Impact of Rituximab Therapy on Response to Tetanus Toxoid Vaccination in Kidney-Transplant Patients

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Abstract

Objectives: **Rituximab is used after kidney transplant to prevention or treat kidney-allograft rejection. However, the impact of rituximab on the ability of patients to respond to tetanus toxoid vaccination has not yet been studied.**

Materials and Methods: **The response to tetanus toxoid vaccination was analyzed in 39 kidney-transplant recipients immunosuppressed by corticoids, antiproliferative agents, and/or calcineurin inhibitors. Thirteen patients had previously received rituximab (group 1), 26 patients had not (group 2). Response to control bacterial antigens and immunologic parameters (lymphocyte count, B-cell subsets, serum immunoglobulin level) were analyzed before and at 1 month after vaccination. Thirty healthy blood donors were used as controls for the before-vaccination immunologic parameters.**

Results: **Before vaccination, neither patient group differed from controls in serum levels of immunoglobulins and antibodies against bacterial antigens, but they did display lower levels of CD4 T cells and B cells compared with controls. Responders to the tetanus toxoid vaccination were slightly fewer in group 1 (4/13) than in group 2 (16/26), but the intensity of the anti-tetanus toxoid response was not significantly different between these 2 groups. None of the parameters studied at the time of vaccination (anti-tetanus toxoid level, peripheral B or CD4 T-cell count, memory B-cell subsets, treatment with rituximab, time since transplant) were associated with an ability to respond to vaccination. The ability to respond to vaccination and graft outcomes were not correlated in each patient group.**

Conclusions: **Rituximab impaired the secondary immune response after tetanus toxoid vaccination, but did not abolish it in all patients.**

Key words: *Immunosuppressive regimen, B-cell memory, Lymphocyte subsets, Immunoglobulin level, Secondary immune response*

Introduction

Rituximab has been used recently in kidney transplant to treat acute kidney-allograft rejection and to prevent acute rejection in highly HLA-sensitized patients (1). Rituximab is a chimeric anti-CD20 monoclonal antibody that depletes CD20-positive B-cells (ie, pre-B cells, and normal and malignant B-cells) leaving CD20-negative plasma cells intact. Rituximab is indicated in the treatment of B-cell malignancies, including non-Hodgkin lymphoma, and in rheumatoid arthritis, as well as other autoimmune diseases where autoantibodies or antigen peptide presentation by B cells are involved in the pathogenic process.

Recipients of solid-organ transplant are at risk of severe infection due to their life-long immunosuppression. There is emerging evidence that vaccinations are safe and effective among immunosuppressed patients, except for live vaccines. Recommended vaccines in transplant recipients include immunization against pneumococcus,

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hepatitis A and B viruses, influenza virus, and tetanus and diphtheria toxoids (2). Immunosuppressed transplant patients display a low response to influenza virus, hepatitis A virus, and diphtheria toxoid vaccines, but a normal response to tetanus toxoid vaccination, compared to healthy controls (3, 4).

In chronically immunosuppressed kidney-transplant recipients, the impact of rituximab on the ability to respond to vaccination has not yet been studied. To evaluate the effect of B-lymphocyte depletion (caused by anti-CD20 therapy) on the capacity to develop a secondary immune response against tetanus toxoid, we compared 2 groups of kidney-transplant patients who had been vaccinated against tetanus toxoid in childhood: 1 group received rituximab before vaccination, the other did not. Both groups of patients were compared with a control group of healthy individuals to evaluate the impact of immunosuppressive treatment on global immunologic parameters, including T-lymphocyte and B-lymphocyte counts, memory B-cell subsets, Ig level, and antibody levels against various antigens.

Materials and Methods

Subjects

The 39 kidney-transplant recipients who were vaccinated against tetanus toxoid in childhood, and who were receiving various immunosuppressive treatments were immunized with tetanus toxoid and influenza virus. The 2 patient groups were defined according to their immunosuppressive therapy at that time. The first group (group 1) consisted of kidney-transplant patients whose immunosuppression relied on antiproliferative agents and/or calcineurin inhibitors (either cyclosporine or tacrolimus) plus steroids, and who had received rituximab in the previous months (median, 9 months; interquartile range [IQR], [4-11.5]; Table 1). In this group, rituximab was injected once a week for 4 consecutive weeks. Indications for rituximab treatment were transplant glomerulopathy (n=8), acute humoral rejection (n=3), and chronic humoral rejection (n=2). The times since their last rituximab injection are shown in Table 1. The second group (group 2) of kidney-transplant patients received mycophenolate mofetil (MMF) associated with calcineurin inhibitors, plus steroids. The demographic and clinical characteristics of these 39 patients are described in Table 1. All patients were studied before vaccination (D0) and at 1 month after (M1). Patient outcome was evaluated at M1: a favorable outcome was defined as an absence of graft rejection and maintenance of a functional graft. Conversely, a poor outcome was defined as acute or chronic rejection or progressive renal failure.

