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Original Article

The role of interleukin-21 in COVID-19 vaccine–induced B cell–mediated immune responses in patients with kidney disease and kidney transplant recipients

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ABSTRACT

T-cell–mediated help to B cells is required for the development of humoral responses, in which the cytokine interleukin (IL)-21 is key. Here, we studied the mRNA-1273 vaccine–induced SARS-CoV-2–specific memory T-cell IL-21 response, memory B cell response,

Abbreviations: BAU, binding antibody unit; CKD, chronic kidney disease; COVID-19, coronavirus disease-2019; IL, Interleukin; IL-21R, interleukin-21 receptor; IR, immune response; Ig, immunoglobulin; KTR, kidney transplant recipients; MMF, mycophenolate mofetil; NK, natural killer; PBMC, peripheral blood mononuclear cell; SARS-CoV, severe acute respiratory syndrome coronavirus.

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and immunoglobulin (Ig)G antibody levels in peripheral blood at 28 days after the second vaccination by ELISpot and the fluorescent bead–based multiplex immunoassay, respectively. We included 40 patients with chronic kidney disease (CKD), 34 patients on dialysis, 63 kidney transplant recipients (KTR), and 47 controls. We found that KTR, but not patients with CKD and those receiving dialysis, showed a significantly lower number of SARS-CoV-2–specific IL-21 producing T cells than controls ($P < .001$). KTR and patients with CKD showed lower numbers of SARS-CoV-2–specific IgG–producing memory B cells when compared with controls ($P < .001$ and $P = .01$, respectively). The T-cell IL-21 response was positively associated with the SARS-CoV-2–specific B cell response and the SARS-CoV-2 spike S1–specific IgG antibody levels (both Pearson $r = 0.5$; $P < .001$). In addition, SARS-CoV-2–specific B cell responses were shown to be IL-21 dependent. Taken together, we show that IL-21 signaling is important in eliciting robust B cell–mediated immune responses in patients with kidney disease and KTR.

1. Introduction

Severe acute respiratory syndrome coronavirus (SARS-CoV)-2–neutralizing antibodies are regarded as an important correlate of protection against severe coronavirus disease 2019 (COVID-19).[1] However, low and waning levels of neutralizing antibodies have often been observed in patients with kidney disease and in kidney transplant recipients (KTR) in particular, which are associated with severe COVID-19 in these patients.[2,3] An essential factor in the development of humoral responses is interleukin (IL)-21. IL-21 is a proinflammatory cytokine and produced by different T-cell subsets, including follicular helper T cells, T helper 17 cells, $CD8^+$ T cells, and natural killer (NK) T cells.[4] It can also be produced by NK cells and stromal cells.[4,5] IL-21 is involved in T cell–dependent B cell activation in germinal centers, which leads to rapid proliferation and subsequent differentiation of B cells into memory and long-lived plasma cells.[6,7] In addition, IL-21 promotes cytotoxic activity of NK cells; stimulates proliferation, differentiation, and maintenance of CD8 T cells; and regulates macrophage activation, which in turn stimulates $CD4^+$ T-cell proliferation.[8-10] We hypothesize that the IL-21 response is lower in patients with a severely impaired kidney function due to the immunosuppressive effect of uremia and impaired in KTR due to specific immunosuppressive medications, compared with healthy indivuduals.[11,12] This could explain both the poor humoral and cellular responses to vaccination observed in these patients.[13,14]

Although neutralizing antibodies are regarded an important correlate of protection, the levels of neutralizing antibodies after repeated COVID-19 vaccination are often low in patients with kidney disease and KTR. It has been shown that the absence of neutralizing antibodies does not correlate to an absence of cellular immune responses (IRs), which were recently shown to be important in protection against severe COVID-19 in macaques.[15,16] These SARS-CoV-2–specific cellular memory responses could balance the low antibody levels in preventing or limiting severe COVID-19, which is especially important in patients with kidney disease and KTR since they are at increased risk of severe COVID-19 after SARS-CoV-2 breakthrough infections.[17] The fact that vaccine efficacy has been largely maintained with regards to severe disease, hospitalization, and death, despite the loss of neutralizing capacity of vaccine–induced antibodies against the newly-emerged Omicron sublineages, underlines the potential important role for SARS-CoV-2–specific T cells.[18]

Currently, there are no data available on the COVID-19 vaccine–induced IL-21 response. Here, we studied the induction of SARS-CoV-2 spike (S)–specific memory T-cell IL-21 response, memory B cell response, and SARS-CoV-2 spike S1–specific immunoglobulin (Ig)G antibodies after 2 doses of the mRNA-1273 vaccine in patients with chronic kidney disease (CKD), patients receiving dialysis, and KTR and compared that with controls. We show that IL-21 signaling is important in eliciting robust B cell–mediated IRs in patients with kidney disease patients and KTR.

2. Materials and methods

2.1. Study design

In this study, we assessed the SARS-CoV-2–specific memory T-cell IL-21 response, memory B cell response and SARS-CoV-2 spike S1–specific IgG antibodies in patients with CKD, patients receiving dialysis, KTR, and controls. Participants were randomly selected from a prospective controlled multicenter cohort study (the RECOVAC IR study).[19] Additional samples from KTR were measured because this group previously showed an impaired vaccine–induced antibody response in the RECOVAC IR study.[19] Ethical approval was obtained from the Dutch Central Committee on Research Involving Human Subjects (CCMO, NL76215.042.21), the local ethics committees of the participating centers in the context of the prospective controlled multicenter cohort study (NCT04741386), and the local ethics committee of our center (MEC2018-1623) for the studies of health care workers' blood samples.

2.2. Participants

In total, 47 control subjects (without kidney disease, estimated glomerular filtration rate of >45 mL/min/1.73m^2), 40 patients with CKD G4/G5 (estimated glomerular filtration rate of <30 mL/min/1.73m^2), 34 patients treated with dialysis (included patients receiving both hemodialysis and peritoneal dialysis), and 63 KTRs were included. All participants received 2 doses of mRNA-1273 (Moderna Biotech Spain, S.L.), with an interval of 28 days according to the manufacturer's instructions. To ensure that the cellular memory responses were purely vaccine-induced, we excluded participants with a prior SARS-CoV-2 infection based on IgG SARS-CoV-2 spike S1–specific antibody level of ≥10 binding antibody unit (BAU)/mL at baseline. In addition, participants who reported a SARS-CoV-2 infection between baseline and 28 days after the second vaccination were also excluded.[19]

2.3. SARS-CoV-2–specific memory T-cell IL-21 response

The SARS-CoV-2–specific memory T-cell IL-21 response was measured in all 184 participants at baseline and 28 days after the second vaccination (see also materials and methods in Supplementary Appendix). Measurement of this response was performed by a commercially available IL-21 ELISpot according to the manufacturer's instructions (U-CyTech biosciences).[20] Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood using Ficoll Paque (GE Healthcare) and stored at −140 °C until analysis. Defrosted cells were added to a 96-well PVDF plate (300 000 PBMCs/well) precoated with a coating antibody capable of capturing IL-21. Then, these cells were stimulated with SARS-CoV-2 antigens for 44 hours using a combination of S1 and S2 peptide pools (JPT Peptide Technologies), consisting of 15-mer peptides overlapping 11 amino acids that cover the entire S protein. Next, biotinylated detection antibody, streptavidin-horseradish peroxidase and 3-amino-9-ethylcarbazole were added to stain the IL-21–producing cells. Finally, spots were counted by an ELISpot reader (Bioreader 6000-V; Bio-Sys). The memory T-cell IL-21 response was defined as the number of SARS-CoV-2–specific memory T-cell IL-21–producing cells (spots) per 1 million (10^6) PBMCs.

