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Perturbations of the T-cell receptor repertoire in response to SARS-CoV-2 in immunocompetent and immunocompromised individuals

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Abstract

Background—Functional T-cell responses are essential for virus clearance and long-term protection after severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, whereas certain clinical factors, such as older age and immunocompromise, are associated with worse outcome.

Objectives—We studied the breadth and magnitude of the T-cell responses in COVID-19 patients and in individuals with inborn errors of immunity (IEI) who had received COVID-19 mRNA vaccine.

Methods—Utilizing high-throughput sequencing and bioinformatics tools to characterize the T-cell receptor β (TRB) repertoire signatures in 540 individuals after SARS-CoV-2 infection, 31 IEI recipients of COVID-19 mRNA vaccine, and in healthy controls, we quantified HLA class-I- and class II-restricted SARS-CoV-2-specific responses and also identified several HLA allele-clonotype motif associations in COVID-19 patients, including a sub-cohort of anti-type I interferon (IFN-I)-positive patients.

Results—Our analysis revealed that elderly COVID-19 patients with critical disease manifested lower SARS-CoV-2 T-cell clonotype diversity as well as T-cell responses with reduced magnitude,

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Competing interests

T.M.S. holds equity in Adaptive Biotechnologies. All the other authors declare no competing interests.

whereas the SARS-CoV-2-specific clonotypes targeted a broad range of HLA class I- and class-II-restricted epitopes across the viral proteome. The presence of anti-IFN-I antibodies was associated with certain HLA alleles. Finally, COVID-19 mRNA immunization induced an increase in the breadth of SARS-CoV-2-specific clonotypes in patients with IEI, including those who had failed to seroconvert.

Conclusions—Elderly individuals have impaired capacity to develop broad and sustained T-cell responses after SARS-CoV-2 infection. Genetic factors may play a role in the production of anti-IFN-I antibodies. COVID-19 mRNA vaccines are effective in inducing T-cell responses in patients with IEI.

Clinical Implications—SARS-CoV-2 infection induces virus-specific T-cell clonotypes, but elderly individuals were impaired in their capacity to sustain an expanded state of overall T-cell response. Use of COVID-19 mRNA vaccine allows T-cell responses also in patients with inborn errors of immunity.

Capsule summary

Elderly individuals have impaired T-cell response to SARS-CoV-2 infection. Use of COVID-19 mRNA vaccine induces T-cell responses also in immunodeficient patients.

Keywords

COVID-19; SARS-CoV-2; T-cell receptor repertoire; inborn errors of immunity; COVID-19 mRNA vaccine; anti-type I interferon antibodies

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected more than 500 million individuals worldwide, causing a spectrum of COVID-19 disease severity that ranges from asymptomatic to life-threatening pneumonia and multiorgan failure[1]. Older age (especially in males) and comorbidities, including icompromised immunity, such as in patients with congenital defects affecting type I interferon (IFN-I) immunity, or neutralizing autoantibodies to type I IFNs[2–11], have been identified as risk factors associated with worse outcomes[12]. While innate immune responses play a critical role in containing viral replication and spread in the very first days after infection, long-term protection against SARS-CoV-2 relies upon adaptive immune responses[13–19]. The induction of a robust and persistent humoral response after immunization to SARS-CoV2 is crucial for improving outcomes of COVID-19 both in the general population and in patients with comorbidities[20–25]. However, the titers of neutralizing antibodies elicited by vaccination tend to wane over time[26]; furthermore, new viral variants may emerge that escape antibody recognition[27, 28]. Functional T-cell responses are essential for controlling and clearing many respiratory viruses[29], including SARS-CoV and Middle East Respiratory Syndrome coronavirus (MERS-CoV)[30, 31] even in the absence of detectable antibodies[32]. SARS-CoV-2-specific CD4+ and CD8+ T cells have been associated with milder manifestations of disease in humans[17] and have been shown to persist for at least 10 months in convalescent COVID-19 patients[33]. In addition, there is increasing body of evidence that SARS-CoV-2 variants of concern (VOCs) rarely escape T cell reactivity[34], due to a wide range of T-cell

