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Kidney transplant monitoring by anti donor specific antibodies

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Summary. Donor-specific anti-HLA antibodies were studied by cytotoxicity crossmatching (CTXM) and flow cytometry crossmatching (FCXM) in 117 kidney transplant candidates; the same study was carried out in 33 cadaver-donor kidney recipients, during the first 3 post-transplant months, for which donor cells were available. Pre-transport evaluation showed that 82.9% of subjects were CTXM negative/FCXM negative, 6.8% of patients were positive in both tests, and 10.3% were CTXM negative/FCCM positive. Post-transplant monitoring for donor-specific antibodies (Abs-DS) showed that nine recipients (27.3%) were FCXM positive; six of them were IgG + and three IgM +. In comparing these results with the clinical course, a significant association between FCXM IgG + and rejection episodes was observed ($P < 0.01$).

Key words: Cytotoxicity crossmatching – Flow cytometry crossmatching – Donor-specific antibodies – Kidney transplantation

Specific anti-HLA antibodies (directed against "non-self" HLA antigens present on transplanted allograft cells) are an important element for the success of kidney transplantation. The presence of these antibodies is inevitably associated with acute or early rejection episodes; positive crossmatching, performed with the standard NIH technique – cytotoxicity crossmatching (CTXM) – is a definite contraindication to kidney transplantation. In various transplant centers, however, cases of acute or early rejection episodes have been observed in CTXM negative subjects as well [1]. These results have stimulated the study of pre-transplant anti-HLA antibody screening, which has led to the development of techniques that are more sensitive and reliable than CTXM. Among these, the flow cytometry technique (FCXM) has been proven to be one of the most interesting [2]. Donor-specific antibodies (Abs-DS) can be directed against T and B lymphocytes, they may or not be complement fixing, they can recognize class I or II antigens and, finally, they may belong to IgG or IgM classes. The CTXM does not allow for easy and rapid antibody characterization and does not provide a full picture of the recipient's pre-transplant immune state. The FCXM, however, has proven to be a more sensitive technique for pre-transplant Abs-DS screening; it has been shown that those transplanted subjects with a negative CTXM, but a positive FCXM, have a higher incidence of acute rejection episodes during the post-transplant period [3, 4]. Furthermore, after transplantation, the FCXM allows the monitoring of specific humoral immunoreactivity directed against the transplanted organ.

The present study reports our experience in the employment of FCXM, both in the screening of kidney transplant candidates and in the monitoring of post-transplant Abs-DS.

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Materials and methods

Pre-transplant screening. From June 1988 to June 1991, 117 kidney transplant candidates were studied, both by CTXM and FCXM. The organ assignement was based on a high degree of HLA-A, B, DR compatibility and on a negative result using CTXM; a positive FCXM did not contraindicate transplantation. We performed 60 transplants from cadaver-donors and all recipients were on their first transplant.

Abs-DS post-transplant monitoring. Of the 60 transplanted patients, 33, whose donor's lymphocytes were available, were studied for Abs-DS appearance during the first 3 post-transplant months. All patients had undergone an immunosuppressive protocol consisting of azathioprine, cyclosporine and prednisone; rejection episodes were treated with 1 g methylprednisolone/day for 3 days.

Complement-mediated cytotoxicity cross-matching. All sera were incubated with donor lymphocites for 60 min at room temperature. After the addition of complement, the sera were again incubated for 60 min at room temperature [5].

Flow cytometry cross-matching. This was performed by double staining, using an anti-CD3 and anti-CD20 phycoerytrin monoclonal

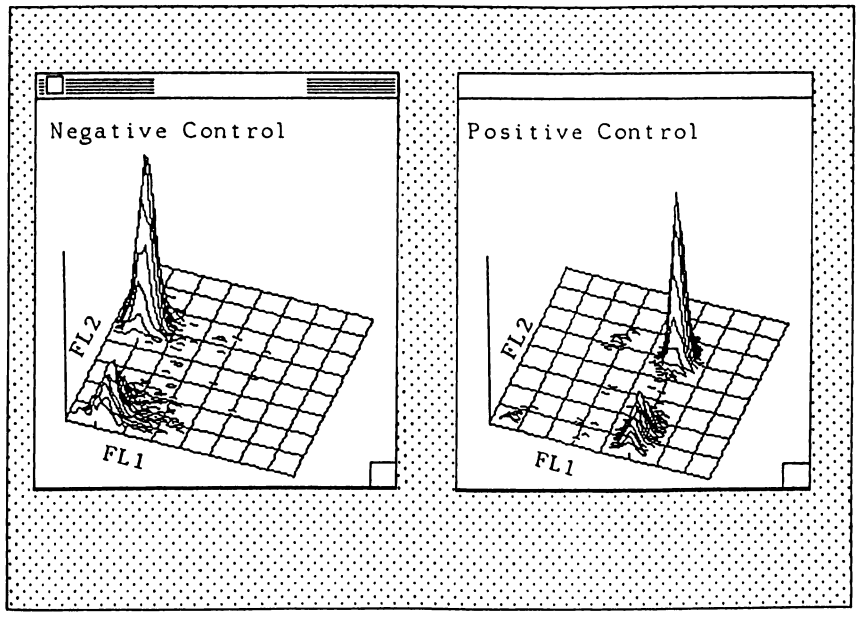


Fig. 1. FCXM. Fl1 = anti IgG o IgM; Fl2 = anti CD3 o CD20

Table 1. CTXM and FCXM results obtained from 117 patients selected for cadaver-donor kidney transplant

