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IMPACT OF DONOR-SPECIFIC ANTIBODIES ON CHRONIC REJECTION OCCURRENCE AND GRAFT LOSS IN RENAL TRANSPLANTATION: POSTTRANSPLANT ANALYSIS USING FLOW CYTOMETRIC TECHNIQUES[1]

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Background. Improvements in immunosuppressive therapy have greatly reduced acute rejection (ARj) episodes, ensuring better short-term graft outcome, but have not modified long-term survival in renal transplantation. It is now well accepted that chronic rejection (CRj) can be determined by both immune and/or nonimmune mechanisms. The aim of this study was to evaluate the importance of the posttransplant humoral immune response towards mismatched HLA graft antigens in CRj occurrence and graft outcome.

Methods. Serum samples from 120 nonpresensitized renal transplant recipients were prospectively screened for 1 year after surgery by means of flow cytometry cross-match (FCXM) and FlowPRA beads (microbeads coated with purified HLA class I and class II antigens) assays. All transplants were followed-up for 2 years or until graft removal.

Results. FCXM monitoring identified donor-specific

antibodies (DS-Abs) in 29 (24.2%) of 120 transplanted patients. Correlation with clinical data highlighted a higher incidence of ARj in DS-Abs–positive patients compared to negative patients (62% vs. 13%, *P*<0.00001). Furthermore, graft failure occurred more frequently among FCXM-positive patients than among negative patients (34% vs. 1%, *P*<0.00001). The deleterious effect of DS-Abs on graft function was confirmed by serum creatinine levels 2 years after transplantation. These were in fact higher in subjects producing DS-Abs than in subjects with only ARj (mean creatinine: 2.5±1.3 mg/dL vs.1.7±0.5 mg/dL, *P*=0.04). Flow-PRA analysis of DS-Ab HLA specificity highlighted the presence of anti-HLA class I antibodies in 85% of FCXM-positive patients, who also presented with a higher incidence of HLA-B mismatches than FCXM-negative patients (1.23±0.66 vs. 0.92±0.59, *P*=0.02).

Conclusions. **Flow cytometric techniques are precious tools for investigating the activation of the humoral response against HLA antigens of the graft in renal transplantation. DS-Abs production has a worse impact on organ function and survival than ARj episodes. These findings represent further proof of the threat posed by DS-Abs on long-term graft function and draw attention to the need for a specific immunosuppressive therapy aimed at counteracting the different kinds of immune activation toward graft.**

The clinical use of cyclosporine in renal transplantation has improved short-term allograft survival (*1*) but has not produced similar results for long-term survival (*2*). Several studies have shown that the occurrence of early acute rejection (ARj) episodes and the appearance of donor-specific antibodies (DS-Abs) are the most important risk factors for the development of chronic graft failure (*3–5*). Recent studies have emphasized that the production of cytotoxic DS-Abs constitutes a negative prognostic factor for 1-year graft survival.

As far as the methodology to reveal the presence of alloantibodies is concerned, flow cytometry cross-match (FCXM) has shown to be more sensitive than complement-dependent lymphocytotoxicity (CDC) (*6–8*). The higher sensitivity of FCXM is due to its ability to identify complement-fixing and nonfixing antibodies and to simultaneously define the class of the detected immunoglobulin (IgG and/or IgM) and the different types of target cells (T and/or B lymphocytes) (*9*). More recent is the FlowPRA screening test, a cytometric technique that uses microbeads coated with purified HLA antigens and allows the characterization of anti-HLA class I or II specificity (*10*).

Plenty of data are available concerning the clinical usefulness of flow cytometry cross-matching before renal transplantation. Several studies have shown that a positive FCXM before transplantation leads to a higher incidence of rejection episodes in primary renal transplantation and early graft loss in re-graft recipients (*11–14*). There is, on the other hand, limited information concerning the importance of posttransplant FCXM in monitoring donor-specific humoral immune response (*15–17*) and predicting the occurrence of chronic graft failure (*18–19*).