epitopes across the entire viral proteome[35–41] which tend to be conserved across VOCs. The development of high-throughput sequencing (HTS) methods with sufficient depth has enabled highly detailed quantitative characterizations of the immune repertoire diversity and composition[42], providing adaptive immune response signatures, such as breadth (proportion of T-cell clonal lineages) and frequency profiles of antigen-specific clonotypes. Longitudinal tracking of SARS-CoV-2-specific T-cell receptor (TCR) clonotypes provides opportunities to characterize the dynamic nature of the antiviral responses. Studying virus-specific T-cell responses is especially important in patients with congenital or acquired forms of compromised immunity that hamper specific antibody production and to better understand the role of T-cell-mediated immunity in protecting against severe disease.

Here, we performed bulk immune repertoire sequencing to characterize the SARS-CoV-2 T-cell receptor repertoire in 584 individuals after natural infection, and to study the impact of inborn errors of immunity (IEI) on the virus-specific T-cell responses after immunization.

Methods

Characteristics of patients and controls of the COVID-19 cohort

Deidentified blood samples (n=677) were obtained from 540 patients who were admitted between February 25, 2020 and September 9, 2020 in 5 hospitals in Italy (following positive nasopharyngeal swab polymerase chain reaction (PCR) for SARS-CoV-2 infection. All patients and their guardians provided written informed consent in accordance with the Declaration of Helsinki and protocols approved by local ethical committees (see: Online Repository). The maximum severity of COVID-19 for each patient was ascertained per the Diagnosis and Treatment Protocol for Novel Coronavirus pneumonia (trial version 7), released by the National Health Commission & State Administration of Traditional Chinese Medicine on March 3, 2020[43]. Demographics, COVID-19 disease severity, presence and nature of comorbidities, time of each sample collection since the onset of symptoms, levels of anti-Spike (anti-S) and anti-Nucleocapsid (anti-N) antibodies, and human leukocyte antigen (HLA) allele composition of the 540 subjects in the COVID-19 cohort are reported in Table E1 in the Online Repository. For further details, see: Online Repository.

The control repertoire sample set for the COVID-19 cohort consisted of 140 healthy subjects with matching ethnicity (all Caucasians) who had been sampled prior to COVID-19 pandemic, and whose characteristics were obtained from Adaptive Biotech's ImmuneACCESS database at https://clients.adaptivebiotech.com/immuneaccess.

Characteristics of immunodeficient patients and health care workers (HCW) of the SARS-CoV-2 vaccine cohort

Peripheral blood samples were obtained from thirteen HCW (samples, n=38) and 31 patients (samples, n=63) with various forms of immunodeficiency, before and at various time points after administration of COVID-19 mRNA vaccines, upon informed consent and according to protocols approved by the local Institutional Review Boards (see: Online Repository). Demographics, the time of sample collection (pre1: baseline; pre2: 1 to 6 days prior to the second dose of the vaccine; post2: at a median of 30 days after the second dose of

the vaccine), and the levels of anti-N and anti-S antibodies are reported in Table E2 in the Online Repository. HCW had a median age of 54 years (interquartile range [IQR] of 16.75, 41.75– 58.50 years), and 10 out of 13 (76.92%) were females. Patients with IEI had a median age of 38 years (IQR of 22.5, 28.5– 51 years), and 17/31 (54.84%) of them were females. All HCW received two doses of the mRNA-based vaccine (Pfizer-BioNTech) 21 days apart. All the 31 patients with immunodeficiency received mRNA-based vaccines Pfizer-BioNTech (n=13) or Moderna (n=18), per manufacturer guidelines.

Measurement of SARS-CoV-2 specific anti-S and anti-N antibodies and of anti-IFN-I antibodies.

Testing for anti-S and anti-N IgG was performed via luciferase immunoprecipitation systems assay[44]. Anti-IFN-I antibodies were measured using a multiplex particle-based assay[45]. For further details, see: Online Repository.

