

STUDY OF PRE-SENSITIZATION BY FLOW CYTOMETRY IN CADAVERIC KIDNEY RECIPIENTS

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Summary

We evaluated the role of crossmatching by the fluorescent activated cell sorter (FACS) on the outcome of 117 cadaveric kidney recipients transplanted in 1983. Although all patients were T cytotoxic crossmatch (XM) negative at the time of transplant, 38 per cent were FACS T positive. In the transfused patients (n=103), the graft survival at one year was 70 per cent with a negative FACS T XM versus 48 per cent with a positive FACS T XM ($p \leq 0.04$). Among patients reactive against the panel (PRA >10%), the effect of a negative FACS T XM was associated with 72 per cent graft survival at one year versus 36 per cent for those FACS T XM positive ($p \leq 0.04$). Patients with B warm XM negative and FACS T XM negative patients had 72 per cent graft survival in the first year and 40 per cent if they were B warm XM positive and FACS T XM positive ($p \leq 0.04$). This study confirms the high sensitivity of the FACS crossmatch. It shows a worse outcome for those patients bearing antibodies detected by the FACS against T cells. The effect of a positive FACS T XM is more evident in those patients reactive against the panel.

Introduction

It is well recognized that pre-sensitization as characterized by the presence of preformed anti-donor antibodies in the serum of recipients can be responsible for hyperacute and accelerated rejection [1]. With the ability to separate lymphocytes into T and B cell populations, it was shown that the antibodies responsible for these types of rejections are mainly those directed against the T cell [2]. While some authors have shown that a B positive crossmatch can improve graft survival [3], others have not found any beneficial effect of these antibodies [4]. These conflicting observations may be due to the large heterogeneity of specificities among anti-B cell antibodies. Flow cytometry is able to accurately separate antibodies binding to either T or B cells and is up to 250 times more

sensitive in detecting antibodies than enhanced (antiglobulin) cytotoxicity cross-matches [5]. In this study we evaluated the role of the FACS XM in detecting states of pre-sensitization that could influence initial cadaveric graft outcome.

Material and methods

All cadaveric recipients transplanted at the UCSF Medical Center during 1983 were included. Prior to transplantation the T cell cytotoxic crossmatch had to be negative by the antiglobulin technique using the most current and the most reactive serum. FACS T and B crossmatch as well as the B warm cytotoxic crossmatch were studied retrospectively. Each patient was also studied prospectively for panel reactive antibodies. Among the 119 cadaveric kidney transplants performed during this period, two had to be excluded because of non-availability of frozen donor lymphocytes.

The group of patients (n=117) examined included 43 females, mean age 36.5 ± 1.9 years (31 multiparas) and 74 males, mean age 37.8 ± 1.42 years. Ninety-nine were first transplants and 18 were second or third transplants. HLA typing gave an average of 0.34 matches for DR and 0.77 matches for the A and B loci per patient. One hundred and three received previous blood transfusions and 36 patients had five or more units. The post-transplant immunosuppressive therapy consisted of oral prednisone 2.0mg/kg and azathioprine 2.0mg/kg at the time of transplant. Standard rejection therapy consisted of intravenous prednisolone (8.0mg/kg) for three days, or oral prednisone (3.0mg/kg) and rabbit ATG or ATGAM in those patients with steroid resistant rejection.

Fluorescent activated cell sorting (FACS) crossmatch

Instrumentation Flow cytometry was performed utilizing a modified Becton-Dickinson (Sunnyvale, CA) Fluorescent Activated Cell Sorter (FACS II) with a 5-watt argon-ion laser at 488nm and dual log amplification, with forward and right angle scatter and two fluorescent detectors. A DEC MINC ELEVEN with LSI 11/03 CPU (Digital Equipment Corp, Maynard, MA) was interfaced for storage and histogram analysis.