Table 1. Patient characteristics at the time of vaccination.

	Group 1 (antiproliferative agent +/- CNI + steroids + previous rituximab	Group 2 (MMF + CNI + corticoids)	*P* value
Number of patients	13	26	
Sex (male/female)	10/3	15/11	.3
Age (median [IQR], y)	55 [40-64]	48 [36-59]	.34
Number of patients who had a previous transplant	4 (31%)	5 (19%)	.44
Time since transplant (median [IQR], y)	6.9 [2.5-15.3]	2.3 [2-4]	.01
Time since last rituximab injection (median, [IQR], mo)	9 [4-11.5]		NA
Creatinine clearance at vaccination (median [IQR], mL/min)	42.4 [39-48]	60.5 [48-69]	.007
Number of patients with induction treatment:			
Anti-lymphocyte serum	8	7	.08
Anti-IL2 Rα	2	10	.26
Anti-CD3	1	0	.69
No induction	2	9	.27
Number of patients with immunosuppressive treatment:			
MMF	11	26	.1
CNI	10	26	.03
sirolimus	1	0	.33
leflunomide	2	0	.1

Abbreviations: CNI, calcineurin inhibitor; IQR, interquartile range; MMF, mycofenolate mofetil.

The control group consisted of 30 healthy blood donors: 20 men and 10 women, aged 24 to 66 years (median, 48.5 years). This group did not receive the tetanus toxoid vaccination and was used as control for the before-vaccination immunologic parameters.

Vaccination

Each patient was immunized with tetanus toxoid (1 vaccine dose) and influenza A and B strains, which contained the A/New Caledonia/20/1999 (H1N1)-analog, the A/Wisconsin/67/2005 (H3N2)-analog (NYMC X-161B), and the B/Malaysia/2506/2004-souche analog strains (Tetagrip, Sanofi Pasteur). This was administered via a subcutaneous route, as recommended (5).

Serology

Serum IgG, IgA, and IgM concentrations were quantified by nephelometry (Immage, Beckman

Coulter, Roissy, France). Specific antibodies against tetanus toxoid (IgG and IgG1 subclass), pneumococcal capsular polysaccharides (PCP, IgG, and IgG2 subclass), diphtheria toxoid (DT) and *Haemophilus influenzae b* (Hib) were measured with an enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's instructions (Binding Site, Saint Egrève, France). Because results of anti-tetanus toxoid IgG and IgG1, and of anti-PCP IgG and IgG2 were correlated, only results of anti-tetanus toxoid IgG and anti-PCP IgG are shown. Protective levels were 0.15 IU/mL for tetanus toxoid (6), 1 mg/L for Hib (7), and 0.1 IU/mL for DT (8). A protective level of serum antibody for PCP has not yet been strictly defined (9). The ratio between antibody concentration after vaccination and that observed before vaccination was calculated for each patient. Significant response to tetanus toxoid vaccination was defined as a 4-fold increase of this ratio.

It has been previously shown that tetanus seroprotection rates and titers are not influenced by concomitant vaccination against influenza virus (10).

In our study, the response to influenza virus was not studied because inhibition caused by the hemagglutination method is semiquantitative, and because ELISA does not allow for good quantification owing to the great variability of virus antigens. Indeed, the original antigenic imprint from the first exposure to an influenza virus dominates all subsequent responses. Therefore, it is not possible to accurately explore the secondary immune response of an individual if previous immunization virus strains are ignored (11).

Flow cytometry

The absolute counts of peripheral T lymphocytes (CD3+CD4+, CD3+CD8+), B lymphocytes (CD19+), and natural killer (NK) cells (CD3-CD16+CD56+) were determined by flow-cytometry on blood samples (TruCount tubes and Multiset software, BD Biosciences, Le Pont-de-Claix, France).