This study was aimed at characterizing posttransplant humoral immune response against mismatched HLA antigens of the graft to investigate the clinical relevance of such DS-Abs and evaluate the role of these alloantibodies in chronic rejection (CRj) occurrence and renal graft survival. For this purpose, 120 cadaveric renal-transplanted patients were prospectively monitored with FCXM during the first year after transplantation.

MATERIALS AND METHODS

Patient population. Among the patients who underwent cadaveric renal transplantation at the Transplant Unit of Clinical Surgery, Tor Vergata University of Rome, between the years 1992–1997, only 120, who were negative for the presence of preformed antibodies against donor cells using both CDC-XM and FCXM and whose donor lymphocytes were available, were enrolled in the study. All but four patients had received kidney transplantation for the first time. Organs were assigned according to the best donor-recipient HLA compatibility; matching priority was HLA-DR, HLA-B, and HLA-A. Pretransplant patient characteristics are shown in Table 1.

Immunosuppression protocol. All patients received a triple-therapy regimen that consisted of cyclosporine/prednisone/azathioprine (Aza) or mycophenolate mofetil (MMF). Oral cyclosporine was administered twice daily, starting with 8 mg/kg/day from the day after transplantation, and it was subsequently adjusted according to serum cyclosporine levels. On the day of surgery, 500 mg of methylprednisolone was administered. Successively, corticosteroid therapy consisted of 20 mg/day of prednisone, reduced to 10 mg/day at 1 month and to 5 mg/day after 3 months. Aza (1.5 mg/kg/day) or MMF (1500 mg/day) doses were adjusted according to the patient's leukocyte count. Only 16 of 120 subjects assumed MMF instead of Aza as an immunosuppressant; three of the subjects started it from the day of surgery, whereas the other 13 changed to MMF after the first rejection episode. ARj treatment consisted of three boluses of methylprednisolone (0.5-1 g/dose).

Clinical outcome parameters. All patients were followed-up for at least 2 years after transplantation or until removal of the transplant. Graft function was monitored by studying serum creatinine levels and ARj occurrence. The latter was assessed by clinical symptoms and, in all but three cases, confirmed by needle core biopsy. Histological diagnosis of CRj was made in five cases. We moreover considered as graft failure, because of CRj, the six cases in which serum creatinine levels were persistently higher than 4 mg/dL at several subsequent controls.

Graft failure or patient death due to infectious or cardiovascular causes was recorded in one FCXM−/ARj+ patient, one FCXM+/ARj− patient, and four FCXM−/ARj− patients.

FCXM. Patients' sera were screened for the presence of DS-Abs with flow cytometry cross-match. Samples were collected before transplantation, at regular intervals during the first year after transplant (on days 7, 14, 30, 60, 90, 120, 150, 180, 270, and 365), and each time clinical symptoms led us to suspect the occurrence of ARj.

Donor lymphocytes were collected from lymph nodes or spleen fragments, cryopreserved by a 10% dimethyl sulfoxide solution, and stored in liquid nitrogen until used.

FCXM monitoring was performed using a three-color fluorescence technique (*9*). Briefly, 2.5×10^5 donor lymphocytes were incubated with 75 μl of undiluted serum for 30 min. at 25°C. After two wash steps with phosphate-buffered saline solution that contained 5%

TABLE 1. Pretransplant recipient characteristics

Age, mean±SD (yr)	39.3±10.9
Gender (M/F)	74/46
Transplant no.	
1	116
2	4
Transfused patients (%)	50
No. of transfusions/patient, mean±SD	3.6±3.3
HLA-AB mismatch, mean±SD	1.9±0.9
HLA-DR mismatch, mean±SD	0.6±0.6

TABLE 2. Posttransplant patient population characteristics[a]

ARj episodes	
0	90 (75%)
1	24 (20%)
2	6 (5%)
Time to first ARj	
<3 months	25 (20.8%)
>3 months	5 (4.2%)
Graft failure	
Arj	1 (0.8%)
CRj	10 (8.3%)
Other	6 (5%)
DS-Ab–positive patients	29 (24.2%)
Time to DS-Ab appearance	
<3 months	24 (20%)
>3 months	5 (4.2%)