2.4. SARS-CoV-2–specific memory B cell response

The SARS-CoV-2–specific memory B cell response was measured at baseline and 28 days after the second vaccination (see also materials and methods in supplementary appendix). The memory B cell response could be measured in only 115 of the 184 participants due to limited availability of PBMCs. Measurement was performed by a commercially available B cell ELISpot, according to the manufacturer's instructions (U-CyTech biosciences).[21] Defrosted PBMCs were added to a 24 well plate (2 000 000 PBMCs/well) and were activated polyclonally by preincubating with IL-2 and R848 for 7 days. Then, cells (200 000 cells for SARS-CoV-2 antigen–specific and 10 000 cells for total IgG–producing cells) were added to a 96-well PVDF plate, precoated with a coating antibody capable of capturing IgG, for 20 hours. Next, biotinylated detection antibodies were added, including IgG biotin, to estimate the total IgG-producing cells and recombinant SARS-CoV-2 spike His-tag biotin protein to estimate the SARS-CoV-2 antigen–specific IgG-producing cells. Hereafter, horseradish peroxidase and 3-amino-9-ethylcarbazole were added to stain the total IgG-producing cells and SARS-CoV-2 antigen–specific IgG-producing cells. Finally, spots were counted by an ELISpot reader (Bioreader 6000-V). The memory B cell response was defined as the number of SARS-CoV-2 antigen–specific IgG-producing cells (spots) per 1 million (10^6) PBMCs.

2.5. Blocking the IL-21 signaling pathway

To investigate the effect of blocking IL-21 signaling on SARS-CoV-2–specific memory B cell response, we first measured whether indeed IL-2+R848 was able to induce IL-21 secretion. For this, PBMCs from 4 health care workers who were vaccinated twice with a COVID-19 mRNA vaccine were used and treated under the same condition of the B cell ELISpot assay. Next, to detect the proportion of IL-21–secreting cells, we used the IL-21 ELISpot assay in which we stimulated these PBMCs with the standard concentration of IL-2+R848. After this, we measured the SARS-CoV-2–specific memory B cell response in the absence and presence of a human monoclonal anti–interleukin-21 receptor (IL-21R) recombinant antibody (ATR-107; Absolute Antibody) in the B cell ELISpot. ATR-107 has been shown to be a potent IL-21 pathway inhibitor by binding to the IL-21R.[22] The IL-21R antagonist was added in 4 different concentrations (1, 5, 10, and 20 μg/mL) to the B cell ELISpot assay while simultaneously adding IL-2 and R848. This was done in samples from 7 health care workers and 5 control subjects of the RECOVAC IR study (in total, N = 12), who were vaccinated twice with a COVID-19 mRNA vaccine. Subsequently, we measured both the SARS-CoV-2 memory B cell response and total IgG-producing cells (which served as positive control) at 28 days.

2.6. SARS-CoV-2 spike S1–specific antibody responses

SARS-CoV-2 spike S1–specific IgG antibody levels were already measured in the context of the multicenter cohort study (RECOVAC IR) at baseline, 28 days after the second vaccination, and 6 months after the second vaccination and were available for all 184 participants. These were measured in serum samples by a validated fluorescent bead–based multiplex immunoassay, with a specificity and sensitivity of 99.7% and 91.6%, respectively.[23] Concentrations were expressed as international BAUs per milliliter. Serological responders were defined as participants with an S1-specific IgG antibody level of ≥10 BAU/mL after vaccination.

2.7. Statistical analysis

We analyzed the data using 5 steps. First, the baseline characteristics in each group are described. Continuous data were presented as median with interquartile range in case of nonnormal distribution and as mean ± SD in case of normal

distribution. Categorical data were presented as numbers and percentages. Second, we illustrated the memory T-cell IL-21 and memory B cell response by means of boxplots at baseline and 28 days after the second vaccination. Regarding the positive controls, we compared these between the different cohorts to account for potential influence of these on the measured outcomes. Differences between each cohort and the control group were examined using a Mann-Whitney *U* test. IRs were non-normally distributed and were therefore $\log_{10}$-transformed. Third, we investigated the correlation between memory T-cell IL-21 and memory B cell response (both at 28 days); memory T-cell IL-21 (at 28 days) and antibody response (at 28 days and 6 months); and memory B cell (at 28 days) and antibody response (at 28 days and 6 months) after the second vaccination, using a Pearson correlation coefficient (Pearson *r*). We determined these correlations for all groups together. Moreover, we counted the number of participants with a memory T-cell IL-21 and memory B cell response who were defined as a serological nonresponder at 28 days after the second vaccination, to find out whether the absence of antibodies necessarily means an absence of cellular memory IRs. Responders and non-responders were classified using the lower limit of detection of the cellular responses and the cutoff value for the IgG antibody level as described earlier. We also examined the IL-21 response in participants with and without a SARS-CoV-2 breakthrough infection. Fourth, we performed univariate quantile regressions between several important baseline characteristics and memory T-cell IL-21 response at 28 days after the second vaccination to identify baseline characteristics relevant for explaining the memory T-cell IL-21 response. We built this in a stepwise manner for each variable (eg, sex, age, and cohort) sequentially. In addition, we performed a multivariate quantile regression including all relevant baseline characteristics. Further, in the KTR cohort, we performed univariate quantile regressions. As the sample size was small, we did not perform multivariate regressions. Finally, we presented the data of the effect of blocking IL-21 on memory B cell response in box-and-whisker plots for increasing IL-21R antagonist dose. The percentage of inhibition was calculated by the following formula: 1 − (median number of SARS-CoV-2–specific B cell spots per 10^6 PBMCs with adding IL-21R antagonist in a specific concentration/median number of SARS-CoV-2–specific B cell spots per 10^6 PBMCs without adding IL-21R antagonist) multiplied by 100. Statistical analyses were performed with SPSS (version 28), R studio (version 4.1.2), and GraphPad Prism 28. A 2-sided *P* value of $<.05$ was considered statistically significant.

3. Results

3.1. Baseline characteristics

Baseline characteristics are described in Table 1. At baseline, no major differences between the groups were noted in SARS-CoV-2–specific memory T-cell IL-21 (Fig. 1A and Supplementary Table S1), memory B cell (Fig. 1C and Supplementary Table S1), and spike S1–specific antibody response (Supplementary Table S1 and Supplementary Fig. S2A).

