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Original Article
Clinical Investigation

Immunogenicity and safety of two doses of SARS-CoV-2 mRNA vaccine in kidney transplant recipients with low-dose rituximab

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Abbreviations & Acronyms
COVID-19 = coronavirus disease 2019
eGFR = estimated glomerular filtration rate
ELISpot = enzyme-linked immunospot assay
KT = kidney transplantation
KTR = kidney transplant recipient
MMF = mycophenolate mofetil
mRNA = messenger ribonucleic acid
PBMC = peripheral blood mononuclear cell
SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2
UP/C = urine protein creatinine ratio

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Objectives: We evaluated whether the treatment history of low-dose rituximab affected safety profiles, and humoral and cellular responses induced by severe acute respiratory syndrome coronavirus 2 messenger ribonucleic acid vaccine in healthy controls and kidney transplant recipients.

Methods: We enrolled 10 healthcare workers as controls, 22 kidney transplant recipients with rituximab, and 36 kidney transplant recipients without rituximab without history of coronavirus disease 2019 who received two doses of vaccine. We assessed anti-severe acute respiratory syndrome coronavirus 2 spike antibody and the antigen-specific T cells using enzyme-linked immunospot against spike protein at baseline and after two doses of vaccine.

Results: All controls showed anti-severe acute respiratory syndrome coronavirus 2 antibody seroconversion and enzyme-linked immunospot positivity. Only 19/58 (33%) kidney transplant recipients experienced anti-severe acute respiratory syndrome coronavirus 2 antibody seroconversion and 31/58 (53%) kidney transplant recipients developed enzyme-linked immunospot assay positivity after vaccination. The anti-severe acute respiratory syndrome coronavirus 2 antibody seroconversion rate and enzyme-linked immunospot assay positivity rate after vaccination were not significantly different between kidney transplant recipients with or without rituximab. Multivariate regression analysis demonstrated rituximab was not associated with a lack of humoral and cellular responses to the vaccine.

Conclusions: Low-dose rituximab in kidney transplant recipients did not affect humoral or cellular responses to the severe acute respiratory syndrome coronavirus 2 messenger ribonucleic acid vaccine without severe systemic adverse events including the deterioration of kidney function.

Key words: COVID-19, kidney transplantation, mRNA vaccine, rituximab, SARS-CoV-2.

INTRODUCTION

The development of several vaccines for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) including messenger ribonucleic acid (mRNA) vaccines is an important response to the current coronavirus disease 2019 (COVID-19) pandemic.[1–3] Although solid organ transplant recipients have not been enrolled in phase 3 trials of the BNT162b2 and mRNA-1273 vaccines, evidence of the effectiveness of mRNA SARS-CoV-2 vaccines in solid organ transplant recipients is gradually accumulating.[4,5] Several independent studies showed that only 4% to 48% of kidney transplant recipients (KTR) developed detectable anti-spike antibodies after two doses of the vaccine.[4] Moreover, previous treatment with rituximab was associated with significantly reduced mRNA vaccine-induced immunogenicity.[6–8] The dose of rituximab administered to KTR is generally low, and whether low-dose rituximab affects immune responses induced by mRNA vaccines has not been determined. We evaluated whether the treatment history of low-dose rituximab affected safety profiles, and humoral and cellular responses, induced by the SARS-CoV-2 mRNA vaccine in KTR.

METHODS

Patients

The study included three cohorts. The control group was composed of 10 healthcare workers (none of them receiving immunosuppressive treatment) from our institution. Sixty-four adult KTR (aged >20 years) with a functioning graft who were routinely followed at our institution and who had a routine visit during the study period were divided into two groups according to a history of rituximab treatment (KTR with rituximab therapy: RIT group; KTR without rituximab therapy: non-RIT group). All participants completed a two-dose course of SARS-CoV-2 mRNA-1273 vaccine (Moderna) or BNT162b2 SARS-CoV-2 mRNA vaccine (Pfizer-BioNTech) between April 2021 and October 2021. The research was conducted following the principles outlined in the Declaration of Helsinki. All the participants provided written informed consent. The ethics committee of the Yamagata University Faculty of Medicine approved the study (approval no. 2020–377).

Blood sample collection

Blood samples were obtained within 2 weeks before the first dose, and within 2 to 4 weeks after the second dose of the vaccine. Serum creatinine and the urine protein creatinine ratio (UP/C) recorded on the day of the blood sample collection were retrieved from patient records.

Anti-SARS-CoV-2 antibody detection

The blood samples were tested using an anti-SARS-CoV-2 S enzyme immunoassay (Elecsys anti-SARS-CoV-2 S RUO; Roche Diagnostics) that detects antibodies against the receptor-binding domain of the SARS-CoV-2 spike protein, according to the manufacturer's instructions. The assay is semiquantitative and has been consistently correlated with neutralizing immunity.[9] Values below 0.8 U/ml were considered negative.

