B and T Cell Responses after a Third Dose of SARS-CoV-2 Vaccine in Kidney Transplant Recipients

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ABSTRACT

Background: Accumulating evidence suggests solid organ transplant recipients, as opposed to the general population, show strongly impaired responsiveness toward standard SARS-CoV-2 mRNA-based vaccination, demanding alternative strategies for protection of this vulnerable group.

Methods: In line with recent recommendations, a third dose of either heterologous ChAdOx1 (AstraZeneca) or homologous BNT162b2 (BioNTech) was administered to 25 kidney transplant recipients (KTR) without humoral response after two doses of BNT162b2, followed by analysis of serological responses and vaccine-specific B- and T-cell immunity.

Results: Nine out of 25 (36%) KTR under standard immunosuppressive treatment seroconverted until day 27 after the third vaccination, whereas one patient developed severe COVID-19 infection immediately after vaccination. Cellular analysis 7 days after the third dose showed significantly elevated frequencies of viral spike-protein receptor-binding domain-specific B cells in humor responders as compared with nonresponders. Likewise, portions of spike-reactive CD4+ T helper cells were significantly elevated in patients who were seroconverting. Furthermore, overall frequencies of IL-2+, IL-4+, and polyfunctional CD4+ T cells significantly increased after the third dose, whereas memory/effector differentiation remained unaffected.

Conclusions: Our data suggest a fraction of transplant recipients benefit from triple vaccination, where seroconversion is associated with quantitative and qualitative changes of cellular immunity. At the same time, the study highlights that modified vaccination approaches for immunosuppressed patients remain an urgent medical need.

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Vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have proven high effectiveness among at-risk populations, such as the elderly,1 patients on maintenance hemodialysis,2 or individuals suffering from chronic inflammatory diseases under selected immunosuppressive regimens.3 In contrast to the latter, kidney transplant recipients (KTR) receiving immunosuppressive medication exhibit strongly reduced humoral and impaired B and T cell responses after mRNA-based vaccination.

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vaccination.4–6 These observations are in line with reports of breakthrough infections with severe disease courses in fully vaccinated solid organ transplant recipients, underlining the urge of adapted vaccination protocols for this at-risk population.7,8 To address this clinical challenge, a third vaccination for solid organ transplant recipients has recently become standard of care in France and Israel.9 The first case series reported seroconversion in only 33% of patients who were anti–S1 IgG negative after a third vaccine dose, regardless of whether a vector- or an mRNA-based vaccine was used.8 These findings are in line with recent data from a larger cohort, where seroconversion increased from 40%–67% after three doses of mRNA vaccine.5 In this study, we extend these observations by reporting results in 25 KTR receiving a third vaccination dose of either heterologous ChAdOx1 (Vaxzevria) or homologous BNT162b2 (Comirnaty). In addition to monitoring vaccine-induced humoral responses, we provide comprehensive data on quantitative and qualitative changes within the spike-antigen–specific B and T cell compartments.

**MATERIALS AND METHODS**

**Study Protocol and Participants**

After standard two-dose BNT162B2 (BioNTech/Pfizer) immunization (21 days apart), 25 KTX patients without anti–spike S1 IgG response received a third vaccination as a standard of care clinical measure under strict monitoring by nephrologists with heterologous ChAdOx1 (n=11, 90±7 days after first vaccine) or homologous BNT162b2 (n=14, 127±1 days after first vaccine), depending on availability. All participants gave written informed consent for sample collection according to the approval of the ethics committees of the Charité-Universitätsmedizin Berlin (EA2/010/21, EA4/188/20), of the county of Saxony-Anhalt (EA7/21) and the University of Greifswald (B019/21). Patient demographics are summarized in Table 1. Peripheral blood and serum samples were collected 7±2 days after the second and third vaccination for serological, B, and T cell analysis, and 19–27 days after third vaccination for serology only.