3.2. Vaccine–induced SARS-CoV-2–specific memory T-cell IL-21 response

All 184 participants showed a memory T-cell IL-21 response at 28 days after the second vaccination (Fig. 1B). We found a significantly lower median number of SARS-CoV-2–specific IL-21–producing memory T cells at 28 days after the second vaccination in KTR compared with controls (200 [50-398]/10^6 PBMCs vs 501 [398-794]/10^6 PBMCs; $P < .001$). However, this was not the case for patients with CKD (501 [200-794]/10^6 PBMCs; $P = .31$) and patients receiving dialysis (398 [200-631]/10^6 PBMCs; $P = .09$). The positive control (PBMCs stimulated with phytohemagglutinin) was not significantly different between the different cohorts (Supplementary Fig. S1A, B). An illustration of IL-21 spots measured by ELISpot is shown in Figure 1E. As transplant patients are prescribed immunosuppressive drugs, which may influence cytokine responses, we analyzed the correlation between dose and trough levels of both tacrolimus and mycophenolate mofetil (MMF)/mycophenolic acid and SARS-CoV-2–specific memory T-cell IL-21 response. Our analysis indeed showed that the tacrolimus trough level measured within 1 month before baseline correlated with the SARS-CoV-2–specific memory T-cell IL-21 response at 28 days after the second vaccination in KTRs (Pearson $r = -0.4$; $P = .02$) (Fig. 2). We also found a negative trend toward both a higher tacrolimus (Pearson $r = -0.2$; $P = .18$) (Supplementary Fig. S3A) and MMF dose at baseline (Pearson $r = -0.2$; $P = .16$) (Supplementary Fig. S3B). In total, 5 of the 184 participants had a breakthrough infection during a follow-up period of 9 months after the second vaccination, all of whom were KTR (Supplementary Fig. S4). We found a lower median number of SARS-CoV-2–specific IL-21–producing memory T cells at 28 days after the second vaccination in participants who had a breakthrough infection compared with participants who had not (125 [32-291]/10^6 PBMCs vs 398 [157-707]/10^6 PBMCs). Since only 5 participants had a breakthrough infection, we did not perform statistical analysis.

3.3. Vaccine–induced SARS-CoV-2–specific memory B cell response

In total, 98% (113/115) of the participants had a detectable memory B cell response at 28 days after the second vaccination. Two KTRs did not develop detectable memory B cells (Fig. 1D). Additionally, we found a significantly lower median number of SARS-CoV-2–specific IgG–producing memory B cells at 28 days after the second vaccination in KTRs than that in controls (25 [5-50]/10^6 PBMCs vs 158 [63-631]/10^6 PBMCs; $P < .001$). To a lesser extent, patients with CKD also had significantly reduced SARS-CoV-2–specific IgG–producing memory B cells compared with controls (79 [25-316]/10^6 PBMCs; $P = .01$), while this was not the case for patients on dialysis (100 [20-501]/10^6 PBMCs; $P = .12$). The positive control (total IgG-producing cells) was not significantly different between the different cohorts (Supplementary Fig. S1C, D). An illustration of B cell spots measured by ELISpot is shown in Figure 1E. To assess the role of IL-21 in B cell activation, we investigated the correlation between the memory T-cell IL-21 and memory B cell response. We found a

Table 1
Baseline characteristics of participants in whom IL-21 ELISpot was performed.

Characteristic	Control (n = 47)	CKD G4/5 (n = 40)	Dialysis (n = 34)	KTR (n = 63)	Total (N = 184)
Age (y), median (IQR)	61 (52-69)	64 (55-68)	61 (49-77)	55 (44-65)	59 (49-68)
Sex, n (%)					
Male	21 (45)	24 (60)	25 (74)	38 (60)	108 (59)
Female	26 (55)	16 (40)	9 (26)	25 (40)	76 (41)
Ancestry, n (%)					
Caucasian	41 (87)	34 (85)	28 (82)	55 (87)	158 (85)
Asian	3 (7)	2 (5)	0 (0)	2 (3)	7 (4)
Black	0 (0)	4 (10)	4 (12)	3 (5)	11 (6)
Other	2 (4)	0 (0)	2 (6)	3 (5)	7 (4)
Not specified	1 (2)	0 (0)	0 (0)	0 (0)	1 (1)
BMI[a], median (IQR)	27 (23-31)	29 (25-31)	26 (24-30)	26 (24-28)	27 (24-30)
eGFR[b], median (IQR)	86 (74-101)	17 (12-23)	NA	51 (40-62)	53 (25-74)
Time after last transplantation (y), median (IQR)	NA	NA	NA	7 (2-13)	NA
Comorbidities, n (%)					
Hypertension	17 (36)	36 (90)	20 (59)	51 (81)	124 (67)
Diabetes mellitus	9 (19)	16 (40)	6 (18)	15 (24)	46 (25)
History of CAD	5 (11)	10 (25)	7 (21)	10 (16)	32 (17)
Heart failure	1 (2)	5 (13)	2 (6)	2 (3)	10 (5)
Chronic lung disease	6 (13)	4 (10)	5 (15)	3 (5)	18 (10)
History of malignancy	6 (13)	6 (15)	9 (27)	7 (11)	28 (15)
Auto-immune disease	0 (0)	3 (8)	1 (3)	3 (5)	7 (4)

BMI, body mass index; CAD, coronary artery disease; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; ELISpot, enzyme-linked immunosorbent spot; IL, interleukin; IQR, interquartile range; KTR, kidney transplant recipient; NA, not applicable.

[a] BMI is the weight in kilograms divided by the square of the height in meters.

[b] eGFR (in mL/min/1.73 m^2) was calculated using CKD-EPI.

positive correlation between these responses at 28 days after the second vaccination for all participants together (Pearson $r = 0.5$; $P < .001$) (Fig. 3).

3.4. Correlations between vaccine–induced memory T-cell IL-21 response and antibody response

The influence of SARS-CoV-2-specific T-cell reactivity on the induction of antibody response was examined by determining the correlation between memory T-cell IL-21 response and SARS-CoV-2 Spike S1-specific IgG antibody levels. The number of memory IL-21–producing T cells was positively correlated with the SARS-CoV-2 spike S1–specific IgG antibody level at 28 days after the second vaccination for all participants analyzed together (Pearson $r = 0.5$; $P < .001$) (Fig. 4A). To assess whether being a serological nonresponder (<10 BAU/mL) means that there is no memory T-cell IL-21 response, we counted the number of participants with a memory T-cell IL-21 response who were defined as a serological nonresponder at 28 days after the second vaccination. Of the 184 participants, 22 (12%) showed a memory T-cell IL-21 response, despite being defined as a serological nonresponder at 28 days after the second vaccination (<10 BAU/mL), of whom 21 (95%) were KTR. A positive correlation was also found between memory T-cell IL-21 response at 28 days and SARS-CoV-2 spike S1–specific IgG antibody level at 6 months after the second vaccination for all participants together (Pearson $r = 0.5$; $P < .001$) (Fig. 4B).

