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# Successful Desensitization of T cell Flow Cytometry Crossmatch Positive Renal Transplant Recipients Using Plasmapheresis and Super High-Dose Intravenous Immunoglobulin

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**Background.** High-dose IVIG (2 g/kg) alone or low-dose IVIG (100 mg/kg) in conjunction with plasma exchange is typically administered as a renal transplantation desensitization therapy. Herein, we monitored changes in T cell and B cell flow cytometry crossmatch (FCXM) to assess the effects of short-term super high-dose IVIG (4 g/kg) administration with plasmapheresis before living-donor renal transplantation. **Methods.** Seventeen patients, each showing positive T cell FCXM (median ratio,  $\geq 1.4$ ) after 2 rounds of double-filtration plasmapheresis, received 4-day regimens of IVIG (1 g/kg per day) over 1-week periods. T cell and B cell FCXM determinations were obtained after every IVIG dose and again up to 4 weeks after initiating IVIG to ascertain negative conversion of T cell FCXM (median ratio  $< 1.4$ ). The primary study endpoint was the percentage of patients achieving T cell FCXM-negative status after the 4-dose IVIG regimen. **Results.** Upon completion (4 g/kg total) or discontinuation of IVIG administration, 8 (47.1%) of 17 patients displayed negative T cell FCXM. Based on Kaplan-Meier estimates, the cumulative T cell FCXM-negative conversion rate 4 weeks after IVIG administration initiation was 60.3%. The T cell FCXM-negative conversion rates after cumulative doses of 1, 2, 3, and 4 g/kg IVIG were 29.4%, 35.3%, 56.3%, and 46.7%, respectively. **Conclusions.** Desensitization of donor-specific antibody-positive renal transplant recipients seems achievable in only a subset of recipients through IVIG dosing (1 g/kg  $\times$  4) within 1 week after double-filtration plasmapheresis. The T cell FCXM-negative conversion rate resulting from a cumulative IVIG dose of 3 g/kg or greater surpassed that attained via conventional single-dose IVIG (2 g/kg) protocol. This short-term high-dose IVIG desensitization protocol may be an alternative to conventional protocols for recipients with donor-specific antibody.

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Renal transplantation in patients with end-stage kidney disease improves both duration and quality of life.<sup>1-3</sup> Using advanced immunosuppressive therapeutics, the incidence of T cell-mediated rejection can be reduced, and graft survival rates increased.<sup>4</sup> However, recipients with donor-specific

antibodies (DSA) before renal transplantation (ie, sensitized renal transplantation) show higher rates of antibody-mediated rejection (AMR).<sup>5-7</sup> AMR is difficult to prevent with conventional immunosuppressive drugs in DSA-positive patients and is a major cause of renal allograft loss.<sup>8</sup>

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Recently, development in desensitization protocol gives DSA-positive patients more opportunities to receive successful renal transplantation.<sup>9,10</sup>

In general, 2 protocols are implemented for desensitization before renal transplantation: either high-dose IVIG (2 g/kg) alone or low-dose IVIG (100 mg/kg) in combination with plasma exchange (PE).<sup>11-14</sup> Although it is unknown which of these 2 protocols is superior, IVIG plays a key role in desensitization protocols. Rituximab (anti-CD20 antibody) has also proved beneficial in such protocols.<sup>15,16</sup> A 4-month regimen of IVIG (2 g/kg per month) is advocated by guidelines for clinical use of IVIG published by public agencies of countries.<sup>17-21</sup> IVIG has similarly helped in resolving posttransplantation episodes of steroid-resistant rejection.<sup>10</sup> Such rescue therapies generally involve high-dose IVIG (2 g/kg) given as a single dose<sup>22</sup> or over the course of several days.<sup>23</sup>

At present, preferred protocols for preemptive desensitization appear to be transplant center-specific, with limited or no randomized prospective studies to compare efficacies.<sup>9</sup> Moreover, there have been no definitive studies on dosages and timing of IVIG administration. In this study, we monitored changes in T cell flow cytometry crossmatch (FCXM) as part of a desensitization protocol in which recipients demonstrating positive T cell FCXM received IVIG (1 g/kg) for 4 days within a 1-week period (total of 4 g/kg) after double-filtration plasmapheresis (DFPP) in preparation for living-donor transplantation. T cell FCXM was measured after each IVIG dose and up to 4 weeks after initiating IVIG administration, allowing us to better establish the timing of conversion and the effectiveness of cumulative IVIG dosing. This super high-dose IVIG-based desensitization protocol may be an alternative to conventional protocols for recipients with DSA.

