Differential kinetics of T cell and antibody responses delineate dominant T cell epitopes in long-term immunity after COVID-19

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Abstract

Long-term immunity to SARS-CoV-2 is crucial for the development of herd immunity and the aim of vaccination approaches. Reports on rapidly decreasing antibody titers question the efficacy of humoral immunity. The relevance of T cell memory after COVID-19 is yet unclear. Longitudinal analysis of SARS-CoV-2 immunity in convalescents up to six months post-infection revealed decreasing and stable spike and nucleocapsid antibody responses, respectively. In contrast, T cell responses remained robust and even increased in frequency and intensity. Single epitope mapping of T cell diversity over time identified ORF-independent, dominant T cell epitopes mediating long-term SARS-CoV-2 T cell responses and may be fundamental for vaccine design.

Main Text

The SARS-CoV-2 pandemic poses a serious threat to the world population with dramatic socioeconomic consequences. Immunity after SARS-CoV-2 infection is crucial for individual long-term protection upon virus re-exposure, but even more important to reduce transmission rates and ultimately achieve herd immunity. Moreover, elucidation of the immunological mechanisms underlying the potential development of protective long-term immunity in the course of COVID 19 will guide the design of effective SARS-CoV-2 vaccines and treatment.

Long-term immunity is generally mediated by the adaptive immune system. Memory B and T cells persist after infection and enable more rapid and effective responses upon re-challenge with the same pathogen (1). However, the persistence of cellular and humoral immunological memory differs between pathogens, and experience with the other two zoonotic coronaviruses – SARS CoV-1 and MERS-CoV – revealed early loss of humoral immunity (2, 3). So far, data on long-term immunity to SARS-CoV-2 are lacking. Available reports, up to three months after COVID-19 are partially conflicting, but overall point towards a decrease and even loss of SARS-CoV-2-specific antibody responses (4-8) and thus raise concerns regarding long-term humoral immunity. In SARS-CoV-1, T cell immunity was identified as important determinant for recovery and long-term protection (9-12), with long-lasting memory T cell responses detected in convalescents even 17 years after infection (13). Likewise, T cell immunity also appears to play a key role in COVID-19, with several studies reporting on T cell responses in acute infection up to three months after convalescence. This comprises evidence for potential preexisting immunity mediated by cross-reactive T cells to human common cold coronaviruses (6, 13-20). We and others recently characterized the T cell epitopes mediating these specific and cross-reactive SARS-CoV 2 T cell responses in convalescents as well as unexposed individuals and provided evidence that the development of immunity requires recognition of multiple epitopes (13, 15, 16, 20-22). In the light of the available data on SARS-CoV-2 immune responses, persistence of SARS-CoV-2 T cell immunity may be crucial for long-term protection after COVID-19, with respective consequences for vaccine development. Here we conducted the first longitudinal analysis comparing T cell and antibody responses in SARS-CoV-2 convalescents up to six

months post infection. We report on the differential kinetics of cellular and humoral immunity after COVID-19 and delineate dominant T cell epitopes for long-term immunity.

Longitudinal follow-up of SARS-CoV-2 convalescents

Clinical and immunological analysis of convalescents after mild or moderate SARS-CoV-2-infection (SARS donors, n = 51, Tables S1 and S2) was conducted 35 - 56 days (median 40 days, time point 1, T1) (21) and 141 - 183 days (median 159 days, time point 2, T2) after positive SARS-CoV-2 PCR (Fig. 1A). Persisting or newly arisen post-infectious symptoms were reported by 27% of SARS donors at T2, with fatigue (64% of symptomatic donors) as well as anosmia and ageusia (64% of symptomatic donors) being most common (Fig. 1B, Table S1). Kinetics of SARS-CoV-2-directed T cell immunity was determined longitudinally with regard to both, (i) intensity (SARS group A, n = 29) and (ii) diversity (percentage of detected T cell epitopes per donor; SARS group B, n = 23) of $CD4^+$ and $CD8^+$ T cell responses (Fig. 1C). To standardize determination of changes in SARS-CoV-2 T cell response intensity over time, we employed broadly applicable human leukocyte antigens (HLA) class I and HLA-DR SARS-CoV-2 epitope compositions (EC) comprising dominant (recognized by ≥ 50% of SARS donors) and subdominant (recognized by < 50% of SARS donors) specific T cell epitopes recognized exclusively in COVID-19 convalescents or cross-reactive T cell epitopes recognized by both, convalescents and individuals never exposed to SARS-CoV-2 (Table S3) (21). The number of convalescents with detectable SARS-CoV-2 T cell responses was found to increase over time. In detail, the percentage of donors with detectable T cell responses to SARS-CoV-2-specific EC (HLA class I: 45% T1 vs. 69% T2; HLA-DR: 90% T1 vs. 100% T2; Fig. 1D) as well as to cross-reactive EC (HLA class I: 31% T1 vs. 38% T2; HLA-DR: 93% T1 vs. 100% T2; Fig. 1E) increased from 93% (T1) to 100% (T2) as assessed by *ex vivo* IFN-g ELISPOT assays (Fig. 1, D and E).