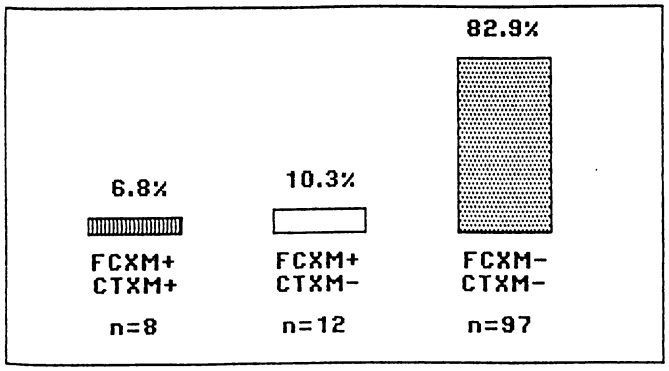
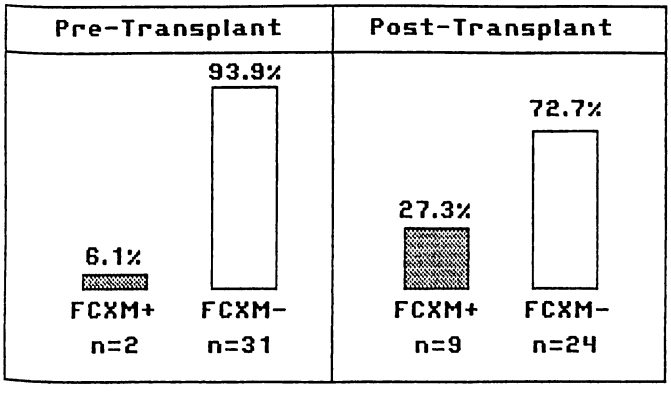


Table 2. Pre- and Post-Transplant FCXM in 33 cadaver-donor kidney transplants



antibody to identify T and B lymphocytes and using fluorescein stained anti-human IgG or IgM F(ab')2 antibodies to identify anti-lymphocyte antibodies present in the serum [6]. Positive and negative control sera were obtained, respectively, from a serum pool with a high reactivity to a cell panel (> 90%) and from a negative serum pool obtained from nontransfused male subjects. Samples were analyzed on a FACScan flow cytometer (Becton-Dickinson) and data were collected using "Lysis" software (Becton-Dickinson) (Fig. 1). The sera that presented a shift greater than 10 channels from the fluorescence 1 (fluorescein) mean curve were considered positive.

Results

Pre-transplant screening

Of the patients selected for transplantation 82.9% were CTXM negative/FCXM negative; 6.8% were positive on both tests, and 10.3% were CTXm negative/FCXM positive (Table 1).

Post-transplant monitoring

We studied 33 transplanted patients of whom 6.1% were FCXM positive before the transplant, while, at 3-month follow-up, 27.3% were positive for FCXM IgG or IgM (Table 2). During our study, 11 rejection episodes occurred and one graft was lost due to irreversible rejection. The relationship between acute rejection and the presence of Abs-DS demonstrated a significant association between these two factors ($P < 0.05$); moreover, we observed a significant association between FCXM IgG + and acute rejection ($P < 0.01$) (Table 3).

Discussion

Compared to the CTXM the FCXM has been proven to have a higher sensitivity in identifying transplant patients with Abs-DS. If the concentration of Abs-DS is lower than is required for a complement-metiated cytotoxic reaction, or if they do not fix complement, the CTXM gives

Table 3. Relationship between donor-specific antibody class and rejection episodes in 33 cadaver-donor transplants

	FCXM −	FCXM +	
		IgG	IgM
No patients	24	6	3
No rejections	5	5	1

$P < 0.01$

a negative or dubious result. The detection of Abs-DS by FCXM suggests the presence of a low titer sensitization state, which does not necessarily lead to a secondary immune response after transplantation. The clinical value of detecting these antibodies before transplantation must be correlated both to the donor-recipient HLA compatibility level and to the immunosuppressive protocol [3, 7].

In our study, monitoring in FCXM-positive recipients showed a constant decrease in the Abs-DS titer during the first post-transplant months. However, the post-transplant detection of Abs-DS by FCXM represented specific sensitization against the transplanted organ. The significant relationship, which we found between rejection episodes and post-transplant positive FCXM, confirmed the negative prognostic value of the appearance of Abs-DS. The cell immune response occurring during a rejection episode is undoubtedly accompanied by a humoral immune response. The appearance of Abs-DS is an expression of the patient's immune state, and their persistence may have a negative prognostic value in the long-term success of the transplant. Furthermore, of extreme importance is the Abs-DS class, since IgM antibodies (unlike IgG antibodies) do not seem related to the risk of acute rejection episodes.

In conclusion, compared to the CTXM technique, the FCXM technique had great advantages both before the transplant, providing a better and more exhaustive immunologic evaluation of the patient, and after the transplant, for identifying those subjects at a higher risk of rejection. In our experience, Abs-DS monitoring by FCXM was an important immunological test, providing useful information for a more effective immunosuppression.

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