3.5. Correlations between vaccine–induced memory B cell response and antibody response

The relationship between vaccine–induced memory B cells and antibody formation was illustrated by the positive correlation between memory B cell response and SARS-CoV-2 spike S1–specific IgG antibody level at 28 days after the second vaccination for all participants together (Pearson $r = 0.5$; $P < .001$) (Fig. 5A). The durability of this relationship was demonstrated by a positive correlation between memory B cell response at 28 days and SARS-CoV-2 spike S1–specific IgG antibody level at 6 months after the second vaccination for all participants

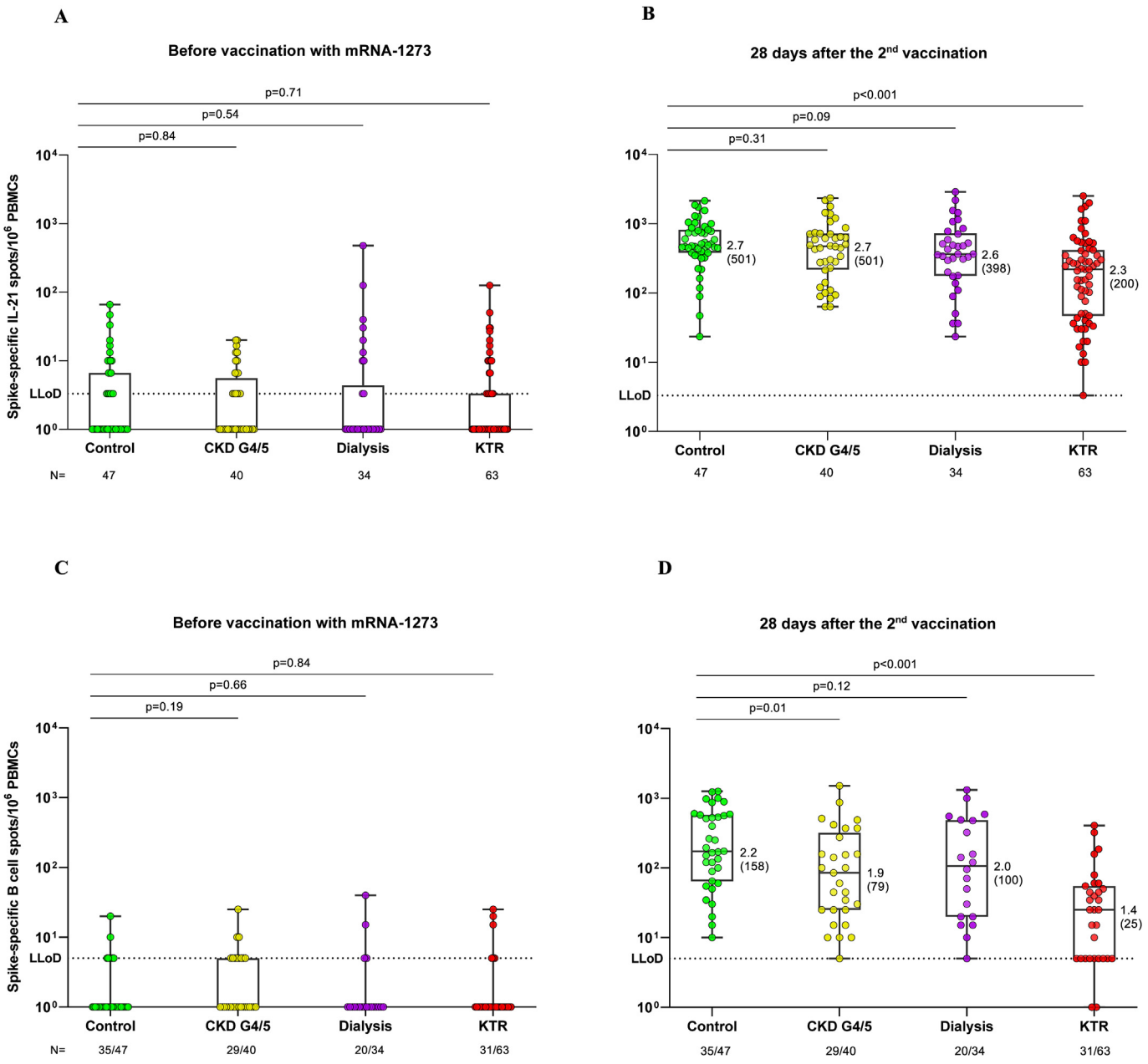


Figure 1. SARS-CoV-2–specific memory T-cell IL-21 and memory B cell response measured by ELISpot. Data are presented in box-and-whisker plots. The horizontal line and numbers within the whisker indicate the medians and the tops and bottoms indicate interquartile ranges. Mann-Whitney *U* tests were applied to compare medians. LLoD stands for the lower limit of detection and was 3.3 spots (or 0.52 if $\log_{10}$-transformed) for memory T-cell IL-21 response and 5 spots (or 0.7 if $\log_{10}$-transformed) for memory B cell response. Each symbol represents an individual. SARS-CoV-2–specific memory T-cell IL-21 response at (A) baseline and (B) 28 days after the second vaccination. SARS-CoV-2–specific memory B cell response at (C) baseline (n = subset of participants) and (D) 28 days after the second vaccination (n = subset of participants). (E) Illustration of spots measured by ELISpot. The top right number in red indicates the number of spots measured by the ELISpot assay (IL-21 producing cells per 300 000 PBMCs/well, total IgG–producing cells per 10 000 PBMCs/well or SARS-CoV-2–specific B cell spots per 200 000 PBMCs/well) and the top left number in red indicates the location on the 96-well PVDF plate. CKD, chronic kidney disease; KTR, kidney transplant recipient; PBMC, peripheral blood mononuclear cell.

together (Pearson $r = 0.4$; $P < .001$) (Fig. 5B). Finally, to assess whether being a serological nonresponder (<10 BAU/mL) means that there is no memory B cell response, we counted the number of participants with a memory B cell response who were defined as a serological nonresponder at 28 days after the second vaccination. Of the 113 participants, 10 (9%) showed a memory B cell response, despite being defined as a serological nonresponder, of whom 9 (90%) were KTRs.