**Serological Assessment**

SARS-CoV-2 S1 domain-specific IgG and IgA was determined by ELISA (Euroimmun). Previous or current SARS-CoV-2 infection was excluded for all, but one patient on the basis of medical history in combination with negativity in a SARS-CoV-2 nucleoprotein specific ELISA (Euroimmun). Samples were considered positive with OD ratios of ≥1.1 as per manufacturer’s guidelines. An OD ratio value was determined by calculating the ratio of the OD of the respective test sample over the OD of the internal calibrator provided with the ELISA kit. Virus neutralization capacity of sera was analyzed using a surrogate SARS-CoV-2 neutralization test (GenScript), with >30% being defined as a positive response as described previously.10,11

**Characterization of Antigen-specific B and T Cells**

All experiments have been performed as previously described.4,6,12 In brief, peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation using Ficoll–Paque PLUS (GE Healthcare Bio-Sciences, Chicago, IL, USA).

B cells were detected within PBMC flow cytometry and gated as CD19+CD3 CD14+ among single live lymphocytes (gating shown in Supplemental Figure 3). Antigen-specific B cells were identified by double staining with recombinant purified RBD (DAGC149, Creative Diagnostics, 3033, 2021).

**Significance statement**

Protection of solid organ transplant recipients against SARS-CoV-2 by vaccination remains an unmet need, given the low immunogenicity of available vaccines in the presence of immunosuppression. Administration of a third dose to 25 kidney transplant recipients (KTR) resulted in seroconversion in 36% of patients, associated with significant quantitative and functional changes within the spike-antigen–specific B cell and CD4+ T-helper cell compartment. Our data support the need for individual humoral monitoring of immunosuppressed individuals after vaccination and continued efforts to adapt vaccination protocols for this at-risk group.

**Table 1. Characteristics of enrolled patients who received a kidney transplant (n=25)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs (mean±SD)</td>
<td>59.7 (13.8)</td>
</tr>
<tr>
<td>Females (%)</td>
<td>11 (44.0)</td>
</tr>
<tr>
<td>White patients (%)</td>
<td>25 (100)</td>
</tr>
<tr>
<td>Clinical parameters</td>
<td></td>
</tr>
<tr>
<td>Time since Tx, yrs (mean±SD)</td>
<td>10.4 (8.69)</td>
</tr>
<tr>
<td>Retransplantation (%)</td>
<td>5 (20.0)</td>
</tr>
<tr>
<td>Acute graft rejection (%)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>IS medication</td>
<td></td>
</tr>
<tr>
<td>CS+ Tac+ MMF (%)</td>
<td>13 (56.0)</td>
</tr>
<tr>
<td>CS+ CyA+ MMF (%)</td>
<td>7 (28.0)</td>
</tr>
<tr>
<td>mTOR+ MMF+ CS (%)</td>
<td>3 (12.0)</td>
</tr>
<tr>
<td>CyA+ mTOR (%)</td>
<td>1 (4.0)</td>
</tr>
<tr>
<td>CS+ MMF (%)</td>
<td>1 (4.0)</td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>23 (92.0)</td>
</tr>
<tr>
<td>Coronary heart disease (%)</td>
<td>6 (24.0)</td>
</tr>
<tr>
<td>History of myocardial infarction (%)</td>
<td>2 (8.0)</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>5 (20.0)</td>
</tr>
<tr>
<td>History of liver disease (%)</td>
<td>4 (16.0)</td>
</tr>
<tr>
<td>History of malignancy (%)</td>
<td>6 (24.0)</td>
</tr>
</tbody>
</table>

Tx, transplant; IS, immunosuppression; CS, corticosteroids; Tac, tacrolimus; MMF, mycophenolate mofetil; CyA, cyclosporin A; mTOR, mammalian target of rapamycin inhibitor.

*Until 19–27 days after third vaccination.
New York, USA) conjugated to AF647 or AF488, respectively.