[a] Abbreviations used in table: ARj, acute rejection; CRj, chronic rejection; DS-Abs, donor-specific antibodies.
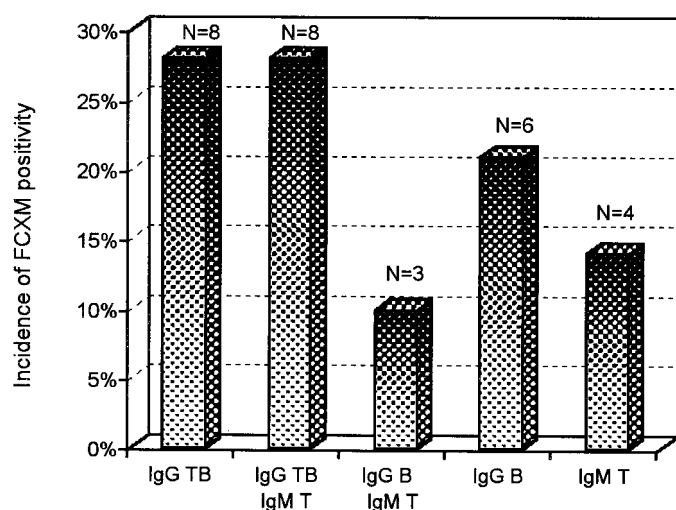

FIGURE 1. Analysis of FCXM positivity in relation to antibody specificity for donor T and/or B lymphocytes and the class of detected immunoglobulins.

fetal bovine serum and 0.1% sodium azide (PBS-Flow), 50 μl of pretitred fluorescein isothiocyanate (FITC)-conjugated F(ab')$_2$ fragment of goat anti-human IgG or IgM (Dakopatts, Denmark), 5 μl of peridinin chlorophyll protein–conjugated anti-CD3 monoclonal antibody (mAb), and 5 μl of phycoerythrin-conjugated anti-CD20 mAb were added to each tube. After a 30 min. incubation at 4°C in the dark, the cells were washed twice with cold PBS-Flow and resuspended in 200 μl PBS plus 1% paraformaldehyde until analysis. For all FCXMs, donor lymphocytes were incubated with the patient's test serum, a positive control serum (a pool of >5 patients' sera, each containing >90% panel-reactive antibodies) and a negative control serum (a pool of >5 sera from healthy, nontransfused male subjects). Flow cytometric analysis was performed with a FACScan flow cytometer and Cell Quest software (Becton Dickinson, CA). Data were collected by logarithmic amplification, and fluorescence intensity was displayed on 1,024 channels. From each sample, 10,000 cells were captured in the lymphocyte gate, and analysis was performed on the fluorescence 1 (FL1) histogram (anti-human IgG or IgM) using gates on CD3- and CD20-positive cells. A positive cross-match was defined as a median channel shift of more than 2 SD above the predetermined median channel shift from the negative control. To exclude false positives caused by the presence of auto-reactive

antibodies, all positive samples were screened for the presence of such antibodies using the previously described technique.

FlowPRA. All sera that were IgG positive with the FCXM technique were investigated using the FlowPRA class I and II screening test (One Lambda, Inc., CA), which consists of a pool of microbeads coated with purified HLA class I or class II antigens from 30 cell lines covering all common HLA antigens. According to the manufacturer's suggested protocol, class I and II microbeads (5 μl of each type) were mixed with 20 μl of sample serum. The mixture was then incubated for 30 min. at 25°C. After three washes, 100 μl of properly diluted FITC-conjugated goat anti-human IgG (Fcγ) was added, and the samples were incubated again in the same conditions. The microbeads were then washed twice and resuspended in 0.5 ml of fixing solution. Negative and positive control sera were tested in the same manner. From each test tube, 10,000 events were collected and a two-color analysis was performed using a FACScan flow cytometer and Cell Quest software. Two gates were set on the fluorescence 2 (FL2) histogram to analyze class I (FL2-negative particles) and class II (FL2 high-fluorescent particles) beads separately. The percentage of beads that had shifted to the right of the cut-off point set on the FL1 histogram of the negative control serum represented the amount of class I and/or II antibodies detected in each serum sample.