## MATERIALS AND METHODS

### Study Design

A phase II/III, open-label, single-arm multicenter prospective trial was performed in Japan between November 2013 and September 2015. The study protocol was approved by the institutional review board of each participating institution and conducted in accordance with the Good Clinical Practice described in the Helsinki Declaration. Patients provided written informed consent before registering in this study. ClinicalTrials.gov Identifier: NCT02032095.

### Patient Demographics and Characteristics

A total of 17 patients were enrolled for study in preparation for living-donor renal transplantation. Each participant displayed the following: (1) negative T cell complement-dependent cytotoxicity, (2) positive T cell FCXM, and (3) stage-5 chronic kidney disease. Relevant patient characteristics were collected for analysis.

### Study Protocol

Patients showing positive T cell FCXM after 2 rounds of DFPP were administered 1 g/kg per day IVIG (Venoglobulin IH 5%; Japan Blood Products Organization, Tokyo, Japan) for 4 days over a 1-week period. T cell and B cell FCXM were measured the day after each of the 4 IVIG doses. For patients showing positive T cell FCXM after the fourth administration, FCXM was measured weekly until negative conversion was confirmed, for up to 4 weeks after initiating IVIG. The primary study endpoint was percentage of patients

converting to T cell FCXM-negative status after the fourth IVIG dosage. Patients who prematurely discontinued IVIG dosing were evaluated according to last observation carried forward. Secondary study endpoints were percentage of patients showing conversion to T cell and B cell FCXM-negative status (last observation carried forward) 4 weeks after initiating IVIG administration and percentage of patients converting to T cell and B cell FCXM-negative status after each dose of IVIG (1 g/kg). This study plan aimed to confirm the potential of IVIG as a desensitization therapy and did not require renal transplantation, but planned for transplantation to be performed in cases in which FCXM-T became negative.

### Donor-specific Crossmatching Analysis

FCXM determinations were conducted at a single clinical laboratory (ReproCELL Inc., Yokohama, Japan) equipped with a cell analyzer (FACSCalibur; Becton, Dickinson and Co., Franklin Lakes, NJ). Class I- and II-positive control sera (One Lambda, Inc., Canoga Park, CA) were purchased, whereas prospective donor plasma served as a negative control. Median ratio was calculated by dividing median fluorescence intensity of patient specimens by median fluorescence intensity of the negative control. Median ratio less than 1.4 for T cells and less than 1.7 for B cells defined negative. These cutoff values of FCXM were determined by +2SD of the measurements of 34 cases which negative Flow panel-reactive antibody (PRA) screening outcomes.

### Monitoring of Adverse Events

Adverse events occurring within 4 weeks after initiating the IVIG regimen were recorded and assessed for relevance to treatment, applying codes stipulated in the Medical Dictionary for Regulatory Activities (v18.1). Adverse events having a causal association with IVIG administration were considered side effects.

### Statistical Analysis

All computations relied on standard software (SAS v9.3; SAS Institute, Cary, NC). To assess IVIG efficacy in this protocol, a full-analysis set was applied. A 95% confidence interval (CI) is shown for percentage of patients showing conversion to T cell or B cell FCXM-negative status. In Kaplan-Meier estimates, the endpoint was projecting duration (days) required for conversion of 25%, 50%, and 75% of patients to FCXM-negative status and 95% CI. In addition, the cumulative conversion rates (with 95% CI) for conversion to FCXM-negative status 4 weeks after initiating IVIG were calculated.