Intensity of SARS-CoV-2 T cell responses over time

Longitudinal *ex vivo* IFN-g ELISPOT analysis of T cell responses at T1 and T2 with standardized EC (SARS group A, n = 29, Fig. 2A, Fig. S1) revealed robust intensities of HLA class I SARS-CoV-2-specific and cross-reactive T cell responses (median calculated spot counts: 20 (T1) vs. 18 (T2) and 74 (T1) vs. 61 (T2), respectively), whereas the intensities of SARS-CoV-2 T cell responses to HLA-DR specific and cross-reactive EC (median calculated spot counts: 29 (T1) vs. 53 (T2) and 35 (T1) vs. 75 (T2), respectively) was significantly increased over time (Fig. 2, B and C). A high inter-individual heterogeneity of longitudinal T cell response intensity was observed (52% and 45% of donors with new or ≥ 2-fold increased T cell responses, 24% and 45% with stable (fold-change 0.6-1.9) T cell responses, as well as 24% and 9% showed ≥ 2-fold decreased or lost T cell responses to HLA class I specific and cross-reactive EC at T2, respectively (Fig. 2D, Fig. S2A)). For HLA-DR, longitudinal increase of T cell response intensity in individual donors was even more pronounced (66% and 55% of donors with new or ≥ 2-fold increased T cell responses, 24% and 31% with stable (fold-change 0.6-1.9) T cell responses, as well as 10% and 14% showed ≥ 2-fold decreased or lost T cell responses to HLA-DR specific and cross-reactive EC at T2, respectively (Fig. 2E, Fig. S2B)). Interestingly, the three SARS donors showing the most pronounced

decrease of T cell responses to the HLA-DR specific EC all still suffered from post-infectious symptoms (Fig. 2E). Characterization of long-term SARS-CoV-2-directed T cells at T2 using *ex vivo* flow cytometry-based assessment of surface markers and intracellular cytokine staining (ICS) revealed that T cell responses to HLA class I cross-reactive EC were predominantly mediated by $CD8^+$ T cells, whereas T cell responses to HLA-DR specific and cross-reactive EC were mainly mediated by $CD4^+$ T cells. The vast majority of T cell responses to HLA class I SARS-CoV-2-specific EC was mediated by both, $CD8^+$ and $CD4^+$ T cells (Fig. 2, F and G, Fig. S3). $CD8^+$ T cells targeting HLA class I specific EC were mainly positive for CD107a, whereas $CD4^+$ and $CD8^+$ T cells targeting HLA-DR SARS-CoV-2-specific as well as HLA-DR and HLA class I cross-reactive EC displayed positivity for several of the markers IL-2, TNF, IFN-g, and CD107a (Fig. 2, H and I, Fig. S3).

Dynamics of SARS-CoV-2 antibodies in relation to T cell responses and post-infectious clinical status

Two independent assays were employed to longitudinally assess SARS-CoV-2 antibody responses in convalescents (n = 51) at T1 and T2 to determine (i) ratios of IgG and IgA antibodies targeting the S1 domain of the spike protein including the immunologically relevant receptor binding domain (RBD, EUROIMMUN; Fig. 3, A and B, Fig. S4, A and B) as well as (ii) anti-nucleocapsid antibody titers (Elecsys® immunoassay including IgG; Fig. 3C, Fig S4C). Both, anti-S1 IgG and IgA response significantly decreased over time (median 3.8 vs. 2.6 and 2.6 vs. 1.6, respectively; Fig. 3, A and B, Fig. S4, A and B), whereas anti-nucleocapsid antibody titers remained stable from T1 to T2 (median 29 vs. 25; Fig. 3C, Fig. S4C). Loss or ≥ 2-fold decrease of anti-S1 IgG and IgA was observed in 31% and 44% of SARS donors, respectively, whereas loss or ≥ 2-fold decrease of anti-nucleocapsid antibody titers was documented in only 13% of convalescents. Among the convalescents still suffering from post-infectious symptoms at T2, 36% (5/14) and 50% (7/14) presented with ≥ 2-fold decrease or loss of anti-S1 IgG and IgA, respectively, whereas none showed ≥ 2-fold decrease of anti-nucleocapsid antibody titers (Fig. 3, D to F). Anti-S1 IgG antibody responses moderately correlated with the intensity of T cell responses to SARS-CoV-2-specific and cross-reactive HLA-DR EC as well as cross-reactive HLA class I EC at T2 (Fig. S5). Longitudinal T cell and antibody responses as well as symptoms during and after COVID-19 varied among the donors (Fig. 3G). Neither the intensity of SARS-CoV-2-specific nor that of cross-reactive T cell responses to HLA class I or HLA-DR EC at T2 correlated with demographics (gender, age, or BMI; Table S4). High anti-nucleocapsid antibody titers at T2 were associated with a higher prevalence of post-infectious symptoms (Fig. 3, H and I). In contrast, neither intensity nor longitudinal kinetics of SARS-CoV-2 T cell responses were associated with post-infectious symptoms (Fig. 3, J and K).

