The Magnitude and Functionality of SARS-CoV-2 Reactive Cellular and Humoral Immunity in Transplant Population Is Similar to the General Population Despite Immunosuppression

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Background. The ability of transplant (Tx) patients to generate a protective antiviral response under immunosuppression is pivotal in COVID-19 infection. However, analysis of immunity against SARS-CoV-2 is currently lacking. **Methods.** Here, we analyzed T cell immunity directed against SARS-CoV-2 spike-, membrane-, and nucleocapsid-protein by flow cytometry and spike-specific neutralizing antibodies in 10 Tx in comparison to 26 nonimmunosuppressed (non-Tx) COVID-19 patients. **Results.** Tx patients (7 renal, 1 lung, and 2 combined pancreas-kidney Txs) were recruited in this study during the acute phase of COVID-19 with a median time after SARS-CoV-2-positivity of 3 and 4 d for non-Tx and Tx patients, respectively. Despite immunosuppression, we detected antiviral CD4$^+$ T cell-response in 90% of Tx patients. SARS-CoV-2–reactive CD4$^+$ T cells produced multiple proinflammatory cytokines, indicating their potential protective capacity. Neutralizing antibody titers did not differ between groups. SARS-CoV-2–reactive CD8$^+$ T cells targeting membrane- and spike-protein were lower in Tx patients, albeit without statistical significance. However, frequencies of anti-nucleocapsid–protein-reactive, and anti-SARS-CoV-2 polyfunctional CD8$^+$ T cells, were similar between patient cohorts. Tx patients showed features of a prematurely aged adaptive immune system, but equal frequencies of SARS-CoV-2–reactive memory T cells. **Conclusions.** In conclusion, a polyfunctional T cell immunity directed against SARS-CoV-2 proteins as well as neutralizing antibodies can be generated in Tx patients despite immunosuppression. In comparison to nonimmunosuppressed patients, no differences in humoral and cellular antiviral-immunity were found. Our data presenting the ability to generate SARS-CoV-2–specific immunity in immunosuppressed patients have implications for the handling of SARS-CoV-2–infected Tx patients and raise hopes for effective vaccination in this cohort.

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INTRODUCTION

The emergence of the COVID-19 pandemic in late 2019 led to >1 million deaths attributed to COVID-19 as of November 2020. Since certain patient cohorts are at increased risk for critical courses, the identification and protection of such vulnerable groups are a major concern.[1-3] Transplant (Tx)-patients were defined to be of high-risk by national health agencies early after the COVID-19 outbreak. This assumption is mainly based on the immunosuppressive treatment after Tx, and the subsequently higher susceptibility to infectious diseases including viral infections.[4-6] In addition, Tx patients also suffer from higher rates of comorbidities compared to the general population.[3,4,6,7]

However, case series report diverse outcomes of immunosuppressed COVID-19 patients, with some suggesting an increased risk and others a decreased fatality rate compared to the general population.[3,7-14] A reduction of COVID-19 symptoms and incidence of severe courses might be explained by the milder reaction of the suppressed immune system, therefore diminishing immunopathogenesis.[8] In fact, immunosuppressive drugs used in transplantation, in particular the steroid dexamethasone, have been shown to improve the outcome of critically ill COVID-19 patients.[15] Interestingly, studies revealed an inhibiting effect of the immunosuppressive drugs cyclosporine A and antimetabolites on coronavirus replication in vitro.[16-18] On the other hand, the impairment of antiviral immunity by immunosuppressive medication is well documented, and community-acquired respiratory viruses pose a greater risk to Tx patients as compared to the general population.[19-21]

Therefore, there is a need to understand the SARS-CoV-2–reactive adaptive immunity in Tx recipients.[6,9] Here, we provide data on the adaptive immune responses in SARS-CoV-2 infected Tx patients. We show that T cell and neutralizing antibody responses of Tx patients are similar to non-Tx patients, with polyfunctional and memory T cell reactivity. Thus, we suggest that Tx patients can mount a protective SARS-CoV-2–reactive adaptive immune response.

MATERIALS AND METHODS

Patient Samples

The study was approved by the ethical committee of the Ruhr-University Bochum (20-6886) and University Hospital Essen (20-9214-BO). Blood samples of 10 Tx patients and 26 non-Tx patients were collected after written informed consent was obtained. For reasons of limited patient material, resources, and the time that would be needed to generate a new cohort, the non-Tx patient control group was formed from patient samples already included in another study.[22] In 8 Tx patients and 20 non-Tx patients, multiple blood samples (up to 5 visits) were collected, so that in total 27 Tx patient and 60 non-Tx patient samples were included in this study. In all but 1 patients, membrane- (M), nucleocapsid- (N), and spike- (S) protein reactive T cells were analyzed. In 1 patient, 2 of 3 blood samples could be analyzed for N- and S-protein reactive T cells only because of the limited amount of collected blood and lymphopenia. The clinical characteristics of the patients are presented in Table 1. COVID-19 severity was evaluated according to a guideline of the German Robert Koch Institute, as previously described.[23] Of patients with multiple samples, the worst disease classification of this patient was reported. Samples of patients with moderate and severe COVID-19 were collected shortly after symptom-onset and positive SARS-CoV-2 PCR. Samples of critical COVID-19 patients were included at the time of ICU admission.