ELISpot analysis

To analyze cellular responses, an enzyme-linked immunospot assay (ELISpot) measuring interferon-gamma produced by specific SARS-CoV-2 T cells was performed before and after vaccination. Peripheral blood mononuclear cells (PBMCs) were isolated by specific gravity centrifugation using Ficall-Paque Premium (Cytiva) and cryopreserved until analysis. Stimulation was conducted with individual sequences containing 11 amino acids overlapping a 15-mer peptide pool derived from a peptide scan of the full-length sequence of the vaccine (BNT162b2), which encoded the receptor-binding domain of the SARS-CoV-2 spike glycoprotein (2 μg/ml/peptide; JPT Peptide Technologies). Tests were performed in duplicate. Negative control wells lacked peptides, and positive control wells included anti-CD3 monoclonal antibody (1:1000; Mabtech). Then, 2×10^5 PBMCs per well were stimulated, placed in a plate pre-coated with anti-IFN-γ (Human INF-γ ELISpotPro kit; Mabtech) in a 37°C humidified incubator with 5% CO_2, and incubated for 48 h. The cells were removed, and the plates were washed five times with phosphate-buffered saline. Then, 100 μl of 200-fold diluted secondary anti-INF-γ antibody conjugated with horseradish peroxidase (ELISpotPro kit; Mabtech) was added to each well and incubated at room temperature for 2 h. After five washes with phosphate-buffered saline, the tetramethylbenzidine substrate was added. ELISpot analysis was performed using an ELISpot Reader (Autoimmun Diagnostika). Cytokine activity was calculated from the spot size and intensity values using a previously described formula.[10] The rate of change in cytokine activity in each test was calculated using the formula below, and the mean value of the two measurements was used as the measured value.

The rate of change in cytokine activity = 100 × (cytokine activity in peptide-stimulated wells − cytokine activity in negative control wells)/cytokine activity in negative control wells.

The cutoff value was determined by calculating the mean ± 2 standard deviations in a group of healthcare workers obtained prior to the first vaccination and was determined as a rate of change in cytokine activity greater than 164.

Vaccine safety

Adverse reactions were obtained using a specific questionnaire that included local reactions (pain, redness, and swelling at the injection site) and systemic reactions (fever, fatigue, headache, chills, myalgia, arthralgia, vomiting, and diarrhea) after each dose of the vaccine. Participants were also asked to rate their symptoms on an ordinal scale of none, mild, moderate, or severe. Mild symptoms were defined as those that did not interfere with daily activities, whereas moderate symptoms were those that caused some interference with daily activity, and severe symptoms were those that prevented daily activity.

Clinical episodes of acute rejection and acute elevation of the estimated glomerular filtration rate (eGFR) and UP/C after two doses of the vaccine were also evaluated. eGFR was calculated with a formula modified for Japanese patients, as regulated by the Japanese Society of Nephrology (eGFR = 194 serum creatinine mg/dl$^{1.094}$ × age$^{0.287}$ × 0.739 [if female]).

Statistical analysis

All clinical data were collected from patient records and analyzed retrospectively. A statistical analysis of various parameters was performed for each group using Fisher's exact test for categorical variables and the Mann–Whitney *U* test/Wilcoxon signed-rank tests for continuous variables. The significance level was set at 0.05. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University), a graphical user interface that is a modified version of R commander designed to add statistical functions frequently used in biostatistics (The R Foundation for Statistical Computing).[11]

RESULTS

Demographic characteristics of the study participants

One KTR withdrew consent during the study period. No participants had a clinical episode of COVID-19 infection before vaccination. No participants had anti-SARS-CoV-2 antibodies