Diversity of SARS-CoV-2 T cell immunity identifies epitopes mediating long-term T cell responses

In various viral diseases including SARS-CoV-2, diversity of T cell responses, i.e. the recognition of multiple T cell epitopes, has been implicated as a prerequisite for effective immunity (21, 23). We longitudinally analyzed the diversity of SARS-CoV-2 T cell responses by single epitope mapping using dominant and subdominant HLA-DR (n = 20) and HLA-A*24 (n = 6) SARS-CoV-2 T cell epitopes (21). To enable detection of low-frequent peptide-specific T cell populations, we used *in vitro* 12-day pre-

stimulation for expansion of peptide-specific T cells. Longitudinal diversity of HLA-DR and HLA-A*24 T cell responses decreased across all donors and T cell epitopes over time (median T cell recognition per donor 59% and 50% at T1, 48% and 17% at T2, respectively; Fig. 4A, Fig. S6A). The decrease in HLA-DR T cell diversity was confirmed in subgroup analyses for specific and cross-reactive (Fig. S7A), dominant and subdominant T cell epitopes (Fig. S7B) as well as for epitopes derived from structural or non-structural (Fig. S7C) and nucleocapsid vs. non-nucleocapsid viral open reading frames (ORF, Fig. S7D). However, donor- and epitope-specific assessment identified a subset of T cell epitopes derived from different ORFs, which sustain a persisting T cell response (10/20 HLA-DR T cell epitopes; 2/6 HLA-A*24 T cell epitopes; Fig. 4, B and C, Fig. S6, B and C, Tables S5 and S6). In particular the eight dominant T cell epitopes (7 HLA-DR T cell epitopes, 1 HLA-A*24 T cell epitope) identified to mediate a persisting T cell response in up to 100% of convalescents appear to be essential for long-term T cell immunity to SARS-CoV-2 and may thus enable the development of effective vaccination approaches.

Discussion

The SARS-CoV-2 pandemic results in dramatic worldwide consequences for economy, health care, and daily life. To enable the development of therapeutic and prophylactic approaches for COVID-19, elucidation of the mechanisms underlying SARS-CoV-2-directed immune responses is of utmost importance. This holds particularly true for the assessment of immunological memory, which requires a detailed longitudinal analysis of cellular and humoral immune responses. Accumulating evidence obtained from patients and convalescents regarding frequency, intensity, and diversity of T cell responses and their correlation with SARS-CoV-2 antibody titers as well as clinical characteristics point to a central role of T cell immunity in COVID-19 (6, 13, 17, 18, 21, 24). Here we provide a six-month longitudinal follow-up of $CD4^+$ and $CD8^+$ T cell and antibody responses in SARS-CoV-2 convalescents, which constitute core issues of SARS-CoV-2 immunity.

We observed robust and increasing intensity of SARS-CoV-2 T cell responses targeting HLA class I and HLA-DR T cell epitopes, respectively, over time. This observation is in line with and extends the findings of a report on SARS-CoV-2 immune memory up to three months post-infection as well as data obtained from SARS-CoV-1 (3, 6, 13). The increase in SARS-CoV-2 HLA-DR T cell responses could be due to ongoing antigen exposition (25-27) and/or persisting cross-stimulation by different components of the immune system (28, 29). Although the underlying mechanisms remain to be elucidated, these data clearly indicate an important role of SARS-CoV-2 $CD4^+$ T cell responses in the natural course of infection, which is in line with previous reports on acute and chronic viral infection (21, 30, 31).

In contrast to the kinetics of the T cell response, both IgG and IgA antibody responses to the S1 domain of the spike protein significantly declined during the six-month follow-up. In line with several reports on decreasing antibody titers after SARS-CoV-2 infection (5-8, 30, 31), this raises concerns that humoral immunity against SARS-CoV-2 may not provide long-term protection. It needs to be taken into consideration that the protective efficacy of the antibodies analyzed in our study remains unclear, even if RBD antibody levels reportedly correspond to virus-neutralizing activity (32). Epidemiologic studies

employing neutralizing assays in large cohorts are required to thoroughly unravel the relevance of long-term SARS-CoV-2 humoral immunity. Nevertheless, our finding that anti-S1 antibody responses decrease over time, whereas nucleocapsid antibody titers persist is important in the context of vaccine development, as several ongoing approaches are focusing on the induction of immune responses to the RBD of the spike protein (33, 34).