Phenotyping of Whole Blood Samples

Whole blood from EDTA collection tubes was stained for 10 min at room temperature in the dark with CD45-Alexa Fluor 488 (clone 2D1) (Biolegend [BL]), CD3-BV785 (clone OKT3) (BL), CD4-Alexa Fluor 700 (clone OKT4) (BL), CD8-V500 (clone RPA-T8) (Becton Dickinson), CD19-BV605 (clone HIB19) (BL), and CD56-PerCP-Cy5.5 (clone NCAM) (BL). VersaLyse (Beckman Coulter) was used for erythrocyte lysis for 15 min at room temperature in the dark. Samples were measured on a CytoFlex flow cytometer (Beckman-Coulter) after addition of propidium iodide (Thermo Fisher Scientific). Measured absolute counts were calculated as cells/nanoliter (nL).

SARS-CoV-2 Overlapping Peptide Pool Stimulation and Flow Cytometric Analysis of peripheral Blood Mononuclear Cell

Stimulation of peripheral blood mononuclear cell (PBMC) with SARS-CoV-2 M-, N-, and S-protein was performed as previously described.[22] Briefly, PBMC were isolated from EDTA collection tubes (Sarstedt) and stored at –80 °C to allow analysis in batches. PBMCs were left resting overnight after thawing and afterward stimulated with SARS-CoV-2–PepTivator peptide-pools solved in water (Miltenyi Biotec). Staphylococcal-enterotoxin-B (Sigma-Aldrich) treated and untreated PBMCs were used as positive and negative control, respectively. After 2 h of stimulation, Brefeldin-A (Sigma-Aldrich) was added and the stimulation stopped after 16 h. Surface- and intracellular-staining for flow cytometry was performed using fixation and permeabilization (Thermo Fisher Scientific) and antibodies listed in Table S1 (SDC, http://links.lww.com/TP/C185). Samples were measured on a CytoFlex flow cytometer (Beckman-Coulter).

SARS-CoV-2 Neutralizing Antibody Measurement

Assessment of neutralizing antibodies was performed in 8 Tx patients (Table 1) and 20 non-Tx patients as previously described.[22] In brief, complement factors in patient sera were inactivated by incubation at 56°C for 30 min. Quadruplicates of 2-fold serial dilutions of patient sera were incubated with a propagation-incompetent VSV*ΔG(FLuc)-pseudovirus-system bearing the SARS-CoV-2 S-protein in the envelope. Afterwards, Vero-E6-cells $(1 \times 10^4$ cells / well) were infected with the pseudovirus in DMEM + 10% FBS (Life Technologies). Firefly-luciferase-reporter-activity was determined using a GloMax plate-reader (Promega) after addition of 25 µL of firefly luciferase ONE-Glo substrate (Promega) 18 h postinfection and the reciprocal antibody dilution causing 50% inhibition of the luciferase-reporter calculated.

TABLE 1.

Clinical characteristics, COVID-19 treatment, and monitoring strategy of individual transplant patients

Patient	A	B	C	D	E	F	G	H	I	J
Age (y)	37	63	62	54	58	29	35	69	55	52
Gender	Male	Female	Female	Male	Male	Female	Male	Male	Male	Female
Transplanted organs	Kidney	Kidney	Kidney	Kidney	Lung	Kidney	Kidney	Kidney-pancreas	Kidney	Kidney-pancreas
Transplant age (d)	4420	7002	243	118	NA	3957	123	4657	1625	3191
No. of previous transplants	0	1	0	0	0	0	0	0	0	0
Donor type (living/deceased)	Living	Deceased	Deceased	Deceased	Deceased	Deceased	Deceased	Deceased	Deceased	Deceased
Initial IS	Tac (2.5 ng/mL) Pred (5 mg)	Tac (18.3 ng/mL) Pred (10 mg)	Tac (7.9 ng/mL) Pred (10 mg)	Tac (11.3 ng/mL) Pred (5 mg)	Unknown	Tac (5.8 ng/mL) Pred (5 mg)	Bela (350 mg) Pred (10 mg)	Tac (40 ng/mL) MMF (2 × 1 g)	Everol (3.27 ng/mL) Pred (10 mg)	Tac (3.57 ng/mL) MMF (2 × 1 g) Pred (5 mg)
IS during disease	Withdrawn	No changes	No changes	No changes	Unknown	Pred	HC	HC	HC	HC
COVID-19 severity	Moderate	Moderate	Severe	Moderate	Critical	Moderate	Moderate	Critical	Moderate	Moderate
COVID-19 treatment	Anticoag.	Anticoag. RDV and plasma therapy	Antib. anticoag. and plasma therapy	Anticoag.	Antib.	Anticoag.	Antib. and anticoag.	Antib. HCQ and anticoag.	Antib. HCQ and anticoag.	Antib. and anticoag.
Outcome (discharged/deceased)	Discharged	Discharged	Discharged	Discharged	Deceased	Discharged	Discharged	Discharged	Discharged	Discharged
ICU? (yes/no)	No	No	No	No	Yes	No	No	Yes	No	No
Invasive ventilation? (yes/no)	No	No	No	No	Yes	No	No	No	No	No
Hospitalization length (d)	15	10	10	30	11	2	7	28	15	13
AKI (yes/no)	No	Yes	No	Unknown	Yes	Unknown	No	Yes	Yes	Yes
SCr at admission (mg/dL)	HD	2.17	1.06	HD	1.72	1.19	2.30	1.60	2.60	1.00
SCr at discharge (mg/dL)	HD	1.90	1.12	HD	n.a.	1.10	2.30	1.10	1.60	0.90
First T cell measurement (d after first positive PCR)	2	4	5	6	2	8	28	-2	1	1
No. of T cell measurements	2	3	3	3	1	1	2	5	4	3
Neutralizing antibody assay	Done	Done	Done	Done	Not done	Done	Done	Done	Done	Not done