3.6. Associations between baseline characteristics and the vaccine–induced memory T-cell IL-21 response

We performed univariate quantile regressions to identify baseline characteristics associated with the vaccine–induced memory T-cell IL-21 response at day 28 after the second vaccination. Univariate quantile regressions showed that female sex was associated with a higher memory T-cell IL-21 response

E

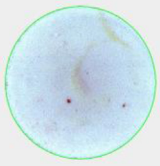
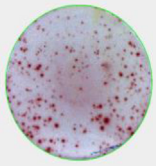
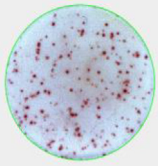
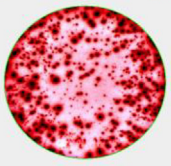
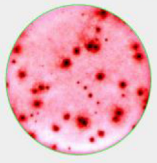
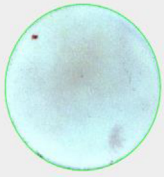
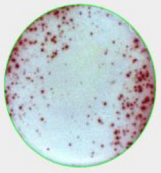
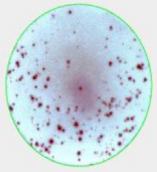
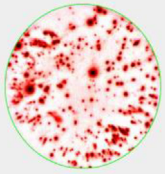
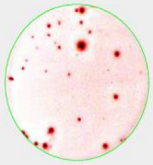
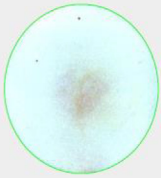
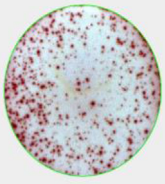
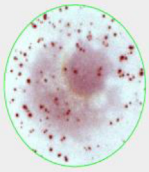
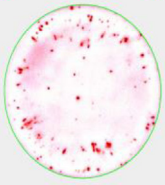
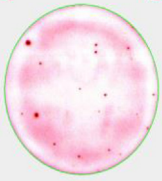
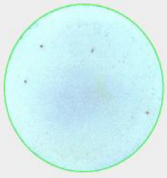
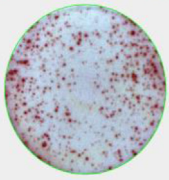
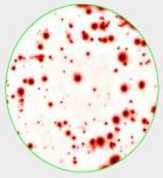
Cohort	Negative control (DMSO)	Positive control (PHA)	Stimulated with SARS-CoV-2 antigen (SARS-CoV-2 specific IL-21 spots)	Total IgG producing cells (positive control)	Stimulated with recombinant SARS-CoV-2 Spike His-tag Biotin Protein (SARS-CoV-2 specific B cell spots)
Control	H4 2 	H6 184 	H7 145 	H7 322 	H3 66 
CKD G4/5	H2 5 	H3 168 	H8 113 	B7 212 	B1 24 
Dialysis	A5 2 	A6 312 	B4 97 	F9 61 	F2 12 
KTR	A4 3 	A6 255 	C5 11 	F9 110 	F2 4 

Figure 1. (*continued*).

($\beta = 0.18$; 95% CI= 0.09 to 0.31; $P < .01$), while being a KTR was associated with a lower response ($\beta = -0.35$; 95% CI $= -0.60$ to -0.23; $P < .01$) (Table 2). Multivariate quantile regressions confirmed that being a KTR was associated with a lower response ($\beta = -0.40$; 95% CI $= -0.67$ to -0.20; $P < .01$), but the association with sex (female) disappeared ($\beta = 0.08$; 95% CI $= -0.10$ to 0.26; $P = .34$). Univariate quantile regressions were unable to show baseline characteristics that were associated with the memory T-cell IL-21 response at day 28 after the second vaccination in KTR (Table 3).

3.7. The effect of blocking IL-21 signaling on SARS-CoV-2–specific memory B cell response

The importance of IL-21 in the functional interaction between antigen-activated T cells and B cells was established by IL-21R–blocking experiments. We first demonstrated that IL-2+R848 was able to induce the secretion of the ligand IL-21 (Fig. 6A). After this, we illustrated that 20 μg/mL of the IL-21R antagonist inhibited the induction of SARS-CoV-2–specific memory B cells by a median of 69% compared to adding no

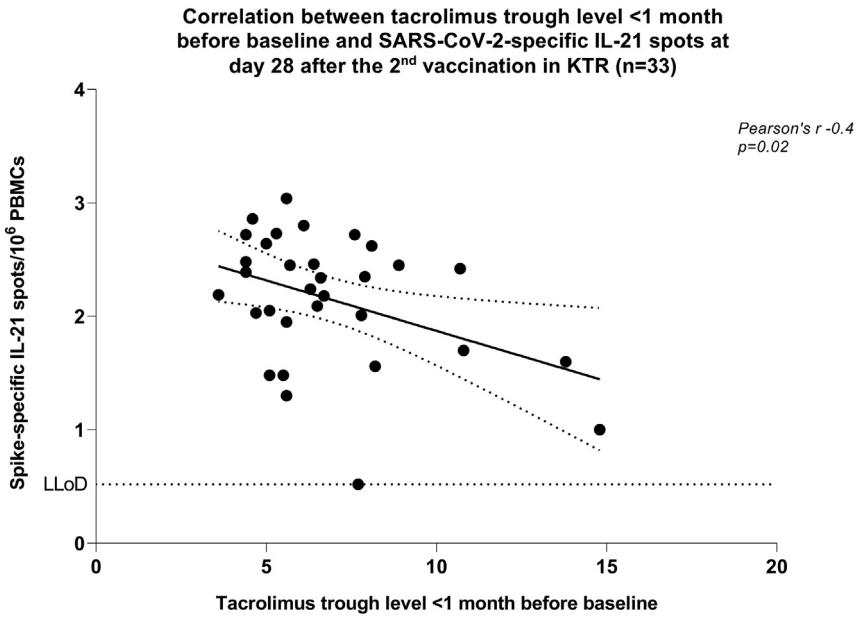


Figure 2. Correlation between tacrolimus trough level within 1 month before baseline and vaccine–induced memory T-cell IL-21 response in kidney transplant recipients (KTRs) at 28 days after the second vaccination (Pearson $r = -0.4$; $P = .02$). The horizontal dotted line represents the lower limit of detection of memory T-cell IL-21 response (= 3.3 spots or 0.52 if $\log_{10}$-transformed).