## RESULTS

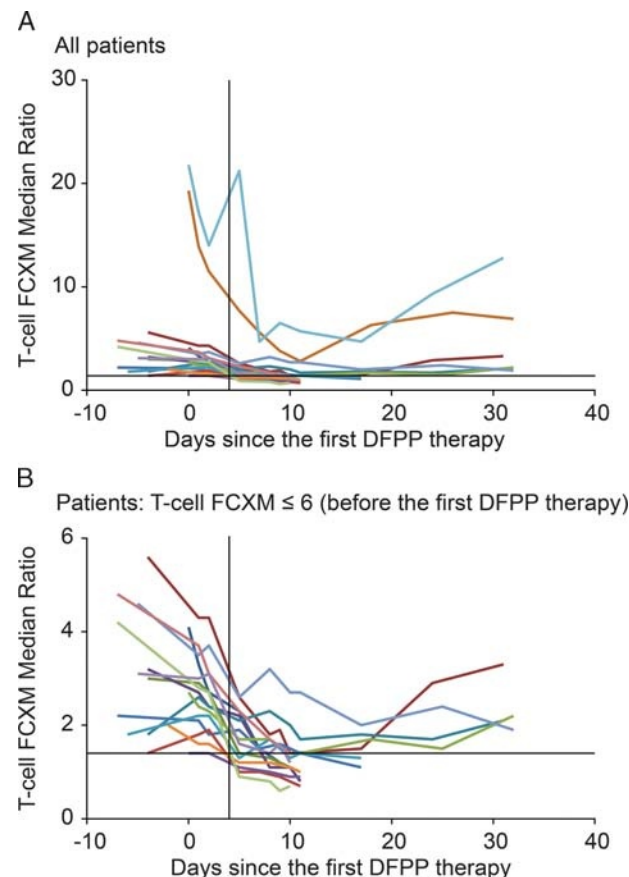
### Patient Demographics and Characteristics

Demographics and characteristics of the patients studied are summarized in Table 1. Four men and 13 women (mean age, 50.5 ± 8.6 years) were enrolled in this study. Pertinent historical exposures included the following: pregnancy (n = 12), blood transfusion (n = 6), and transplantation (n = 4). At baseline, 6 patients had never undergone dialysis, and 16 patients showed positive B cell FCXM. T cell FCXM median values before and after DFPP (before IVIG dosing) were 3.1 (range, 1.4-21.8) and 2.7 (range, 1.4-14.0), respectively. Median values of B cell FCXM were 4.3 (range, 1.6-26.0) and 3.6 (range, 1.6-41.1), respectively.

**TABLE 1.**  
Baseline demographic data and characteristics of patients

Characteristics		
No. patients		17
Sex	Male/Female	4 (23.5%)/13 (76.5%)
Age, y		52.0 (33-66)
Body weight (kg)		52.00 (42.9-63.6)
Primary disease	IgA nephropathy	4 (23.5%)
	Polycystic kidney disease	2 (11.8%)
	Nephrosclerosis	2 (11.8%)
	Unknown	2 (11.8%)
	Other	7 (41.2%)
History of transplantation		4 (23.5%)
History of blood transfusion		6 (35.3%)
History of pregnancy		12 (92.3%)
History of dialysis		11 (64.7%)
Blood type	Compatible (identical)	7 (41.2%)
	Compatible (nonidentical)	5 (29.4%)
	Incompatible	5 (29.4%)
T cell FCXM median ratio	Before DFPP	3.1 (1.4-21.8)
	After DFPP	2.7 (1.4-14.0)
B cell FCXM median ratio	Before DFPP	4.3 (1.6-26.0)
	After DFPP	3.6 (1.6-41.1)

Median (range).