AKI, acute kidney injury; Antib., antibiotics; Anticoag., anticoagulants; Bela, belatacept; COVID-19, coronavirus disease 2019; Everol, everolimus; HC, hydrocortisone; HCQ, hydroxychloroquine; HD, hemodialysis; ICU, intensive care unit; MMF, mycophenolate mofetil; n.a., not applicable; NA, not available; PCR, polymerase chain reaction; Pred, prednisolone; RDV, remdesivir; SCr, serum creatinine; Tac, tacrolimus.

Flow Cytometry Data Analysis

FlowJo version 10.7.1 (BD Biosciences) was used for analysis of flow cytometry data. Single stains and fluorescence-minus-1 controls were used for gating. Gates of each individual were adjusted according to the negative control. The gating strategy is presented in Figure S1 (**SDC**, http://links.lww.com/TP/C185). CD4^{+} T cells expressing CD154 and CD137 and CD8^{+} T cells expressing CD137 in combination with production of at least 1 of IL2/IL4/IFNγ/TNFα/GrzB were defined as reactive T cells. Unspecific activation in unstimulated controls was subtracted from stimulated samples to account for SARS-CoV-2–specific activation in the presented frequencies. Of patients with multiple samples, the mean value is presented. Negative values were set to 0. Stimulation index (SI) was calculated by dividing the measured T cell subset response by the respective negative control. If the negative control was 0, the minimum value across that subset was used for calculation. SI below 1 was set to 1. SI >3 was considered detectable response. Of patients with multiple samples, the maximum value is presented for analysis of detectable responses. Boolean gating of IL2, IL4, IFNγ, TNFα, and GrzB producing T cells in combination with CD154 for CD4^{+} and CD137 for CD8^{+} T cells was used to calculate polyfunctional T cells. Composition of polyfunctional T cells was analyzed by calculating the relative contribution of each subset to the total polyfunctional cells of each patient and then the mean contribution across all patients.

Statistical Analysis

Statistical analysis was performed using R, version 4.0.2,[24] and GraphPad Prism v7, which was also used for graphical representation. Categorical variables are summarized as numbers and frequencies; quantitative variables are reported as median and interquartile range (IQR). Normal distribution was assessed using D'Agostino-Pearson omnibus normality test and parametric or nonparametric tests were then used accordingly. For the characterization of demographic, treatment, and clinical outcome, differences between groups were calculated using Fisher's exact test for categorical variable and Mann-Whitney U test for quantitative variables. Characterization of absolute lymphocyte subset counts and immune responses of Tx and non-Tx patients was performed employing Mann-Whitney U test. Thereafter, bivariate-regression-analysis was performed with age and transplantation status as independent variables (without interactions) and was considered significant if a significant effect of transplantation status was found. Only differences significant for both tests are reported in this work; the *P* in the figures correspond to the Mann-Whitney U test. Ratio of memory cells among T cells and chronologic age was compared using unpaired t-test. *P* values <0.05 were considered significant; only significant *P* are reported.

RESULTS

Study Participants

Samples of 10 Tx patients and 26 nontransplant (non-Tx) patients were analyzed for this study. All patients were hospitalized and tested positive for SARS-CoV-2 infection. The median age of Tx patients was 55 (IQR 41–61) and

significantly lower than that of non-Tx patients (median 69, IQR 58-82; *P* = 0.006) (**Table S2, SDC**, http://links.lww.com/TP/C185). Seven (70%), 1 (10%), and 2 (20%) of Tx patients and 6 (23%), 10 (38.5%), and 10 (38.5%) of non-Tx patients had moderate, severe, and critical COVID-19 severity, respectively (*P* = 0.043). Relatively more Tx patients were treated with anticoagulation as compared to non-Tx patients. There were no significant differences in the time between diagnosis and sample analysis between Tx and non-Tx patients (**Table S2, SDC**, http://links.lww.com/TP/C185). The clinical characteristics and details on the COVID-19 disease course of the individual Tx patients are listed in Table 1. Seven patients had a kidney Tx, 2 combined kidney-pancreas Tx, and 1 lung Tx. The immunosuppression of 2 patients remained unchanged during the COVID-19 treatment, 5 patients received glucocorticoid monotherapy and the immunosuppression of 2 patients was completely discontinued. Typically for COVID-19 patients,[23,25] the majority of study participants were lymphopenic with low absolute counts of T cells, CD4^{+} and CD8^{+} T cell subsets, B cells, and NK cells without statistically significant differences between the Tx and non-Tx groups (**Figure S2, SDC**, http://links.lww.com/TP/C185).

Polyfunctional SARS-CoV-2–reactive T Cell Responses and Neutralizing Antibodies Do Not Differ in Tx Patients and Nonimmunosuppressed Patients

Specific and highly functional T cells play a pivotal role in viral control.[26] Detection of SARS-CoV-2–specific T cells according to the expression of activation markers and cytokines after stimulation of PBMC with SARS-CoV-2 membrane- (M), nucleocapsid- (N), and spike- (S)-protein overlapping peptide pools has been described before by us and other groups (**Figures S1 and S3, SDC**, http://links.lww.com/TP/C185).[22,27] In this study, we used this approach to compare the magnitude and functionality of cellular immunity in Tx COVID-19 patients to a general, non-Tx COVID-19 patient cohort.