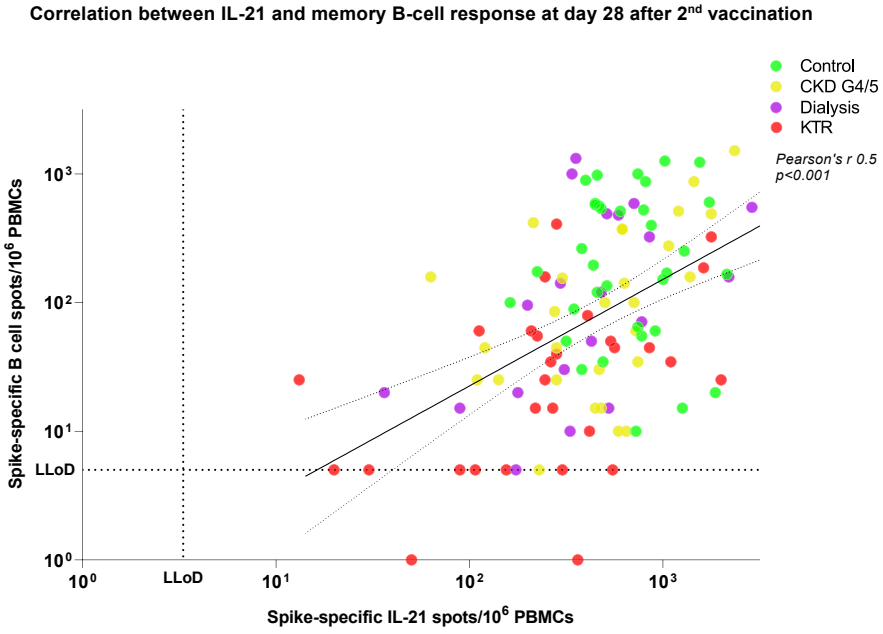


Figure 3. Correlation between vaccine–induced memory T-cell IL-21 response and memory B cell response at 28 days after the second vaccination (Pearson $r = 0.5$; $P < .001$). The horizontal dotted line represents the lower limit of detection of memory B cell response (=5 spots or 0.7 if $\log_{10}$-transformed) and the vertical dotted line represents the lower limit of detection of memory T-cell IL-21 response (=3.3 spots or 0.52 if $\log_{10}$-transformed).

antagonist (Fig. 6B), which was comparable to the percentage of inhibition of the total IgG-producing cells (median 65%) (Fig. 6C).

4. Discussion

IL-21 is an essential factor in the development of humoral responses including the development of (neutralizing) antibodies. This is the first study, to our knowledge, to investigate the mRNA-1273 vaccine–induced SARS-CoV-2–specific memory T-cell IL-21 response, memory B cell response, and SARS-CoV-2 spike S1–specific IgG antibodies in patients with CKD, patients on dialysis and KTRs and compared those with controls.

We demonstrated that the absence of antibodies does not necessarily mean the absence of a memory T-cell IL-21 and memory B cell response in patients at higher risk for severe COVID-19. This suggests that focusing on antibody responses alone is insufficient to assess vaccine efficacy. This finding may

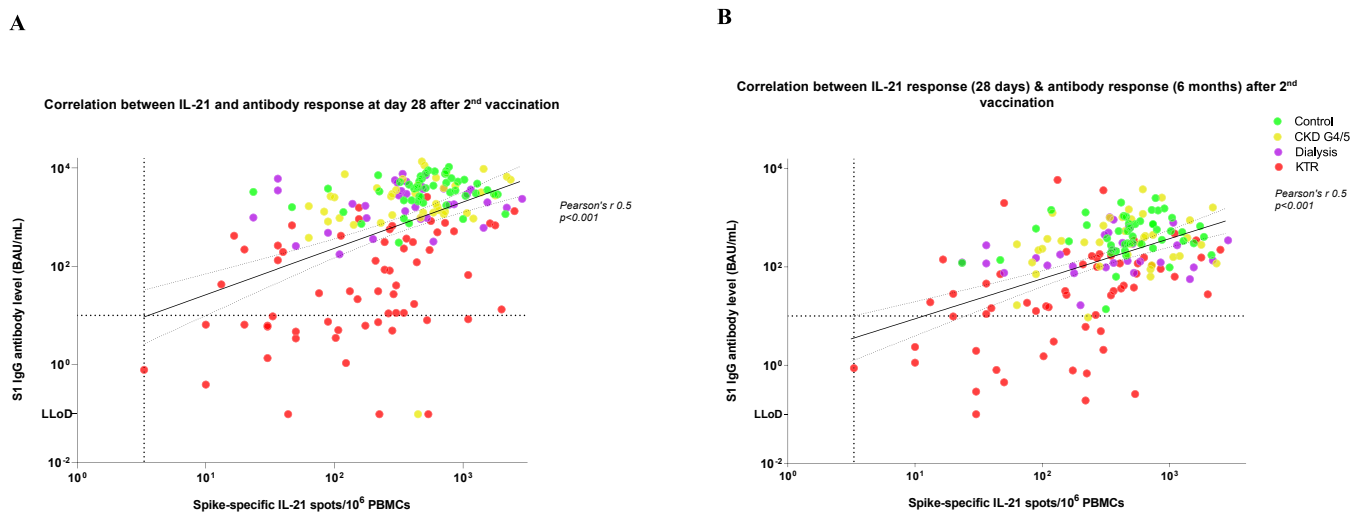


Figure 4. Correlation between vaccine–induced memory T-cell IL-21 response and antibody responses. (A) Correlation between memory T-cell IL-21 response and S1 IgG antibody levels both at 28 days after the second vaccination (Pearson $r = 0.5$). (B) Correlation between memory T-cell IL-21 response at 28 days after the second vaccination and S1 IgG antibody levels at 6 months after the second vaccination (Pearson $r = 0.5$). The horizontal dotted line represents the cutoff value for being a serological responder ($\geq$10 BAU/mL or $\geq$1 if $\log_{10}$-transformed), and the vertical dotted line represents the lower limit of detection of IL-21 response (=3.3 spots or 0.52 if $\log_{10}$-transformed). Each symbol represents an individual.