For analysis of vaccine-specific T cells, $3–5 \times 10^6$ PBMC were stimulated for 16 h with overlapping 15-mers encompassing the complete SARS-CoV-2 spike protein (1 ug/ml per peptide; JPT, Berlin, Germany). Specific CD4+ T helper cells were identified on the basis of CD137 and CD154 coexpression as depicted in Supplemental Figure 4. For detection of surface molecules, antibodies against CD3 (SK7, Biolegend, Carlsbad, CA, USA), CD4 (SK3, Becton Dickinson, Franklin Lakes, NJ, USA), CD8 (SK1, Ebioscience, San Diego, CA, USA), CD45RO (UCHL1, BioLegend), CD62L (DREG-56, BioLegend), and PD1 (EH12.1, Becton Dickinson) were used. Unwanted cells were excluded via a “dump” channel containing CD14 (M5E2, BioLegend), CD19 (HIB19, BioLegend), and dead cells (fixable live/dead, BioLegend). After stimulation, cells were fixed in FACS Lysing Solution (Becton Dickinson), permeabilized in FACS Perm II Solution (Becton Dickinson) and intracellularly stained with anti-CD154 (24–31, BioLegend), anti-CD137 (4B4–1, BioLegend), anti–TNF-α (MAb11, BioLegend), anti–IFN-γ (4SB3, Ebioscience), anti–IL-2 (MQ1–17H12, BioLegend), anti–Ki67 (B56, Becton Dickinson), and anti–IL-4 (MP4–25D2, BioLegend). All flow cytometric analyses were performed using a BD FACS Fortessa ×20 (BD Biosciences, Franklin Lakes, NJ, USA).

FACS Data Analysis and Statistics

Flow cytometric data analysis was conducted with FlowJo 10 (Becton Dickinson). The gating strategies for analysis of antigen-specific B and T cells are illustrated in Supplemental Figures 3 and 4. Coexpression of cytokines was analyzed via Boolean gating. Statistical examination and composition of ELISA and FACS data derived graphs were performed using GraphPad Prism 8 (GraphPad, La Jolla, CA, USA). Parameter distribution was assessed using the Kolmogorov–Smirnov test.
Depending on normal distribution or not, a t test or Mann–Whitney test was used for two-group comparisons; for multiple comparisons, a two-way ANOVA with Šidák’s post-test or Kruskal–Wallis test with Dunn’s post-test were chosen. For analysis of contingency tables, Fisher’s exact test was applied.

RESULTS

Vaccination-induced Humoral and B-cell Immunity

KTR who received a two-dose BioNTech/Pfizer BNT162B2 immunization 3 weeks apart and who did not show a humoral response (anti–S1 IgG) were revaccinated with a heterologous ChAdOx1 (n = 11, 90 ± 7 days after first vaccine) or homologous BNT162b2 (n = 14, 127 ± 1 days after first vaccine) protocol. In addition to medical history data, humoral, B, and T cell responses were evaluated 7 ± 2 days after the second and third vaccination, and humoral responses were additionally investigated 19–27 days after each vaccination. Anti–S1 specific IgG data were available for all patients, whereas specific IgA and neutralization capacity were only examined in samples from 20 individuals. Patients were on a stable immunosuppressive therapy without changes between or after the vaccination.

One patient developed severe coronavirus disease 2019 (COVID-19) (World Health Organization ordinal scale 6) 10 days after the third vaccination (indicated in red, throughout the figures). Three out of 25 (12%) KTR developed anti–S1 IgG 7 ± 2 days after the third dose, whereas nine out of 25 (36%) seroconverted until 19–27 days, and were categorized as responders. Of these, three patients had received BNT162b2 and six ChAdOx1 as third vaccine; however, anti–S1 IgG was highly positive (OD ratio > 5) only in three responders (two BNT162b2 and one ChAdOx1) (Figure 1A and Supplemental Figure 1A). One of these patients was the only one in our study without mycophenolate mofetil as an immunosuppressant (indicated in blue throughout the figures). Anti–S1 IgA (Figure 1B and Supplemental Figure 1A) only increased 19–27 days after the third vaccination, whereas neutralization capacity was increased 7 ± 2 days and 19–27 days after the third vaccination, as compared with day 7 after the second vaccination, respectively (Figure 1C and Supplemental Figure 1A) in seven out of 20 patients. The patient with the isolated high anti–S1 IgA had high anti–S1 IgA already before third vaccination. The SARS-CoV-2 nucleoprotein–specific ELISA was negative at all samplings.