The frequencies of SARS-CoV-2–reactive CD4^{+} T cells were similar among Tx patients and non-Tx patients. The number of patients with detectable responses in both cohorts was between 70% and 90% after stimulation with M-, N-, and S-protein without significant differences between the patient groups (Figure 1A). In general, no significant differences were observed regarding the frequencies of cytokine-producing CD4^{+} T cells (Figure 1B–F). Only for IL4, we detected significantly higher frequencies of N-protein reactive CD4^{+} T cells in Tx patients (Figure 1G). However, the production of TH1-cytokines greatly exceeded the production of the TH2 cytokine IL4, and the difference in IL4 production between the cohorts could be clinically irrelevant.

Neutralizing antibodies are another arm of adaptive immunity crucial for antiviral defense. Like T cells, B cells are also susceptible to immunosuppressive therapy. Therefore, we compared the antibody-dependent capacity of SARS-CoV-2 neutralization between Tx and non-Tx patients. In accordance with the CD4^{+} T cells, which are required for the generation of effective humoral immunity,[28] we observed that antibodies of Tx patients and non-Tx patients have a similar SARS-CoV-2 neutralizing
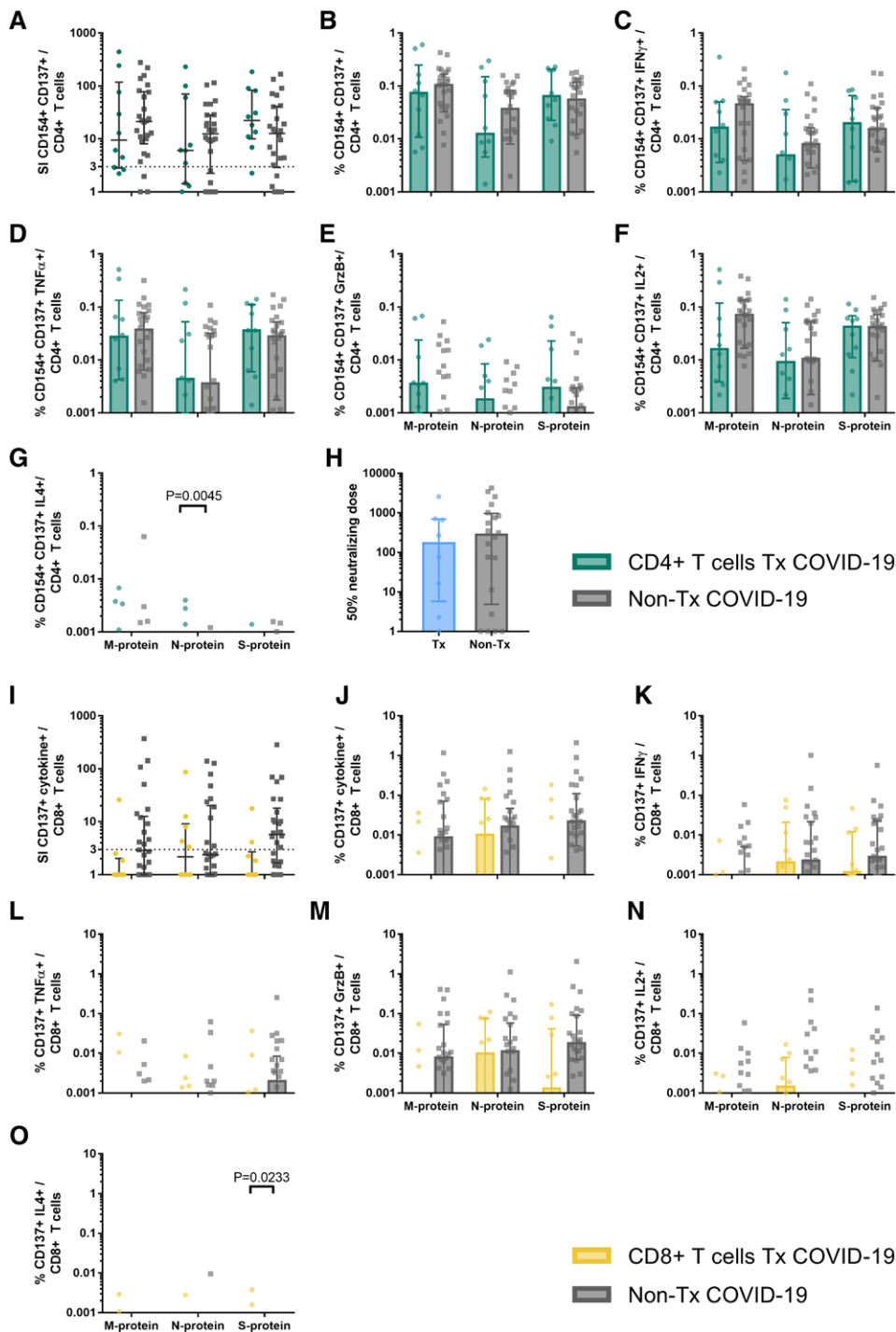
FIGURE 1. Characterization of SARS-CoV-2-reactive T cells in transplant (Tx) and non-Tx COVID-19 patients. PBMCs of 10 Tx and 26 non-Tx COVID-19 patients were stimulated overnight with overlapping peptide pools of SARS-CoV-2 membrane (M), nucleocapsid (N), and spike (S)-protein and analyzed by flow cytometry. A, Stimulation index (SI) of activation markers CD154 and CD137 expressing CD4$^+$ T cells (SARS-CoV-2 specific CD4$^+$ T cells). SI was calculated by dividing the measured T cell subset response by the respective response in the negative control. Values >3 are considered above detection limit. For patients with multiple samples, the maximal response was calculated. Scatter plot with line at median and interquartile range. B–G, Frequencies of SARS-CoV-2 specific CD4$^+$ T cells (B) and SARS-CoV-2 specific CD4$^+$ T cells expressing IFNγ (C), TNFα (D), GrzB (E), IL2 (F), or IL4 (G). Negative controls were subtracted from specifically stimulated samples to exclude unspecific activation. For patients with multiple samples, the mean response was calculated. Bars show median, error bars show interquartile range. H, SARS-CoV-2 spike neutralizing antibody dose (ND50) in patient sera of 8 Tx patients and 20 non-Tx patients. For patients with multiple samples, the maximal response was calculated. Scatter plot with line at median and interquartile range. I, Stimulation index (SI) of activation marker CD137 and at least 1 of the cytokines IFNγ, TNFα, IL2, IL4, or effector molecule GrB expressing CD8$^+$ T cells (SARS-CoV-2 specific CD8$^+$ T cells). SI was calculated by dividing the measured T cell subset response by the respective response in the negative control. Values >3 are considered detectable. For patients with multiple samples, the maximal response was calculated. Scatter plot with line at median and interquartile range. J–O, Frequencies of SARS-CoV-2 specific (CD137$^+$ cytokine$^+$) CD8$^+$ T cells (B) and CD137$^+$ CD8$^+$ T cells expressing IFNγ (C), TNFα (D), GrzB (E), IL2 (F), or IL4 (G). Negative controls were subtracted from specifically stimulated samples to exclude unspecific activation. For patients with multiple samples, the mean response was calculated. Bars show median, error bars show interquartile range. Statistical comparison was done with Mann-Whitney U test and controlled by multivariate analysis for the influence of transplantation status and age. $P < 0.05$ was considered significant. PBMC, peripheral blood mononuclear cell.