Peripheral blood mononuclear cells (PBMCs) were isolated from ethylenediaminetetraacetic acid (EDTA) blood, and frozen in liquid nitrogen. Thawed PBMCs with a viability > 90% were then analyzed with the following combinations of monoclonal antibodies: tube 1: CD27-FITC, CD5-Percp Cy5.5, CD19-APC; and tube 2: CD27-FITC, IgD-PE, CD19-Percp Cy5.5, and IgM-APC (all from BD Biosciences). Analyses were performed on a FACs Calibur with CellQuest software (BD Biosciences) on 20 000 events for tube 1 and 3000 CD19+CD27+ for tube 2. Memory B-cells were defined as CD19+CD27+, and switched and unswitched memory B-cells were defined as CD19+CD27+IgM-IgD- and CD19+CD27+IgM+IgD+, respectively.

Statistical analyses

Quantitative parameters were compared between the groups using the Kruskal-Wallis test or Mann-Whitney *U* test. Qualitative parameters were studied using the Fisher exact test. Differences between baseline and postvaccination were analyzed using the Wilcoxon matched-paired test. The influence of quantitative and qualitative parameters on response to tetanus toxoid vaccination (expressed as a ratio of antibody titers obtained after compared to those obtained before vaccination) was assessed by ANCOVA. Values of $P < .05$ were taken to be statistically significant.

Results

Immunologic parameters of patients before vaccination

We evaluated the impact of immunosuppressive regimen on the immunologic parameters of 39 kidney-transplant recipients before vaccination. At the time of vaccination, neither of the 2 patient groups differed significantly from the healthy controls for IgG, IgA, IgM serum levels, or CD8 T-cell counts (Figures 1 and 2A). In contrast, both patient groups displayed lower peripheral CD3+CD4+ and lower CD19+ counts than healthy blood donors (Figure 2A). Patients from group 2 displayed lower NK cell (CD3-CD16+CD56+) counts than blood donors (Figure 2A). As expected, patients from group 1 (rituximab) displayed lower CD19 than those from group 2 ($P < .0001$). However, the 2 patient groups did not differ in their CD4, CD8, or NK counts. Of note, complete CD19+ B-cell depletion, that is, B-cell counts of $< 5/mm^3$, occurred for all patients who had received rituximab therapy, that is, group 1.

We further analyzed B-cell subsets and observed that before vaccination, patients who had received rituximab (group 1) had a similar percentage of CD19+CD5+ cells as the healthy controls. In contrast, patients from group 2 displayed lower CD19+CD5+ cells than healthy controls and group 1 patients

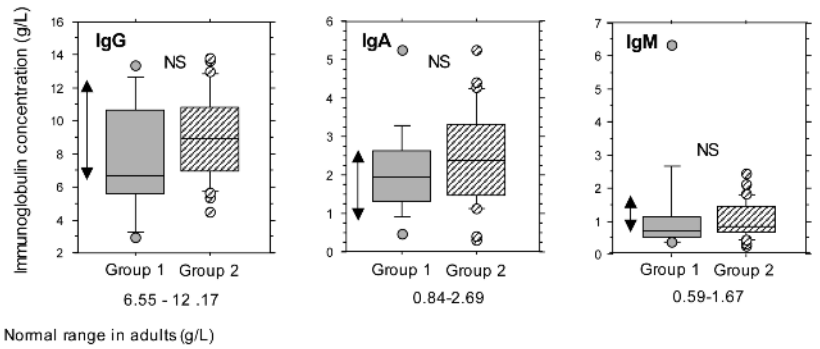


Figure 1. Serum immunoglobulin (IgG, IgA, IgM) concentrations in 39 kidney-transplant recipients before vaccination.
Serum concentrations of immunoglobulins G, A, and M were obtained by nephelometry. The data show the median values and the 10th, 25th, 75th, and 90th percentiles. All values above the 90th or below the 10th percentile are detailed. Arrows show the normal range in healthy adults. Statistical analyses between each patient group and the control group are shown. NS: not significant.