HLA typing

HLA genotypes of COVID-19 patients were determined from whole genome sequencing data using HLA*LA[46] (see: Online Repository for further details).

TRB repertoire analysis

Sequences of complementarity determining region 3 (CDR3) of TCR-β (TRB) chains present in the human peripheral blood mononuclear cell samples were studied using HTS through the ImmunoSEQ assay after amplification of the extracted DNA from each sample in a bias-controlled multiplex PCR. The resulting CDR3 sequences were collapsed and filtered to quantify the absolute abundance and frequency of each unique TRB CDR3 region with the immunoSEQ Analyzer tool from Adaptive Biotechnologies.

To identify the SARS-CoV-2-specific signatures in the repertoire data, we utilized the criterion of exact TRB CDR3 matching with the SARS-CoV-2-specific TRB CDR3 sequences reported in the ImmuneCODE[37, 47–49] [developed using the Multiplex Identification of T-cell Receptor Antigen Specificity (MIRA) assay[50] and VDJdb[51] databases. To further confirm SARS-CoV-2 clonotype specificity, we computed the incidence rate of those clonotypes among the 540 SARS-CoV-2 infected individuals and among 140 uninfected controls (Fig. E1 in the Online Repository).

SARS-CoV-2-specific breadth of each sample was computed using the approach described in the literature[37], where breadth is the proportion of distinct T-cell clonal lineages in a repertoire that are SARS-CoV-2 specific, quantified as the fraction of the ImmuneCODE CDR3 matches at the unique clonotype level. For each sample, we also computed the median and summed frequencies of the SARS-CoV-2-specific T-cell clonotypes, as alternative repertoire metrics which take clonotype abundance levels into account to complement the breadth metric. In the larger cohort of COVID-19 samples, in order to mitigate the impact of potential noise across the control and case samples, we set a minimum abundance of two copies (per clonotype) while computing the values of these metrics.

We used GLIPH2 (Grouping of Lymphocyte Interaction by Paratope Hotspots 2)[52] for clustering the *TRB* CDR3 sequences to identify potential sequence motifs and patterns present in the TCR repertoire data associated with the COVID-19 positive and the anti-IFN-I antibody positive states. For additional details, see: Online Repository.

All the *TRB* sequencing data can be accessed with the following credentials:

Notarangelo-review@adaptivebiotech.com Password: notarangelo2023review

Statistical methods

TCR repertoire statistics were computed using the v0.7.0 Immunarch package (https://github.com/immunomind/immunarch) in R (v4.1.1). The reported Chao1 diversity per sample reflects the median value across 100 randomized instances of downsampling, where each instance utilized the set of repertoire sequences that were downsampled to the minimum sequencing depth (total cell count) in the larger cohort.

To compute potential associations between the HLA alleles and the anti-IFN-I positive state, SARS-CoV-2-specific CDR3 clusters derived from the anti-IFN-I positive repertoire samples were assigned Fisher's exact test-based p-values (using the fisher.test function in R) with respect to the anti-IFN-I-negative repertoire dataset.

The R packages ggpubr (v0.4.0.999)[53] and ggstatsplot (v0.11.1) were used for visualization of the results with box and scatter plots. Furthermore, the bar plots and dot plots were generated using the R packages ggplot (v3.4.2)[54], whereas the upset plots were generated with the R package UpSetR (v1.4.0)[55].

The associations between disease severity, positive serology, vaccination, case-control status, and SARS-CoV-2-specific repertoire characteristics were computed with linear regression using the lm function in R, while controlling for potential confounding factors including age, gender, and the number of days since onset of symptoms of the infection.

Results

Characteristics of patients and controls in the COVID-19 cohort

We studied 540 COVID-19 patients whose blood samples were obtained within 60 days since the onset of symptoms and 140 healthy controls with matching ethnicity who had been samples prior to COVID-19 pandemic. Because older age (especially >65 years) has been identified as a risk factor for severe COVID-19[56, 57], patients and controls were divided into three different age groups: 18–55 years, 56–65 years, and >65 years (Table 1). A total of 311 patients (57.6%) had critical disease, while 196 (36.3%) had moderate-severe and 33 (6.1%) had mild disease.