Cell preparation The target lymphocytes of the cadaveric donors were obtained from previously frozen lymphnode or spleen preparation using Ficoll-Hypaque density gradient separation. The cells were suspended in RPMI 1640 media containing Hepes buffer and four per cent heat inactivated fetal calf serum at one million cells/ml. The incubating and staining technique was performed as previously described [5]. Briefly, one millilitre of cell suspension was added to Fisher tubes and centrifuged at 2000 x G for one minute. The supernate was aspirated and cells resuspended in the residual supernate. Serum to be tested was added (0.1ml) and incubated for 30 minutes at 22°C. The cells were then washed twice by adding phosphate buffered saline (PBS) containing 0.1% sodium azide. Fluoresceinated goat F (ab') antihuman immunoglobulin, which had been centrifuged at 100,000 x G for 60 minutes (Beckmann-Airfuge) to

remove aggregated material, was added ($10\mu\text{g}$) and the mixture incubated for 20 minutes at 4°C and then analysed on the FACS II. Negative controls consisted of donor lymphocytes incubated in RPMI 1640 containing Hepes buffered four per cent heat inactivated fetal calf serum, or incubated with normal human sera (NHS) or uraemic sera and positive control consisted of donor lymphocytes incubated with a known HLA-A,B antibody. All samples were tested in duplicate. The pre-transplant sera tested included the most current sera, the serum exhibiting the highest degree of screening panel reactivity and one to four other pre-transplant sera (average of 4.15 sera per patient). FACS histograms were generated after gating on the lymphocyte population and counting 10,000 cells. The result is expressed as the number of channels by which the mean of the histogram shifted to the right (increasing fluorescence). Since B cells naturally bear surface immunoglobulin, two different peaks corresponding to T lymphocytes and B lymphocytes (plus monocytes) are observed. Increased amounts of antibodies bound to the T and or B cells increases their fluorescence and causes a shift of each peak to the right. On a log scale a doubling of antibodies gives a 10 channel shift against the reference negative controls. The reference point against which any shift was measured, was the lowest reading of either the NHS control, uraemic sera or one of the patient's own sera.

B cytotoxicity crossmatch

T and B cell separation of the lymphnode and spleen cells of the donor was performed by the nylon-wool technique. T cell cytotoxicity was tested by the NIH, Amos and Antiglobulin technique. The B cytotoxic crossmatch was evaluated through the Terasaki-NIH microcytotoxicity test with a two hour incubation time at 37°C .

Statistical analysis We used the Cox proportional model of hazard in order to evaluate the factors influencing the graft outcome in this patient population [6]. Actuarial survival curves were calculated based on each of these factors as a function of survival. When comparisons were made between groups the χ^2 test was used to evaluate statistical significance. Statistics for two-way tables were by the χ^2 test. Means are expressed as the mean \pm SE.

Results

Sensitization Based on our previous study, we have considered nine or more channel shifts on a log scale as indicating the presence of antibody on the target cell. Forty-three (37%) of the patients had a positive FACS T crossmatch on peak sera and 69 (59%) had a positive FACS B crossmatch. Table I shows the results of FACS crossmatch and the distribution of the patients according to the degree of channel shift of the FACS histogram on the serum providing the largest channel shift. Only two patients had a larger shift in serum other than the peak serum.

TABLE I. Largest channel shift in FACS crossmatch

	FACS T	FACS B
Negative: 0-5	44	16
5-10	29	32
	} 62%	} 41%
Positive: 10-15	22	34
≥15	23	35
	} 38%	} 59%

Clinical outcome In general the deleterious effect of a positive FACS T XM on graft survival could be observed in the first month post-transplant and from this point it remained constant. The first year graft survival for all transfused patients with FACS T positive was 48 per cent against 70 per cent if they were FACS T negative ($p \leq 0.04$) (Figure 1). There were too few patients ($n=14$) that had not

