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Donor-reactive HLA antibodies in renal allograft recipients: Considerations, complications, and conundrums

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ABSTRACT

Whether sensitized patients wait for a compatible crossmatch with a deceased donor, enter a paired exchange program with the hope of finding a compatible living donor, or go through a desensitization protocol depends on a number of factors, not the least of which is the overall philosophy of the transplant center. Centers such as ours take the position that donor-directed antibodies detected by solid phase assays (even those that are "weak") present an unacceptable risk factor to the patient. This philosophy is predicated on the biologic role of the immune system, specifically that antibodies were generated in response to a non-self (allo) antigen and that a successful immune response eliminates that which caused its stimulation. Although obviously an oversimplification, this philosophy mandates a comprehensive evaluation of HLA antibodies in sensitized recipients. This article addresses the challenges and conundrums associated with human leukocyte antigen antibody identification.

1. Introduction

Following the landmark report of Patel and Terasaki [1], a positive cytotoxic crossmatch between donor cells and recipient serum was considered a contraindication to renal transplantation. The high rate of immediate graft loss among patients undergoing transplantation across this barrier was unacceptable. It soon became apparent that the clinically relevant antibodies in a lymphocyte crossmatch were those directed against antigens encoded by the human major histocompatibility complex (MHC), henceforth referred to as the human leukocyte antigen (HLA) complex. The paradigm to not cross the positive crossmatch barrier was modified when it was recognized that cytotoxic crossmatches resulting from non-HLA antibodies (*e.g.,* autoantibodies) had no impact on allograft survival and could be safely ignored (reviewed in [2]). However, there was a growing appreciation that complement-fixing, donor-directed HLA antibodies undetectable by standard cytotoxicity assays were clinically relevant (reviewed in [3]). Such observations led to the development of more sensitive antibody detection tests including the antiglobulin-enhanced cytotoxicity (AHG-CDC) and flow-cytometric crossmatch assays [4–8]. Although these tests were more sensitive, their shortcoming was their relative lack of specificity [9,10]. Subsequently, the development and implementation of solid phase antibody detection systems (SPADS) that specifically identified HLA antibodies represented a major advancement [3,11,12]. These sensitive and specific assays provided the transplant community with analytical tools not previously available and, in many situations, actually changed how crossmatch data were interpreted. For example, in a single-center study by Kerman *et al.,* no differences were initially reported in the number of rejection episodes and/or graft losses among recipients undergoing transplantation with renal allografts from donors whose flow crossmatches were positive or negative as long as the AHG-CDC crossmatches were negative [13]. Subsequently, retesting of sera from these patients by solid phase technology revealed that several of the "positive" flow-cytometric crossmatches were actually caused by non-HLA antibodies [14]. Importantly, the above patients experienced no rejection episodes or graft loss during the study period. Thus, it should not be surprising that SPADS have supplanted cell-based assays and have become the gold-standard for identifying HLA antibodies.

Nonetheless, how information from solid phase assays is translated into clinical practice is quite controversial. For example, in terms of risk, should "weak" (low titered, low fluorescence intensity) donor-directed antibodies be given the same clinical significance as "strong" (high titered, strong fluorescence intensity) donor-directed antibodies [15–18]? Can non–complement-fixing donor-directed HLA antibodies be considered less problematic than complement-fixing antibodies, as suggested by Bohmig *et al.* [19], or should they be considered to confer the same degree of risk to long-term graft survival as proposed by Cai and Terasaki [20]? Such disparate data beg the question of how antibodies are classified as "strong" or "weak" or as complement fixing/non–complement fixing. Indeed,

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we recently demonstrated that HLA antibodies considered to be non–complement fixing by AHG-CDC assays were in fact complement fixing when assayed by a more sensitive flow-cytometry–based assay [21]. Thus, the fact that an antibody does not fix complement *in vitro* should not automatically be taken as evidence that the same antibody will not fix complement *in vivo*.

There are numerous other controversies involving antibodies detected by SPADS, with literature supporting either side of the argument. Can donor-directed antibodies detected in a solid phase assay that do not result in a positive lymphocyte crossmatch, even by the most sensitive of assays, be ignored [22,23]? Are antibodies directed against certain HLA antigen specificities less problematic than others (reviewed in [24])? Are donor-directed class II antibodies more tolerable than donor-directed class I antibodies [25,26]?

Over the past 3 years, the concept of virtual crossmatching (*i.e.*, predicting the crossmatch outcome without actually performing a physical crossmatch) has taken center stage as an approach to more efficient allocation and distribution of deceased-donor organs [27–29]. Although virtual crossmatching has proved successful for predicting actual negative crossmatches between specific donor:recipient combinations, it can be argued that, if the threshold for calling an antigen "unacceptable" is set too low, those patients will never be considered for donors that might be suitable. Without question, not all patients with low, moderate, or even high levels of donor-directed antibody have poor outcomes if they receive donor organs that express the corresponding antigens. Unfortunately, other patients with characteristics virtually identical to those just described will experience severe episodes of rejection and may lose their grafts if they undergo transplantation. There are currently no assays that unequivocally categorize patients into one group or the other.