before the first vaccination. ELISpot activity was positive in 5 of 63 KTR before the first vaccination, and these five KTR were excluded from the study cohort. Finally, 10 healthcare workers and 58 adult KTR with a functioning graft were enrolled in this study. The characteristics of each KTR are shown in Table S1. The KTR were significantly older than the controls (age ranges 54 ± 15 and 40 ± 6 years, respectively), whereas the sex balance was similar between the groups. All participants in the control group received the BNT162b2 vaccine, 57 of 58 (98%) KTR received the BNT162b2 vaccine, and only one (2%) KTR received the mRNA1273 vaccine. Only one participant in the control group had hypertension, and the others had no comorbidities. Table 1 shows the characteristics of the KTR group: 53 of 58 recipients (91%) were living donor KTR, and 18 of 58 (31%) received an ABO-incompatible kidney transplantation (KT). At vaccination, all recipients were treated with calcineurin inhibitors: 51 (88%) with mycophenolate mofetil (MMF), two (3%) with mizoribine, 10 (17%) with everolimus, and 51 (88%) with steroids. The first dose of vaccine was administered 81 ± 70 (3 to 302) months after KT. Twenty-two of fifty-eight recipients received rituximab therapy. All KTR received rituximab for the induction of ABO-incompatible and/or sensitized KT. One of twenty-two recipients with rituximab received 200 mg/body twice, and the other 21 recipients received 200 mg/body once. ABO-incompatible KT was higher in the RIT group (74% vs. 3%, $p < 0.001$) compared with the non-RIT group. More KTR in the RIT group received steroid therapy (100% vs. 81%, $p = 0.037$) and 2 to 5 sessions of plasmapheresis before KT (55% vs. 3%, $p < 0.001$), whereas fewer KTR received postoperative local graft irradiation (9% vs. 22%, $p = 0.009$). The proportion of KTR who received early preoperative immunosuppressant initiation (7 days before KT) was similar between the two groups (100% vs. 72%, $p = 0.290$). Duration from rituximab therapy to the first vaccination was 60 ± 40 months, and 4 of 22 KTR received their first vaccination within 12 months after rituximab therapy.

TABLE 1 Demographics of kidney transplant recipients

	With RIT (n = 22)	Without RIT (n = 36)	p-value
Age, years (mean, SD)	55 (15)	53 (15)	0.700
Sex			
Male (%)	17 (77%)	22 (61%)	0.256
Vaccine type			1
BNT162b2 vaccine (%)	22 (100%)	35 (97%)	
mRNA-1273 vaccine (%)	0 (0%)	1 (3%)	
Time since KT, months (mean, SD)	60 (40)	94 (82)	0.252
Donor type			0.146
Living donor (%)	22 (100%)	31 (85%)	
Deceased donor (%)	0 (0%)	5 (15%)	
Retransplantation (%)	4 (17%)	1 (3%)	0.134
ABO incompatible KT (%)	17 (74%)	1 (3%)	<0.001
DSA positive before vaccine	5 (22%)	3 (8%)	0.311
Plasmapheresis before KT	12 (55%)	1 (3%)	<0.001
Local graft irradiation after KT	2 (9%)	8 (22%)	0.009
Early IS initiation before KT	22 (100%)	26 (72%)	0.290
IS medication			
Steroid (%)	22 (100%)	29 (81%)	0.037
Tacrolimus (%)	20 (91%)	30 (3%)	0.697
Cyclosporine (%)	2 (9%)	6 (15%)	0.697
MMF (%)	21 (95%)	30 (83%)	0.235
Mizoribine (%)	1 (5%)	1 (3%)	1
Everolimus (%)	2 (9%)	8 (22%)	0.290
Indication of RIT			
Induction (%)	22 (100%)		
Treatment (%)	0 (0%)		
Dose of RIT			
200 mg/body	21 (95%)		
400 mg/body	1 (4%)		
Time since RIT, months (mean, SD)	62 (41)		
Comorbidities			
Hypertension (%)	14 (64%)	24 (67%)	1
Diabetes (%)	10 (45%)	11 (31%)	0.275
Cardiovascular diseases (%)	4 (18%)	5 (14%)	0.718
History of malignancy (%)	2 (9%)	7 (19%)	0.459
eGFR, ml/min/1.73 m^2 (mean, SD)	47 (13)	54 (20)	0.239
UP/C (mean, SD)	0.2 (0.2)	0.2 (0.3)	0.712

Abbreviations: ABMR, antibody-mediated rejection; DSA, donor specific anti-human leukocyte antigen antibody; eGFR, estimated glomerular filtration rate; IS, immunosuppression; KT, kidney transplantation; MMF, mycophenolate mofetil; RIT, rituximab; SD, standard deviation; UP/C, urine protein to creatinine ratio.

Anti-SARS-CoV-2 antibody levels after vaccination

After two doses of the vaccine, all controls and 19 of 58 (33%) KTR ($p < 0.001$) were positive for anti-SARS-CoV-2 antibodies (Figure 1a). Moreover, anti-SARS-CoV-2 antibody titers in KTR were significantly lower than in the controls (89 ± 358 U/ml vs. 1367 ± 853 U/ml, $p < 0.001$). The humoral response rate to vaccination in the RIT group was not significantly different, but it was lower than that in the non-RIT group (23% vs. 39%, $p = 0.256$) (Figure 1a). Anti-SARS-CoV-2 antibody titers were not significantly different, but they were lower in the RIT group than in the non-RIT group (27 ± 116 U/ml vs. 126 ± 442 U/ml, $p = 0.155$) (Figure 1b). Anti-SARS-CoV-2 antibody was not detected in all four KTR who received rituximab within 12 months. In the univariate model, age ≥60 years, living KT, ABO-incompatible, and immunosuppression with MMF were associated with a lack of humoral response to the vaccine (Table 2). Multivariate regression analysis accounting for age ≥60 years, and immunosuppression without MMF confirmed these associations. Rituximab was not associated with a lack of humoral response to the vaccine in univariate and multivariate regression analyses.