activity (Figure 1H). Thus, the sera of Tx patients had equal inhibitory effects on the viral infectivity of susceptible cell culture cells as sera from non-Tx patients.

The number of patients with detectable SARS-CoV-2–specific CD8^{+} T cell responses in Tx patients was 1 (10%), 5 (50%), and 2 (20%) after stimulation with M-, N-, and S-protein, respectively. These numbers were lower than in the non-Tx patients cohort, in which 12 (46%), 10 (38%), and 17 (65%) showed detectable responses after stimulation with M-, N-, and S-protein, respectively (Figure 1I), without reaching statistical significance. Accordingly, the frequency of activated and IFNγ and GrzB producing SARS-CoV-2–specific CD8^{+} T cells was lower after

stimulation with M- and S-protein, but not after stimulation with N-protein, and not reaching statistical significance (Figure 1J, K, and M). Similar to CD4^{+} T cells, there was a statistically significant difference in the frequency of S-protein reactive CD8^{+} T cells producing IL4. However, the very low frequencies of these cells, as well as of TNFα and IL2 producing CD8^{+} T cells, dissent a relevant role in this setting (Figure 1L, N, and O).

T cells producing multiple cytokines are correlates of effective viral control.[26] Interestingly, polyfunctional CD4^{+} and CD8^{+} T cells were not diminished in patients receiving immunosuppression (Figure 2A–D). CD4^{+} bifunctional and trifunctional T cells produced mainly IL2, IFNγ, and
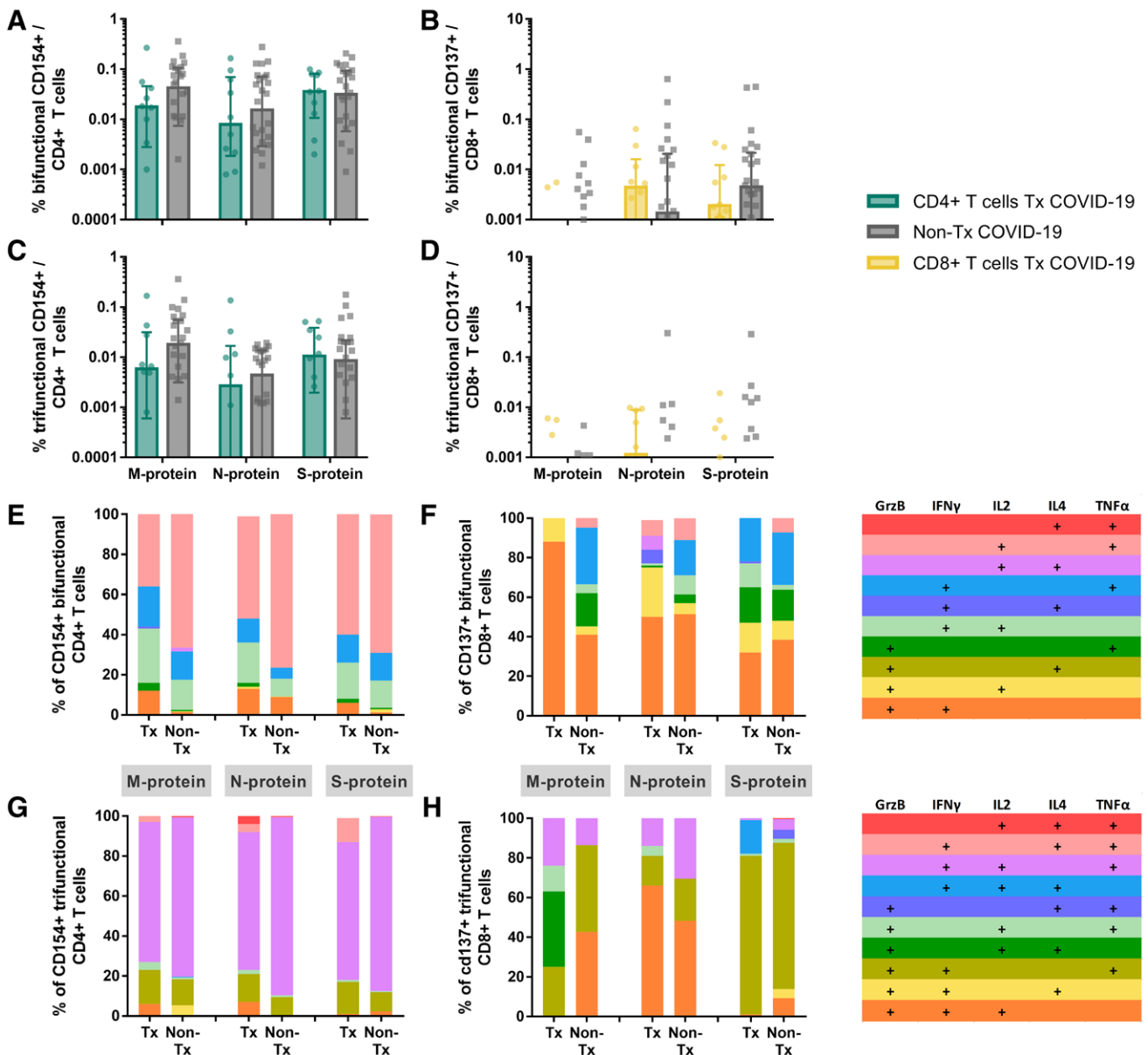

FIGURE 2. Composition of polyfunctional CD4^{+} and CD8^{+} T cells in transplant (Tx) and non-Tx COVID-19 patients. SARS-CoV-2 M-, N-, and S-protein reactive CD154^{+} CD4^{+} (A, C) and CD137^{+} CD8^{+} (B, D) polyfunctional T cells of 10 Tx and 26 non-Tx patients were analyzed by Boolean gating of production of IFNγ, TNFα, GrzB, IL2, and IL4. A–D, Bifunctional (A) and trifunctional (C) CD154^{+} CD4^{+} T cells and bifunctional (B) and trifunctional (D) CD137^{+} CD8^{+} T cells were calculated negative controls were subtracted from specifically stimulated samples to exclude unspecific activation. For patients with multiple samples, the mean