As meanwhile more than 14 million people have recovered from COVID-19, increasing evidence for the prevalence and nature of post-infectious symptoms and secondary damages is arising (35-41). In line, 27% of our convalescents suffered from fatigue, anosmia and ageusia, etc. The pathomechanism underlying persistence of these symptoms after SARS-CoV-2-infection is matter of debate and investigation. Microangiopathic cerebral lesions (42), effects directly mediated by the virus (38, 43), and immune-mediated inflammatory syndromes (44) are proposed to play a role. Previous studies reported on the correlation of high antibody titers with more severe course of acute COVID-19 (5, 21). Here we could show that high nucleocapsid antibody titers at six-month follow-up also associate with an increased prevalence of post-infectious symptoms. No correlation of post-infectious symptoms with intensity or longitudinal dynamics of anti-SARS-CoV-2 T cell responses was observed. Together with recent data providing evidence that the intensity of T cell responses does not correlate with acute COVID-19 severity (15, 21), this finding is of high relevance for the design of vaccines, as it provides evidence that disease-aggravating effects might not hamper the development of vaccination approaches aiming to induce SARS-CoV-2-specific T cell responses.

Previous work on viral diseases including SARS-CoV-2 implicates diversity of T cell responses, *i.e.* recognition of multiple T cell epitopes, as important prerequisite for effective immunity (21, 23). Identification of respective T cell epitopes that induce potent and long-lasting SARS-CoV-2 T cell responses is fundamental for both, detection of immunological memory and also vaccine design. Our longitudinal analysis of T cell response diversity using a single epitope-based approach allowed for discrimination of T cell epitopes capable or incapable to induce persisting SARS-CoV-2 T cell responses. This enabled the characterization of ORF-independent, dominant T cell epitopes that may govern long-term SARS-CoV-2 T cell immunity. These SARS-CoV-2 T cell epitopes constitute the basis of a multi-peptide vaccine for induction of T cell immunity to SARS-CoV-2 to combat COVID-19, which we will evaluate in an upcoming first-in-man clinical trial (NCT04546841).