Cellular response after vaccination

Cytokine activity in positive control wells were similar before and after mRNA vaccine in KTR (pre; 112 874 ± 37 415, post; 117 442 ± 30 091, $p = 0.146$). After two doses of the vaccine, all controls showed positive ELISpot activity, whereas 31 of 58 (53%) KTR ($p = 0.005$) had positive ELISpot activity (Figure 2a). The rate of change in cytokine activity in KTR was significantly lower than in the controls (303 ± 434 vs. 808 ± 615, $p = 0.001$) (Figure 2b).

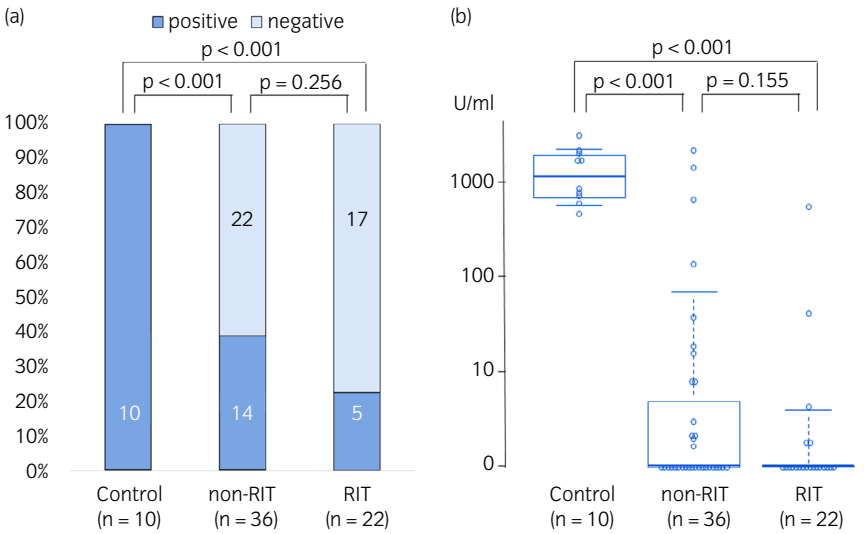

FIGURE 1 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) anti-spike antibody positive rate (a) and antibody titer (b) after two doses of SARS-CoV-2 messenger ribonucleic acid vaccine. RIT, kidney transplant recipients with rituximab therapy; non-RIT, kidney transplant recipients without rituximab therapy.

TABLE 2 Factors associated with a negative antibody response after two doses of vaccine in kidney transplant recipients

	Univariate			Multivariate		
	OR	95% CI	*p*-value	OR	95% CI	*p*-value
Sociodemographics						
Age ≥60 years	3.92	1.07–16.86	0.027	5.24	1.27–21.60	0.026
Male (ref: female)	0.92	0.23–3.40	1			
Body mass index ≥25	0.24	0.03–1.42	0.099			
Transplantation characteristics						
KT <1 year	3.86	0.44–187.00	0.252			
Living donor (ref: deceased donor)	Inf	Inf–0.46	0.003	3.35×10^8	0–Inf	0.994
Retransplantation	0.30	0.02–2.84	0.318			
ABO incompatible	5.76	1.11–58.22	0.029	2.60	0.46–14.60	0.278
Plasmapheresis before KT	8.00	0.96–67.00	0.055			
Local graft irradiation after KT	5.40	0.63–46.20	0.124			
Early IS initiation before KT	4.04	0.98–16.60	0.053			
IS medication						
Steroid	3.12	0.47–24.03	0.201			
Tacrolimus (ref: cyclosporin)	2.30	0.38–14.14	0.418			
MMF	16.59	1.77–823.03	0.004	23.90	2.21–257.00	0.009
Everolimus	0.25	0.05–1.27	0.065			
Rituximab	2.13	0.58–9.13	0.256	0.30	0.03–2.81	0.293
Comorbidities						
Hypertension	1.62	0.44–5.85	0.557			
Diabetes	1.35	0.37–5.30	0.773			
Cardiovascular diseases	0.97	0.18–6.77	1			
History of malignancy	0.97	0.19–6.77	1			

Abbreviations: CI, confidence interval; Inf, infinity; IS, immunosuppression; KT, kidney transplantation; MMF, mycophenolate mofetil; OR, odds ratio; ref, reference.