Effects of age and disease severity on SARS-CoV-2-specific TCR repertoire.

To characterize the TCR repertoire signatures of COVID-19 patients, we used genomic DNA as a template and performed bulk *TRB* repertoire sequencing in 677 peripheral blood samples derived from the 540 COVID-19 patients (Fig. E2 in the Online Repository) and

from the 140 uninfected controls. Upon matching the TRB CDR3 sequences obtained from COVID-19 patients with the sequences from the ImmuneCODE[37] and VDJdb[51] databases, we identified over 42,000 unique clonotypes (with ≥2 copies per clonotype per sample). Within each age group, uninfected individuals had a higher diversity of TCR repertoire (as defined by the Chao1 index) compared to COVID-19 patients (Fig. 1A). A low fraction of unique SARS-CoV-2 specific clonotypes (representative of the breadth of virus-specific T-cell response) was identified in uninfected controls, possibly reflecting previous exposure to seasonal coronaviruses, with no difference by age group (Fig. 1B). By contrast, a significantly higher fraction of unique SARS-CoV-2 specific clonotypes was detected in infected individuals for each age group (Fig. 1B). Furthermore, among the younger (18–55-year-old) infected individuals, the breadth of the SARS-CoV-2-specific repertoire increased with disease severity. Among patients with critical disease, older age (56–65 and >65 years) was associated with reduced breadth of SARS-CoV-2 TRB clonotypes compared to the younger (18–55 years) age group. (Fig. 1B). To further analyze the magnitude of the SARS-CoV-2 specific T cell response, we computed the median frequency of all SARS-CoV-2 specific TRB clonotypes as well as the summed frequency of the 100 most expanded SARS-CoV-2-specific clonotypes. As expected, within each age group, COVID-19 patients had a higher median frequency of SARS-CoV-2 specific clonotypes compared to uninfected controls (Fig. 2A). Analysis of the correlation between the median frequency of SARS-CoV-2 clonotypes and the number of days since the onset of symptoms, revealed a progressive reduction over time in patients with moderate/severe and especially in those with critical disease (Fig. E3 in the Online Repository). Moreover, when we analyzed the correlation between the median frequency and the breadth of the SARS-CoV-2 TRB clonotypes at various time intervals (days 1–15, 16–30, and 31–60 since onset of symptoms), a positive correlation throughout these time intervals was observed in patients with moderate/severe disease, but not at the latest time interval (31–60 days) in patients with critical disease (Fig. E4 in the Online Repository). Finally, among individuals >65-year-old, a lower value of the summed frequency of the top 100 SARS-CoV-2 specific clonotypes was observed in patients with critical COVID-19 compared to patients with moderate/severe disease, and a similar trend was detected also in comparison to patients with mild disease (Fig. 2B). A similar pattern of the summed frequency of the top 100 clonotypes was also observed in the entire *TRB* repertoire of elderly individuals (Fig. 2C). Furthermore, when we used TRCMatch to predict *TRB* clonotypes recognizing SARS-CoV-2, cytomegalovirus (CMV) and Epstein-Barr virus (EBV) epitopes, patients with critical COVID-19 showed higher values of the breadth and of the median frequency of TRB clonotype hits, but lower values of the summed frequency of clonotype hits for each of the these viruses, when compared to patients with moderate-severe or mild disease (Fig. E5A–C in the Online Repository). Overall, these results showed that the elderly individuals with critical COVID-19 were impaired in their capacity to sustain an expanded state of overall T-cell response, especially at late interval (days 31–60 after onset of symptoms).