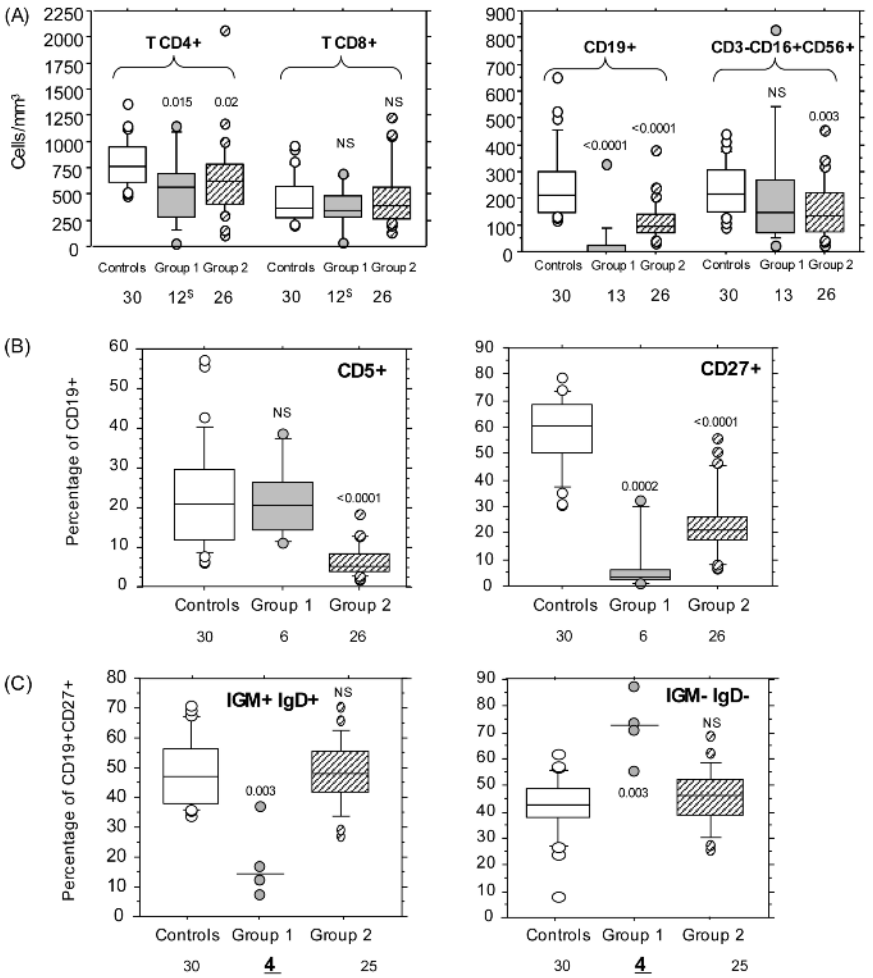


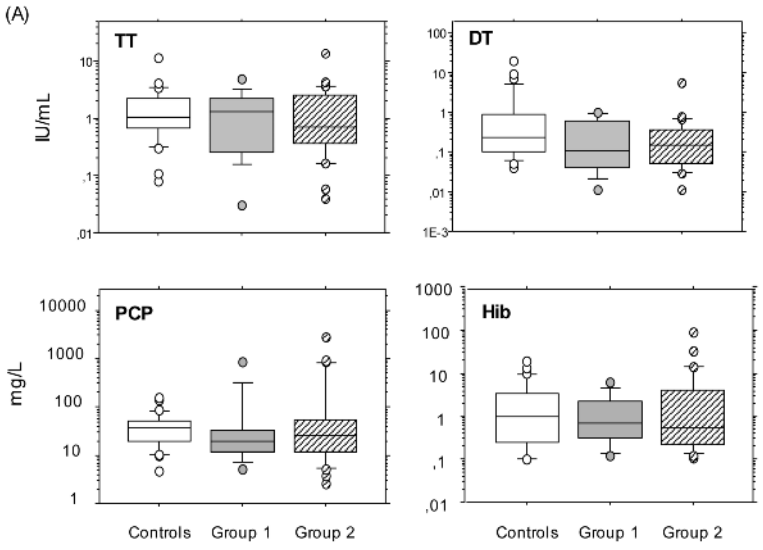
Figure 2. Peripheral lymphocyte subsets in kidney-transplant recipients before vaccination according to their immunosuppressive treatment.
(A) Absolute counts of peripheral CD3+CD4+, CD3+CD8+, CD19+, and CD3-CD16+CD56+ cells were obtained with TruCount tubes and the following combinations of monoclonal antibodies: CD3-FITC/CD16+CD56-PE/CD45-PerCP/CD19-APC and CD3-FITC/CD8-PE /CD45-PerCP /CD4-APC. (B) Percentages of peripheral CD19+ B-cells expressing CD5 or CD27 were analyzed with CellQuest software and the following combination of monoclonal antibodies: CD27-FITC/CD5-PerCP/CD19-APC. (C) Percentages of unswitched (IgM^+ IgD^+) and switched (IgM^- IgD^-) among CD19+CD27+ memory B-cells were analyzed with CellQuest software and the following combination of monoclonal antibodies: CD27-FITC/IgD-PE /CD19-PerCP/IgM-APC.
Healthy blood donors (white bars), patient group 1 (gray bars), and patient group 2 (hatched bars).
[$]Data are missing for one patient from group 1.
The data show the median values and the 10th, 25th, 75th, and 90th percentiles. All values above the 90th or below the 10th percentile are detailed.