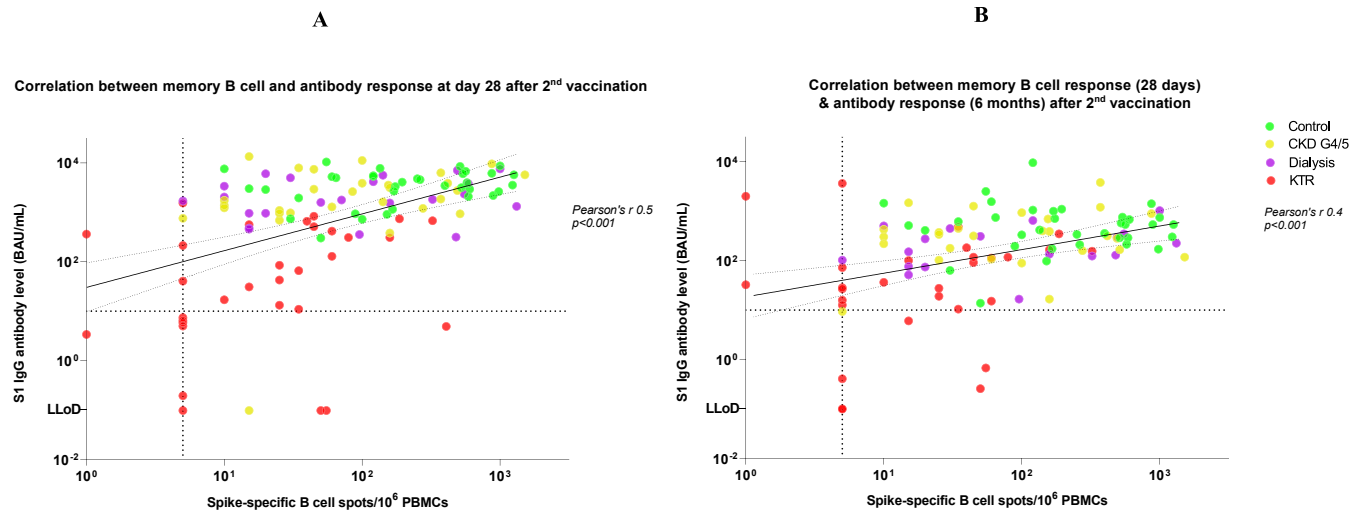


Figure 5. Correlation between vaccine–induced memory B cell response and antibody responses. (A) Correlation between memory B cell response and S1 IgG antibody levels at 28 days after the second vaccination (Pearson $r = 0.5$). (B) Correlation between memory B cell response at 28 days after the second vaccination and S1 IgG antibody levels at 6 months after the second vaccination (Pearson $r = 0.4$). The horizontal dotted line represents the cutoff value for being a serological responder ($\geq$10 BAU/mL or $\geq$1 if $\log_{10}$-transformed), and the vertical dotted line represents the lower limit of detection of memory B cell response (=5 spots or 0.7 if $\log_{10}$-transformed). Each symbol represents an individual.

explain that administration of 2 doses of an mRNA vaccine was successful in preventing hospitalization and death in solid organ transplant recipients with a breakthrough SARS-CoV-2 infection, despite the fact that these patients had impaired antibody responses after this vaccination strategy in other studies.[19,24-27] This is also supported by our data suggesting that the vaccine–induced memory T-cell IL-21 response may affect clinical outcomes because participants with a breakthrough infection had a lower response compared with participants who had not. These findings underline the potential important role for cellular memory IRs in protection against severe COVID-19. In addition to compensating for an impaired antibody response, cellular memory IRs may even have some advantages over antibody responses. First, cellular responses may be more long-lived because SARS-CoV-2–specific T-cell responses have been observed in peripheral blood $\geq$6 months after vaccination in 69% of patients with CKD, in 53% of patients on dialysis, and in 13% of KTR, compared with 75% in control subjects without a kidney

Table 2
Quantile regression on log_{10}-transformed vaccine–induced memory T-cell IL-21 response at day 28 after the second vaccination in all participants.

Covariate	Univariate[a], β (95% CI)	Multivariate[b], β (95% CI)
Sex (male as reference)		
Female	0.18 (0.09 to 0.31)**	0.08 (−0.10 to 0.26)
Age (y)	0.003 (−0.003 to 0.006)	0.001 (−0.01 to 0.007)
Cohort (control as reference)		
CKD G4/5	−0.03 (−0.24 to 0.12)	0.03 (−0.24 to 0.11)
Dialysis	−0.13 (−0.27 to 0.03)	−0.15 (−0.32 to −0.008)
KTR	−0.35 (−0.60 to −0.23)[c]	−0.40 (−0.67 to −0.20)[c]
Ethnicity (Caucasian as reference)		
Asian	0.06 (−1.20 to 0.47)	−0.01 (−1.15 to 0.43)
Black	−0.06 (−0.20 to 0.39)	−0.01 (−0.19 to 0.35)
Other	−0.02 (−0.09 to 0.11)	0.04 (−0.09 to 0.29)
BMI (kg/m^2)	−0.003 (−0.02 to 0.02)	0.001 (−0.02 to 0.009)
Past malignancy (no malignancy as reference)	0.11 (−0.16 to 0.30)	0.18 (−0.12 to 0.23)

BMI, body mass index; CKD, chronic kidney disease; KTR, kidney transplant recipient.

[a] Univariate quantile regressions, crude estimates.

[b] Multivariate quantile regression.

[c] Significant at $P < .05$.

Table 3
Quantile regression on log_{10}-transformed vaccine–induced memory T-cell IL-21 response at day 28 after the second vaccination in KTRs.

Covariate	Univariate[a], β (95% CI)
Sex (male as reference)	
Female	0.12 (−0.19 to 0.68)
Age (y)	−0.0003 (−0.02 to 0.007)
Time after last transplantation (y)	0.01 (−0.01 to 0.04)
Donor type of last transplant (living donor as reference)	0.25 (−0.12 to 0.69)
No. of transplants received	0.07 (−0.75 to 0.16)
Absolute lymphocyte count	−0.09 (−0.29 to 0.16)
Steroids (no steroids as reference)	−0.13 (−0.21 to 0.81)
Mycophenolate mofetil[b] (no mycophenolate mofetil as reference)	NA
Calcineurin inhibitor[b] (no calcineurin inhibitor as reference)	NA
Baseline eGFR	0.01 (−0.004 to 0.02)

eGFR (in mL/min/1.73 m^2) was calculated using CKD-EPI.
eGFR, estimated glomerular filtration rate; IL, interleukin; KTR, kidney transplant recipients; NA, not applicable.

[a] Univariate quantile regressions, crude estimates.

[b] The distribution of the users versus nonusers of mycophenolate mofetil (18/63 nonusers, 29%) or calcineurin inhibitors (11/63 nonusers, 17%) was insufficient to estimate in the quantile regressions. Hence, estimates could not be estimated or tested as possible predictors of memory T-cell IL-21 response.

disease.[3] Next to this, SARS-CoV–specific memory T cells have been detected more than 17 years postinfection, demonstrating the durability of coronavirus-specific T cells.[28] In contrast, the average half-life of SARS-CoV-2–neutralizing antibodies was determined to be 20 days.[29] Second, virus-specific T cells can recognize a broad array of viral peptides, which makes it harder for emerging variants to escape T-cell recognition.[30] In contrast, antibody neutralization of variants can be compromised because variants harbor mutations within the receptor-binding domain of the spike protein.[31] PBMCs consist of other immunocompetent cells than just T cells, which can also produce IL-21. Therefore, we cannot exclude that stimulation of PBMCs with SARS-CoV-2