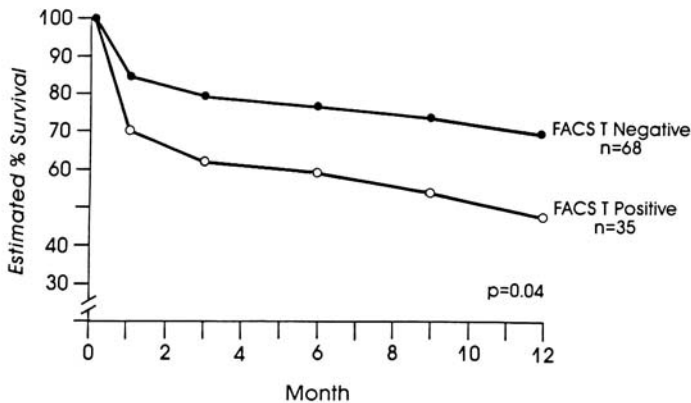


Figure 1. Influence of FACS T crossmatch on graft outcome of transfused patients

been transfused to analyse this population separately. In the transfused patients reactive against >10 per cent of the panel the graft survival at one year was 72 per cent if they were FACS T XM negative and only 36 per cent if they were FACS T XM positive (Figure 2). In this study the B warm crossmatch by itself did not significantly influence the survival of the graft. However, if associated with B warm antibodies there were FACS T antibodies, the graft outcome was significantly worse than in the group lacking both antibodies, 40 per cent versus 72 per cent graft survival at one year respectively.

In examining the incidence of FACS reactivity, those patients with PRA greater than 10 per cent were more often FACS T positive; in contrast, patients with PRA lower than 10 per cent were more often FACS T negative ($p < 0.05$).

Cytotoxic B warm antibodies were found in 49 (43.7%) of patients. There was a significant association between the likelihood of finding B warm antibodies in patients who were pre-sensitized ($p < 0.05$). The occurrence of FACS T

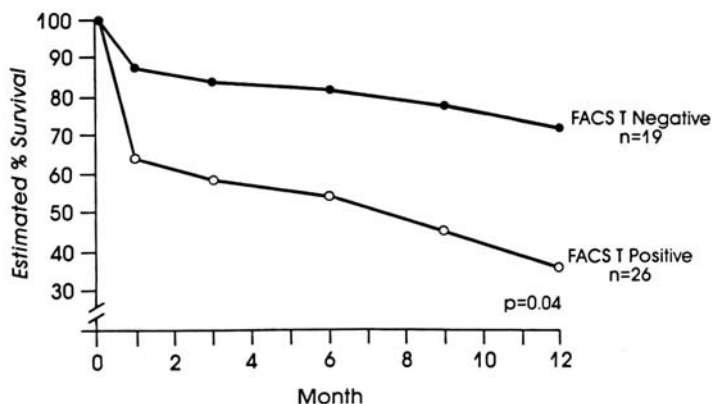


Figure 2. Influence of FACS T crossmatch on graft survival of patients with PRA >10%

crossmatch was also higher among the positive B warm crossmatch patients. This is consistent with the observation that many weak HLA-ABC antibodies may be detected on B lymphocytes but missed by T cytotoxic tests.

Discussion

The increased sensitivity of the FACS crossmatch technique was again confirmed by finding FACS T positivity in over a third of patients who had a negative T cytotoxic crossmatch. The presence of these FACS T antibodies appears to be clinically relevant since the outcome was significantly worse when they were present (48% survival versus 70% FACS T negative). There were no significant differences between patients who possessed or lacked B warm antibodies. However, when B warm antibodies were accompanied by FACS T cell antibodies there was a significant decrease in graft survival (72% versus 40%). Hence it may be important to determine when B warm antibodies are due to weak HLA-A,B specificities and when to 'true' B cell (HLA DR, DQ, DP) specificities. Our data are also consistent with the finding of lower graft survival in patients with B warm antibodies that could be removed by platelet absorption [4]. Whereas those patients with positive B warm cytotoxic antibodies but negative FACS T crossmatch may be bearing enhancing antibodies [3]. FACS crossmatching is relatively simple to perform, often requiring no more than two hours of cell preparation and staining time. Its increased sensitivity and ease of use offers a new approach in our search for the continued improvement of kidney transplant survival.

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