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Brief communication

Chronic humoral rejection mediated by anti-HLA-DP alloantibodies: Insights into the role of epitope sharing in donor-specific and non-donor specific alloantibodies generation

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ABSTRACT

We report the case of a renal transplanted patient, in whom the detection of a unique anti HLA-DP antibody response preceded the development of chronic humoral rejection. In addition to donor-specific anti-DP alloantibodies, the patient displayed reactions against several non-donor-specific DP antigens (NDSA). Interestingly, we found that all the DP molecules recognized by the alloantibodies displayed the same amino-acid sequence suggesting that epitope sharing between unrelated HLA molecules was the mechanism underlying NDSA generation.

This case highlights the pathogenicity of anti-DP alloantibodies and suggests that it could be more meaningful to match the epitopes than the HLA antigens for the prevention of rejection.

1. Background

It is widely accepted that antibodies directed against donor HLA antigens (donor-specific alloantibodies, DSA) have a deleterious impact on graft survival. However, because this conclusion has mainly been drawn from studies focused on alloantibodies directed against A, B or DR molecules, the clinical significance of other anti-HLA specificities has remained unclear [1,2].

In the other hand, it has been recurrently observed that some transplant recipients develops antibodies against HLA molecules not expressed by their graft (known as non-donor specific alloantibodies, NDSA) but the immunological mechanism underlying the generation of the NDSA remains elusive [3].

2. Case report

A 38-year-old woman (HLA-A3,30;B7,18;DR3,15;DPB1*0401,*0402) received a first cadaveric kidney graft (HLA-A3,30;B7,18;DR2,3;DPB1*0301,*0401) in November 1990 for the treatment of an end-stage renal failure related to a Goodpasture's disease. In 1997 she gave birth to her unique child. She was back in hemodialysis in August 2000, due to chronic allograft nephropathy (Fig. 1A). In March 2002 the first graft was removed to control a severe hemolytic anemia. She received a second cadaveric kidney transplant in April 2003 (HLA-A2,3;B7,60;DR1,15;DPB1*0401,*1401). The complement-dependant cytotoxicity cross-match was negative for T and B cells at the time of the transplantation. The follow up of this second transplantation (Fig. 1A) was complicated by the occurrence of a biopsy proven chronic active antibody-mediated rejection episode in December 2006. Luminex assays revealed the presence of anti-HLA class II alloantibodies, the reactivity of which was restrained to the DP molecules. No reactivity against HLA-I or other HLA-II molecules was detected. Single antigen analysis showed that these anti-DP alloantibodies were directed against the DPB1*0301 allele shared by the first donor and her child (Fig. 1C). The cross-reactivity of these antibodies with the DPB1*1401 allele expressed by the second graft (DSA) and with some other DP alleles (NDSA) was explained by the presence of a peculiar amino-acid sequence shared by all these DP alleles (Fig. 1D). A retrospective analysis on frozen sera indicated that anti-DP reactivity appeared in 2002. We concluded that the patient gets immunized against the DEAV epitope at the time of her first transplantation and/or her pregnancy, and that she

