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Clinical relevance of circulating antibodies and B lymphocyte markers in allograft rejection

Patrice Vallin [a], Olivier Désy [a], Stéphanie Béland [a], Eric Wagner [b], Sacha A. De Serres [a,*]

[a] Transplantation Unit, Renal Division, Department of Medicine, CHU de Québec, Faculty of Medicine, Laval University, Québec, QC, Canada
[b] Immunology and Histocompatibility Laboratory, CHU de Québec, Faculty of Medicine, Laval University, Québec, QC, Canada

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ABSTRACT

The main challenge in solid organ transplantation remains to tackle antibody-mediated rejection. Our understanding of the antibody-mediated response and the capacity to detect it has improved in the last decade. However, the sensitivity and specificity of the current clinical tools to monitor B cell activation are perfectible. New strategies, including the refinement in the characterization of HLA and non-HLA antibodies, as well as a better understanding of the circulating B cell phenotype will hopefully help to non-invasively identify patients at risk or undergoing antibody-mediated allograft damage. The current review discusses the current knowledge of the B cell biomarkers in solid organ transplantation, with a focus on circulating antibodies and peripheral B cells.

1. Introduction: historical perspective

In the 60s, Kissmeyer-Nielsen and colleagues described for the first time the involvement of antibodies in the ABO-independent rejection of a solid organ transplant in patients previously transfused or with a past history of pregnancy [1]. In 1969, Terasaki et al. developed the complement-dependent cell cytotoxicity (CDC) assay and demonstrated the involvement of antibodies that react against the donor cells in hyperacute rejection. These antibodies are directed against human leukocyte antigens (HLAs) expressed on nucleated cells [2–4]. In 1978, Soulillou et al. showed an association between the development of circulating anti-HLA-DR donor-specific antibodies (DSAs) and graft rejection [5]. The emergence of new detection techniques using solid phase assays, first by ELISA, followed by flow cytometry and by Luminex technology, has allowed enhancing the sensitivity threshold in comparison to the CDC assay [6–8]. The relative usefulness and clinical relevance of the different assays available for antibody detection have been reviewed recently [9].

Despite the early recognition of the crucial importance of anti-HLA antibodies in hyperacute and acute rejection, their involvement in chronic allograft damage processes has been neglected for a long time, in part because of a lack of adequate tools to monitor antibody-mediated responses post-transplant. In the early 1990s, studies showed the association between the presence of DSA and poor graft outcomes [10,11]. A few years later, Colvin's group developed a C4d staining method on renal biopsies. They observed that only patients with antibody-mediated rejection (ABMR) were positive for this marker, allowing the distinction with acute cellular rejection [12]. C4d staining has then been incorporated into the Banff classification of renal allograft pathology. In 2001, using immunofluorescence for C4d staining of frozen sections from patients with chronic allograft rejection, this group demonstrated that C4d deposits identify chronic ABMR from the so-called chronic allograft nephropathy (CAN) [13]. Although it is now recognized that some cases of ABMR are C4d negative, these observations induced a paradigm shift in our understanding of CAN and opened a new era of diagnostic and therapeutic opportunities. Subsequently, several studies have confirmed that ABMR was a frequent cause of chronic rejection and late graft loss [14,15].

Since then, research focusing on the diagnosis of ABMR and the monitoring of B cell activation has been one of the most intensive fields in transplantation (Table 1). New technologies available to characterize circulating antibodies revealed how complex it is to predict rejection and graft outcomes. The direct phenotyping of B cells, as well as cytokine secreted by B cells, such as BAFF, are also under investigation for clinical purposes. Here we present a review of the most recent literature

* Corresponding author at: Transplantation Unit, Renal Division, Department of Medicine, CHU de Québec - L'Hôtel-Dieu, Faculty of Medicine, Laval University, 11 Côte du Palais, Québec, QC G1R 2J6, Canada.
E-mail address: sacha.deserres@crchuq.ulaval.ca (S.A. De Serres).