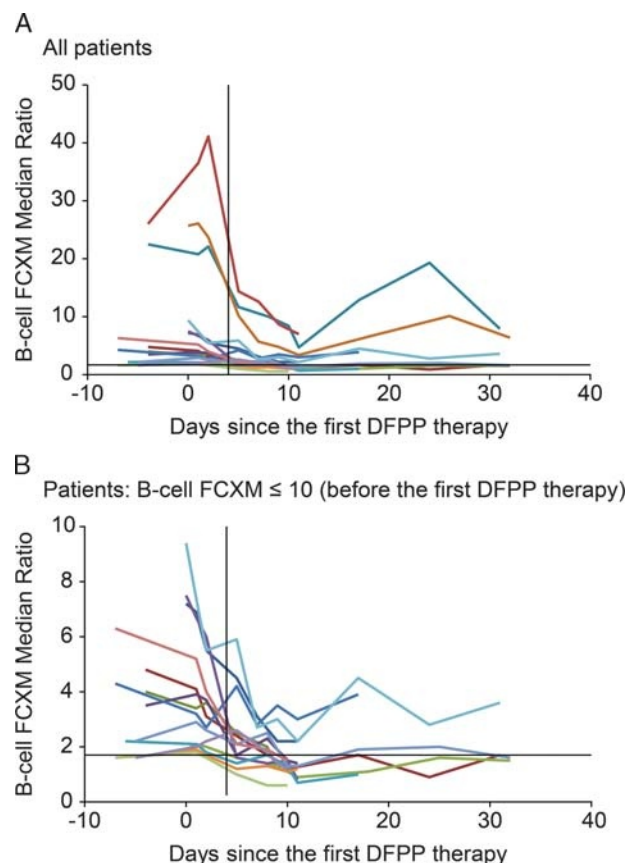


**FIGURE 1.** Changes in T cell FCXM median ratios: (A) T cell FCXM median ratios of all patients studied and (B) patients with median ratios  $\leq 6$  before initial DFPP. Vertical auxiliary line denotes initiation of IVIG protocol (1 g/kg  $\times$  4 in 1 week). Horizontal auxiliary line indicates T cell FCXM median ratio cutoff value of 1.4.

## Efficacy

In Figures 1 and 2, T cell and B cell FCXM changes in each patient studied and in specific patient subsets (FCXM  $\leq 6$  or  $\leq 10$  before first DFPP) are plotted. Successive conversion rates for IVIG doses 1 to 4 were as follows: 29.4% (5/17) after the first dose; 35.3% (6/17) after the second dose; 56.3% (9/16) after the third dose; and 46.7% (7/15) after the fourth dose (Table 2).

As primary endpoint, 8 of 17 patients became T cell FCXM negative after the fourth dose of IVIG or at discontinuation, for a conversion rate of 47.1% (95% CI, 23.0-72.2%). Two of 8 patients who were T cell FCXM positive after the fourth dose of IVIG converted to negative T cell FCXM 1 week later. As a result, 10 of 17 patients became T cell FCXM negative within 4 weeks after initiating IVIG administration (58.8% conversion rate; 95% CI, 32.9-81.6%). On the other hand, 8 of 15 patients given 4 doses of IVIG did not become T cell FCXM-negative after the fourth dose; and of these patients, 4 had T cell FCXM median ratio of 1.4, falling short of the criterion set ( $<1.4$ ). One week later, median ratios in 2 of these 4 patients eventually met the criterion for negative T cell FCXM. The mean values of the T cell FCXM median ratio decreased to 74.7%, 60.0%, 54.6%, and 50.5% after the first, second, third, and fourth administration of IVIG, respectively, compared with the values after DFPP (before IVIG administration) (Figure 3A).



**FIGURE 2.** Changes in B cell FCXM median ratios: (A) B cell FCXM median ratios for all patients studied and (B) patients with median ratios  $\leq 10$  before initial DFPP. Vertical auxiliary line denotes initiation of IVIG protocol (1 g/kg  $\times$  4 in 1 week). Horizontal auxiliary line indicates B cell FCXM median ratio cutoff value of 1.7.

**TABLE 2.****Percentage of patients converting to T cell and B cell FCXM-negative status after each administration of IVIG**

IVIG administration	T cell FCXM			B cell FCXM		
	N <sup>a</sup>	Negative patients	% conversion	N <sup>a</sup>	Negative patients	% conversion
First dose	17	5	29.4	16 <sup>b</sup>	4	25.0
Second dose	17	6	35.3	16	3	18.8
Third dose	16	9	56.3	15	9	60.0
Fourth dose	15	7	46.7	15	9	60.0

<sup>a</sup> N is the number of patients completing each IVIG administration (1 g/kg).<sup>b</sup> Number of B cell FCXM-positive patients after 2 rounds of DFPP therapy.

In Kaplan-Meier estimates, the cumulative rate of conversion to T cell FCXM-negative status 4 weeks after initiating IVIG administration was 60.3% (95% CI, 38.2-83.0%), with 25% and 50% points reached on day 4 (95% CI, 1-6) and day 13 (95% CI inestimable due to so few patients), respectively. The 75% point could not be estimated (Figure 4A). Of 15 patients with T cell FCXM median ratio of 6.0 or less before desensitization, 10 became T cell FCXM-negative 4 weeks after initiating IVIG administration, for a conversion rate of 66.7%. In the other 5 patients, median ratios declined to 1.4 for 3 patients and to 1.7 and 1.9 for the 2 remaining. Two patients with particularly high median ratios (19.3 and 21.8) did not convert to negative T cell FCXM, but these values did fall substantially (to 2.8 and 4.7, respectively).