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Declarations

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Figures

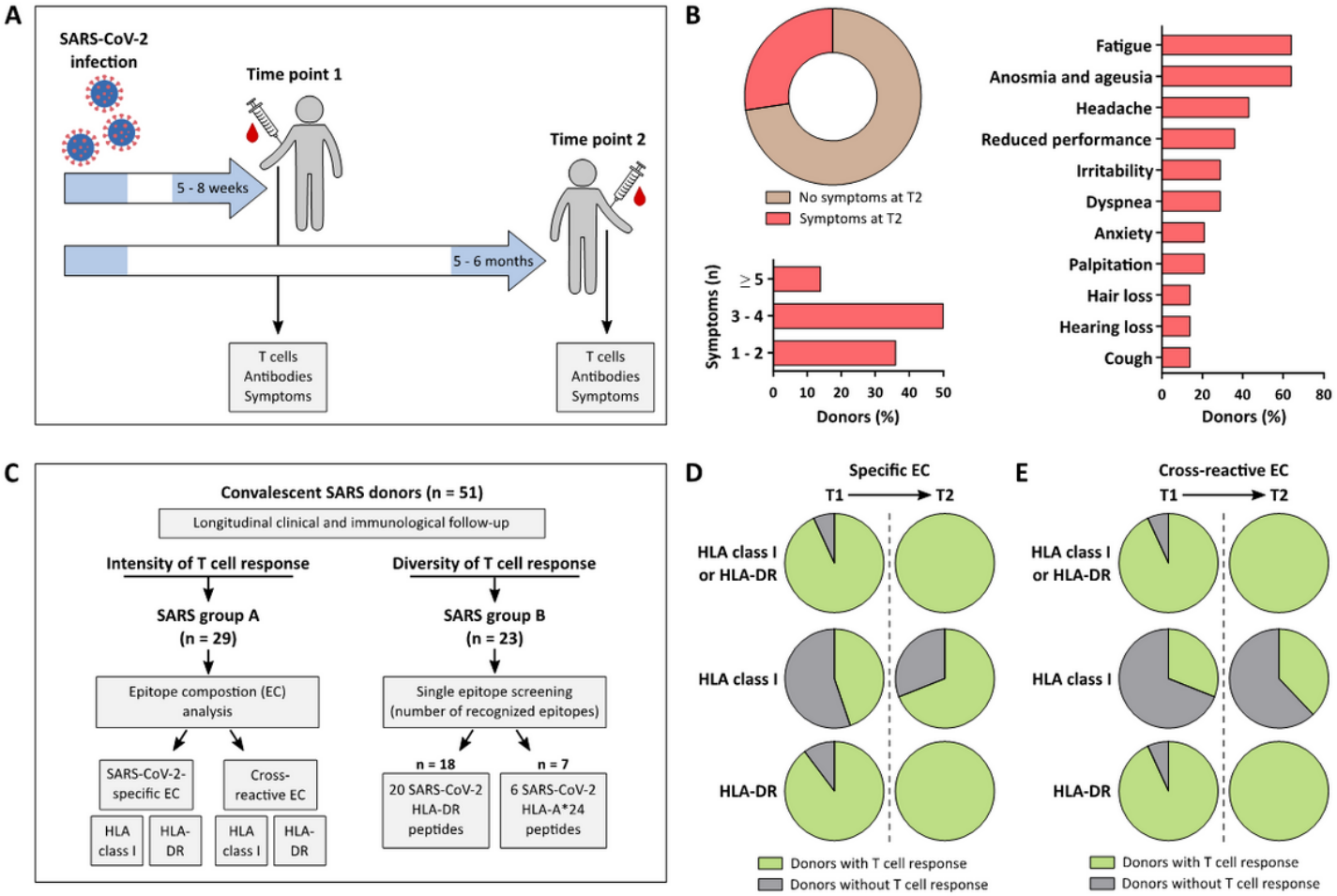

Figure 1

Longitudinal clinical and immunological follow-up analysis of convalescent donors after SARS-CoV-2 infection. (A) Schematic overview and timeline of the experimental setting for the longitudinal analysis of convalescent donors after SARS-CoV-2 infection (SARS donors). Blood samples were collected at two time points: 5 - 8 weeks (T1) and 5 - 6 months (T2) after positive SARS-CoV-2 PCR. At both time points, assessment of clinical characteristics was performed and blood and serum samples were analyzed for T cell- and antibody-mediated immune responses. (B) Prevalence, quantity and character of post-infectious symptoms in convalescent SARS donors (total n = 51, symptomatic n = 14) at T2. (C) Schematic overview of the experimental workflow for the longitudinal analysis of immune responses in convalescent SARS donors (n = 51). Intensity of T cell responses was assessed in SARS group A (n = 29) using SARS-CoV 2-specific and cross-reactive epitope compositions (EC) in ex vivo IFN-γ ELISPOT assays. Diversity of T cell responses was analyzed in SARS group B (n = 23) by single epitope screening using 20 HLA-DR and 6 HLA-A*24 SARS-CoV-2-derived peptides. (D and E) Proportion of convalescent donors with T cell responses to (D) SARS-CoV-2-specific and (E) cross-reactive EC at T1 and T2.