response was calculated. Bars show median, error bars show interquartile range. Statistical comparison was done with Mann-Whitney U test and controlled by multivariate analysis for the influence of transplantation status and age. *P* < 0.05 was considered significant. E and F, Analysis of the relative contribution of individual cytokines and effector molecules to the pool of polyfunctional cells. Calculation was done for each patient and then the mean contribution across all patients was determined.

TNFα (Figure 2E and G). CD8^{+} bifunctional and trifunctional T cells produced mainly GrzB in combination with IFNγ, IL2, or TNFα (Figure 2F and H).

Tx Patients Develop SARS-CoV-2–specific Memory T Cells

Memory T cells are hallmarks of adaptive immunity and convey long-term protection against pathogens. The diminished repertoire of naive T cells (T_{NAIVE}) in elderly patients has been suggested as a factor contributing to critical COVID-19 course.[29,30] Of interest, an expansion of CD4^{+} effector-memory T cells (T_{EM}) and reduction of T_{NAIVE} during end-stage renal disease and after transplantation has been described earlier.[31-34] In line with these results, we observed higher frequencies of T_{EM} and lower frequencies of T_{NAIVE} in Tx patients as compared to non-Tx patients in CD4^{+} T cells (Figure 3A). This pattern was interestingly not observed in CD8^{+} T cells (Figure 3H). The immunologic age as defined by the memory T cell ratio among CD4^{+} T cells[30,35] in comparison to the chronologic age of Tx patients was significantly higher than that of non-Tx patients (Figure 3B and H). However, the SARS-CoV-2–specific memory composition was nearly identical between both groups. Despite the overall smaller pool of T_{NAIVE} that can progress into memory-phenotype T cells, there was no significant reduction of SARS-CoV-2–specific central-memory (T_{CM}), T_{EM}, and T_{EMRA} in CD4^{+} (Figure 3C–F) and CD8^{+} T cells (Figure 3I–L). This finding demonstrates that Tx patients form SARS-CoV-2–specific memory cells early after infection, either by new formation from T_{NAIVE} or by crossreactivity of existing memory T cells.