(Figure 2B). Both patient groups displayed lower percentages of memory CD19+CD27+ cells than healthy controls, with group 1 (rituximab) having the lowest level, which also was significantly lower than those of group 2 (P = .0069). Memory B-cells were either switched (IgM-IgD-) or unswitched (IgM+IgD+). Patients from group 2 displayed a similar percentage of switched-memory B-cells as the healthy donors, whereas the rituximab group displayed a higher percentage of switched memory B-cells than donors or group 2 (Figure 2C).

Levels of antibodies against various pathogens also were analyzed before vaccination. Neither of the patient groups differed significantly from the healthy controls for anti-tetanus toxoid, -DT, -PCP, or -Hib antibody levels before vaccination (Figure 3A). The 2 patient groups also did not differ in their anti-tetanus toxoid levels before vaccination (P = .78). Similarly, the percentages of patients with protective antibody levels against tetanus toxoid, DT, and Hib were not significantly different in the patient groups or the healthy controls (Figure 3B).

Anti-tetanus toxoid humoral response

The percentage of responders to tetanus toxoid vaccination in all patients was 51%: 4/13 among group 1 (31%) and 16/26 among group 2 (61%). This percentage was not significantly different between the 2 patient groups (P = .096). Also, the percentage of protected individuals against tetanus toxoid after vaccination did not differ between the 2 groups (12 protected individuals [92%] in group 1, and 25 protected individuals [96%] in group 2). The 4 responders from group 1 had an increase in anti-tetanus toxoid level (from 1.37 to 8.65 IU/mL), though this was not significantly different from responders in group 2 (from 1.09 to 5.85 IU/mL) (P = .3) (Figure 4A). However, group 2 patients (both responders and nonresponders) had a significant increase in anti-tetanus toxoid antibodies (from 0.7 to 3.7 IU/mL) (Figure 4B) in contrast with group 1 patients (from 1.3 to 1.9 IU/mL) (Figure 4B). Thus, anti-tetanus toxoid postvaccination titers observed in group 2 patients tended to be higher than those of group 1 patients (P = .06). Antibody production



(B)

Protected individuals	Healthy controls	Group 1	Group 2
n=	30	13	26
TT	28 (93%)	12 (92%)	24 (92%)
DT	23 (77%)	7 (54%)	15 (57%)
Hib	11 (37%)	4 (31%)	10 (38%)

Figure 3. Antibody concentration in healthy blood donors and kidney-transplant recipients before vaccination according to their immunosuppressive treatment. (A) Concentrations of antibodies against tetanus toxoid, DT, PCP, and Hib were measured by ELISA in healthy blood donors (white bars), group 1 (gray bars), and group 2 (hatched bars). (B) Numbers and percentages of protected individuals against each pathogen within the 3 groups (see Materials and Methods for protective levels).