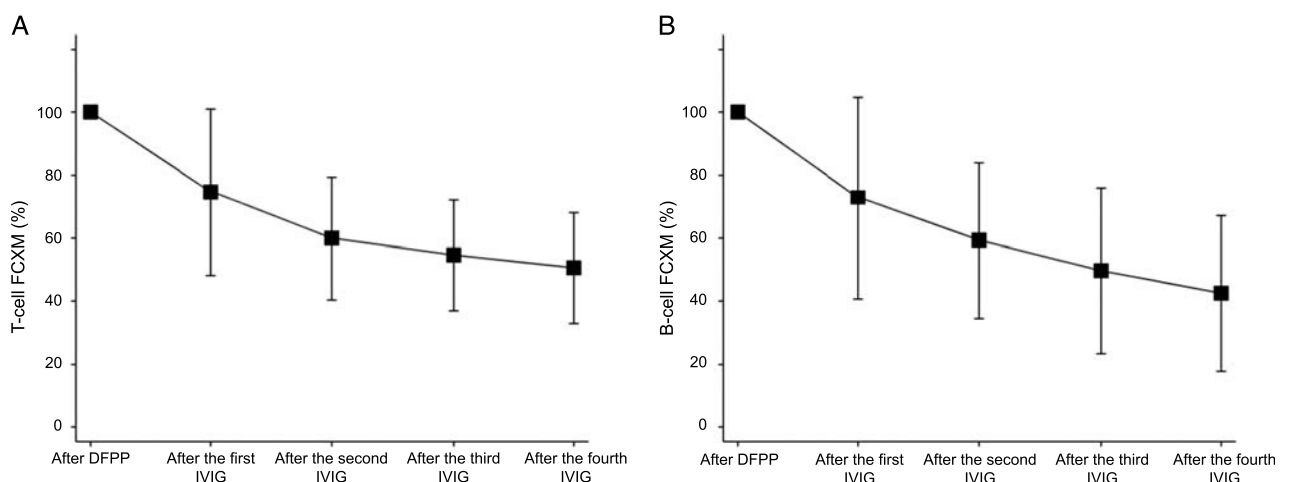
Nine of 16 patients became B cell FCXM-negative 4 weeks after initiating IVIG administration, for an overall B cell FCXM-negative conversion rate of 56.3% (95% CI, 29.9-80.2%). Also, negative B cell FCXM was observed in 6 of 10 patients who eventually converted to T cell FCXM-negative status. After IVIG administrations 1 to 4, B cell FCXM conversion rates were 25.0% (4/16 patients), 18.8% (3/16 patients), 60.0% (9/15 patients), and 60.0% (9/15 patients), respectively (Table 2). In Kaplan-Meier estimates, the cumulative rate of conversion to B cell FCXM-negative status 4 weeks after the first IVIG dose was 66.7% (95% CI, 37.4-92.4%). The 25% and 50% points were reached on day 5 (95% CI, 1-6) and day 17 (95% CI inestimable due to so few patients), respectively; and the 75% point could not be estimated (Figure 4B). Mean values of B cell FCXM median ratio declined to 72.8%, 59.3%, 49.6%, and 42.5% after first, second, third, and

fourth administrations of IVIG, respectively, compared with values after DFPP (before IVIG dosing) (Figure 3B). Overall, the mean serum IgG levels after DFPP (before IVIG administration), after completing the IVIG regimen, and 4 weeks after initiating IVIG administration were  $547.9 \pm 205.0$  mg/dL,  $4923.3 \pm 635.7$  mg/dL, and  $2,733.3 \pm 455.7$  mg/dL, respectively. Among those 10 patients who eventually converted to T cell FCXM-negative status after desensitization therapy, 4 received living-donor renal transplants within the time frame of this study, and none of them experienced AMR. In 2 of 6 remaining cases, living donor kidney transplant was done after the end of the time frame of this study, and there was no AMR observed. In the remaining 4 cases, kidney transplant was not done due to nonmedical reasons.