Statistical analysis. The χ^2 test, Mann-Whitney two-sample test, and Student t test were used for statistical comparison. $P<0.05$ was considered significant.

RESULTS

Patients' characteristics and renal allograft outcome. Posttransplant characteristics of the patients are listed in Table 2. Thirty subjects experienced at least one ARj episode, and six of them had two ARjs. Patients who suffered ARj were analyzed according to the early (within 3 months after transplantation) or late (after 3 months) onset of ARj; therefore, 25 of 30 subjects were classified as early onset and the other five as late onset.

Graft failure due to CRj occurred in 10 patients. Only one subject lost the graft because of severe ARj. Moreover, in four cases, failure was caused by severe viral and/or mycotic infections, and two patients died of acute myocardial ischemia.

FCXM results and graft outcome. Analysis of FCXM monitoring during the first posttransplant year showed that 29 (24.2%) patients produced alloantibodies specific for T and/or B donor lymphocytes and that in 24 of them, DS-Abs made a precocious appearance, being detectable within the third month after transplantation. Data in Figure 1 shows that all but four FCXM-positive patients produced IgG DS-Abs, whereas the other four subjects (14%) showed exclusive IgM FCXM positivity. As far as antibody target cells are concerned, we found that just six (21%) of the 29 FCXM-positive

TABLE 3. ARj incidence, graft outcome, and serum creatinine levels (2 years after transplantation) according to FCXM status

	FCXM positive (N=29)	FCXM negative (N=91)	Significance
Acute rejection			
Positive	18 (62%)	12 (13%)	$P<0.00001$
Negative	11 (38%)	79 (87%)	
Graft outcome			
Graft failure	10 (34%)	1 (1%)	$P<0.00001$
Good function	19 (66%)	90 (99%)	
Serum creatinine (mg/dL)[a]	2.46±1.27	1.57±0.54	$P<0.0001$

[a] Data are given as mean±SD.

TABLE 4. 2-year graft outcome based on simultaneous analysis of FCXM and ARj data

	FCXM+/ARj+ (n=18)	FCXM−/ARj+ (n=12)	FCXM+/ARj− (n=11)	FCXM−/ARj− (n=79)
Good function	55.6%	91.7%	81.8%	100%
Graft failure[a]	44.4%[b]	8.3%[b]	18.2%	0%

[a] Considering only failures due to ARj or CRj.
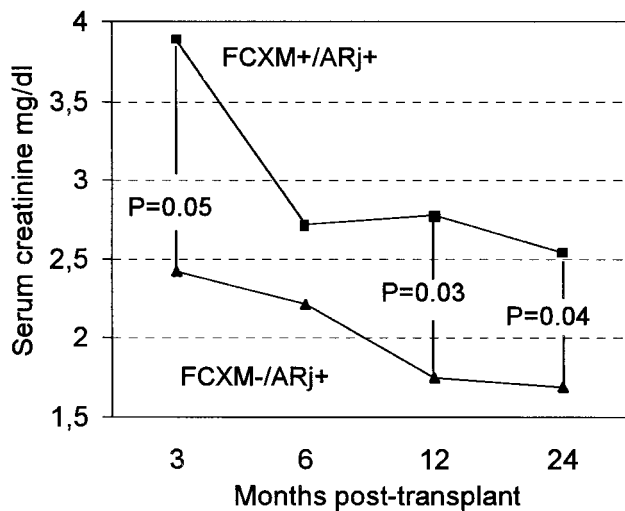[b] $P=0.043$.

FIGURE 2. The importance of DS-Abs production expressed as FCXM positivity in graft function during our follow-up period in subjects with ARj episodes. Mean serum creatinine levels in FCXM-positive patients (■) appeared significantly higher than those of FCXM-negative ones (▲) throughout the observation period.

patients showed IgG DS-Abs exclusively against donor B lymphocytes.

No correlation between the amount and type of FCXM positivity and graft outcome was established.