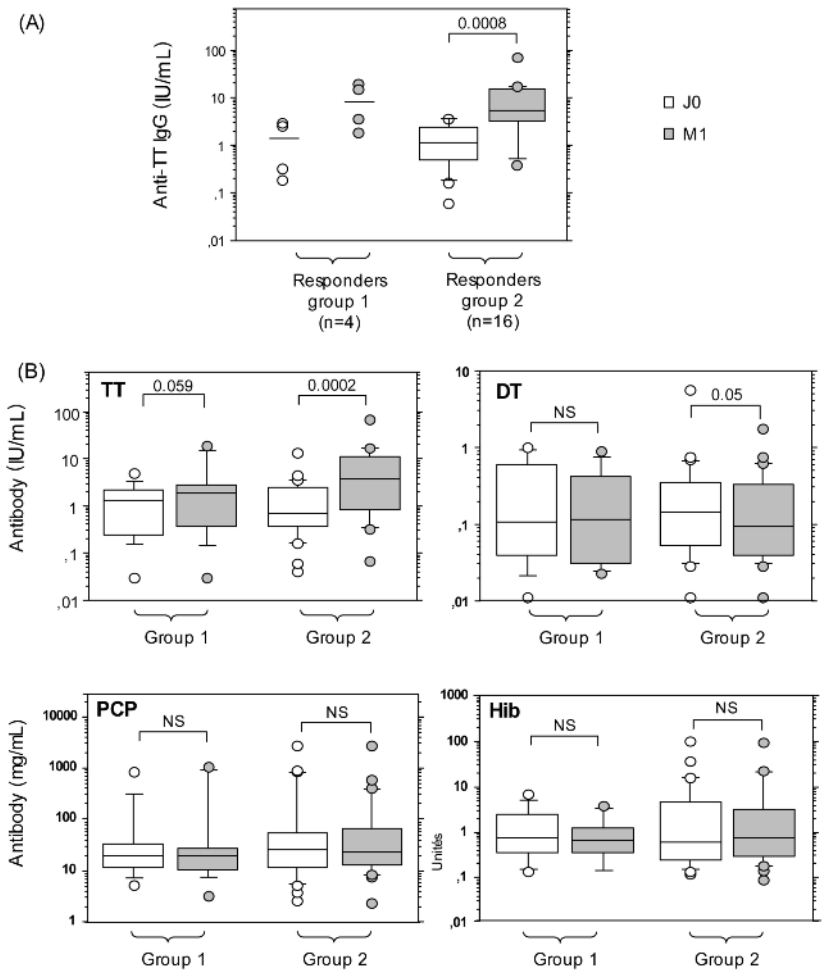


Figure 4. Response to tetanus toxoid vaccination in kidney-transplant recipients.
(A) Concentration of anti-tetanus toxoid before (white bars) and at 1 month after vaccination (gray bars) in patients who responded to tetanus toxoid vaccination. (B) Concentration of anti-tetanus toxoid, -DT, -PCP, and -Hib antibodies as measured by ELISA in the 2 patient groups before (white bars) and at 1 month after vaccination (gray bars).

against control antigens (DT, PCP, and Hib) did not significantly change after tetanus toxoid vaccination in either of the 2 patient groups (Figure 4B).

Because the 2 patient groups differ slightly in the time since transplant, we checked if the latter was correlated with the response to tetanus toxoid vaccination, and found no correlation between both parameters (P = .8, r^2=0.005 for group 1 and P = .89, r^2=0.003 for group 2). Also, the time since last rituximab injection was not correlated with response to tetanus toxoid vaccination in group 1 (P = .85, r^2=0.003).

We also looked at the influence of B-cell count in response to vaccination and found no correlation. Among the 7 patients from group 1 who had no blood B-cells in their blood (< 5/mm^3 at the time of vaccination), 3 responded to tetanus toxoid-vaccination. In contrast, none of the 5 patients who had between 8-30 B-cells/mm^3 at the time of vaccination responded. Only 1 patient from group 1 had blood B-cells > 30/mm^3 at the time of vaccination, and he did respond to vaccination. The 4 patients from group 1 who did respond to tetanus toxoid vaccination, did not differ significantly from the 9 who did not, either in their immunologic parameters before vaccination (anti-tetanus toxoid level, B-cell count, CD4 or CD8 T cell count, CD4/CD8 ratio, NK count, Ig level) or in their ages, time since transplant, or in time since last rituximab injection (4, 9, 11, and 18 months). Among the 4 responders from group 1, two were treated with leflunomide and 1 with sirolimus at the time of vaccination.

The ability to respond to vaccination (expressed as the ratio of tetanus toxoid antibodies after to that before vaccination) was not predictable (an

ANCOVA test did not reveal any significant parameters) from either the immunologic parameters studied at D0 (anti-tetanus toxoid level, IgG, IgA, IgM serum levels, CD4+ T cells, B-cell counts or memory B-cell subsets), the clinical parameters (sex, age, time since transplant, time since last rituximab injection), or the type of immunosuppressive treatment (rituximab or not).

The ability to respond to vaccination was not correlated with graft outcome in either patient group (Table 2). The frequency of patients with a favorable outcome (absence of graft rejection and maintenance of a functional graft) did not differ significantly between responders and nonresponders to tetanus toxoid vaccination, in both patient groups ($P = .99$ for both groups, Fisher exact test). In addition, the 2 patient groups did not vary in the number of patients who had a favorable outcome (Table 2).

Table 2. Patient graft outcome after tetanus toxoid-vaccination.