However, the positive rate of ELISpot activity was not significantly different between the RIT group and the non-RIT group (59% vs. 50%, $p = 0.592$) (Figure 2a). The rate of change in cytokine activity in the RIT group was also not significantly different from that in the non-RIT group (249 ± 245 vs. 336 ± 517, $p = 0.943$) (Figure 2b). Positive ELISpot activity was detected in 3 of 4 KTR who received rituximab within 12 months. ELISpot activity was positive in all five KTR with rituximab whose anti-SARS-CoV-2 antibody turned positive after vaccination. In the univariate and multivariate logistic regression models, only KT within 1 year was associated with a lack of positive ELISpot activity after vaccination (Table 3).

Correlation between humoral and cellular responses

All controls were anti-SARS-CoV-2 antibody and ELISpot activity positive. However, only 5 of 22 (23%) KTR with RIT and 9 of 36 (25%) KTR without RIT were anti-SARS-CoV-2 antibody and ELISpot activity positive. We found 36% of KTR in the RIT group and 25% in the non-RIT

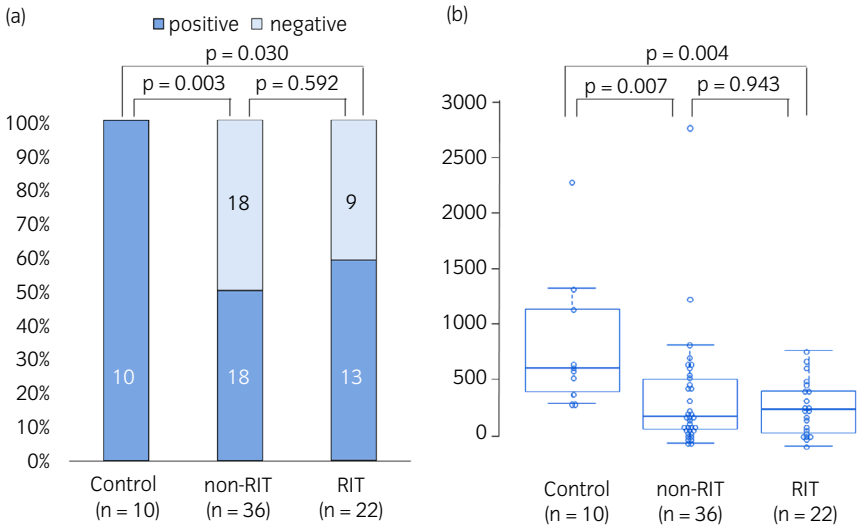

FIGURE 2 Enzyme-linked immunospot assay activity positive rate (a) and the rate change in cytokine activity (b) after two doses of severe acute respiratory syndrome coronavirus 2 messenger ribonucleic acid vaccine. RIT, kidney transplant recipients with rituximab therapy; non-RIT, kidney transplant recipients without rituximab therapy.

TABLE 3 Factors associated with a negative cellular response after two doses of vaccine in kidney transplant recipients

	Univariate			Multivariate		
	OR	95% CI	*p*-value	OR	95% CI	*p*-value
Sociodemographics						
Age ≥60 years	1.30	0.41–4.17	0.793			
Male (ref: female)	1.3	0.38–4.62	0.781			
Body mass index ≥25	0.34	0.03–2.14	0.263			
Transplantation characteristics						
Transplantation <1 year	10.12	1.16–487.30	0.020	10.5	1.20–91.74	0.034
Living donor (ref: deceased donor)	0.75	0.06–7.12	1			
Retransplantation	0.26	0.01–2.91	0.360			
ABO incompatible	0.89	0.25–3.11	1.000			
Plasmapheresis before KT	0.65	0.19–2.31	0.508			
Local graft irradiation after KT	1.93	0.48–7.73	0.354			
Early IS initiation before KT	1.38	0.35–5.52	0.649			
IS medication						
Steroid	2.40	0.35–27.06	0.432			
Tacrolimus (ref: cyclosporin)	1.53	0.26–10.90	0.582			
MMF	0.62	0.08–4.10	0.694			
Everolimus	1.91	0.39–10.44	0.490			
Rituximab	0.7	0.21–2.29	0.592	0.56	0.17–1.73	0.328
Comorbidities						
Hypertension	1.10	0.33–3.77	1			
Diabetes	0.79	0.23–2.64	0.786			
Cardiovascular diseases	1.52	0.29–8.66	0.720			
History of malignancy	1.52	0.29–8.66	0.720			

Abbreviations: CI, confidence interval; IS, immunosuppression; KT, kidney transplantation; MMF, mycophenolate mofetil; OR, odds ratio; ref, reference.

group were anti-SARS-CoV-2 antibody negative and ELISpot activity positive. No KTR in the RIT group and 14% in the non-RIT group were anti-SARS-CoV-2 antibody positive and ELISpot activity negative. We evaluated the relationship between anti-SARS-CoV-2 antibody titer and the rate of change in cytokine activity using Spearman's correlation coefficient. There was a weak correlation between anti-SARS-CoV-2 antibody titer and the rate of change in cytokine activity for all participants ($r = 0.509$), KTR without RIT ($r = 0.441$), and KTR with RIT ($r = 0.253$). There was no correlation in the control group ($r = -0.394$) (Figure 3).

