, have been introduced which utilize solubilized or recombinant HLA antigens as targets instead of intact lymphocytes. Autoantibodies against non-HLA targets and immune complexes do not interfere in these test systems, and they have a higher sensitivity than CDC in detecting HLA antibodies.

Luminex-Supported Single Antigen Bead Methodology

In highly sensitized patients with antibodies against many different HLA alleles, the Luminex-supported single antigen bead (L-SAB) test, due to its high ability of resolution, is currently the only technique which allows the precise characterization of HLA antibody specificities. In this flow cytometric method, microbeads coated with recombinant single antigen HLA molecules are employed. The system is capable of differentiating antibody reactivity in two reaction tubes against approximately 100 different HLA class I and 100 different HLA class II alleles. A crude approximation of the strength of antibody reactivity is derived from the mean fluorescence intensity (MFI). L-SAB test kits are currently offered by two vendors. In addition to antibody reactivity against HLA-A, -B, -C, -DR and -DQB antigens, L-SAB is capable of detecting antibodies against HLA-DQA, -DPA, and -DPB antigens that are not detectable by the available ELISA assays.

There are additional Luminex kits for detection of non-HLA antibodies, such as major histocompatibility complex class I-related chain A (MICA) and human neutrophil antibodies, and kits that utilize, instead of recombinant HLA molecules, affinity purified pooled human HLA molecules obtained from multiple cell lines (screening test to detect the presence of HLA antibodies without further specification) or phenotype panels in which each bead population bears either the HLA class I or HLA class

II proteins of a cell line derived from a single individual (panel reactivity, PRA-test). The L-SAB technique, in which each bead population is coated with a molecule representing a single cloned allelic HLA antigen (class I) or heterodimeric antigen (class II), is the only test that enables precise antibody specificity analysis due to its high power of resolution.

A representative example of detection of HLA antibodies by three different techniques (CDC-PRA, ELISA-PRA and L-SAB) in the serum of a patient who developed DSA against the donor HLA mismatch antigen B27 on a previously failed kidney transplant is shown in figure 1.

Determination of Unacceptable HLA Antigen Mismatches

According to the recently published consensus guidelines on the testing of HLA antibodies in organ transplantation, determination of UAMs in the pretransplant period should be part of the organ allocation procedure [4]. Using this information, negative cross-match prediction or 'virtual cross-matching' is possible when a potential donor's complete HLA typing is available. Determination of UAMs is a critical decision step for the patient because with an increasing number of UAMs the patient's chance to receive a donor organ offer diminishes. Especially in highly sensitized patients, designation of many HLA antigens as UAM can result in extremely prolonged waiting times, and as a result patients often die on the waiting list before they can be transplanted. Conversely, unrecognized UAMs, due to insensitive or incorrect testing, result in futile organ shipments because the cross-match test in the recipient center is positive or, if transplantation is carried out, in inferior graft survival.

With the introduction of these more sensitive antibody assays, it remains presently unclear which antibody test at what sensitivity level should be used for the determination of UAMs. Donor-specific IgG HLA antibodies detected by CDC are considered a contraindication to transplantation, whereas DSA detected by other assays are thought to represent varying degrees of risk [5]. While there are good data that preexisting DSA as determined by L-SAB are associated with an increased risk of rejection [5–9], it is debatable whether antibodies that go undetected in CDC and ELISA and are detectable exclusively with the sensitive L-SAB technique have a deleterious effect on graft outcome [10, 11]. In a recent analysis of 118 patients with kidney graft loss within the first 3 years after transplantation and whose pretransplant sera were negative in CDC as well as ELISA testing, we did not observe a higher incidence of L-SAB-detected DSA as compared to matched control patients without graft loss [11].

Several studies indicate that SABs often carry denatured antigens on their surface, and these can lead to false-positive antibody test results [12–16]. As a result, HLA antibodies have been found in sera of healthy blood donors without a history of a sensitizing event. In the study of Morales-Buenrostro et al.