When we analyzed the length of FCXM positivity, four (16%) subjects showed a persistent positivity throughout our observation and all of them suffered graft loss during the same period. Fifteen of the remaining 25 patients no longer presented serum DS-Abs after 1 month of observation, whereas in the other 10 patients, the disappearance of antibodies took place over a period of clinical monitoring that ranged from 1.5 to 9 months. As far as graft failure is concerned, no significant difference was observed between subjects showing antibody positivity for a short period and those presenting DS-Abs for a long time. This may be explained by the fact that DS-Abs, although still produced, may be bound to the graft or masked into immune complexes formed with soluble HLA antigens released from the graft cells.

The correlation between FCXM-monitoring results and clinical follow-up revealed a strong correlation between antibody production and graft function. In fact, a significantly higher incidence of both ARj and immunologically determined graft failure was observed in FCXM-positive patients compared with negative ones (ARj: 62% vs. 13%, $P<0.00001$; CRj or removal of the graft: 34% vs. 1%, $P<0.00001$). This finding was moreover supported by serum creatinine levels with mean values of 2.46 ± 1.27 mg/dL in FCXM-positive pa-

tients and 1.57 ± 0.54 mg/dL in the negative ones, 2 years after transplantation, thus resulting in a $P<0.0001$ (Table 3).

Noteworthy, in 9 of 18 FCXM+/ARj+ patients, DS-Abs appearance preceded any clinical manifestation of ARj by 10 to 1 days.

Further evidence of the clinical value of FCXM in evaluating graft function was obtained by dividing the patients into four groups according to FCXM-monitoring results and ARj occurrence (group 1: FCXM+/ARj+; group 2: FCXM−/ARj+; group 3: FCXM+/ARj−; and group 4: FCXM−/ARj−) (Table 4). Our data highlighted a significantly higher incidence of graft failure in FCXM+/ARj+ patients than in subjects showing only ARj occurrence without DS-Abs production (44.4% vs. 8.3%, $P=0.043$). A higher incidence of graft failure was, furthermore, ascertained in FCXM+/ARj− subjects (18.2%) than in FCXM−/ARj+ subjects (8.3%).

Renal function, evaluated 3, 6, 12, and 24 months after transplantation, also appears to be more closely related to DS-Abs production than to ARj. In fact, FCXM+/ARj+ patients have always shown higher mean serum creatinine levels than FCXM−/ARj+ ones (3 months: 3.9 ± 2.6 mg/dL vs. 2.4 ± 1 mg/dL, $P=0.0499$; 6 months: 2.7 ± 1.3 mg/dL vs. 2.2 ± 0.8 mg/dL, $P=$n.s.; 12 months: 2.8 ± 1.6 mg/dL vs. 1.8 ± 0.5 mg/dL, $P=0.0333$; and 24 months: 2.5 ± 1.3 mg/dL vs. 1.7 ± 0.5 mg/dL, $P=0.0409$) (Fig. 2). Thus, 2 years after transplantation, FCXM-positive subjects had higher mean serum creatinine levels than FCXM-negative subjects, regardless of the occurrence of ARj (Fig. 3).

We also investigated the possible role of other covariables in graft loss among FCXM-positive subjects. No significant variations in donor/recipient age, weight, and posttransplant acute tubular necrosis were found between FCXM-positive and -negative patients. Delayed graft function had a higher incidence in FCXM-negative patients (29/91, 31.9%) than in the FCXM-positive patients (6/29, 20.7%); thus, this variable
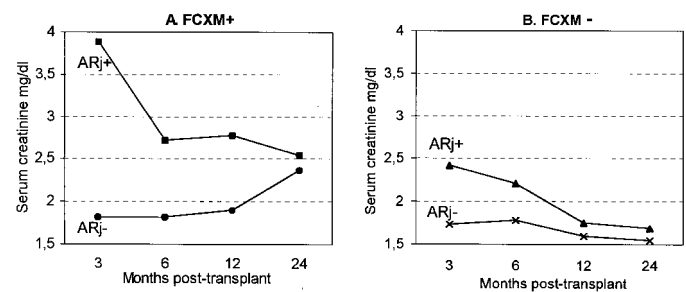

FIGURE 3. Comparison of mean serum creatinine levels in FCXM-positive and -negative patients according to ARj occurrence. In panel A (FCXM+ patients), a higher mean serum creatinine is apparent after 2 years, regardless of ARj occurrence, whereas in panel B (FCXM−), all patients are stable with low serum creatinine at the same period, notwithstanding ARj status.

does not constitute a higher risk factor for graft loss in those patients who produce DS-Abs.