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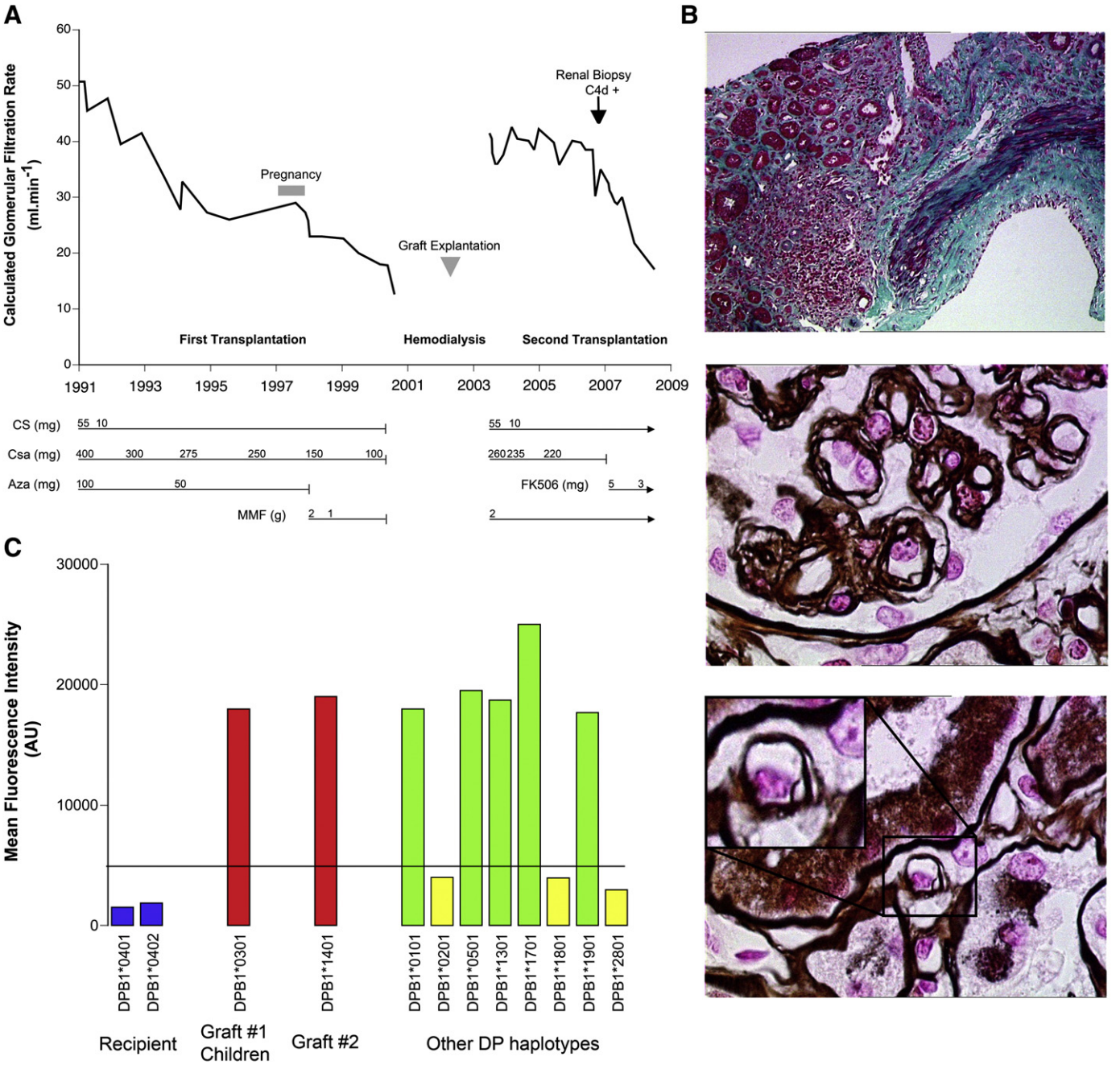
subsequently developed a chronic active antibody-mediated rejection of her second graft due to the presence of preformed antibodies able to cross react with the DP allele of the donor. The negativity of the complement-dependant cytotoxicity cross-match at the time of the second transplantation could be related to the low level of expression of DP molecules on the lymphocyte surface.



D

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                              10        20        30        40        50        60        70        80        90       100
                              |         |         |         |         |         |         |         |         |         |
DPB1*040101 RATPENYLFQ GRQECYAFNG TQRFLERYIY NREEFARFDS DVGEFRAVTE LGRPAAEYWN SQKDILEEKR AVPDRMCRHN YELGGPMTLQ RRVQPRVNVS
DPB1*0402   ---------- ---------- ---------- -----V---- ---------- ----DE---- ---------- ---------- ---------- ----------

DPB1*030101 -------VY- L--------- ---------- -----V---- ---------- ----DED--- ----L----- -----V---- ---DEAV--- -----K----
DPB1*1401   -------VH- L--------- ---------- -----V---- ---------- ----DED--- ----L----- -----V---- ---DEAV--- -----K----

DPB1*010101 -------VY- ---------- ---------- ----Y----- ---------- ---------- ---------- -----V---- ---DEAV--- -----K----
DPB1*020102 ---------- ---------- ---------- -----V---- ---------- ----DE---- --------E- ---------- ---------- ----------
DPB1*0501   ---------- ---------- ---------- ----LV---- ---------- ----E----- ---------- ---------- ---DEAV--- -----K----
DPB1*1301   -------VY- L--------- ---------- ---------- ---------- ---------- --------E- -----I---- ---DEAV--- -----K----
DPB1*1701   ******--VH- L--------- ---------- -----V---- ---------- ----DED--- --------E- ---------- ---DEAV--- --********
DPB1*1801   ********VY- ---------- ---------- -----V---- ---------- ----DE---- ---------- ---------- ---VGPM--- **********
DPB1*1901   ---------- ---------- ---------- -----V---- ---------- ----E----- --------E- -----I---- ---DEAV--- -----K----
DPB1*2801   ******----- ---------- ---------- ---------- ---------- ----DE---- ----L----- ---------- ---VGPM--- --********
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3. Discussion