### Safety

No deaths occurred during the study period. Adverse events, moderate or mild (total of 52), were observed in 13 patients (76.5%), and side effects (total of 38) were recorded in 11 patients (64.7%). Table 3 lists the spectrum of side effects. The chief side effects included headache (29.4%), hepatic dysfunction (17.6%), rash (17.6%), and nausea (11.8%).

Four patients (23.5%) suffered serious adverse events (total of 6), and serious side effects (total of 5) were recorded in 3 patients (17.6%). Serious side effects included leukopenia (5.9%), neutropenia (5.9%), thrombocytopenia (5.9%), headache (5.9%), and aggravation of renal function (5.9%), all of which were predictable. The protocol was discontinued in 2 patients after the third dose of IVIG. One became cytopenic (leukopenia, neutropenia, thrombocytopenia), without need of

**FIGURE 3.** Decline in the T cell FCXM median ratio (A) or B cell FCXM median ratio (B), expressed as ratio of value after each IVIG dose to value after DFPP (before initiating IVIG).

granulocyte colony-stimulating factor or platelet transfusions, and the other experienced headaches. In another patient, hemodialysis was initiated for the first time and continued until the point of transplantation to address deteriorating renal function after IVIG administration. There was no thromboembolism which is a concern due to the large dose of IVIG.

## DISCUSSION

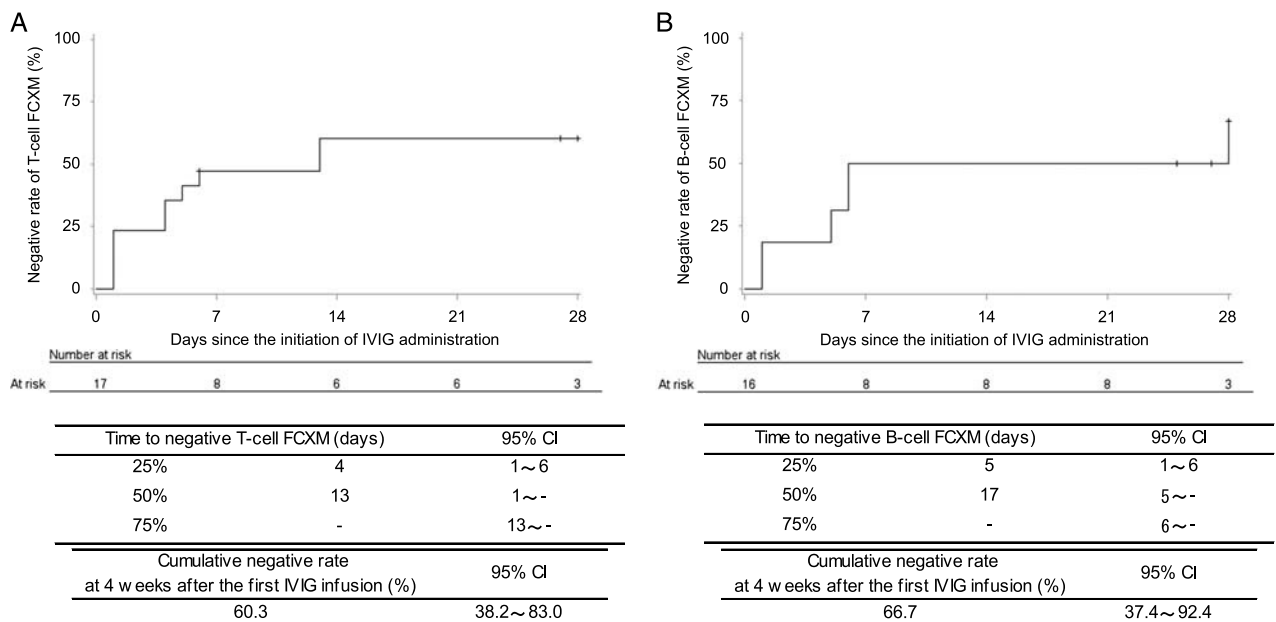
In renal transplantation recipients with DSA, AMR occurs at a high rate, and the posttransplantation prognosis is poor.<sup>8</sup> Desensitization protocols developed over the past several years to overcome this immunologic barrier generally have entailed high-dose IVIG alone or low-dose IVIG combined with PE.<sup>11-14</sup> Recently, regimens incorporating rituximab<sup>16</sup> or bortezomib<sup>24</sup> have been tested as well. Although IVIG plays a key role in desensitization protocols, it is unknown whether high-dose IVIG alone or low-dose IVIG combined with PE is superior. Furthermore, there is no protocol to date where the number of IVIG administrations and intervals have proven optimal. In this study, we measured changes in T cell and B cell FCXM after each dose of IVIG (1 g/kg × 4) administered to sensitized renal transplant recipients within 1 week before transplantation to determine the effects of such treatment.