Recent Data on the Clinical Relevance of Luminex-Supported Single Antigen Bead-Detected Preformed Donor-Specific HLA Antibodies

Recently, Otten et al. [7] reported that the simultaneous presence of class-I and class-II IgG DSA as detected by L-SAB in pretransplant sera of cross-match-negative kidney recipients is indicative of an increased risk of graft failure. In contrast, graft loss in patients with only HLA class I or class II DSA was not significantly higher than in patients without HLA antibodies. These findings are in complete agreement with ELISA antibody findings published by us previously in 2002 [21] and 2009 [22], which indicated that presensitization of first kidney transplant recipients against either HLA class I or class II is of no clinical consequence, whereas sensitization against both HLA class I and class II resulted in increased rejection of kidney grafts. This 'double-positivity' effect was HLA mismatch-dependent, indicating that DSA directed against donor HLA antigens were responsible for this phenomenon. Because 'double-positive' patients benefit greatly from HLA well-matched grafts, we believe that this should be considered in donor kidney allocation systems. In our own center, we accept for 'double-positive' patients only relatively well-matched grafts with a maximum of 2 HLA-ABDR mismatches, and we follow special risk-reducing measures [20].

There is further debate concerning the clinical significance of L-SAB-detected C1q-fixing DSA, which are believed to be an indicator of complement-activating HLA antibodies [7, 23]. While Chin et al. [23] reported that a positive C1q assay predicts antibody-mediated rejection early after heart transplantation, no clinical significance of C1q-fixing IgG-DSA was found in the study of Otten et al. [7] in kidney recipients, possibly due to the low prevalence of such antibodies.

Utilization of Luminex-Supported Single Antigen Bead Technology for Transplantation of Highly Sensitized Patients and Surveillance of Desensitization Therapy

Highly sensitized patients, e.g. patients with a PRA $\geq 85\%$, often have positive pretransplant CDC cross-matches with their respective donors due to the high number of preexisting HLA alloantibodies. In general, positive CDC cross-match results are considered a contraindication to transplantation since patients with a positive CDC cross-match often reject their graft early after transplantation. With the introduction of more sensitive antibody detection techniques, such as the L-SAB, more patients are considered sensitized or even highly sensitized. However, due to the lower specificity of L-SAB-detected antibodies for allograft loss, these antibodies are not considered a contraindication but a risk factor for transplantation. Because the L-SAB method allows the precise characterization of HLA antibody specificities, it represents, in combination

with other antibody detection techniques and knowledge of the immunization history of the patient, an important tool in the working up and management of sensitized or highly sensitized patients before and after transplantation.

Highly sensitized patients with preformed L-SAB-detected DSA can be transplanted in two different ways: i) by the selection of a donor towards whom the recipient possesses no (and never possessed) HLA alloantibodies, or ii) after desensitization, e.g. after removal of alloantibodies before transplantation. A combination of both can be practiced to further facilitate successful transplantation of these otherwise difficult to transplant patients [20].

Special programs, such as the Eurotransplant Acceptable Mismatch Program, have been effective in allowing the successful and timely transplantation of patients with a (virtual) PRA of $\geq 85\%$. As mentioned above, patients transplanted in this way receive organs from donors who possess HLA antigens towards which the recipient never formed HLA alloantibodies [18, 19].

In the case of living-donor transplantation, paired donor exchange programs exist, i.e. in the Netherlands, the UK, the USA, and Australia, where blood group- and/or HLA-incompatible donor recipient couples are brought together to obtain a compatible constellation. However, experience has shown that in these programs highly sensitized patients with many different UAMs (as well as blood type O recipients) are disadvantaged and accumulate on the 'exchange list' [24].

As an alternative to the search for a compatible donor, especially in the case of living-donor kidney transplantation, patients with DSA against the donor can be desensitized by lowering DSA levels prior to transplantation and in the immediate postoperative period by means of plasmapheresis with or without low-dose intravenous immunoglobulins or immunoadsorption, together with potent immunosuppression to prevent an antibody rebound. The L-SAB method allows the precise monitoring of antibody levels; in general, if pretransplant DSA levels can be decreased below 1,000 MFI, the transplantation is performed [25].

Additional measures applied in these patients include the administration of an anti-CD20 antibody (rituximab) to deplete B cells and to prevent de novo antibody synthesis, the administration of the proteasome inhibitor bortezomib that has an effect on plasma cells, and more recently the administration of the anti-C5 inhibitor eculizumab which prevents the deleterious action of complement upon antibody binding to the endothelium. L-SAB appears to be an excellent method for monitoring de novo DSA development in its earliest phases.

It comes to the question whether living-donor transplantation following desensitization provides a benefit to the patient in comparison to waiting for an HLA-compatible kidney from a deceased donor. This question has been answered by a landmark study from Montgomery and coworkers in the year 2011 [26]. Although graft survival was not optimal in desensitized living-donor kidney recipients, they had a clear (patient) sur-

vival benefit as compared to patients waiting for a compatible deceased donor organ.