HLA-DP antigens were first discovered 30 years ago [4]. Since then it wasn't long before antibodies to DP were identified [5]. Anti-DP alloantibodies are less common than anti-DR and anti-DQ, but are still found in 7–12% of transplanted recipients [1,6,7], mainly women or patients that have lost a previous graft [7]. Since DP antigens are constitutively expressed on renal microvasculature, a negative impact of anti-DP alloantibodies on graft survival was anticipated but this hypothesis long remained controversial [1,2] (mainly because of the lack of reliable test to identify them). The recent development of single antigen beads technology that efficiently identifies anti-DP alloantibodies represents therefore a valuable tool to address this issue. Qiu et al. [6] found a negative correlation between the presence anti-DP and renal graft survival. However, since most patients with anti-DP alloantibodies also produce anti-DR and/or -DQ [6,7], the pathogenic potential of anti-DP is difficult to assess. The most direct evidence for the deleterious role of anti-DP alloantibodies therefore depends on the few cases of graft loss reported in patients with isolated anti-DP reactivity [8,9]. According to this scarce literature, including our own case, it appears that anti-DP alloantibodies can mediate humoral allograft rejection with the full range of kinetics.

Another interesting finding is the observation that all the HLA-DP molecules recognized by the alloantibodies shared a common amino-acid sequence, suggesting that all these reactions were in fact directed against a single epitope. This case highlights the concept of epitope sharing within HLA antigens and suggests that NDSA reactions are due to reactions of DSA against non-donor antigens that share epitopes with donor antigens. It could therefore be more meaningful, as long suggested by Terasaki et al. [10], to match the epitopes that cause antibody response rather than the HLA antigens. Indeed, in organ transplantation the degree of HLA compatibility is generally determined by counting the number of mismatched HLA antigens between the donor and the recipient. This is inadequate because previous works, based on serological cross-reactivity between HLA antigens [11], have demonstrated that i) HLA molecules have multiple epitopes, i.e. short sequences involving polymorphic amino acid residues in antibody-accessible positions, and ii) that some epitopes are shared by distinct HLA antigens. Based on these findings Duquesnoy has proposed HLAMatchmaker (http://HLAMatchmaker.net), a matching algorithm founded on two principles: i) each HLA antigens represents a distinct string of structurally defined epitopes as potential immunogens that can induce specific antibodies, and ii) patients can not make antibodies against epitopes that are expressed by their own HLA molecules. A few studies have addressed the impact of epitope matching on transplant outcome. For instance, an analysis of the UNOS and Eurotransplant kidney transplant databases has shown that serologically HLA-A,B mismatched kidneys that are compatible at the epitope level exhibit almost identical graft survival rates as the zero HLA-A,B antigen mismatched [12]. This strategy is particularly interesting for highly sensitized patients, where it allows a significant reduction of the waiting time for a suitable kidney while ensuring an excellent graft survival [13]. Accordingly, epitope matching through HLAMatchmaker is now routinely used in Eurotransplant [14].

We concluded that DP tissue typing should be performed for regrafts and female patients. Avoiding presensitized DP alleles (or better HLA-DP epitopes) in these recipients may translate into better transplantation outcome. More generally this case suggests that it could be more meaningful to match the epitopes that cause antibody response rather than the HLA antigens.

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Fig. 1. A: Summary of the patient's clinical history from the first transplantation to the last follow-up. Graft function is evaluated by the calculated glomerular filtration rate according to the MDRD formula. Changes in immunosuppressive regimen are indicated at the bottom of the graph. Abbreviations are: CS, corticosteroid; Csa, cyclosporine; FK506, tacrolimus; Aza, azathioprine; MMF, mycophenolate mofetil. B: Representative findings of the histological analysis of the second graft are presented. Masson's trichrome demonstrates tubular atrophy with interstitial fibrosis, nodular infiltration of inflammatory cells in the interstitium, and prominent vascular lesions (upper panel). Silver staining shows glomerular double contours (middle panel) and capillary basement membrane multilayering (Lower panel).These features fulfilled the criteria for the diagnosis of chronic active antibody-mediated rejection as assessed by the Banff 2007classification. C: We performed an HLA-DP genotyping of the recipient (HLA-DPB1*0401, 0402), her husband (HLA-DPB1*0301, 1101), her children (HLA-DPB1*0301, 0401), the first graft (HLA-DPB1*0301, 0401), and the second graft (HLA-DPB1*0401, 1401). A Luminex single antigen analysis was carried out to determine the specificity of the alloantibodies. As expected, no antibody directed against the DP alleles of the recipient was detected (blue bars). In contrast, a strong reactivity was evidenced against DPB1*0301 allele (shared by the first graft and her children) and the DPB1*1401 allele of the second graft (red bars). Interestingly, we also evidenced a strong reactivity against some DP alleles (green bars) for which the immune system of the patient was considered as naïve. Other DP alleles were not recognized by the antibodies (yellow bars). D: amino acid sequence alignment of the various DP alleles unravels the molecular basis for the cross reactivity of the anti-DP alloantibodies. All the alleles recognized by the antibodies (highlighted in green) share a peculiar DEAV sequence in position 84–87 (violet). When this sequence was absent, the DP molecules (highlighted in yellow) were not recognized (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)