Adverse reactions after vaccination

Adverse events at the injection site did not differ between the controls and KTR after the first (100% vs. 97%, $p = 1$) or second dose (100% vs. 97%, $p = 1$) of the vaccine (Figure 4a). Systemic adverse events after the first dose were similar between the two groups (50% vs. 34%, $p = 0.480$), whereas systemic adverse events after the second dose occurred more often in the controls than in KTR (90% vs. 47%, $p = 0.015$) (Figure 4b). Systemic adverse events classed as greater than moderate were increased after the

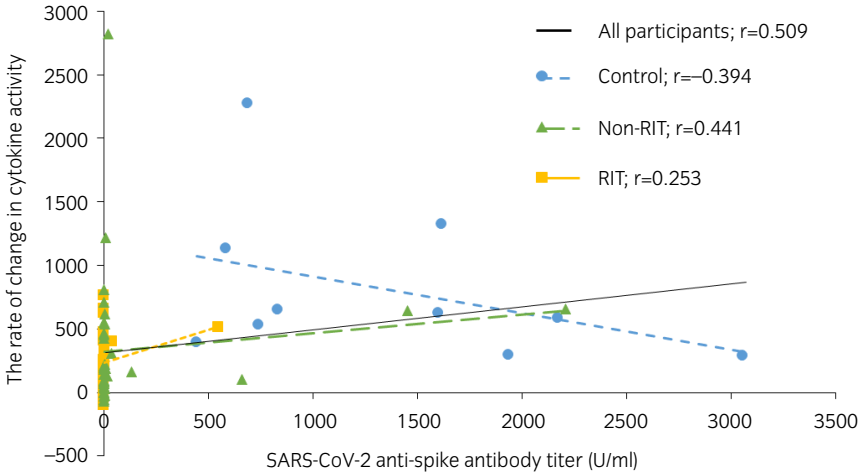

FIGURE 3 Correlation between severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) anti-spike antibody titers and enzyme-linked immunospot assay activity against the SARS-CoV-2 spike protein. Spearman's correlation coefficient was used to evaluate the relationship between anti-SARS-CoV-2 antibody titer and the rate of change in cytokine activity in each group. The blue circles represented controls, green triangles represented non-RIT, and yellow square represented RIT. RIT, kidney transplant recipients with rituximab therapy; non-RIT, kidney transplant recipients without rituximab therapy.