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Table 1

Study	Organ and sample size	Follow-up (mean years)	Type	Main result
Pretransplant DSA				
Lefaucheur et al. [17]	402 kidney transplant recipients	8	Observational	Presence of pretransplant DSA correlates with a higher risk of ABMR and graft loss.
Singh et al. [19]	237 CDC-XM-negative kidney transplant recipients	2	Retrospective	Class-I and -II DSAs are associated with increased ABMR
Otten et al. [16]	837 kidney transplant recipients	7	Retrospective	Pretransplant class-I plus -II DSAs are indicative for an increased risk of graft failure in crossmatch-negative patients
O'Leary et al. [29]	1270 liver transplant recipients	n/a	Retrospective	Patients with preformed class-II DSA showing high MFI (>5000) have a higher risk of graft rejection at 6 months post-transplant and a lower 5 years graft survival
De novo DSA				
Hidalgo et al. [40]	145 kidney transplant recipients	n/a	Prospective, cross-sectional	There is a predominance of class-II or class-I and -II, compared with class-I alone in de novo DSA
Wiebe et al. [35]	315 kidney recipients without pre-transplant DSA	6	Retrospective	Patients with de novo DSA have a lower 10-year graft survival.
Willicombe et al. [39]	505 DSA-negative kidney transplant recipients	3	Retrospective, longitudinal	Mismatch at both HLA-DR and -DQ loci increases the incidence of developing de novo DSA and ABMR, compared with a single mismatch at -DR or -DQ
Everly et al. [38]	189 primary kidney transplant recipients	8	Longitudinal	Patients with a HLA-DQ mismatch are the most likely to develop de novo DSA
Antibody strength				
Dieplinger et al. [42]	24 non-sensitized kidney transplant recipients	2	Prospective, longitudinal	Patients with an impaired graft function following DSA development have a higher MFI and a stronger increase in MFI than stable patients with DSA
Anti-HLA -C, -DQ and -DP DSA				
DeVos et al. [49]	347 kidney transplant recipients	3	Prospective	Anti-HLA-DQ DSAs are the most common type of DSA and they are associated with worse graft outcome
Ling et al. [52]	251 sensitized patients	0.5	Retrospective	Prevalence and strength of anti-HLA-C and -DP were lower than -A, -B, -DR and -DQ
Freitas et al. [51]	203 of 284 primary kidney transplant recipients	6	Retrospective	De novo persistent HLA-DQ DSA increases the risk of ABMR and graft loss
Complement-binding DSA				
Yabu et al. [63]	31 kidney recipients	n/a	Retrospective	C1q-binding DSA is more specific for transplant glomerulopathy but less sensitive for C4d deposition than IgG DSA
Loupy et al. [62]	1016 kidney recipients	5	Retrospective	Post-transplant C1q-binding DSA is associated with lower 5-year graft survival, and ABMR, compared with non-C1q-binding DSA
Sicard et al. [71]	69 kidney recipients	2	Retrospective	C3d-binding DSA shows a better association with graft loss than C1q-binding assay
IgG subclasses				
Honger et al. [75]	74 kidney recipients transplanted in the presence of HLA-DSA	>2	Retrospective, longitudinal	IgG isotype subclasses are found in DSA-positive patients with the following prevalence: IgG1 78%, IgG2 49%, IgG3 36%, and IgG4 20%
O'Leary et al. [77]	1270 primary liver transplant recipients	>5	Retrospective, longitudinal	Development of IgG3 DSA is a better predictor of death than C1q-binding assay positivity.

CDC-XM: complement-dependent cell cytotoxicity crossmatch, n/a: not available.

related to anti-HLA antibodies, non-HLA antibodies and cellular biomarkers of B cell activation.

2. Anti-HLA antibodies

2.1. Pretransplant DSA

The presence of DSA pretransplant represents a risk for poor graft survival and renal function, both in the short and long terms [16]. Lefaucheur et al. demonstrated in an observational cohort of 402 renal transplant patients that pretransplant anti-HLA DSA correlates with ABMR and reduced graft survival [17]. They found that the 8-year graft survival was lower in sensitized kidney recipients with pre-existing anti-HLA DSA, compared with sensitized recipients without DSA and with unsensitized patients (61, 93 and 85% respectively). ABMR has been associated with class I [18] and class II DSA [19]. The dual presence of class I and class II DSA pretransplant substantially reduces the 10-year graft survival compared with patients without DSA (72% vs. 30%) [16]. Interestingly, pretransplant class I-specific antibodies as opposed to class II-specific antibodies can predict acute ABMR and early graft loss [20,21]. Although not clearly established, pre-existing class II-specific antibodies, which persist after transplantation, appear to be associated with chronic forms of renal allograft rejection.

In contrast to these reports, other investigators found no association between pretransplant DSA and graft survival [22,23]. Notably, results of the Collaborative Transplant Study published by Opelz on patients in whom recipient and donor DNAs were available for HLA typing revealed no association between Luminex-detected DSA and graft loss [22]. Even strong DSA, defined by mean fluorescence intensity (MFI) above 2000 or 3000, did not predict graft loss at 3 years post-transplant. This difference could first be explained by the length of follow-up, most negative studies being limited to five years or less post-transplant. Second, the longitudinal follow-up seems to be important, since the same group reported later that persisting pretransplant anti-HLA antibodies are associated with graft loss, especially when they are C1q-binding and whether or not they are donor-specific [24]. Third, the discrepancy between studies could be due to technical issues in the detection of DSA, for instance denatured antigens on some beads that can lead to false-positive results. Denatured antigens present a particular challenge in the HLA laboratory, because they can vary from one