The relative percentage and absolute number of SARS-CoV-2 spike RBD–specific B cells within the CD19+ population did not change between the second and third vaccination (Figure 1D). The relative percentage of SARS-CoV-2 spike RBD–specific CD19 cells did not differ between responders and nonresponders (Figure 1E), whereas absolute numbers of RBD+ B cells were higher among responders compared with nonresponders 7 ± 2 days after the third vaccination (Figure 1E). There were no differences between antigen-specific B cell responses between patients receiving a homologous versus a heterologous boost (Supplemental Figure 1D).

Quantitative and Qualitative Assessment of Vaccination-specific CD4+ T Cell Responses

Vaccine-specific CD4+ T helper cells were identified within PBMC after stimulation with an overlapping peptide mix encompassing the complete spike glycoprotein on the basis of coexpression of CD137 and CD154, as demonstrated earlier,6,12 and depicted in Supplemental Figure 4. A T cell response was defined as positive when peptide pool stimulated PBMC contained more than two-fold higher portions of CD137+CD154+ T cells as compared with the unstimulated control with ≥ 20 events. The overall prevalence of vaccinees displaying spike-specific CD4+ T cell responses was high (>90%) after the second and third inoculation, with no significant changes of antigen-reactive T cell frequencies in cellular responders (Figure 2A). However, individuals who seroconverted after the third vaccination (IgG+) were characterized by significantly higher portions of antigen-reactive T cells than humoral nonresponders (Figure 2A). Analysis of activation-associated markers revealed a significant drop of proliferating Ki67+ and activated PD1+ T cells after the third dose (Figure 2B), whereas no changes in specific memory/effector subset composition were noted between the two timepoints (Figure 2C). Importantly, KTR showed significantly higher frequencies of IL-2 and IL-4 secreting and polyfunctional IFN-γ+TNF-α+IL-2+ (triple+) T cells after the third dose; this observation did not account for IFN-γ or TNF-α alone (Figure 2D). We did not detect significant differences in frequencies of specific T cells, their activation marker expression, memory phenotype, or functional profile in patients who were AstraZeneca versus Biotech/Pfizer boosted (Supplemental Figure 2, A–D). Furthermore, no significant differences in cytokine production by specific T cells were observed in humoral responders versus nonresponders (Supplemental Figure 2E).

DISCUSSION

Investigating the humoral, B, and T cell response after a third vaccination with either ChAdOx1 or BNT162b2 in previously nonresponding KTR, we observed an increase in anti–S1 IgG in nine out of 25 (36%) patients, four out of 14 (28%) after homologous, and five out of 11 (45%) after heterologous vaccination. This observation is in accordance with previously published data of third vaccinations in a heterogeneous group of solid organ transplant recipients.8,9 However, these studies did not stratify patient responses according to the type of transplant, or quantify vaccine-specific cellular T and B cell immunity.
There is increasing evidence that neutralizing capacity is a reliable correlate of protection13-15 and that anti-S1 IgG correlates with neutralizing capacity.10,16 Of the nine responders in our cohort, only three developed high titer anti-S1 IgG (OD IgG >5) whereas one was just above the threshold for positivity. Consistent with lack of protection, one humoral nonresponder developed severe COVID-19 after the third vaccination, underlining the clinical importance of adequate antibody titers in KTR, especially with the emergence of viral variants. It has also been reported that antibody titers decline 3 months after vaccination with an mRNA vaccine, so initial high titers might be necessary for long-term protection.17 The absolute number of antigen-specific B cells significantly increased in responders as compared with nonresponders after the third dose, although it remained largely unchanged compared with the second vaccination. This lack of substantial antigen-specific B and plasmablast induction, as opposed to what has been demonstrated for healthy individuals,4 supports the weak additional mobilizing effect of a third vaccination in the majority of KTR and likely explains its overall limited effectiveness.