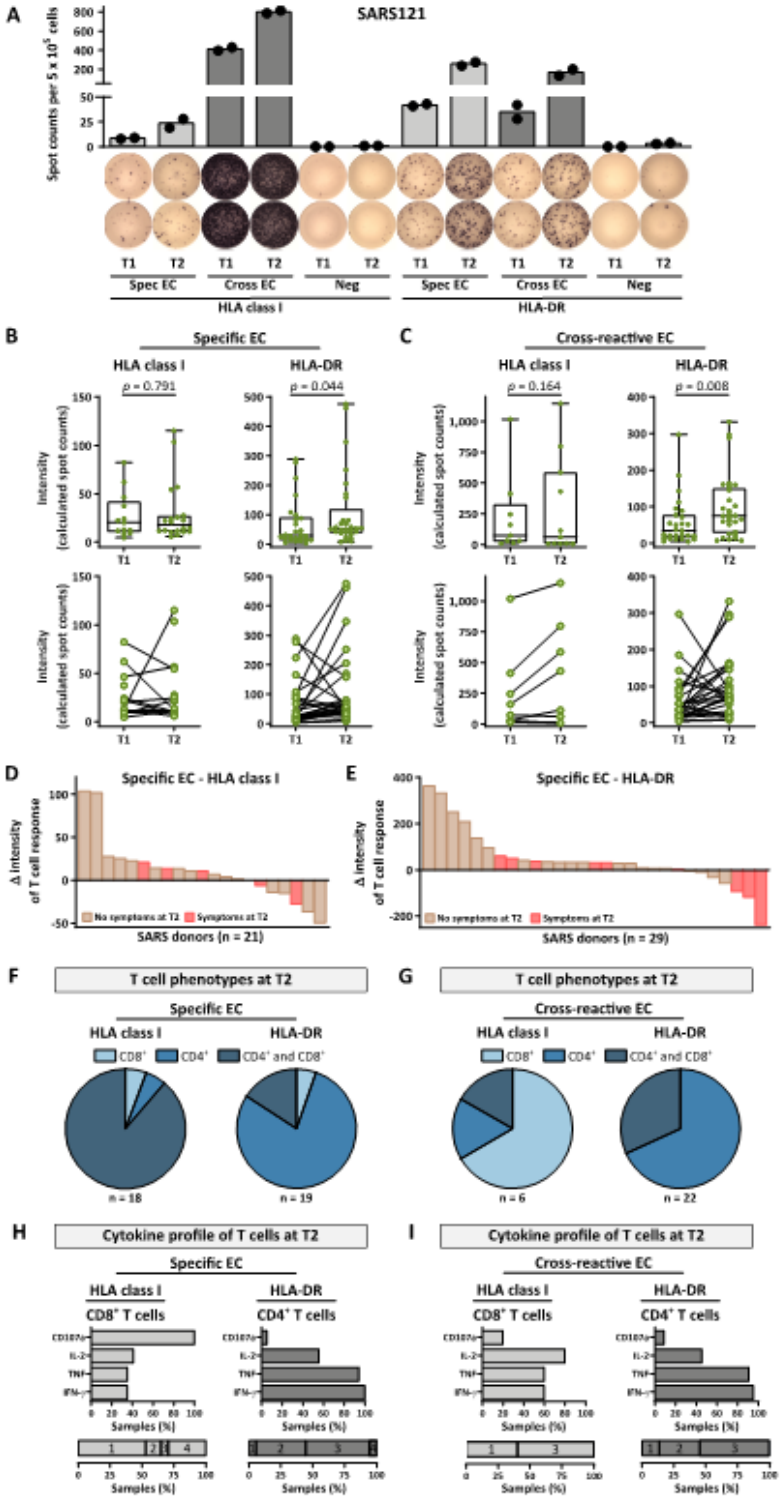

Figure 2

Longitudinal analysis of SARS-CoV-2 T cell response intensity in convalescents. (A) Representative example (SARS121) of T cell responses to HLA class I and HLA-DR SARS-CoV 2-specific and cross-reactive epitope compositions (EC) as assessed by ex vivo IFN-γ ELISPOT assays at T1 and T2. Data are presented as scatter dot plot with bars indicating the mean spot counts of duplicates normalized to 5 x 105 cells. EC, epitope compositions; Spec EC, SARS CoV-2-specific EC; Cross EC, cross-reactive EC; Neg,

negative control. (B and C) Intensities of ex vivo T cell responses to (B) SARS-CoV-2-specific (HLA class I (n = 21), HLA DR (n = 29)) or (C) cross-reactive EC (HLA class I (n = 11), HLA-DR (n = 29)) at T1 and T2. Dots represent individual donors with detectable T cell response. Min/max box plots (top rows) with corresponding line plots (bottom rows), Wilcoxon test. (D and E) Waterfall plots show ΔT2-T1 of T cell response intensity to (D) HLA class I and (E) HLA-DR SARS-CoV-2-specific EC. Donors with post-infectious symptoms are marked in red. (F to I) Flow cytometry-based ex vivo characterization of HLA class I and HLA-DR (F and H) SARS-CoV-2-specific or (G and I) cross-reactive T cell responses. (F and G) CD4+- and CD8+-mediated T cell responses with (H and I) cytokine profiles (IFN-γ, TNF, IL-2) and degranulation marker (CD107a) for CD8+ (left panel) and CD4+ (right panel) cells. Percentage of samples with CD107a+, IL-2+, TNF+, and IFN γ+ SARS-CoV-2 T cell responses are shown in the upper rows. The lower rows display proportion of samples revealing mono- (1), di- (2), tri- (3), or tetra-functional (4) T cell responses.