Immunosuppressant therapy with MMF was moreover considered. In fact, it has been widely demonstrated how this drug, which is a specific inhibitor of de novo purine synthesis, is able to inhibit T- and B-lymphocyte proliferation (*20–21*) and to reduce in vivo antibody formation in both animals and man (*22–23*). Even though only a small number of patients in our study population had been treated with MMF, it was nonetheless possible to observe that 7 of 19 (36.8%) FCXM-positive patients, who had a good graft function 2 years after transplantation, had been treated with this immunosuppressant drug. On the contrary, all 10 FCXM-positive patients, who suffered graft loss, had received only standard therapy with Aza. The better clinical outcome registered in FCXM-positive patients treated with MMF may be due to its ability to block antibody production that in turn led to a stop in antibody-mediated damage of the transplanted organ.

FlowPRA analysis results and HLA matching. The Flow-PRA class I and II screening test was performed in 20 of 25 FCXM-positive patients who produced IgG alloantibodies specifically directed against the donor's T and/or B lymphocytes. Anticlass I antibodies were found in seven (35%) patients, whereas anticlass I and II DS-Abs were found in 11 (55%) cases. Only two (10%) subjects exclusively produced anticlass II antibodies. Therefore, 90% of the examined subjects produced antibodies specifically directed against the HLA class I antigens of the graft (Fig. 4). Evaluation of the patient sera with only anti-B lymphocyte IgG DS-Abs according to the FCXM analysis revealed an interesting pattern of anti-HLA antibody production: two (33.3%) of six patients exclusively produced anticlass II antibodies, one (16.7%) patient formed only anticlass I DS-Abs, and the remaining three (50%) patients presented both anticlass I and II antibodies. The presence of anticlass I DS-Abs in patients who showed only anti-B lymphocyte antibody production according to FCXM analysis may be due to low-titre antibodies against class I antigens. In these cases, it is easier to identify such antibodies when they are bound to B lymphocytes,
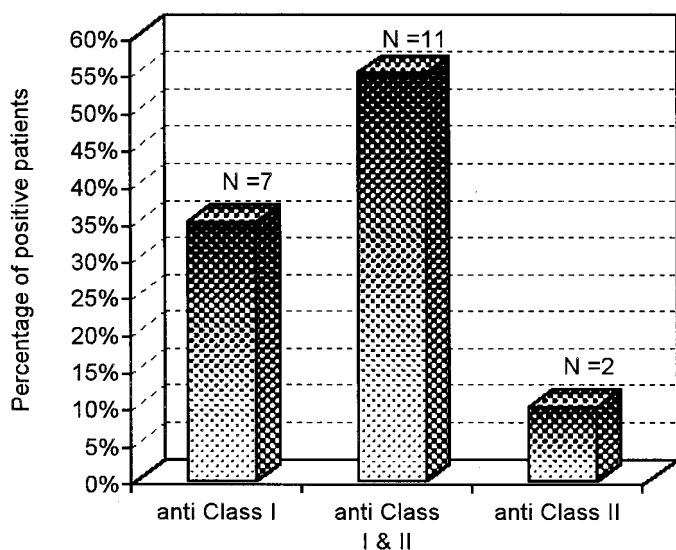
which constitutionally express a higher number of HLA class I antigens than T lymphocytes.

When we analyzed HLA-A, HLA-B, and HLA-DR mismatches in relation to anti-HLA class I and II antibodies, a significant incidence of B-locus mismatches was found in anti-HLA class I–positive patients when compared to negative patients (1.23 ± 0.66 vs. 0.92 ± 0.59, $P=0.0248$) (Fig. 5). No evidence of a correlation between DR mismatches and the presence of anticlass II DS-Abs was found.