The exact contribution of vaccine-induced T cell immunity for protection against SARS-CoV-2 infection is still debated, particularly with respect to direct antiviral effects versus a role in B cell activation. We recently reported that vaccine-specific CD4+ T cells in KTR show broad quantitative and functional limitations.6 In line with data on specific B cells presented herein, spike-reactive T cell frequencies were largely unaffected by a third vaccination. In accordance with the fact that an additional boost does not substantially expand the specific T cell pool in KTR, signs of ex vivo activation, as mirrored...
by high Ki67 or PD1 expression, were significantly reduced after the third dose as compared with the second dose. For reasons that need to be explored further, humoral responders were characterized by a significant increase of spike-specific T cells, a finding supported by recent work of Sahin et al. demonstrating a strong correlation between specific T cell frequencies and antibody titers post vaccination. As opposed to their quantities, vaccine-reactive T cells underwent a functional maturation after the third dose with higher portions of IL-2+, IL-4+, and multifunctional cells. Whereas increased IL-4 secretion could lower the threshold for isotype switching to IgG, patients might potentially benefit from augmented polyfunctionality being associated with potent SARS-CoV-2 clearance.

Antigen-specific T and B cells cannot be considered a routine diagnostic measure because it is costly and time consuming. Also neutralization titers are not broadly established in routine diagnostic laboratories, therefore the determination of anti–S1 IgG, which excellently correlates with neutralization capacity should be used to guide clinical decision for further booster doses in immunocompromised patients. In patients with a negative or low positive anti–S1 IgG after three doses, a fourth dose should be considered. In any case, the patients should be informed they likely are not protected against severe COVID-19. In our cohort, all but one patient received antimetabolite medication. This is in clear contrast to other previously published cohorts of solid organ recipients, for example, the cohort of Boyarsky et al. where only 72% of patients received an antimetabolite and where more patients showed a serological response after two vaccine doses. In the study of Kamar et al. describing a seroresponse in 68% of solid organ recipients after three doses, 66% of patients were on immunosuppressive treatment with an antimitabolite. Also other studies such as the study of Hall et al. and of Werbel et al. describe a significant and comparable increase in seroresponse after three vaccine doses.

The differences in the study populations and their immunosuppressive regimens might explain the different response rates. Our single patient without mycophenolate mofetil showed high IgG titers and high neutralization capacity and a high IL-2 response among antigen-specific T cells, being key for cell proliferation and functional differentiation. This observation supports one potential strategy to improve responder rates by reducing immunosuppression and especially antimitabolite treatment in patients with stable allograft function without previous episodes of rejection or pre-existing HLA immunization before vaccination. Indeed, data from a large multicenter study suggested that withdrawal of mycophenolate mofetil in KTR formerly receiving tacrolimus-based triple immunosuppression does not impair graft or patient survival, thereby supporting short-term drug weaning as an option, especially in patients that are on corticosteroids, as in our cohort.

In conclusion, a third vaccination against SARS-CoV-2 leads to a serological response in a fraction of KTR, whereas the majority of patients still lack protective antibody titers. This lack of serological response can be explained by only marginal improvements in cellular responses among antigen-specific B cells and T cells after a third vaccination. Alternative vaccination protocols are urgently needed to protect this at-risk group.