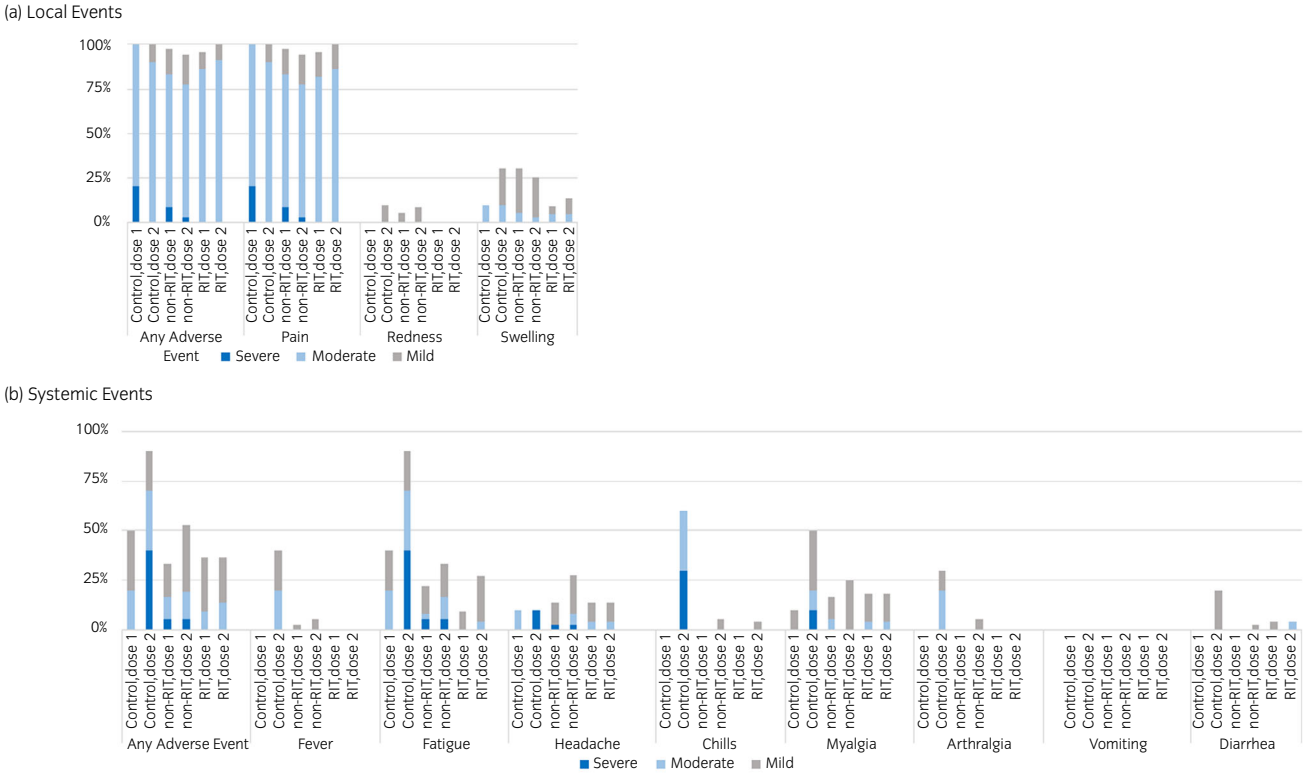

FIGURE 4 Local and systemic reactions after the first and second injection of SARS-CoV-2 mRNA vaccine in each group. Injection-site reactions are shown in (a). Systemic events are shown in (b). RIT, kidney transplant recipients with rituximab therapy; non-RIT, kidney transplant recipients without rituximab therapy.

second dose of the vaccine in the controls (20% vs. 70%, $p = 0.070$) but not in KTR (14% vs. 17%, $p = 0.798$). In KTR, adverse events at the injection site did not differ between the RIT group and the non-RIT group after the first (95% vs. 97%, $p = 1$) and second doses (100% vs. 94%, $p = 0.521$) (Figure 4a). Systemic adverse events were also not different between the RIT group and the non-RIT group after the first (36% vs. 33%, $p = 1$) and second doses (53% vs. 36%, $p = 0.283$) (Figure 4b). Systemic adverse events classed as greater than moderate were not significantly increased after the second dose in both groups.

Kidney function after vaccination

There was no significant deterioration of the eGFR after vaccination in both groups (Table 4). There was no significant elevation of UP/C after vaccination in both groups (Table 4). No patient showed clinical symptoms of acute rejection including acute deterioration of the eGFR and/or elevated UP/C.

DISCUSSION

Seroconversion of anti-SARS-CoV-2 antibody after two doses of the vaccine in KTR was much lower than that of healthy

TABLE 4 Comparison of kidney function before and after two doses of vaccine in kidney transplant recipients

	With RIT (n = 22)			Without RIT (n = 36)		
	Before vaccine	After vaccine	p-value	Before vaccine	After vaccine	p-value
eGFR, ml/min/1.73 m^2 (mean, SD)	47 (13)	48 (14)	0.846	54 (20)	54 (20)	0.935
UP/C (mean, SD)	0.22 (0.17)	0.23 (0.23)	0.800	0.24 (0.8)	0.18 (0.22)	0.032

Abbreviations: eGFR, estimated glomerular rate; IS, immunosuppression; RIT, rituximab; SD, standard deviation; UP/C, urine protein to creatinine ratio.

controls: 2.5% versus 63.3%.[4,12–18] Our data showed a similar anti-SARS-CoV-2 antibody seroconversion rate after two doses of the vaccine in the non-RIT and RIT groups. There are several explanations for our different results regarding humoral responses after vaccination in KTR with rituximab compared with those in a study by Haskin et al., which reported rituximab therapy was a significant risk factor for poor humoral responses.[6] Their indication for rituximab was induction therapy and treatment for rejection, post-transplant lymphoproliferative disease, and glomerulonephritis. They did not report the dose of rituximab administered; however, the total dosage of rituximab is generally much higher for post-transplant lymphoproliferative disease and glomerulonephritis treatment than for induction therapy.[19,20] Furer et al. reported that treatment with rituximab at a mean dose of 1656.1 ± 623.6 mg in autoimmune inflammatory rheumatic disease patients was a major risk factor that reduced BNT162b2-induced immunogenicity.[7] Apostolidis et al. reported that SARS-CoV-2 mRNA vaccine-induced antibody responses were significantly reduced in patients with anti-CD20 monoclonal antibody monotherapy at a mean cycle of 3.2 ± 1.6 compared with healthy controls.[8] The total dosage of rituximab in those studies was much higher than that in our cohort; therefore, a higher total dosage of rituximab might impair humoral responses to mRNA vaccines. Interestingly, the authors of these two studies demonstrated that a shorter interval between the pre-vaccination administration of anti-CD20 antibody to SARS-CoV-2 mRNA vaccination was significantly associated with a lack of humoral response.[12] In our study, no humoral response was induced in all four KTR with rituximab within 12 months before the vaccine, suggesting that a relatively long duration from rituximab therapy to the first vaccination might affect the seroconversion rate in KTR with rituximab in this cohort.