	Group 1 (antiproliferative agent +/- CNI + steroids + previous rituximab	Group 2 (MMF + CNI + corticoids)	*P* value
Number of patients	13	26	
Number of patients with favorable outcome[1]			
In responders to tetanus toxoid	3/4 (75%)	15/16 (94%)	.37
In nonresponders to tetanus toxoid vaccination	6/9 (67%)	10/10 (100%)	.09

Abbreviations: CNI, calcineurin inhibitor; MMF, mycofenolate mofetil.
[1]Absence of graft rejection and maintenance of a functional graft.

Outcome of immunologic parameters after vaccination

Most of the immunologic parameters studied (ie, IgG, IgA, IgM serum levels, T and NK lymphocyte counts, CD4/CD8 ratio) were not modified after vaccination in the 2 patient groups; that is, in either responders or nonresponders to tetanus toxoid vaccination. Only CD19+ B-cell counts were increased after vaccination (medians: $2/mm^3$ before vaccination to $15/mm^3$ after vaccination) in the rituximab group (group 1). An increase of greater than 2-fold occurred in 6 of 7 patients analyzed. The 6 other patients had no detectable peripheral B-cells before vaccination and 5 had no peripheral B-cells after vaccination. In rituximab-treated patients with detectable B-cells, all B-cell subsets increased harmoniously after vaccination (CD27+ switched or not, CD5+ or CD5-); however, the low number of patients within this group excluded statistical analysis. In contrast, CD19 counts and B-cell subsets were not modified after vaccination in group 2 patients.

Discussion

In this study, we have evaluated the impact of 2 immunosuppressive regimens (1 that included rituximab and the other that did not) on immunologic parameters, and the response to tetanus toxoid vaccination in kidney-transplant recipients. We report that peripheral CD4 T-cells and B-cells were lower in these 2 patient groups compared with healthy controls. However, there was no decrease in Ig level and, although rituximab impaired the secondary humoral immune response, some patients who received rituximab (4 out of 13) could develop a secondary immune response.

We observed that the 2 studied immunosuppressive regimens were associated with a low peripheral CD4 T-cell count. CD4 T-cell lymphopenia has been previously reported in patients receiving MMF+ calcineurin inhibitors + corticoids (12). As neither patient group differed in their blood CD4+ T- cell counts, the addition of rituximab did not further deplete blood CD4+ T. This is important to note, because in patients who have lupus nephritis and who have received rituximab therapy, it has been suggested that a decrease in CD4+ T lymphocytes is linked with rituximab-induced T-cell apoptosis (13). Help from CD4 T-cells is required for the development of the B-cell response and B-cell memory to T-dependent peptidic antigens, such as tetanus toxoid. Therefore, CD4 lymphopenia could impair the ability to develop a secondary humoral immune response. However, we did not find any correlation between the ability to respond to tetanus toxoid vaccination and the CD4 lymphocyte count at the time of vaccination.

Both types of immunosuppressive regimens (group 1 and 2) were associated with low levels of B-cells, including memory (CD27+) B-cells. This is not surprising as most of these patients had received MMF: this has been previously associated with low peripheral B-cell counts (14). As expected, the more drastic decrease in peripheral B-cells was observed in rituximab-treated patients. This rituximab group displayed a higher percentage of CD5+ B-cells and of switched memory B-cells than group 2 patients (MMF + calcineurin inhibitor + corticoid group). This

allowed us to deduce that, after rituximab therapy, these 2 subpopulations of B cells are either more resistant or are better able to reconstitute than other B cells.

Despite the low peripheral B-cell count observed in both patient groups, serum levels of immunoglobulins IgG, IgA, and IgM were within the normal range, as observed in rheumatoid arthritis (15) or lymphoma patients treated with rituximab (16). In addition, neither serum levels of antibodies directed against tetanus toxoid, diphtheria toxoid, *Pneumococcus,* and *Haemophilus*, nor the percentage of individuals with protective levels of these antibodies, were significantly different in patients compared to age-matched blood donors. Thus, although associated with a mixed decrease in T-lymphocyte and B-lymphocyte subsets, the 2 immunosuppressive regimens studied did not result in decreased antibody protection against tetanus, diphtheria, and *Haemophilus influenza b*. This is certainly linked to the long-lived nature of plasma cells involved in the production of these antibodies in healthy individuals (17).

We next asked whether these strong immunosuppressive regimens could alter the ability of patients to respond to tetanus toxoid vaccination. We observed that 61% of patients under MMF + calcineurin inhibitor + corticoids (group 2) could respond to tetanus toxoid vaccination, without modification of the number of protected individuals (the response rate was evaluated as 67% in healthy adults; (18). In the present study, the percentage of responders to tetanus toxoid vaccination was lower among patients who had received rituximab (31%) than in patients who had not. However, the intensity of the response was not significantly different between these 2 groups. None of the parameters studied were associated with the ability to respond to vaccination (anti-tetanus toxoid level, peripheral B-cell count, memory B-cell subsets, CD4 or CD8 T cells or NK count, Ig level, treatment with rituximab or not).

