HLA Class II-Specific Antibodies Can React With T Cells in Flow Cytometry Crossmatch: A Case Report

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ABSTRACT

HLA Class I–specific antibodies are usually detected using a flow cytometry HLA crossmatch using T cells. A positive case is shown as a single, right-shifted peak on the anti-IgG FITC histogram of T cells. We report a case showing 2 peaks occurring concurrently, both positive and negative. The positive peak resulted from the binding of HLA Class II–specific antibodies to donor-activated T cells expressing the HLA-DR antigens. This case suggested donor-specific HLA Class II–specific antibodies can bind to some T cells as well as to whole B cells.

LOW cytometry crossmatch (FCXM) is one of the routine techniques of histocompatibility testing.1,2 The HLA antibodies detected using T-cell FCXM are regarded as Class I–specific because HLA Class II molecules are mainly expressed on monocytes and B cells among white blood elements. On the anti-IgG FITC histogram of T cells in this T-cell FCXM, positive cases are shown as a right-shift of a single peak. However, we experienced the curious appearance of 2 peaks in a patient awaiting a renal transplant: 1 negative and the other positive. Further investigation revealed that the positive peak resulted from the binding of HLA Class II–specific antibodies to activated T cells.

CASE STUDY

A 61-year old woman, who was diagnosed with chronic renal failure due to chronic glomerulonephritis beginning 15 years earlier, underwent maintenance hemodialysis and was admitted for a first renal transplantation displaying a serum creatinine value of 10.9 mg/dL.

Histocompatibility Testing

The results for the patient and her son donor showed 1 A, B, DR mismatch with donor DR4. HLA Class II–specific antibodies against DR53, which is the second DR of HLA DR4, were detected in her serum by using a panel-reactive antibody (PRA) test using enzyme-linked immunosorbent assay. The anti-human globulin (AHG)–augmented complement-dependent lymphocyte-toxicity crossmatch, using the total lymphocytes, was negative, although 10% of cells were dead in the 1:1 well. A B-cell FCXM was positive with a patient/control mean fluorescent intensity (MFI) ratio of 8.1 (cutoff, 3.0). However, the T-cell FCXM showed 2 curious peaks on the anti-IgG FITC histogram. Among the 2 peaks, 1 was negative and the other positive with an MFI ratio of 11.9 (cutoff, 2.0) (Fig 1A). Therefore, it was speculated that the population forming the subsidiary positive peak was a T cell one expressing HLA Class II molecules. This hypothesis was confirmed by performing another FCXM using 3-color staining: anti-HLA-DR FITC, anti-IgG PE, and anti-CD3 PE-Cy5. Analysis of the anti-IgG PE/anti-HLA-DR FITC plot of the previously gated T cells revealed that HLA antibodies were bound to the HLA-DR–positive T cells (Figure 1B). An analysis of the donor peripheral blood also confirmed that HLA-DR–positive T cells represented 30% of the total circulating T cells (Fig 2).

Clinical Progress

Plasma exchanges were performed 3 times before the renal transplantation with intravenous administration of Ig to decrease the blood level of HLA antibodies. However, the B-cell FCXM MFI ratio increased from 8.1 before the first exchange to 13.2 and 17.2 after the second and third exchanges, respectively (Fig 3). Induction therapy using anti-thymocyte globulin for 5 days was prescribed to prevent graft rejection. Maintenance immunosuppression included tacrolimus, mycophenolate mofetil, and corticosteroid. However, the creatinine level increased from 4.2 mg/dL before transplantation to 5.8 and 5.3 mg/dL on the 1st and 4th postoperative days, respectively. On the 8th day when the creatinine level was 4.2 mg/dL, the AHG-CDC crossmatch was positive (1:1) and both the T-cell and B-cell FCXM were positive with increased MFI ratios of 14.6 and 22.8, respectively. With the aid of appropriate treatments, the creatinine level normalized on the day 30.
On day 55, the AHG-CDC crossmatch and T-cell FCXM were both negative. Although the B-cell FCXM was still positive, the MFI ratio decreased significantly to 4.3. Currently, on day 118, the function of the transplanted kidney remains good with a creatinine level of 0.7 mg/dL.

**DISCUSSION**

Some circulating T cells in the peripheral blood of healthy people express HLA Class II molecules on their surface. In this case, 3-color flow cytometry revealed HLA antibodies bound to the HLA-DR–positive T cells (Fig 1B). This finding was also supported by the PRA test, which detected a donor-specific HLA Class II–specific antibody (DR53) in the patient serum. Hence, it was concluded that this HLA antibody in the crossmatch was bound to some donor T cells as well as to whole B cells.

Although the graft survival rates of offspring to mother renal transplantations are equivalent to those of other living donors, accelerated rejection can result from an anamnestic reaction after in utero exposure of the mother to the HLA antigens of the fetus with sensitization developing during pregnancy. In this case, strong reactivity to donor B cells persisted when reactivity by the non-HLA-specific antibodies was excluded based on heat inactivation of recipient serum. However, the paradoxically increased MFI ratio after plasma exchanges was not completely understood. Possible causes included an intrinsic inaccuracy in the MFI ratio, a rebound reaction to the plasma exchanges, or the intravenous administration of Ig.

This case appears to represent the detection of HLA Class II–specific antibodies in the T-cell FCXM. However, this phenomenon may be rare because it requires an increase in activated T cells that express HLA Class II molecules in the donor, which are targeted by recipient HLA Class II–specific antibodies. Nevertheless, the obser-
vations in this case might explain other cases in which the sera with HLA Class II–specific antibodies bind to some T cells in FCXM or even AHG-CDC crossmatches.

REFERENCES