DISCLOSURES

F. Halleck reports receiving honoraria from MSD and Novartis. K. Buddle reports having consultancy agreements with AbbVie, Alexion, Astellas, Bristol-Myers Squibb, Chiesi, CSL- Behring, Fresenius, Hansa, Hexal, Novartis, MSD, Otsuka, Pfizer, Quark, Roche, Sandoz, Shire, Veloxis, Vifor, and Vitaris; reports receiving research funding from AbbVie, Alexion, Astellas, Bristol-Myers Squibb, Chiesi, CSL-Behring, Fresenius, Hansa, Hexal, Novartis, MSD, Otsuka, Pfizer, Quark, Roche, Sandoz, Shire, Veloxis, Vifor, and Vitaris; reports receiving honoraria from AbbVie, Alexion, Astellas, Bristol-Myers Squibb, Chiesi, Fresenius, Hexal, Novartis, Otsuka, Pfizer, Quark, Roche, Sandoz, Shire, Veloxis Pharma; and reports being a scientific advisor or member of Astellas, Bristol-Myers Squibb, Chiesi, Hansa, Hexal, MSD, Novartis, Pfizer, Roche, and Veloxis. K. Eckardt reports consultancy agreements with Akebia, AstraZeneca, Bayer, Boehringer Ingelheim, Genzyme, Otsuka, Traver, and Vifor; reports receiving research funding from Amgen, AstraZeneca, Bayer, Fresenius, Genzyme, Shire, and Vifor; reports receiving honoraria from Akebia, AstraZeneca, Bayer, Boehringer Ingelheim, Genzyme, Otsuka, Traver, and Vifor; and reports being a scientific advisor or member of the Editorial Boards of British Medical Journal and Kidney International. K. Kotsch reports receiving research funding from Chiesi, T. Dörnèr reports having consultancy agreements with AbbVie, Bayer Healthcare, Biogen, BMS/Celgene, Boston Pharmaceuticals, Eli Lilly, Janssen, Novartis, and Roche/Genentech; reports receiving research funding from Federal Ministry of Education and Research, Deutsche Forschungsgemeinschaft, Leibnitz Society, Senate of Berlin, and Roche; reports receiving honoraria from AbbVie, Bayer Healthcare, Biogen, BMS/Celgene, Boston Pharmaceuticals, Eli Lilly, Janssen, Novartis, and Roche/Genentech; and reports receiving Speakers Bureau from Eli Lilly, Janssen, Roche, and Samsung/Bioepis. All remaining authors have nothing to disclose.

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F. Halleck, A. Sattler, and E. Schrezenmeier designed the study and wrote the manuscript; H. Rincon-Arevalo, A. Sattler, E. Schrezenmeier, and A.-L. Stefanski performed the experiments (B and T cell analysis); F. Bachmann, K. Buddle, M. Choi, C. Hammett, A. Potekhin, E. Schrezenmeier, and H. Staub-Hohenleicher recruited patients; B.

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Jahrsdörfer and H. Schrezenmeier were responsible for serological studies; K. Budde, T. Dörner, K.-U. Eckardt, F. Hallek, K. Kotsch, and E. Schrezenmeier supervised the work and provided funding; all authors read and approved the manuscript. The authors are grateful to Dr. Michael Moesenthin, Dr. Peter Bartsch (both Dialysezentrum Burg), Dr. Ralf Kühn, and Dr. Dennis Heutling (both Dialyse Tangermünde) for patient recruitment, and Dr. Petra Glander and Pia Hambach for biobanking of samples.

SUPPLEMENTAL MATERIAL

This article contains the following supplemental material online at http://jasn.asnjournals.orglookup/suppl/doi:10.1681/ASN.2021070966/-/DCSupplemental.

Supplemental Figure 1. Humoral immune responses and specific B cell immunity after third vaccination in KTR according to vaccine type.

Supplemental Figure 2. SARS-CoV-2 vaccine specific T helper cell responses in patients who received a KTX stratified according to heterologous/homologous third vaccination or specific IgG serostatus (humoral responder/nonresponder).

Supplemental Figure 3. Detection of SARS-CoV-2 vaccine specific B cells.

Supplemental Figure 4. Detection of SARS-CoV-2 vaccine specific T helper cells.