SARS-CoV-2–reactive T Cells Are Detectable Already at Early Time Points After Symptom Onset

Since immunosuppressive medication in most study patients was reduced in follow-up of COVID-19, no final conclusion on the effect of immunosuppression can be drawn from samples obtained at later time points. To address the influence of immunosuppression on SARS-CoV-2–reactive immunity, we analyzed the frequencies of S-, N-, and M-protein reactive T cells in all patients at the first time point. This assured that the analyzed samples were obtained under immunosuppressive conditions. The time of study inclusion after positive SARS-CoV-2 PCR was in median 3 and 4 d for non-Tx and Tx patients, respectively (IQR 2–6 in Tx patients and 1–9 in non-Tx patients;
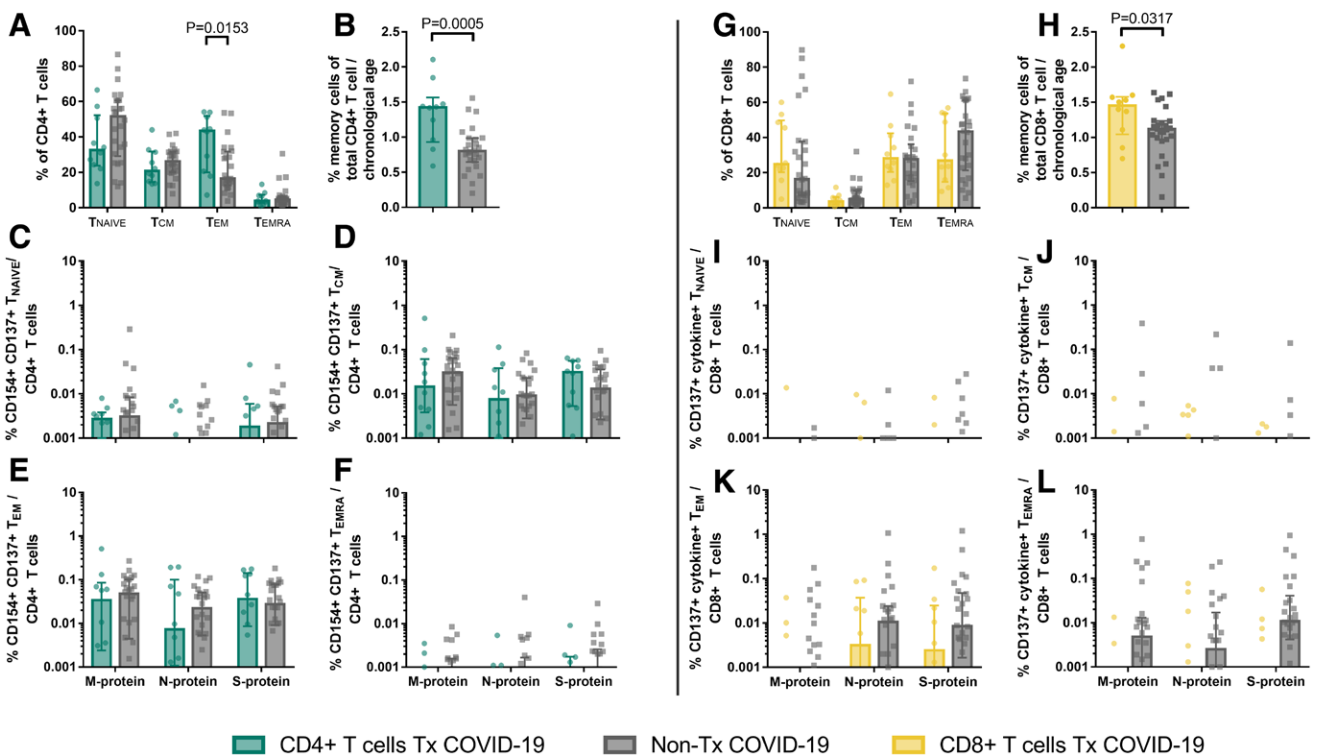

FIGURE 3. Memory phenotypes of SARS-CoV-2-reactive T cells in transplant (Tx) and non-Tx COVID-19 patients. Stimulation of PBMC of 10 Tx patients and 26 non-Tx patients was performed overnight with SARS-CoV-2 membrane (M)-, nucleocapsid (N)-, and spike (S)-protein. Analysis of activation markers CD154 and CD137 in CD4^{+} T cells (SARS-CoV-2-reactive CD4^{+} T cells) and CD137 in combination with production of any of interferon γ, tumor necrosis factor α, granzyme B, interleukin (IL)-2, or IL4 (cytokine^{+}) in CD8^{+} T cells (SARS-CoV-2-reactive CD8^{+} T cells) as well as C-C chemokine receptor type 7 (CCR7) and CD45-RA was performed using flow cytometry. A and G, Quantification of CD45RA^{+} CCR7^{+} (T_{NAIVE}), CD45RA^{−} CCR7^{+} (T_{CM}), CD45RA^{−} CCR7^{−} (T_{EM}), and CD45RA^{+} CCR7^{−} (T_{EMRA}) T cells among total CD4^{+} (A) and CD8^{+} (G) T cells. Bars show median, error bars show interquartile range. Statistical comparison was done with Mann-Whitney U test and controlled by multivariate analysis for the influence of transplantation status and age. B and H, Ratio of the proportion of memory T cells of the total CD4^{+} (B) and CD8^{+} (H) T cells and chronologic age. Bars show median, error bars show interquartile range. Statistical comparison was done with unpaired t-test. C–F and I–L, Frequencies of T_{NAIVE}, T_{CM}, T_{EM}, and T_{EMRA} among SARS-CoV-2-reactive CD4^{+} (C–F) and CD8^{+} (I–L) T cells. Negative controls were subtracted from specifically stimulated samples to exclude unspecific activation. Bars show median, error bars show interquartile range. Statistical comparison was done with Mann-Whitney U test and controlled by multivariate analysis for the influence of transplantation status and age. For patients with multiple samples, the mean response was calculated. $P < 0.05$ was considered significant.