Epitope landscape of the SARS-CoV-2 specific clonotypes

In order to map along the SARS-CoV-2 proteome the epitopes targeted by the SARS-CoV-2 specific clonotypes identified in our patient population, we used the ImmuneCODE database[37, 47] that includes the experimentally identified associations between TRB CDR3

sequences and a comprehensive set of 810 SARS-CoV-2 epitopes, which are classified as major histocompatibility complex (MHC) class I- and class II-restricted as predicted by NetMHCpan[58, 59]. The ImmuneCODE database contains 564,836 clonotype-class I-restricted epitope pairs and 31,400 clonotype-class II-restricted epitope pairs that had been tracked by the MIRA assay[50]. Fig. 3 illustrates the distribution of the number of unique clonotypes recognizing the HLA class-I and class II-restricted epitopes across the SARS-CoV-2 structural and non-structural proteins, where each bar corresponds to a MIRA interval of amino acid residues containing one or more contiguous epitopes. HLA class I-restricted epitopes were scattered throughout the entire viral proteome, whereas a more limited set of HLA class II-restricted epitopes were detected, none of which were in the Open Reading Frame 1ab (ORF1ab) non-structural protein (NSP). Of note, no ORF1ab-specific HLA class II hits are reported in the ImmuneCODE database. All patients included in the study underwent whole genome sequencing, enabling definition of their HLA genotype. We utilized GLIPH2 to identify the HLA restriction patterns of TRB CDR3 clonotypes in an antigen-specific manner. GLIPH2-based clustering of the receptor sequences resulted in 104 clusters representing SARS-CoV-2 specific CDR3 motifs that were present in ≥4 samples, associated with a unique SARS-CoV-2 antigen, while having a strong association (HLA score: $p<0.05$) with at least one HLA allele. Figure 4 shows the most significant CDR3 motif hit ($p<0.05$) for each of 33 HLA alleles identified in our patient population. Fig. E6 and E7 in the Online Repository show the results for all of the 104 clusters with their most significant HLA allele hits, and the top HLA allele per CDR3 cluster (with up to 5 most significant hits per SARS-CoV-2 protein), respectively. Furthermore, we identified approximately 2000 hits when we looked for exact HLA allele-CDR3 matched between our data and VDJdb database as shown in Table E3.

Next, we focused our attention on the Spike protein-specific T-cell responses. For this purpose, we analyzed the percentages of the HLA class I- and HLA class II-restricted SARS-CoV-2 specific clonotypes recognizing distinct Spike protein epitope intervals, as defined by the MIRA assay and reported in the ImmuneCODE database[47]. As shown in Fig. 5, T-cell clonotype specificities were broadly distributed along the Spike protein for both class I- and class II-restricted epitopes. However, some intervals were more frequently targeted, such as amino acid 320–338 within the receptor binding domain (RBD) among class I-restricted epitopes, and the amino acid 849–907 interval outside of the RBD among class II-restricted epitopes. We used a similar approach to identify the HLA class I- and class II-restricted epitopes targeted by TRB clonotypes for the nucleocapside, membrane, and envelope proteins of SARS-CoV-2 (Fig. E8–E10 in the Online Repository). Of note, some of amino acid intervals targeted by SARS-CoV-2-specific clonotypes correspond to previously recognized immunodominant epitopes (such as the class-I restricted $S_{269–277}$, $S_{1208–1271}$, $N_{105–113}$, $N_{322–331}$ and the class II-restricted $S_{167–180}$ and $S_{870–878}$)[60].

HLA association and SARS-CoV-2 specific clonotypes in COVID-19 patients with anti-IFN-I antibodies

Neutralizing anti-IFN-I antibodies have been identified as an important risk factor for life-threatening COVID-19 pneumonia, accounting for approximately 20% of deaths[7, 61], as well as for vaccine breakthrough hypoxemic COVID-19 pneumonia[62]. However, whether

