Donor-Specific Antibody in Solid Organ Transplantation: Where are We?

V. Pietroni\textsuperscript{1}, A. Toscano\textsuperscript{1}, F. Citterio\textsuperscript{1,*}

Abstract—The clinical significance of donor specific HLA antibody (DSA), in solid organ transplantation, is still debated. While it is well known that hyperacute antibody-mediated rejection (AMR) may occur in the presence of pre-transplant DSA, the presence of post-transplant de-novo DSA not always causes AMR. We discuss the role of DSA in solid organ transplantation, the methodology to detect DSA and the effect of immunosuppressive therapy on DSA production.

Keywords —anti- HLA, DSA, AMR, Luminex, Solid Phase Assay

I. INTRODUCTION

Definition of donor specific antibody (DSA)

The association between hyperacute rejection after transplantation and the presence of preformed cytotoxic antibodies to donor HLA antigens, has been well known for more than 30 years [1]. DSA antibodies are anti-HLA antibodies, specifically generated against donor cells and, for this reason a positivity at the standard complement-dependent cytotoxicity test (CDC) before transplantation has been always considered a contraindication for transplantation. Recently, the clinical role of DSA has been questioned and it is now debated their role in kidney transplant outcome.

History of DSA

From the early era the main question in organ transplantation has been whether grafts were rejected by antibodies or by cells. For many years the “cellular theory” has been accepted as correct. P.A. Gorler, in 1938, was the first to introduce the role of antibodies in tumor transplantation, demonstrating that hemagglutinating antibodies appeared concomitantly with the rejection of tumor homografts in mice [2,3]. Thus, based on these emergent studies on antibodies, several groups investigated their role in transplantation discovering anti-HLA antibodies. Terasaki, in 2003, showed adequate proofs supporting the “humoral theory” of transplantation, describing the association with anti-HLA antibodies detection, acute rejection or chronic rejection [4]. In 2004 Terasaki and Ozawa suggested monitoring of anti-HLA antibodies as predictive of transplantation outcome showing that the presence of post-transplant anti-HLA antibodies were at higher risk for acute and chronic AMR, compared with those without antibodies (p=0.00003) [5]. Very recently a new definition of acute rejection has introduced the concept that appearance of DSA is critical together with the presence of c4d positive peritubular cells and creatinine increase, to make the diagnosis of acute antibody mediated rejection (AAMR).

Methodology to detect DSA

Pre-transplant presence of anti-donor HLA antibodies in the recipient, has always conditioned allograft allocation. CDC has always been the golden standard to detect the presence of anti-donor antibodies. The limit of CDC is the presence of non-HLA molecules on the cell membrane that could be an unspecific target for the anti-HLA antibodies. Moreover, the CDC assay is based on complement fixation, and the existence of specific IgG antibodies not fixing complement, can lead to a false negative result. The introduction of solid phase assay using isolated HLA molecules, allowed to avoid unspecific reaction, but rose several questions on the specificity and sensitivity of these assays. The first solid phase assay introduced was the enzyme linked immunoassay (ELISA) in 1993 by Kao et al., which was able to detect complement and non-complement fixing antibodies [6]. The implementation of this assay allowed to finally determine the importance of DSA on graft survival [7]. In 1998, Pei et al., developed an anti-HLA antibodies screening method with microbeads coated with purified HLA antigens, detected by flow cytometry [8]. Recently, Luminex assay, based on HLA molecules attached to beads, has become the most applied detection methodology. Lachmann et al. confirmed the detrimental effect of DSA production on the graft outcome with this methodology [9]. A total of 1014 deceased kidney transplant recipient were monitored for development of HLAb using Luminex Single Antigen beads. The presence of DSA in the thirty percent of recipient was associated with a significantly lower graft survival of 49% vs. 83% in the HLAb negative group (p<0.0001). Due to the larger availability of single HLA antigens, this technique is more sensitive and specific, compared to the previous. However, for the same reason this high sensitivity can cause several false positive, affecting the clinical relevance of DSA. Luminex beads in fact, not only accommodate intact HLA molecules, but also denatured molecules, unmasking hidden epitopes. This denatured molecules are able to react also with “natural” HLA antibodies, present in non-immunized
males [10]. On the contrary, false negative results are affected by the presence of IgM HLA specific antibodies, able to inhibit IgG binding to the molecules, masking clinical relevant allosensitization [11]. Moreover, false negative results, are detected with prozone phenomenon, caused by complement component 1 (C1) that competitively can displace the detection antibodies [12]. The extreme sensitivity of Luminex assay can make difficult the assignment of the positive and negative result. So far, there is no an accepted standardization for cut-off values with MFI (mean fluorescence intensity). The mean fluorescence intensity could vary between centers, depending on different company’s reagents and operator’s ability. This is the main reason why CDC assays are still leading in organ’s allocation.