The rate of cellular responses after two doses of vaccine was reported as 30.4% to 92.0%, which was lower than that of the immunocompetent controls but the same or slightly higher compared with the humoral rate response in KTR.[12,13,16] Our data showed that the cellular immune response rate was lower than that of controls but slightly higher than the humoral response rate in KTR after two doses of vaccine as reported by other groups.[12,14,16,17] Moreover, the cellular response rate in the RIT group was similar to that of the non-RIT group. Apostolidis et al. reported that antigen-specific CD4 and CD8 T cell responses were confirmed in all patients with multiple sclerosis treated with anti-CD20 antibody therapy even though the dosage of rituximab was higher than that in our patients.[8] The facilitation of cellular responses related to vaccination might protect against severe COVID-19 in KTR with low seroconversion and low antibody titers, even when they are administered low-dose rituximab therapy.[21]

Although all controls were anti-SARS-CoV-2 antibody and ELISpot activity positive, there was a high probability of discrepancy between anti-SARS-CoV-2 antibody positive and ELISpot activity positive KTRs. The same discrepancy was observed in other studies, which reported that the probability of being only ELISpot activity positive was slightly higher than that of anti-SARS-CoV-2 antibody positive. An immunosuppressive regimen in KT was considered to amplify this phenomenon in KTR. There was a weak correlation between anti-SARS-CoV-2 antibody titer and the rate of change in cytokine activity for all participants, KTR without RIT, and KTR with RIT; however, there was no correlation in controls. Kato et al. also reported a weak correlation between SARS-CoV-2 spike protein specific T-cell responses and SARS-CoV-2 spike protein IgG titers.[22] In this study, the correlation in controls and KTR without RIT was weak, which might have been affected by the low number of participants in each group. Further evaluation in a large cohort is needed.

Our study had several limitations. First, the number of vaccinated patients, especially those who received rituximab therapy, was low. Second, we evaluated immune responses once after vaccination, which meant we could not evaluate whether the immune response was increased or decreased over time compared with immunocompetent people. Third, we could not evaluate the protective effect of vaccination against COVID-19 infection, including newly developed variants such as omicron, because of the low incidence of actual COVID-19 in this cohort. Last, booster vaccination for immunosuppressed patients including KTR has been started because of poor immune responses and insufficient protection induced by the standard two doses of vaccine in these patients.[4,23] Approximately half the KTR experienced anti-SARS-CoV-2 antibody seroconversion and/or cellular responses in this cohort and a booster vaccination was necessary; therefore, we should also evaluate whether the booster vaccination is impaired in KTR with low-dose rituximab.

In conclusion, low-dose rituximab in KTR did not affect humoral or cellular responses to SARS-CoV-2 mRNA vaccines without deterioration of kidney function. Because the vaccine response rate was insufficient, booster vaccination and its efficacy for obtaining humoral and cellular responses in KTR with low-dose rituximab should be studied in a larger cohort in the future.

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AUTHOR CONTRIBUTIONS

Satoshi Takai: Investigation. **Hayato Nishida:** Conceptualization; investigation; methodology. **Hiromi Ito:** Conceptualization; investigation; methodology. **Hiroki Fukuhara:** Supervision; validation. **Takaaki Nawano:** Supervision; validation. **Takafumi Narisawa:** Supervision; validation. **Hidenori Kanno:** Supervision; validation. **Mayu Yagi:** Supervision; validation. **Atsushi Yamagishi:** Supervision; validation. **Toshihiko Sakurai:** Supervision; validation. **Sei Naito:** Supervision; validation. **Tomoyuki Kato:** Supervision; validation. **Keita Morikane:** Investigation; resources; supervision; validation. **Norihiko Tsuchiya:** Conceptualization; supervision; validation.

CONFLICT OF INTEREST

None declared.

APPROVAL OF THE RESEARCH PROTOCOL BY AN INSTITUTIONAL REVIEWER BOARD

The protocol for this research project has been approved by a suitably constituted Ethics Committee of the institution and it conforms to the provisions of the Declaration of Helsinki. Committee of Yamagata University Faculty of Medicine, Approval No. 2020–377.

INFORMED CONSENT

All informed consent was obtained from the subjects.

REGISTRY AND THE REGISTRATION NO. OF THE STUDY/TRIAL

N/A.

ANIMAL STUDIES

N/A.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Characteristics of each kidney transplant recipients.