certain HLA alleles may increase the risk of developing such autoantibodies is unknown. Using a multiplex-based particle assay[63], we detected high levels of anti-IFN-α and IFN-ω antibodies in 26/540 (4.8%) and 31/540 (5.7%) of the patients, respectively. In total, 33 patients were positive for one (9/33) or two (24/33) anti-IFN-I antibodies. Next, for each HLA allele that was present in our patient population, we computed the proportion of patients who tested positive or negative for any of the two types of anti-IFN-I antibodies and shared that HLA allele or other HLA alleles. We identified the following HLA alleles to be associated with the presence of anti-IFN-I antibodies: DRB1*10:10 (p=0.0004), A*02:01 (p=0.0089), C*14:02 (p=0.0256), DRB1*13:01 (p=0.0438), DRB1*11:02 (p=0.0465), and DPB1*03:01 (p=0.0468) (Table E4 in the Online Repository). Results for the entire set of HLA alleles are provided in Table E5 in the Online Repository. We used GLIPH2 to cluster the SARS-CoV-2-specific TRB CDR3 sequences (derived from the samples collected in the first 60 days since the onset of symptoms) and to identify potential HLA restriction patterns associated with these clusters in the anti-IFN-I positive patients. We identified 47 anti-IFN-I positive SARS-CoV-2-specific CDR3 clusters associated with a unique antigen, that were detected in at least 2 samples, in addition to the hla.score and fisher.score p-values <0.20 (Table E6 in the Online Repository). The HLA restriction patterns across the SARS-CoV-2 specific CDR3 clusters for the anti-IFN-I-positive patients is shown in Fig. E11 in the Online Repository. These data suggest a potential genetic susceptibility to the production of neutralizing anti-IFN-I antibodies.

Breadth and epitope specificity of SARS-CoV-2 Spike protein-specific T cell clonotypes in response to COVID-19 mRNA vaccines in HCW and in patients with IEI

In a previous study, we have shown that patients with IEI mount a variable specific antibody response to COVID-19 mRNA vaccines, with lower seroconversion rate after the first dose compared to what was observed in immunized healthy subjects[25, 64]. However, whether T cell responses were also affected was not investigated. To address this, we leveraged bulk sequencing of the *TRB* repertoire in a sub-cohort of the same IEI patients and HCW to characterize the breadth of the HLA class I- and class II-restricted SARS-CoV-2 Spike protein-specific CDR3 clonotypes that were enriched in immunized subjects. Individuals who were infected with SARS-CoV-2 in the period between the baseline time point (before the first vaccine dose) and within one month after the second dose of the vaccine, were excluded from the analysis. To evaluate whether the Spike protein-specific T-cell responses differ in seropositive and seronegative subjects, at each time point, the breadth of Spike protein-specific CDR3 clonotypes was analyzed separately in subjects who tested negative or positive for anti-S antibodies. The breadth of HLA class I- and class II-restricted SARS-CoV-2-specific clonotypes increased from baseline to post-vaccine time-points both in the healthy controls and in IEI patients (Fig. 6). This increase was observed also in patients who failed to seroconvert after immunization.

Given that COVID-19 mRNA vaccines encode for the Spike protein of SARS-CoV-2, to investigate the landscape of epitopes targeted by TRB clonotypes induced upon immunization, we focused our attention on previously experimentally validated class I- and class II—restricted Spike protein epitopes included in the ImmuneCODE database[37, 47] and predicted by NetMHCpan[58, 59]. Of note, these epitopes were not restricted to the

RBD, but included many other epitopes scattered throughout the Spike protein (Fig. E12). A similar pattern of HLA-restricted epitope hits was observed for both HCW and IEI patients, especially for the class I-restricted epitopes (Fig. E12A–B). Furthermore, the Spike protein-specific class I-restricted epitopes that were most frequently targeted upon immunization were the same as the ones that were targeted upon natural infection (compare Fig. E12A–B and Fig. 5A).