Administration of IVIG as a monthly dose of 2 g/kg is commonly considered high-dose therapy. Glotz et al<sup>11</sup> have reported that renal transplantation was possible in 13 of 15 recipients who were desensitized using 3 courses of IVIG (2 g/kg each) given at 4-week intervals. In 11 of these patients, all recipients of deceased-donor renal transplants, the PRA rate had declined from 64% (50-77%) to 14% (0-24%), representing a mean decrease of 80% through desensitization. The remaining 2 patients were crossmatch-negative and underwent living-donor renal transplantation. Jordan et al<sup>13</sup> likewise reported a placebo-controlled, randomized, double-blind controlled trial in patients showing high sensitization (PRA of at

least 50% for 3 months). After receiving of 2 g/kg IVIG per month for 4 months, the level of PRA was significantly lower in patients given IVIG (vs placebo), and the rate of transplantation was significantly higher (IVIG, 16/46, 35%; placebo, 8/46, 17%). In our study, the rate of conversion to T cell FCXM-negative status after cumulative administration of 1, 2, 3, and 4 g/kg IVIG generally increased in a dose-related manner, culminating in a 60.3% cumulative conversion rate 4 weeks after initiating IVIG. In contrast, the rate of conversion to T cell and B cell FCXM-negative states after standard IVIG dosing of 2 g/kg has not been high, and repeated IVIG administration seemed to be required at or after renal transplantation. Using our IVIG regimen, the FCXM-negative conversion rate increased by 20% or greater through cumulative IVIG administration of 3 g/kg or more (ie, cumulative dose >2 g/kg). Thus, effective, short-term desensitization is likely responsible for the extent of patient conversion achieved.

FCXM can be expressed as a median channel shift (MCS) derived through linear scale calculation or as a ratio calculated via log scale. According to the Laboratory Manual of the American Society for Histocompatibility and Immunogenetics,<sup>25</sup> each institution is obliged to define a standard FCXM cutoff value. In the Laboratory Manual of the American Society for Histocompatibility and Immunogenetics, cutoff values in studies of T cells are at 10 to 15 for MCS 256 scale and ratio of 1.0. For B cells, cutoff values are at 25 to 30 for MCS 256 scale and ratio of 1.2. An abundance of previous research has focused on MCS values of FCXM (FXMCS), graft survival rates, and the incidence of AMR in anti-HLA antigen-positive renal transplantation. In these investigations, clinical cutoff values have varied, as have the mean fluorescence intensities of DSA, and no consensus has yet been reached in establishing associations with clinical events such as AMR.

However, Gloor et al<sup>26</sup> have reported good results (37% incidence of AMR and ≥90% graft survival for ~5 years) by



**FIGURE 4.** Kaplan-Meier estimates of time required for conversion to T cell FCXM-negative status (T cell FCXM median ratio < 1.4) (A) or B cell FCXM-negative status (B cell FCXM median ratio < 1.7) (B) after initiating IVIG. "Number at risk" represents patients showing positive T cell or B cell FCXM at a particular point in time, with potential to convert. In 2 patients discontinuing IVIG after the third dose, no measurements were available thereafter ("censored" data).