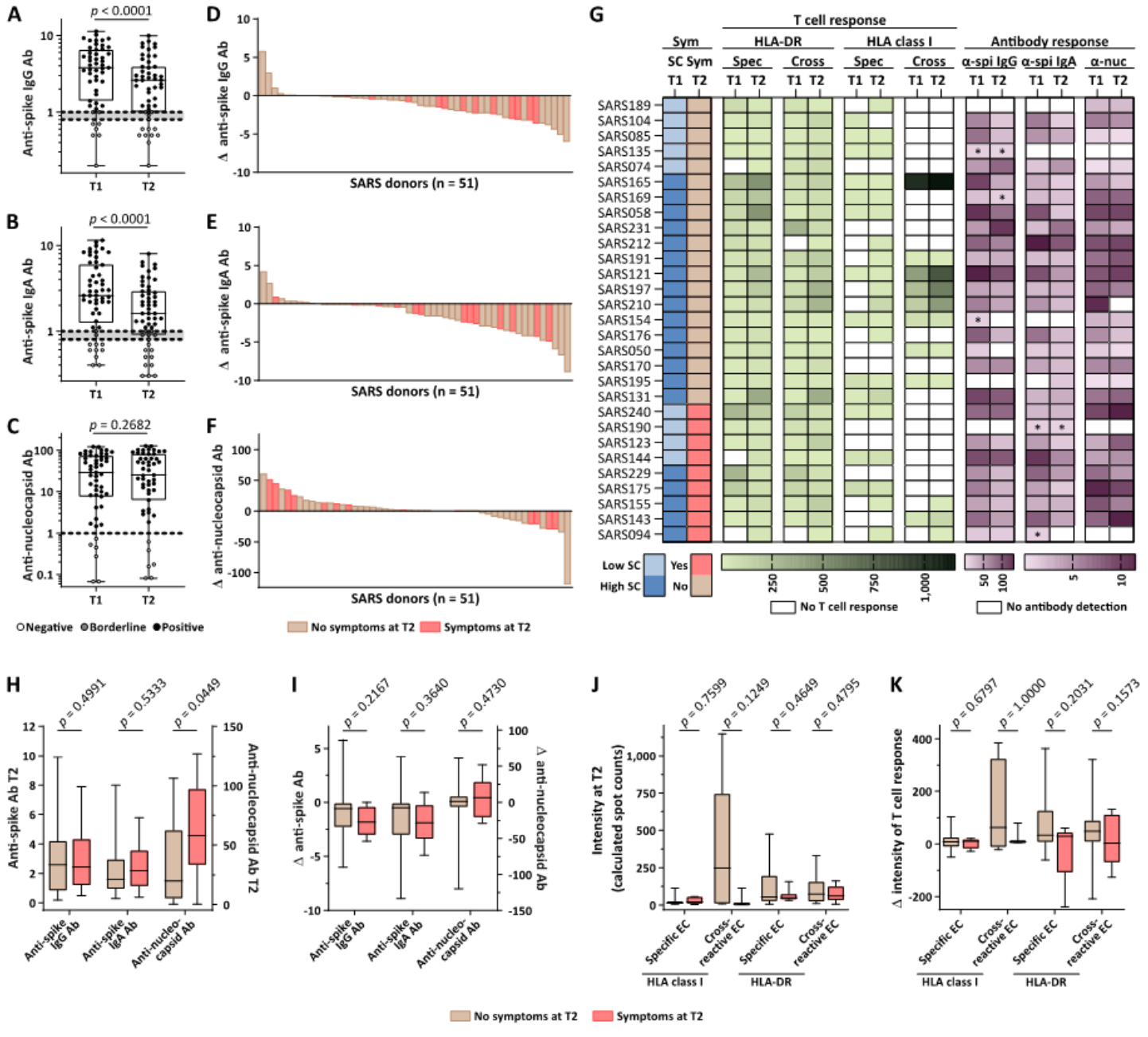

Figure 3

Dynamics of SARS-CoV-2 antibodies in relation to T cell responses and post-infectious clinical status. (A to C) Antibody responses in SARS donors (n = 51) at T1 and T2 for anti-S1 (A) IgG and (B) IgA or (C) anti-nucleocapsid. Donors with negative or borderline responses are marked in white or grey, respectively. Min/max box plots, Wilcoxon test. Ab, antibody. (D to F) Waterfall plots show ΔT2-T1 of anti-S1 (D) IgG and (E) IgA ratios or (F) anti nucleocapsid antibody titers. Donors with post-infectious symptoms are marked in red. (G) Heatmap of COVID-19 (symptom scores (SC)) and post-infectious symptoms, intensities of T cell responses to different EC (color gradient green) and antibody responses (color gradient purple) at T1 and T2 in individual SARS donors (group A, n = 29). Sym, symptoms; Spec, SARS CoV 2 specific EC; Cross, cross-reactive EC; α-nuc, anti-nucleocapsid; α-spi, anti-spike; *, donors with borderline response. (H) Anti-S1 IgG and IgA ratios and anti-nucleocapsid titers at T2 and (I) ΔT2 T1 of respective antibody responses. (J) Intensity of T cell responses at T2 and (K) ΔT2-T1 of intensity to SARS-CoV-2-specific (HLA class I (n = 21), HLA DR (n = 29)) and cross reactive EC (HLA class I (n = 11), HLA-DR (n = 29)). Min/max box plots, Mann-Whitney U test.