Clinical evidence of the detrimental role in renal transplant

One third of waiting list population in kidney transplant, are sensitized patients. The presence of “donor-specific” or “non-donor-specific antibodies” can limit the access to renal transplantation and later on can reduce graft survival. A one year follow-up study associated pre-transplant donor specific antibodies with a risk for development of antibody mediated rejection. Pre-transplant sera from deceased-donor kidney transplant patients, with an high immunological risk, showed an increased hazard ratio for antibody mediated rejection and reduced graft survival, associated with pre-transplant class II DSA (P<0.001) [13]. Monitoring of DSA to predict allograft outcome could be a non-invasive surrogate method compared to graft biopsy. Loupy et al found a correlation between subclinical antibody-mediated rejection biopsy proven at 3 month, with lower mGFR and an higher percentage of DSA Class II at 1 year after transplantation [14]. Several groups investigated on the post-transplantation role of de novo DSA. Mao et al., using single antigen beads technique, analyzed 54 kidney recipients in a five years follow-up, finding a strong association between the production of anti-HLA antibodies, after transplantation, and graft failure [15]. Piazza et al. analyzed serum sample from 120 non-presensitized renal transplant recipients, using Flow Cytometry Crossmatch (FCXCM) and FlowPRA beads detection assay. This study also found a correlation between post-transplant DSA production and worst graft survival, relate to anti-HLA class I antibodies [16], Everly et al. suggested to analyze graft survival from the time of DSA appearance as patients developed donor specific antibodies at different time point [17]. In a 16 years follow-up study Piazza et al. confirmed the Terasaki “humoral theory” showing a very low graft survival rate in patients who developed DSA [18]. The role of specific HLA antigens on the development of graft loss is still on debate. Indeed, HLA-DQ DSA have been associated with AMR, transplant glomerulopathy and allograft loss ( p<0.0001). Patients mismatched at both DR and DQ loci were at risk for developing class II DSA antibodies, resulting in antibody mediated rejection [19,20], as well as the presence of HLA-DP de-novo DSA [21, 22].

Methodology and results of pre-transplant clearance

Different approaches have been proposed to treat patients with circulating DSA: 1) remove circulating DSA by plasmapheresis and immunoadsorption; 2) to block their effect with proteasome inhibitors; 3) to reduce the production with anti-CD20. Several studies showed patients undergone desensitization protocols having a significant survival benefit compared to patients who stayed in dialysis or were on the list for transplantation. Bonomini et al., in 1985, showed the benefits of plasma exchange in pre-sensitized kidney transplant recipient (30% graft loss) vs pre-sensitized kidney transplant recipient receiving only standard immunosuppression (81% graft loss) [23]. Montgomery et al. found better patient survival in transplant patients, after desensitization with plasmapheresis and IVIg treatment, vs the dialysis only group or dialysis-transplantation group [24]. Loupy et al. recently suggested for high risk population a prophylactic immunosuppressive strategy combining IVIg/anti-CD20/plasmapheresis in the first day, associated to a long term graft outcome [25]. To block DSA production with proteasome inhibitors (Bortezomib), Everly et al. treated five kidney transplant patients showing mix acute cellular rejection (ACR) and antibody-mediate rejection (AMR) episodes. DSA levels decreased significantly in all patients and led to prompt ACR and AMR rejection reversal [26]. However, the use of this procedure is still on debate considering that the group of Legendar had an opposite results using Bortezomib as the sole desensitization therapy [27].

Immunosuppression and development of DSA

The balance between immunosuppression and drug toxicity has always been a dilemma in solid organ transplantation. Several studies investigated on the effects of immunosuppressive therapy on anti-HLA antibody production and antibody mediated rejection episodes. In general, excessive minimization of immunosuppressive therapy seems to expose to the risk of enhanced DSA production. Recently, transplant recipients converted from tacrolimus (control group) to sirolimus 3 month after transplantation, showed higher incidence of de novo DSA (17.3% vs 9.6%) [32]. The conversion from cyclosporine to everolimus regimen 3 months after transplantation, also showed increased development of DSA and AMR [33]. Development of donor specific antibody resulted also after conversion from cyclosporine to azathioprine (AZA) but not in patients converted to mycophenolate mofetil (MMF) [34].

Open questions

Nowadays it is very clear that pre and post- transplant donor specific antibodies have to be considered a risk factor more than a contraindication to transplantation, but a number of questions still remain opened. The methodology to detect DSA is a rapid evolving field and better characterization could identify subgroup of DSA positive patients with different prognosis. Some patients with DSA do not have any harm for the presence of antibody, possibly because of the accommodation phenomenon, but we need to recognize them. Therapy to remove or control DSA action are evolving but
large clinical trials are needed to optimize this approach. Finally the major question is still what to do when a patient with normal renal function develops DSA.

References