DISCUSSION

FCXM is undoubtedly a sensitive technique for identifying alloantibodies in renal-transplanted patients. With this technique, we evaluated 120 cadaveric kidney-transplanted patients for DS-Abs production to ascertain the relevance of the donor-specific humoral immune response on renal graft outcome. It is important to emphasize that all patients included in our study did not present with preformed antibodies against donor HLA-mismatched antigens according to both cytotoxic or flow cytometric analyses. Therefore, ARj or CRj episodes that occurred in our study population did not depend on a possible history of presensitization.

Recent findings suggest that posttransplant production of Ds-Abs is strongly associated with ARj (*15–17, 24*). Our data are consistent with this theory, because 62% of FCXM-positive patients suffered ARj episodes compared to only 13% of the FCXM-negative patients ($P<0.00001$). FCXM monitoring revealed the onset of a humoral immune response towards the graft before the appearance of any clinical evidence of rejection in 50% of FCXM+/ARj+ patients, making this procedure an essential tool for an earlier detection of ARj occurrence, as also stated by Scornick et al. (*15*), Utzig et al. (*17*), and Daniel et al. (*25*). On the other hand, our analysis did not confirm a correlation between the type and degree of FCXM positivity and the occurrence of ARj episodes as shown by these same authors.

Further evidence of the strong correlation between FCXM positivity and graft outcome can be seen by comparing serum
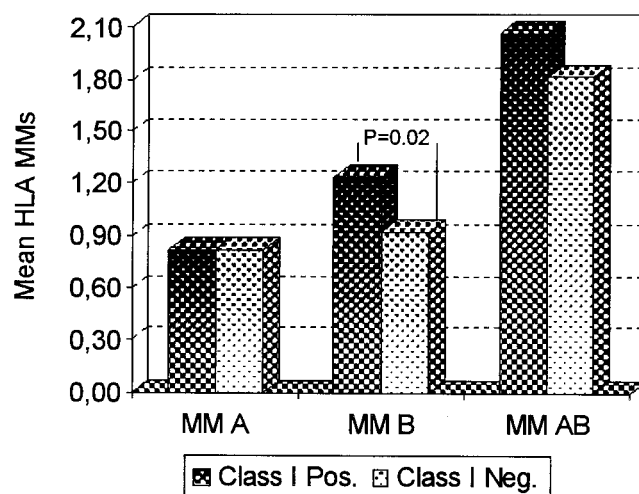

FIGURE 4. Percentages of anticlass I and/or anticlass II antibody–positive patients among IgG FCXM-positive ones.

FIGURE 5. Analysis of mean HLA mismatches (MMs) in anti-HLA class I–positive and –negative patients. It is worth noting that patients who produced class I antibodies showed a prevalence of B-locus mismatches compared with anticlass I–negative patients ($P=0.02$).

creatinine levels and graft function with FCXM status. In fact, FCXM-positive patients constantly showed significantly higher values of serum creatinine throughout our observation period when compared with negative patients.

The production of DS-Abs appears even more important in determining graft failure because of 11 cases of graft loss for ARj or CRj, 91% were among FCXM-positive subjects whereas only 9% occurred in FCXM-negative subjects ($P<0.00001$). It is worth noting that 2 of 10 FCXM-positive subjects who lost the graft during our observation period did not show any evidence of ARj but suffered only a progressive deterioration of renal function, which eventually led to the complete failure of the graft. DS-Abs may, therefore, be considered one of the main causes of CRj, as also stated by Abe et al. (18) and Kirby et al. (26). Our findings do not, however, confirm those reported by other authors. Abe et al. (18) assessed the ineffectiveness of Ds-Abs that appeared within the first month after transplantation in influencing graft survival. Our data do not favor this hypothesis because 4 of 10 FCXM-positive patients who suffered graft loss showed a humoral response within the first month. The other controversial issue is represented by the fact that our data did not confirm a stronger association between anti-B lymphocyte antibodies and CRj, as proposed by the above mentioned authors, because only 3 of 10 FCXM-positive subjects who experienced graft failure demonstrated exclusive anti-B lymphocyte IgG antibody production.