Discussion

In this manuscript, we characterized the TRB repertoire signatures in a large cohort of clinically diverse COVID-19 patients. Upon performing bulk immune repertoire sequencing in a high throughput manner, we have demonstrated that a low fraction of SARS-CoV-2 specific TRB clonotypes were present in uninfected individuals whose samples had been obtained prior to COVID-19 pandemic. These data are consistent with previous reports[65–67]and likely reflect cross-reactivity to T-cell epitopes expressed by other beta-coronaviruses. While a larger fraction of SARS-CoV-2-specific clonotypes was present in each severity group of infected individuals, our results showed reduced overall repertoire diversity in elderly individuals. Contraction of TCR repertoire diversity with ageing has been previously observed after influenza A virus infection[68, 69], with a negative impact on immune responses[70]. Previous studies have also demonstrated that patients with more severe forms of COVID-19 have markedly reduced numbers of circulating $CD4^+$ and $CD8^+$ T cells[71, 72], associated with emergence of an expanded pool of $CD8^+$ T-cells bearing surface markers of T-cell exhaustion (PD-1, TIM-3)[73–75]. Immune senescence has been identified as an important risk factor for increased susceptibility to infections in the elderly[76, 77], and may relate to T-cell lymphopenia and in particular to contraction of the naïve T-cell pool in this age group in COVID-19 patients[78, 79]. By performing a longitudinal analysis of the summed frequency of the top 100 most abundant SARS-CoV-2-specific clonotypes, we demonstrated that patients >65 years with critical COVID-19 were unable to sustain clonotypic expansions at later time points of their disease (31–60 days since the onset of symptoms). Similar TCR response patterns were also identified for CMV- and EBV-specific clonotypes and in the overall TCR repertoire. These observations indicate that T-cell responses with reduced magnitude in elderly patients with critical disease becomes more evident over time. A more frequent use of systemic corticosteroids in patients with critical forms of COVID-19 may have potentially contributed to these observed patterns; however, analysis of the clinical metadata of the patients enrolled in our study indicated that ~50% of the patients equally across all severity groups (mild, severe or critical disease) had received systemic corticosteroids. Unfortunately, information on the dose of steroids and length of treatment for each patient was not available, and therefore we were not able to perform a more in-depth analysis between treated and untreated subjects.

The extent to which TCR diversity, especially in the context of HLA-restricted CDR3 or epitope specificities, contributes to SARS-CoV-2 response is not fully understood. Several studies have focused on the discovery of relevant SARS-CoV-2 epitopes in both $CD4^+$ and $CD8^+$ T cell responses, leveraging *in silico* predictions, stimulation/expansion with peptide pools[35–38, 80], and tetramer binding[39, 40]. Collectively, these studies have identified a number of immunodominant epitopes from across the viral proteome including

structural and non-structural proteins in canonical[35–40, 80] and non-canonical open reading frames[41]. Upon matching the TRB CDR3 sequences obtained from COVID-19 patients with the sequences from the ImmuneCODE and VDJdb databases, we identified over 42,000 unique clonotypes (with ≥2 copies per sample). Consistent with previous observations from the literature[34, 60, 66], our data confirmed that the SARS-CoV-2-specific clonotype hits associated with HLA class I-restricted T-cell epitopes are scattered throughout the entire SARS-CoV-2 proteome, including non-structural proteins. By contrast, a lower number of MIRA intervals corresponding to class II-restricted epitopes were represented in our analysis, driven by the fact that the ImmuneCODE database is biased towards a higher number of class I-restricted epitope-CDR3 pairs. Utilizing HLA genotyping data obtained through whole genome sequencing, we identified antigen-specific HLA restriction patterns in the form of CDR3 motifs across SARS-CoV-2 specific clusters. These data revealed that non-structural proteins represent a frequent target of both class I- and class II-restricted clonotype motifs in an HLA allele-specific manner, which has potential implications for the development of novel vaccines meant to induce adaptive immune responses directed against a broader range of viral epitopes.

The presence of pre-existing neutralizing anti-IFN-I antibodies has been identified as an important risk factor for fatal COVID-19[7, 61]. The proportion of individuals with such autoantibodies increases with age[62], contributing to the higher fatality rate observed in elderly COVID-19 patients. For other anti-cytokine antibodies (anti-IFN-γ in particular), an association with certain HLA alleles has been established[81]; however, this has not been investigated for anti-IFN-I antibodies. In this study, the presence of anti-IFN-I antibodies was associated with certain HLA class I and class II alleles, suggesting that genetic factors may play a role in the production of anti-IFN-I antibodies. However, these initial observations need to be validated in other cohorts, including subjects of different ethnicity.