REFERENCES

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**Supplemental Figure 3:** Detection of SARS-CoV-2 vaccine specific B cells

**Supplemental Figure 4:** Detection of SARS-CoV-2 vaccine specific T helper cells
Humoral immune responses and specific B cell immunity after third vaccination in KTR according to vaccine type. Humoral vaccine-specific immune responses were assessed by ELISA for anti-spike protein S1 IgG (left), spike protein S1 IgA (center) and virus neutralization by a blocking ELISA (right) at the indicated timepoints in KTR after administration of a third dose of either ChAdOx1 (A, n=11, black filled dots) or BNT162b2 (B, n=14, back empty dots). Thresholds defining a positive response are indicated by dotted lines. Relative frequencies (left) and absolute counts (right) of RBD-specific CD19+ B cells in all patients (D) as well as in responders IgG+ and non-responders IgG- 7±2 days after third vaccination with ChAdOx1 or BNT162b2. (A-C) Kruskal-Wallis with Dunn’s post-test. (D) Two way ANOVA test with Šidák post-test. The infected individual is depicted in red. (D, E) Mann-Whitney test.
SARS-CoV-2 vaccine specific T helper cell responses in KTx patients stratified according to heterologous/homologous third vaccination or specific IgG serostatus (humoral responder/non-responder). PBMC of KTx patients were stimulated or not with spike peptide mix. Specific CD4+ T cells were identified and quantified 7 ± 2 days after the third vaccination (AZ or BNT, as indicated) by FACS according to CD137 and CD154 co-expression. Depicted are (A) frequencies of specific CD4+ T cells (left, unpaired t test) and frequencies within humoral responders (anti-S1 IgG+) and non-responders (anti-S1 IgG-) (right, not tested for significance due to low patient numbers/group). (B) Frequencies of antigen-specific CD4+ T cells expressing Ki67 (left, unpaired Mann-Whitney) or PD1 (right, unpaired t test). (C) Memory-effector subset differentiation of spike-reactive CD4+ T cells (T_{CM}: left, unpaired t test; T_{EM}: middle, unpaired t test; T_{eff}: right, unpaired t test). (D) Expression of IFNγ, TNFα (both unpaired Mann-Whitney), IL-2 (unpaired t test) and IL-4 (unpaired Mann-Whitney) in antigen-specific T cells including analysis of IFNγ+TNFα+IL-2+ “triple+” polyfunctional (unpaired Mann-Whitney) cells. (E) Cytokine profile in patients stratified according to humoral response or not. Expression of IFNγ (unpaired t test) TNFα (unpaired Mann-Whitney), IL-2 (unpaired t test) and IL-4 (unpaired Mann-Whitney) in antigen-specific T cells including analysis of IFNγ+TNFα+IL-2+ “triple+” polyfunctional (unpaired Mann-Whitney) cells. Where applicable, graphs show means ± SD.
Detection of SARS-CoV-2 vaccine specific B cells. B cells in PBMCs were detect by flow cytometry. Antigen-specific B cells were identified by double staining with recombinant purified RBD (DAGC149, Creative Diagnostics, New York, USA) conjugated to AF647 or AF488, respectively.
Detection of SARS-CoV-2 vaccine specific T helper cells. PBMC were stimulated or not with SARS-CoV-2 spike overlapping peptide mix for 16 h. Antigen-specific live single CD14-CD19-CD3+ (*dump* negative) specific CD4+ Th cells were detected by FACS according to co-expression of CD137 and CD154. Specific cells were subsequently analyzed for expression of IFNγ, TNFα, IL-2 and/or IL-4, characterized for expression of the activation-related markers Ki67 and PD1, or for their memory phenotype based on CD45RO and CD62L expression (T<sub>CM</sub>: central memory-, T<sub>EM</sub>: effector memory, T<sub>eff</sub>: effector-T cells). Gates for cytokines or activation-induced molecules were set according to the respective unstimulated or unstained controls, respectively.