no significant differences in Mann-Whitney U test). There was no statistically significant difference in COVID-19 severity in both groups at this time point (**Table S2, SDC**, http://links.lww.com/TP/C185). Surprisingly, frequencies of SARS-CoV-2–reactive CD4+ T cells were nearly identical between the Tx patient and the non-Tx patient cohort (**Figure S4, SDC**, http://links.lww.com/TP/C185). Overall CD8+ T cell responses towards SARS-CoV-2 peptides were again lower as compared to CD4+ T cell responses (**Figure S5, SDC**, http://links.lww.com/TP/C185). Although the frequency of S-protein-reactive CD8+ T cells in the non-Tx cohort exceeded the response in the Tx cohort, the difference did not reach statistical significance (**Figure S5b and e, SDC**, http://links.lww.com/TP/C185). There were no differences in the magnitude of M- and N-protein–reactive CD8+ T cells.

DISCUSSION

COVID-19 poses an especially severe risk to vulnerable patients, including the transplantation patient community. A main concern is the insufficient generation of SARS-CoV-2 directed adaptive immunity. The ability to generate efficient immune responses influences, among others, the risk assessment, treatment approaches, and vaccination strategies. The modification of immunosuppressive therapy comes at the cost of rejection risk and potentially reduced Tx survival.[36] At the same time, the effectiveness of this measure in COVID-19 is not known,[6,37,38] because it is unclear if Tx patients are able to mount an effective SARS-CoV-2 directed adaptive immune response after prolonged immunosuppression. With our study, we provide data on SARS-CoV-2–reactive humoral and cellular adaptive immunity in Tx patients early after diagnosis and in short-term follow-up.

Somewhat surprisingly, we did not observe strong differences in the formation of polyfunctional and memory SARS-CoV-2–reactive T cell responses between Tx and non-Tx patients. Following the data on cellular immunity, Tx patients showed similar titers of neutralizing antibodies as compared to non-Tx patients. As in other cohorts,[22,27] we also saw that the SARS-CoV-2 CD4+ exceeded SARS-CoV-2–reactive CD8+ T cell immunity. Cytokine and effector molecule production was diminished in CD8+ T cells of Tx patients as compared to non-Tx patients after stimulation with M and S, but not N-protein. Since N-protein was a strong inducer of CD8+ T cell immunity in previous analyses,[22] it is likely that also Tx patients mount a functional SARS-CoV-2–reactive CD8+ T cell as well as CD4+ T cell response.

Chronic organ failure and transplantation result in an aged immune system with a reduced repertoire of naive T cells and possible dependence on crossreactive memory T cell responses to new immunologic challenges.[32-34,39] Interestingly, a recent study described a lower functional avidity and higher polyclonality of SARS-CoV-2–reactive T cells in hospitalized as compared to nonhospitalized patients, despite higher frequencies of SARS-CoV-2–reactive T cells.[30] This was associated with a higher immunologic age defined as the ratio of memory cells among total CD4+ T cells of the more severely affected patients.[30] Therefore, the higher immunologic age of Tx patients observed in our and previous cohorts,[31-34] might convey a

higher risk of critical COVID-19 as recently hypothesized by Bacher et al.[30]

Despite our encouraging results, one could speculate that the similar SARS-CoV-2–reactive T cell immunity detected in COVID-19 follow-up of Tx patients as compared to nonimmunosuppressed patients might be explained by the reduced or discontinued regimen of immunosuppression. To be able to demonstrate the effect of immunosuppression, we analyzed the SARS-CoV-2–reactive T cells at the time point of COVID-19 diagnosis. Here, we demonstrated that SARS-CoV-2–reactive T cells could be detected at early time points of COVID-19 in Tx patients with a similar magnitude as in the non-Tx cohort. This interpretation is supported by a recent study by Benotmane et al,[40] in which the authors observed that humoral immune response in SARS-CoV-2 infected Tx patients was not significantly impaired. Similarly, Candon et al[41] measured vigorous SARS-CoV-2 cellular and humoral immunity by enzyme-linked immune absorbent spot and ELISA, respectively, in renal Tx and hemodialysis patients. Therefore, although highly preliminary due to the low patient number, in line with previous data[42-44] our study demonstrates a sufficient formation of antiviral response in Tx patients despite immunosuppressive medication.

Several limitations are important to consider regarding the interpretation of our findings. This study was not designed to answer the question of optimal therapy of SARS-CoV-2 infected Tx patients, and thus, the modification of treatment and sample collection was not done systematically. Furthermore, the low number of patients makes robust assumptions impossible. Tx patients were significantly younger than non-Tx patients, which we took account of by bivariate regression analysis. Lastly, the treatment modalities differed between the patients and additional immunomodulatory effects of other interventions cannot be excluded. Our results are likely not transferable to recently transplanted recipients that undergo induction therapy, and thus careful evaluation of transplantation activity in high-prevalence regions remains pivotal.[38,45]

Further studies are required to evaluate the role of individual immunosuppressive regimen on SARS-CoV-2–reactive immunity. Nevertheless, our data show an effective generation of neutralizing antibody and T cell responses towards SARS-CoV-2 in patients with a long history of immunosuppression and chronic disease early after COVID-19 diagnosis.

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