Our experience shows that careful selection of patients and examination of HLA alloantibodies before and after transplantation allows the successful desensitization and transplantation even of highly sensitized patients. In our 'Heidelberg Algorithm' for the transplantation of high-risk patients, a combination of measures is used, such as i) precise characterization of HLA antibodies by different techniques, e.g. CDC, ELISA and L-SAB, and risk categorization based especially on CDC and ELISA, ii) good HLA matching of donor and recipient, iii) inclusion in the acceptable mismatch program, iv) pre- and v) and postoperative desensitization, vi) postoperative HLA antibody monitoring, and vii) protocol biopsies [20].

L-SAB for Posttransplant Monitoring of Preexisting and de novo Donor-Specific HLA Antibodies

Many centers nowadays perform routine HLA alloantibody screening in stable graft recipients at different time points after transplantation in order to diagnose a humoral antibody-mediated rejection in its early stages.

While there is consensus that HLA alloantibodies are responsible for a significant proportion of late graft losses and that HLA antibodies in the context of deteriorating graft function are harmful, the significance of HLA alloantibodies that are detected solely during routine screening is discussed controversially.

For the diagnosis of antibody-mediated kidney graft rejection, histological features of antibody-mediated rejection in the biopsy, together with C4d positivity and/or the detection of a circulating DSA are required. Especially in chronic antibody-mediated rejection, C4d may often be negative (C4d-negative antibody-mediated rejection). Before the introduction of the L-SAB assay, especially in patients with chronic antibody-mediated rejection, there was often no DSA detectable due to the low levels of antibody and the low sensitivity of available HLA antibody detection systems. L-SAB allows detection of DSA with high sensitivity. Recently, Wiebe et al. [27] reported that even weakly reactive L-SAB-detected de novo DSA measured at the low positivity cut-off of 500 MFI is predictive of graft survival.

Early after transplantation acute antibody-mediated rejection occurs in about 1–6% of patients; however, this number may increase up to 21–55% in patients who had detectable DSA already before transplantation and who received desensi-

tization therapy [9, 28, 29]. Persistence or re-emergence of DSA which were detectable already before transplantation is associated with poor allograft outcome.

Einecke et al. [1] reported that, late after transplantation, chronic antibody-mediated rejection is one of the leading causes of graft loss, together with death with a functioning graft, recurrent renal disease, and interstitial fibrosis/tubular atrophy (of unknown origin). Chronic antibody-mediated rejection is found more frequently in patients who are non-adherent to immunosuppressive medication or in whom immunosuppression was reduced for other reasons, e.g. conversion to CNI-free or steroid-free immunosuppressive protocols, recurrent infection, or malignancy [27, 30, 31]. Additional risk factors for the development of de novo DSA and antibody-mediated rejection are class II HLA-DR mismatches between donor and recipient, prior cell-mediated rejection episodes and younger recipient age.

In many patients with late antibody-mediated graft loss, even when HLA class I alloantibodies are detectable, HLA class II de novo DSA are considered to be mainly responsible for rejection. In the recent study of Wiebe et al. [27] on the evolution of HLA alloantibodies after transplantation, de novo DSA were found to appear at a mean of 4.6 years after transplantation, and the prevalence of de novo DSA after 10 years was 20% in adherent as compared to 60% in non-adherent graft recipients. Other antibodies that are discussed in the evolution of chronic antibody-mediated rejection are MICA antibodies, angiotensin II type 1 receptor-activating antibodies, and other anti-endothelial cell antibodies [32, 33]. The exact impact of these antibodies on the outcome of kidney and other organ transplants needs yet to be determined.

In summary, L-SAB is an extremely useful tool for the characterization of HLA antibodies and the surveillance of desensitization therapies pretransplant as well as de novo antibody development and therapy posttransplant. A limitation of the L-SAB assay is that in the pretransplant phase, L-SAB can lead to the unjustified exclusion of patients from transplantation if the critical determination of UAMs is based solely on this technique. Therefore, for the pretransplant determination of UAMs, we recommend the use of L-SAB testing only in combination with other techniques that are not associated with the problem of technically false-positive results.

Disclosure Statement

The authors have nothing to disclose regarding the content of this paper. However, they received honoraries from several pharmaceutical companies for talks on the subject.

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