The time since transplant was greater in group 1 (rituximab) compared with group 2, but this did not influence the impact of rituximab response to vaccination. Indeed, when we considered group 1 and group 2 separately, there was no correlation in either group between the time since transplant and the response to vaccination (expressed as ratio of anti-tetanus toxoid after/before vaccination).

The percentage of patients under calcineurin inhibitors was lower in group 1 (rituximab) compared to group 2. The 3 patients from group 1 who did not received calcineurin inhibitors were treated with MMF plus corticoids (n=2) or sirolimus + MMF + corticoids. This might not have contributed to the impaired anti-tetanus toxoid response observed in rituximab group, because calcineurin inhibitors have been shown to decrease humoral immune response in transplant patients when compared with healthy controls, and because response to vaccination has been shown to be similar between patients treated with calcineurin inhibitors and patients treated with sirolimus (19).

Rituximab treatment is not sufficient to abolish the secondary immune response, as 4 patients responded to tetanus toxoid vaccination; of these, 3 had no peripheral CD27+ memory B-cells. A contradictory observation has been reported in patients after autologous stem-cell transplant in which the number of peripheral B-cells did correlate with antibody response after Hib vaccination (20). An impaired secondary immune response after rituximab therapy has been previously reported in rheumatoid arthritis patients (against 1 out of 3 influenza antigens tested) (21) and in lymphoma-treated patients, though only 4 patients were studied (22).

Thus, although rituximab effectiveness is classically appraised by a decrease in peripheral B-cells, this evaluation is insufficient to predict the ability of patients to develop a secondary immune response. The ability of rituximab therapy to completely deplete peripheral B-cells is well-established, but its effect on B cells located in peripheral lymphoid organs is less clear in humans because it is impossible to routinely assess and seems to be more variable. A single rituximab-dose infusion is associated with decreased B-cells within lymph nodes and the spleen, but B cells may not be completely eliminated, depending on rituximab serum concentrations (23). This confirms previous results obtained in cynomolgus macaques (24). Rituximab therapy completely depletes intrarenal B-cells in more than 96% of tested patients (25). However, the persistence of B cells in the kidney of some patients could influence their clinical outcome as over-expression of B-cell survival factor has been assessed in chronically rejected kidney transplants (26).

In our study, we observed that 4 of the 13 patients who received rituximab (group 1) could develop a secondary immune response. We did not explore B-cells in lymphoid organs, and thus, do not know if the ability to respond to vaccination was linked to the persistence of memory B-cells in such organs. However, this outcome is probable, as patients from the rituximab group who responded to vaccination did not differ from those who did not in their B, CD4, or CD8 T-cell counts, NK count, Ig level, time since transplant, or time since rituximab injection. It should be noted that, among the 4 patients who responded to vaccination, 2 were treated with leflunomide/calcineurin inhibitors, 1 with sirolimus/MMF, and 1 with MMF. Thus, we cannot exclude the possibility that immunosuppressive treatments associated with rituximab received in the recent past influenced these patients' response to vaccination.

The impossibility of rituximab therapy to block the secondary anti-tetanus toxoid humoral response in certain patients suggests that this therapy also may be unable to inhibit a secondary immune response against histocompatibility antigens in these patients. In this study, we were unable to address this problem because recipients were selected according to their HLA donor phenotype to avoid any incompatibility that had previously led to an alloimmune response. Because this strategy prevents the risk of anti-HLA secondary immune responses, it is not surprising that none of our patients (treated or not with rituximab) developed a secondary immune response against HLA donor-specific antigen. Moreover, detection of an eventual secondary humoral immune response against minor histo-compatibility antigens was not possible using the available techniques. Thus, we hypothesized that patients able to develop a secondary immune response against tetanus toxoid have a relative resistance to the immunosuppressive regimen, and therefore, could be capable of developing humoral immune responses against histocompatibility antigens including the minor ones. However, we did not reveal here any correlation between the ability to respond to tetanus toxoid vaccination and the graft outcome in either patient group.

In conclusion, we have shown that although rituximab impaired the secondary immune response in kidney-transplant recipients, it did not abolish it in all patients.

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