As far as the persistence of FCXM positivity is concerned, only four patients unremittingly produced DS-Abs throughout our observation period and all these subjects lost the graft; the other 25 patients showed FCXM positivity for a limited period of time, ranging from 1 to 10 months. Such events may be explained by either the appearance of anti-idiotypic antibodies (27) or by the presence of graft-released–soluble HLA antigens that mask (28) or block DS-Abs in peripheral blood and bind them into immune complexes.

Acute vascular rejection, which is an antibody-mediated event, has been shown to influence graft survival in contrast to acute interstitial rejection (29). To assess the roles of ARj episodes and antibody formation on graft function, we comparatively evaluated the influence of these parameters on graft outcome. FCXM-positive patients who also suffered rejection episodes had a markedly worse outcome than patients whose clinical history was only positive for ARj occurrence. On the other hand, when considering the two parameters individually, FCXM positivity has been shown to be a much worse prognostic factor than ARj occurrence alone; in fact, our data showed that approximately one third (34%) of FCXM-positive subjects lost the graft within 2 years from transplantation compared to only 1% among those who were negative for DS-Abs production ($P<0.00001$). These results lead us to suspect that subclinical antibody-mediated vascular rejection might effectively cause long-term graft loss, even in the absence of clinical symptoms. All of these findings seem to indicate that FCXM positivity, that is to say DS-Abs production, may really represent a more important risk factor than ARj in determining CRj occurrence and, therefore, graft loss.

Further proof of the predominant role of antibody-mediated damage on renal transplant outcome can be gathered by evaluating its impact on serum creatinine levels. FCXM- and ARj-positive patients showed a mean value of 2.5 mg/dL 2 years after transplantation, whereas FCXM-negative subjects were stable at 1.7 mg/dL (FCXM−/ARj+ group) or less (FCXM−/ARj− subjects) during the same period of clinical monitoring. However, the most interesting evidence comes from the FCXM+/ARj− group who showed, during the first posttransplant year, creatinine levels similar to those of FCXM-negative patients but with a subsequent steep rise at the end of the second year, leading to the same levels observed in the FCXM+/ARj+ group. Thus, renal function definitely appears to be more strongly related to DS-Abs production than to ARj occurrence.

Recent data have clearly highlighted the negative influence of anticlass I antibody production on renal graft survival. These antibodies are in fact held responsible for the loss of successive regrafts bearing previously mismatched HLA class I antigens (30). On this basis, we retrospectively analyzed the sera of all IgG-positive patients using class I and class II FlowPRA screening tests to highlight the HLA specificity of the DS-Abs revealed with FCXM analysis. Our results revealed the presence of anticlass I antibodies in a higher percentage (90%) than those reported in literature (31), whereas the presence of anticlass II antibodies alone was observed in a very low percentage (10%) of our subjects.

To investigate the relevance of HLA class I and class II mismatches on antibody production, we analyzed the incidence of HLA-A, HLA-B, HLA-AB, and HLA-DR mismatches in the anti-HLA–positive group of patients and among the anti-HLA–negative patients. No significant variation of mean HLA mismatches was observed when anticlass II antibody production was considered. On the contrary, we noted a strong link between the presence of anticlass I antibodies and B-locus mismatches ($P=0.0248$), notwithstanding the standard matching priority of HLA-DR, HLA-B, and HLA-A. The finding of a high incidence of HLA-B mismatches among anticlass I DS-Abs–positive patients supports the data that concerns the harmful effect of these HLA mismatches on long-term graft survival.

In conclusion, we believe FCXM to be an essential tool in monitoring the onset of an immune response toward renal graft, because early detection of antibody production may be useful in identifying those patients who need a kidney biopsy and in developing appropriate immunosuppressive protocols. In this study, FCXM had a sensitivity of 90.9 and a specificity of 83.3 for CRj and graft failure; thus, the presence of DS-Abs detected by means of flow cytometric techniques constitutes a negative prognostic event and an independent risk factor for medium- and long-term graft survival.

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