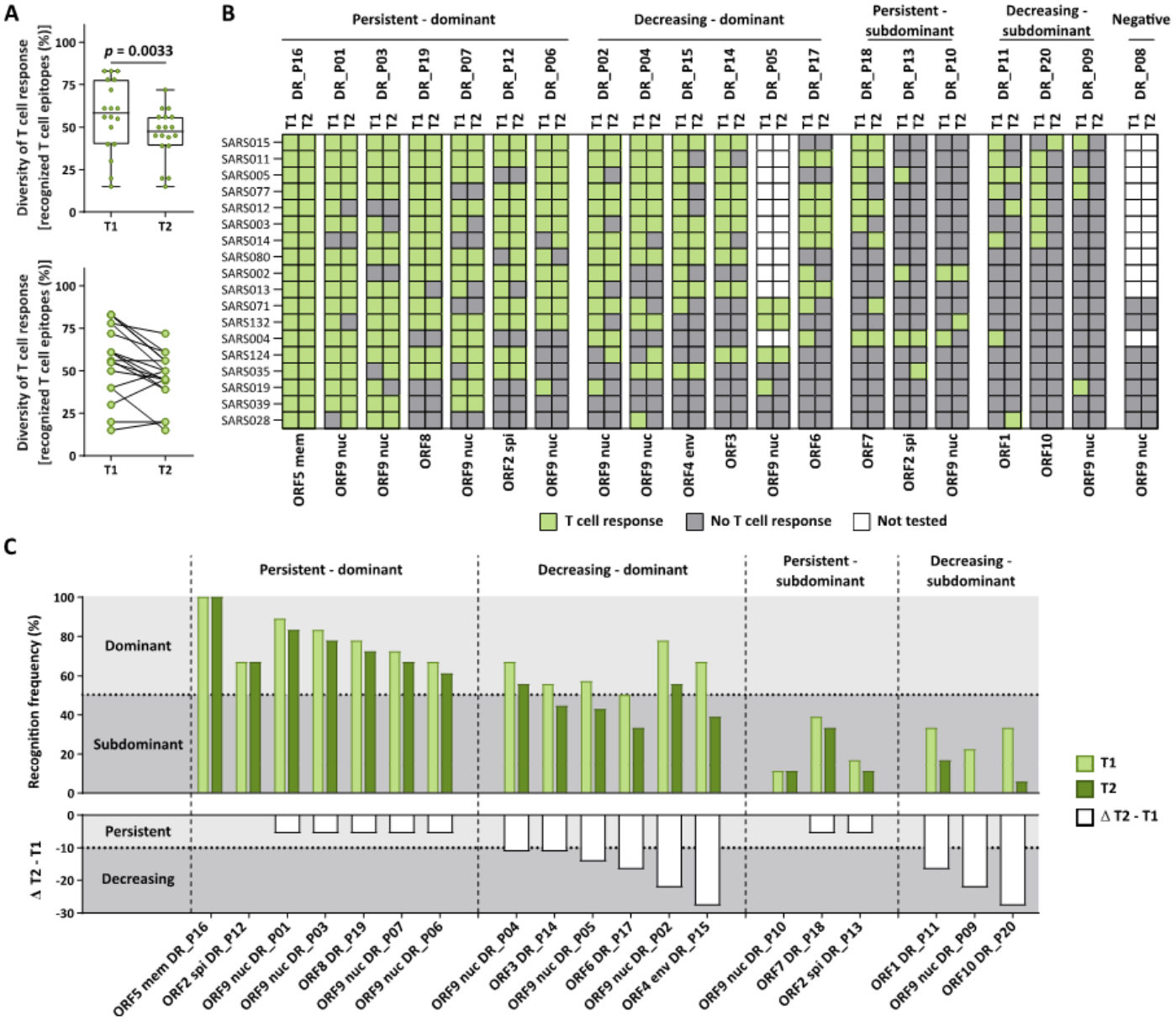

Figure 4

Longitudinal assessment of HLA-DR T cell response diversity in convalescent donors. (A) Diversity of T cell responses, i.e. percentage of recognized HLA-DR-restricted T cell epitopes per SARS donor (n = 18; SARS group B) at T1 and T2 as analyzed by IFN-γ ELISPOT assays after 12-day in vitro pre-stimulation. Dots represent individual donors. The upper row displays min/max box plot. The lower row indicates corresponding line plot, Wilcoxon test. (B) Heatmap indicating positive (green) and negative (grey) T cell responses to 20 HLA DR restricted SARS-CoV-2-derived peptides (DR_P01 – DR_P20) in individual SARS donors (n = 18) at T1 and T2. (C) Recognition frequencies of T cell epitopes at T1 and T2 (top) and their variation over time (bottom, ΔT2-T1) grouped into dominant and subdominant T cell epitopes capable (persistent) or incapable (decreasing) to mediate persisting T cell response over time. ORF, open reading frame; nuc, nucleocapsid; spi, spike protein; env, envelope protein; mem, membrane protein.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterials.pdf