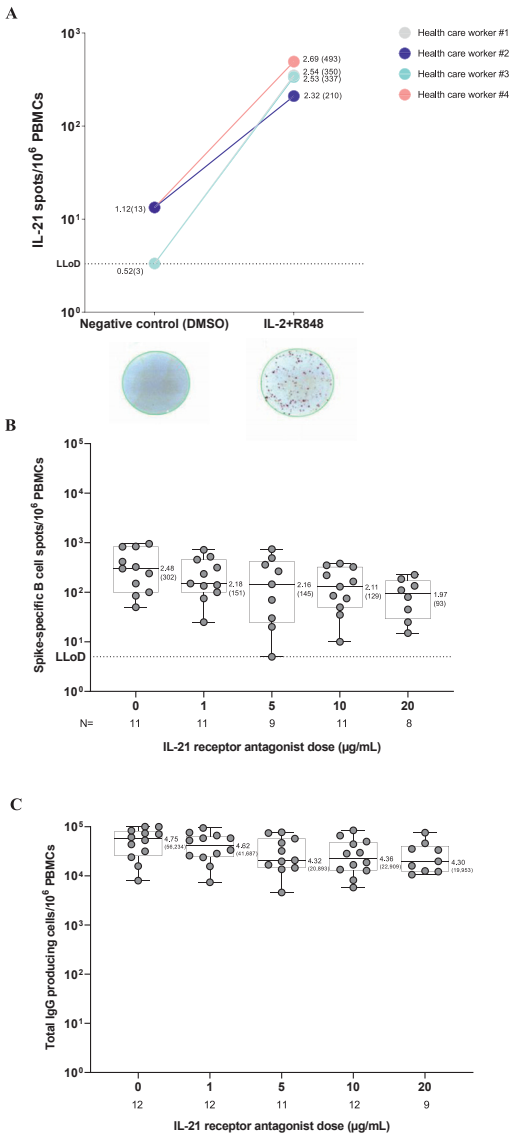


Figure 6. Effect of blocking IL-21 by adding an IL-21 receptor antagonist (ATR-107) on the vaccine–induced SARS-CoV-2–specific memory B cell response. (A) PBMCs from 4 health care workers were used and treated under the same condition of the B cell ELISpot assay and measured in the IL-21 ELISpot assay, in which PBMCs were stimulated with the standard concentration of IL-2+R848 to demonstrate that IL-2+R848 was able to induce the secretion of IL-21. The IL-21R antagonist was added in 4 different concentrations (1, 5, 10, and 20 µg/mL) to the B cell ELISpot in samples from 7 health care workers and 5 controls subjects of the RECOVAC Immune Response study, who were vaccinated twice with a COVID-19 mRNA vaccine. (B) SARS-CoV-2–specific memory B cell response at 28 days after the second vaccination. (C) Total IgG–producing cells at 28 days after the second vaccination, which served as the positive control for the B cell ELISpot. In some experiments, the number of participants was <12 owing to a lack of PBMCs or technical failure. Data are presented in box-and-whisker plots. The horizontal line and numbers within the whisker indicate the medians, and the tops and bottoms indicate interquartile ranges. Mann-Whitney *U* tests were applied to compare medians. LLoD stands for the lower limit of detection and was 5 spots (or 0.7 if $\log_{10}$-transformed) for memory B cell response. Each symbol represents an individual.

antigens induced activation of IL-21–producing non-T cells in the IL-21 ELISpot assay. Our flow cytometric analysis with polyclonally stimulated immunocompetent cells demonstrated that predominantly $CD4^+$ T cells expressed IL-21 (data not shown). This suggests that, in our IL-21 ELISpot assay, $CD4^+$ T cells are largely responsible for the positive signal. An alternative to PBMCs could be the use of purified T cells in studies using the ELISpot assay.[32] Unfortunately, T-cell isolation by negative selection procedures resulted in high background that hampered our studies. Similar to memory T cells, memory B cells play an important role in protective cellular immunity against COVID-19 because these cells are long-lived and can rapidly produce specific antibodies after re-exposure, compensating for waning antibody levels in peripheral blood.[33]

Our finding on the significantly reduced memory T-cell IL-21 and memory B cell response in KTRs is in line with data showing an impaired antibody and T-cell response (measured by interferon gamma production) after 2 doses of mRNA-1273 in these patients.[19] A possible explanation for this may be the use of immunosuppressive drugs. It was previously shown that the IL-21–producing capacity decreases under immunosuppression after transplantation.[14] The use of MMF was identified as an important independent risk factor for being a serological nonresponder after a 2-dose mRNA-1273 regimen in KTR, and the effect of MMF on the humoral response was also dose dependent.[19,34,35] We showed that a high tacrolimus trough level was associated with a lower SARS-CoV-2–specific memory T-cell IL-21 response. Indeed, calcineurin inhibitors were associated with almost complete inhibition of IL-21 mRNA expression in vitro.[36] Thus, these immunosuppressive drugs may affect the vaccine–induced SARS-CoV-2–specific memory T-cell IL-21 response and may, thereby, indirectly affect B cell activation by interfering with T-cell help. A clinical implication of this could be that clinicians could reduce or temporarily discontinue these specific immunosuppressive drugs in patients at high risk for severe COVID-19 (eg, in the period around the next COVID-19 vaccination or during a SARS-CoV-2 infection) or switch to an alternative immunosuppressive drug such as mTOR-inhibitors, to positively affect the memory T-cell IL-21 response, B cell activation, and induction of virus-specific antibodies. Such strategies, where clinicians stop, reduce, or switch specific immunosuppressive drugs to enhance vaccine–induced IRs, have also been studied previously.[37-40]

Compared with KTRs, patients with CKD and patients on dialysis showed a higher memory T-cell IL-21 and memory B cell response in this study. This is consistent with literature showing that the percentage of serological responders in patients with CKD and patients on dialysis was comparable to controls after 2 doses of mRNA-1273. This may explain findings that this vaccination strategy was successful in preventing hospitalization and death in patients with CKD and patients on dialysis.[41-43] This is remarkable because it has been shown that IRs to other vaccines, such as hepatitis B, influenza, and pneumococcal vaccine,

can be considerably lower in patients with severely impaired kidney function.[44] This indicates a relatively strong immunogenicity of the mRNA-1273 vaccine in patients with an impaired kidney function, which may be related to the different vaccine platform, namely mRNA-based vaccines versus inactivated or subunit vaccines. However, compared with controls, patients with CKD showed a lower memory B cell response and patients on dialysis showed both a lower memory T-cell IL-21 and memory B cell response in this study. This may be explained by the immunosuppressive effects of uremia in these patients, which includes impairment of monocyte-derived dendritic cells to activate antigen-specific T cells.[45,46]

We illustrated that the vaccine–induced memory T-cell IL-21 response was positively correlated with the vaccine–induced memory B cell response. This is supported by a study reporting the association between IL-21^{+}CD4^{+} T cells and SARS-CoV-2–specific memory B cell response in patients recovered from mild COVID-19.[47] Together, this indicates that an impaired antibody response can be the result of poor SARS-CoV-2–specific T-cell help to B cells. We confirmed by the IL-21R blocking experiments that B cell activation depends on IL-21. These findings highlight the crucial role of IL-21 in orchestrating humoral responses by T-cell–dependent B cell activation.

The strength of this study is that we assessed cellular memory responses that were purely vaccine-induced, because we excluded participants with a prior SARS-CoV-2 infection. This enabled us to gain mechanistic insights into how mRNA vaccines induce cellular and humoral IRs. Because most people currently have developed a so-called hybrid immunity because of receiving multiple vaccinations in combination with experiencing an infection, nowadays it is difficult to investigate this.

In conclusion, we show that in patients with kidney disease and KTRs, IL-21 signaling is important in eliciting robust COVID-19 vaccine–induced B cell–mediated IRs and that serological nonresponders still have cellular IRs.

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Disclosure

The authors of this manuscript have no conflict of interests to disclose as described by the *American Journal of Transplantation*.

Data availability

The data that support the findings of this study are available from the first author (S.R.K.M.) on reasonable request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajt.2023.05.025.

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