**TABLE 3.**  
The incidence of side effects of IVIG

System organ class	Preferred term	No. patients	Incidence (%)	No. cases
No. patients		17		
Total		11	64.7	38
Blood and lymphatic system disorders		1	5.9	3
	Leukopenia	1	5.9	1
	Neutropenia	1	5.9	1
	Thrombocytopenia	1	5.9	1
Gastrointestinal disorders		2	11.8	3
	Diarrhea	1	5.9	1
	Nausea	2	11.8	2
General disorders and administration site conditions		2	11.8	6
	Chest discomfort	1	5.9	4
	Malaise	1	5.9	1
	Pyrexia	1	5.9	1
Hepatobiliary disorders		3	17.6	3
	Hepatic function abnormal	3	17.6	3
Infections and infestations		1	5.9	1
	Angular cheilitis	1	5.9	1
Investigations		2	11.8	3
	Liver function test abnormal	1	5.9	1
	Hepatitis B core antibody positive	1	5.9	1
	Hepatitis B surface antibody positive	1	5.9	1
Metabolism and nutrition disorders		1	5.9	1
	Hyponatremia	1	5.9	1
Nervous system disorders		5	29.4	7
	Headache	5	29.4	7
Renal and urinary disorders		1	5.9	1
	Aggravation of renal function	1	5.9	1
Respiratory, thoracic and mediastinal disorders		1	5.9	1
	Epistaxis	1	5.9	1
Skin and subcutaneous tissue disorders		6	35.3	8
	Dry skin	1	5.9	1
	Dyshidrotic eczema	1	5.9	1
	Prurigo	1	5.9	1
	Rash	3	17.6	5
Vascular disorders		1	5.9	1
	Internal hemorrhage	1	5.9	1

desensitizing patients with negative T cell antihuman globulin-enhanced complement-dependent cytotoxicity and FXMCS values  $\geq 300$ , lowering FXMCS below 300 (cutoff value of FXMCS-T, 52; FXMCS-B, 104). Jordan et al<sup>27</sup> have also claimed a lowering of AMR to less than 16% within 1 year after renal transplantation, using a DSA-relative intensity scale score of 17 or less (scored according to mean fluorescence intensity), and a FXMCS less than 225 (cutoff value of FXMCS-T: 130, FXMCS-B: 70) to gauge desensitization. Hence, renal transplantation may not be prohibitive, even if negative FCXM is not achieved. The cutoff values used herein less than 1.4 for T cells and less than 1.7 for B cells. When this value of 1.4 or less was defined as negative, the subsequent T cell FCXM conversion rate after cumulative administration of 4 g/kg of IVIG was 73.3%. Neither PRA nor Luminex assays were explored for this study, and we acknowledge that an additional study will be needed.

Rebound of DSA and accompanying AMR have proven to be problematic for desensitization protocols involving PE and IVIG administration, the rate of AMR being as high as 30% to 50%.<sup>9,28</sup> Recent studies have shown better long-term

graft survival rates in recipients desensitized through high-dose IVIG plus rituximab, compared with those subjected to high-dose IVIG alone.<sup>29</sup> Transplantation was planned for patients of ours who were FCXM-negative after cumulative administration of 4 g/kg IVIG. Four of such patients received living-donor renal transplants after treatment with steroids, calcineurin inhibitors, mycophenolate mofetil, plasmapheresis, and rituximab. All of them did well posttransplantation, with no signs of AMR.

Planned desensitization and immunosuppressive therapy in the short term are important for living-donor renal transplantation in DSA-positive recipients. Evaluating a desensitization protocol based on FCXM and PRA determinants is therefore critical. In this study, we have shown that cumulative doses of IVIG  $\geq 3$  g/kg may boost the FCXM-negative conversion rate beyond that attained through conventional IVIG administration (2 g/kg). Although negative conversions of T cell FCXM peaked at IVIG doses of 3 g/kg, there are some cases in which it becomes negative after the fourth administration or 1 week after the fourth administration.

Therefore, in this study, we conclude that IVIG  $\geq 3$  g/kg (3-time administration) is effective.

The primary goal of this study was to monitor changes in T cell and B cell FCXM during an IVIG-based desensitization protocol that differed from the high-dose IVIG alone or low-dose IVIG plus PE protocols typically used. Of the 17 patients studied, only 4 received living-donor renal transplants; and this patient sampling is smaller than that of other published studies.<sup>29</sup> Nonetheless, the results of this limited trial were encouraging. A broader evaluation of our desensitization regimen in greater numbers of transplant recipients is needed to properly assess patient and graft survival at 1 year and longer. This short-term high-dose IVIG desensitization protocol may be an alternative to conventional protocols for recipients with DSA.

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