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Case Report

IgM antibodies towards pre-endothelial cells: strong indication for an association with accelerated rejection. A case report

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Abstract
A 27-year-old woman developed a graft loss due to an accelerated humoral rejection after receiving a blood group identical, human leucocyte antigens (HLA) haploidentical living-related kidney, despite the fact that she did not refer any sensitization event before transplantation. The complement-dependent cytotoxicity and flow cytometry crossmatches were negative for T and B cells. Retrospectively, IgM antibodies against donor precursor endothelial Tie-2^+ cells were detected using a commercially available assay and the pre-transplant serum sample. This case illustrates the necessity of detection of other than the classical HLA-directed antibodies prior organ grafting.

Keywords: accelerated rejection; Tie-2 antibodies

Background

Since 1964, the role of preformed donor-directed human leucocyte antigen (HLA) antibodies has been acknowledged [1]. The crossmatch using lymphocytes and Complement dependent cytotoxicity (CDC) enhanced by anti-human globulin augmented or flow cytometry (FC) crossmatch are performed routinely in our centre before transplantation. In general, 10–20% of the transplanted patients present early rejection episodes despite a negative crossmatch [2] indicating that other molecules than HLA participate in rejection. These molecules, expressed only on the grafts' endothelial cells (EC), include HLA-E, major histocompatibility complex Class I chain-
related genes (MICA/MICB), non-polymorphic tissue-specific antigens and various polymorphic antigens known as endothelial antigens [3]. Consequently, the use of donors' EC as a crossmatch target is necessary for the detection of such antibodies. Several assays have been reported so far for the detection of the anti-EC antibodies (AECA) with no clinical value. Recently, a new assay making use of cells expressing the Tie-2 receptor was reported [4] and subsequently tested in a multicentre study [5]. Tie-2, a receptor tyrosine kinase of angiopoietin which relays signals for EC migration, proliferation and survival, is expressed on 2.0 $\pm$ 0.3% of the total population of peripheral blood mononuclear cells [6]. We report on an accelerated rejection of a kidney graft from a living-related donor that resulted in graft loss and associated with IgM antibodies against the Tie-2^+ donor cells.

Case report

A 27-year-old woman with end-stage renal disease secondary to focal segmental glomerular sclerosis (FSGS) who had a haemodialysis history of 6.5 months received an ABO compatible and HLA haploidentical graft from her father. Patient's HLA and MICA typing was performed using DNA. The recipient had no history of sensitization. No sign of anti-HLA or MICA antibodies was seen when tested with Single Antigen Bead technique (One Lambda, Inc. Canoga Park, CA). To avoid FSGS recurrence, the patient underwent three immunoadsorptions in the perioperative period. Immunosuppressive treatment consisted of mycophenolate mofetil, cyclosporine and methylprednisolone as well as basiliximab on Day 0 and Day 4. Autologous and heterologous IgG and IgM CDC augmented and FC crossmatches performed before transplantation were negative.

The surgery was uneventful but the immediate postoperative course was complicated with slow graft function. The patient did not produce any urine on the day of transplantation. The diethylenetriamine pentaacetic acid scan on the first post-operative day showed good uptake but no excretion. The patient produced ~150 mL of urine/day. At Day 5, she developed acute abdominal pain. The triplex of renal vessels showed thrombosis of renal vein and the patient underwent emergent graftectomy. Histological examination revealed transmural infiltration of the

arteriolar wall by neutrophils and severe intimal arteritis with massive collections of neutrophils into the oedematous intima of small and medium size vessels lifting the endothelium suggestive of severe vascular rejection. Large vessels showed evidence of endarteritis with lymphocytes in the arterial intima in association with focal lymphocytic interstitial infiltrates and focal mild tubulitis. Additional findings were focal cortical haemorrhages, neutrophilic margination in peritubular capillaries and an area of ischaemic necrosis. Although C4d immunohistochemical stain was negative, the histological findings were strongly suggestive for acute antibody-mediated rejection accompanied by cellular rejection.

Retrospectively, a heterologous and autologous IgG and IgM FC crossmatch with Tie-2^+ donor cells (XM-One; Absorber AB, Stockholm, Sweden) was performed with the same pre-transplant serum sample used for the decisive crossmatch. The heterologous IgM-Tie-2 crossmatch was positive with 139 channels shift. The IgM-Tie-2 crossmatch remained positive 5 months post-nephrectomy and was positive when serum was tested against a pool of three unrelated donors. The IgG-Tie-2 crossmatch was negative with the same serum and became positive only after nephrectomy. In that case, the patient had developed de novo IgG donor HLA antibodies identified only with solid-phase assays.

For the retrospective Tie-2 crossmatch, Institutional Review Board approval was obtained from Laikon Hospital and informed consent was obtained from recipient and donor prior to bleeding.

Discussion

This case shows the association of pre-existing IgM non-HLA antibodies with an antibody-mediated accelerated rejection that occurred in the absence of any detectable anti-HLA antibodies. This accelerated rejection can be explained by the pre-existing IgM antibodies against donor Tie-2^+ precursor endothelial cells, not being directed against HLA. The presence of IgM AECA after the nephrectomy against a pool of unrelated donors makes the second transplantation of the patient problematic.

The positive IgG Tie-2 crossmatch observed 5 months after the transplantation is due to the sensitization of the patient against the HLA antigens of the graft, which are expressed on the Tie-2-positive cells.

Recent findings in the literature of a multicentre study show the association of acute rejections with the positive pre-endothelial crossmatch [5]. Furthermore, two case reports [7, 8] show the correlation of the pre-endothelial crossmatch with acute and hyperacute rejection.

The prevalence of donor-reactive preformed AECA in transplantation has been estimated between 2 and 6% [9]. Lucchiari *et al.* [10] refers that 8/25 eluates of kidneys with acute vascular rejection included IgM antibodies to umbilical cord EC. The clinically relevant EC antigens and antibodies remain unclear, although some EC antigens have been identified and characterized with their molecular weight.

Since no screening for these antibodies is currently available, only a direct pre-transplant crossmatch can be performed. In our opinion, this assay is of value and seems to correlate in some cases with high-immunological risk transplants. Further studies are required in order to define the antigens recognized by these antibodies allowing comprehensive screening and crossmatching techniques.

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Conflict of interest statement. None declared.

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