Over the last decade, desensitization strategies have been advocated as an approach to performing transplantations in highly sensitized patients, especially those who present with crossmatch-incompatible donors [30,31]. Albeit an excellent strategy for some patients, there is clear evidence that patients who are

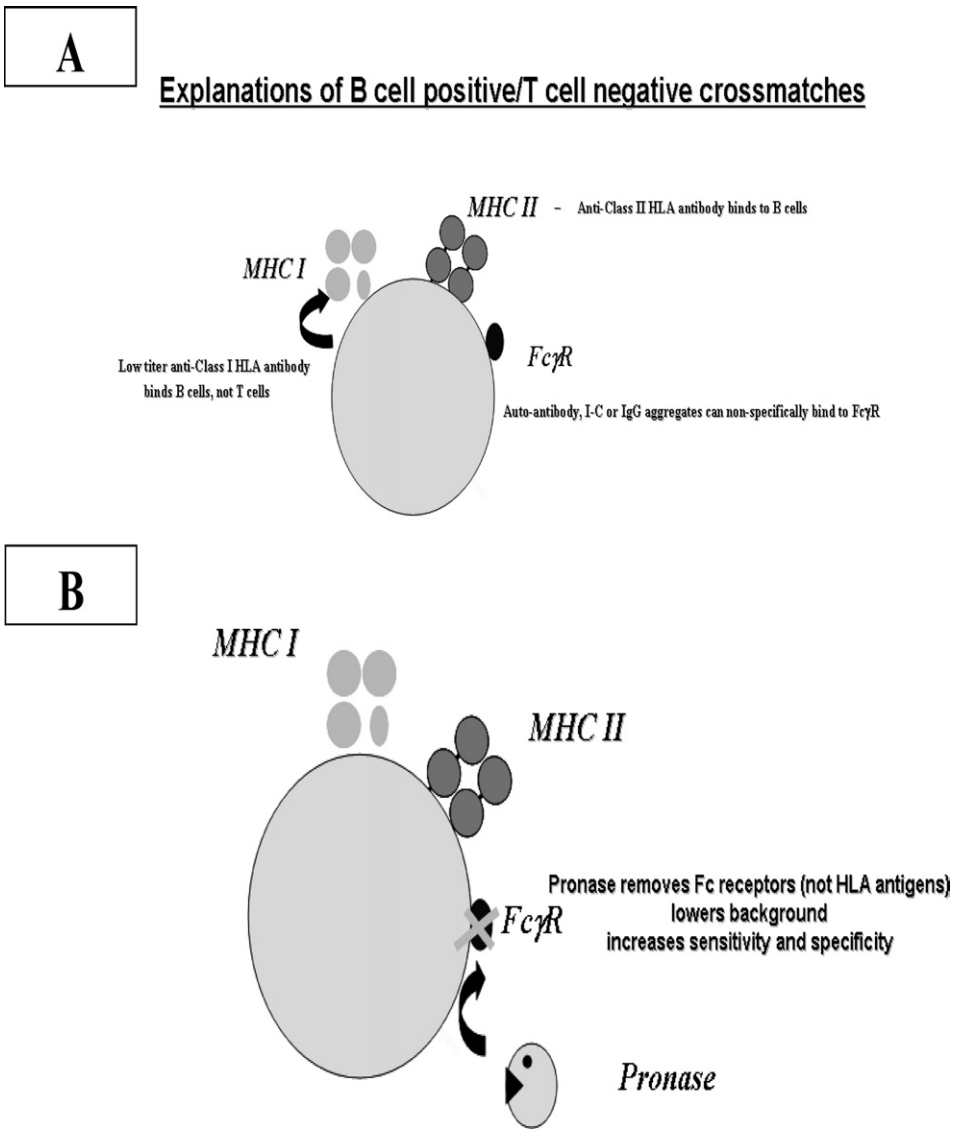

Fig. 1. (A) At least three different types of glycoproteins are expressed on the surface of B cells: namely, MHC class I antigens, MHC class II antigens (which can bind to MHC class II antibodies), and Fc receptors. Low-titer class I antibodies can bind to B cells and not T cells based on B cells expressing more class I than T cells. Antibodies to class II will bind to B cells and not T cells (which, under normal circumstances, do not express class II). Fc receptors (which have a significantly higher expression on B cells than on T cells) can bind the Fc portion of antibodies independent of their antigen specificity. Thus, in a flow-cytometric crossmatch, B cells will have a higher background than T cells. (B) Reduction/elimination of Fc receptors from lymphocytes before performing a crossmatch. Cells are treated with pronase, a proteolytic enzyme that will digest Fc receptors without affecting the expression of class I or class II antigens [44]. Pronase also digests CD20 (a target of Rituxan) from the surface of B cells [58].