Finally, we have analyzed the breadth and epitope specificity of SARS-CoV-2-specific TRB clonotypes following immunization with COVID-19 mRNA vaccines in a cohort of patients with IEI and in HCW as controls. While IEI include a broad range of disorders, it has been reported that COVID-19 in this patient population tends to manifest at a younger age and has a more severe outcome compared to SARS-CoV-2 infection in the general population[82], emphasizing the importance of implementing active immunization against SARS-CoV-2 in patients with IEI. However, multiple studies have shown that IEI patients often mount lower SARS-CoV-2–specific antibody responses compared to healthy controls; in several cases, multiple doses of vaccine were necessary to induce robust humoral immune responses[64, 83, 84]. While low levels of SARS-CoV-2-specific T-cell responses have been reported in patients with primary antibody deficiencies[84–88], our study is the first to use high-throughput sequencing to examine perturbations of the T-cell receptor repertoire in patients with IEI following administration of COVID-19 mRNA vaccines. The results obtained demonstrated an increase in the breadth of SARS-CoV-2 specific T cell responses even in patients who had blunted antibody responses. Furthermore, we demonstrated that the Spike protein epitopes that the same SARS-CoV-2-specific clonotypes were elicited upon immunization in patients with IEI and in HCW, mirroring what is observed after natural infection. Altogether, these data provide evidence of a beneficial role of COVID-19 mRNA vaccine in patients with IEI.

This study has limitations. Unavailability of paired biological specimens before and after SARS-CoV-2 infection precluded investigating the possibility of pre-existing T-cell immunity directed against SARS-CoV-2 as a protective factor against severe disease. Furthermore, our study was based on bulk sequencing of the *TRB* repertoire derived from peripheral blood samples, and no functional validation was performed upon isolating SARS-CoV-2-specific T cells. Because we did not perform single-cell TRA and TRB sequencing, it is also likely that some of the SARS-CoV-2-specific TRB clonotypes present in our data were pairing with TCRα chains that did not contribute to recognition of SARS-CoV-2, thereby limiting the ability to faithfully identify SARS-CoV-2-specific T-cell clonotypes defined by both the TRA and TRB chains. Furthermore, sequencing *TRB* rearrangements using whole blood genomic DNA without sorting for the $CD4^+$ and $CD8^+$ T-cell populations was another limitation, which we attempted to mitigate by utilizing the HLA typing data to characterize the class I- and class II-restricted T-cell responses by utilizing TRB sequences associated with either MHC class based on the experimental literature. A relatively small number of patients with mild COVID-19 disease were included in the study, which posed a challenge when interpreting the data for this group of patients compared to the other disease severity groups. This study was performed in white patients only, limiting the generalizability of our observations. Nonetheless, the large number of COVID-19 patients studied using immune repertoire data and the availability of detailed clinical metadata, helped us characterize the SARS-CoV-2 specific T-cell responses in a wide range of age and severity groups. Finally, although a limited number of patients with selected forms of IEI were included in the study and future investigations are needed to assess efficacy of COVID-19 vaccines in this group of patients, our results suggest that COVID-19 vaccines can induce T-cell responses also in IEI patients who fail to seroconvert.

Supplementary Material

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Abbreviations

CDR3	complementarity determining region 3
CMV	cytomegalovirus
COVID-19	coronavirus disease 2019
EBV	Epstein-Barr virus
GLIPH2	Grouping of Lymphocyte Interaction by Paratope Hotspots 2
HCW	health care workers
HLA	Human Leukocyte Antigen
HTS	high throughput sequencing
IEI	inborn errors of immunity
IFN	interferon
IFN-I	type I interferon

IQR	interquartile range
MHC	Major Histocompatibility Complex
MERS-CoV	Middle-East respiratory syndrome coronavirus
MIRA	Multiplex Identification of T-cell Receptor Antigen Specificity
N	Nucleocapsid protein
NSP	non-structural protein
ORF	open reading frame
PCR	polymerase chain reaction
RBD	receptor binding domain
S	Spike protein
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
STAT1	Signal Transducer and Activator of Transcription 1
TCR	T cell receptor
TRA	T cell receptor α
TRB	T cell receptor β
VOC	variant of concern

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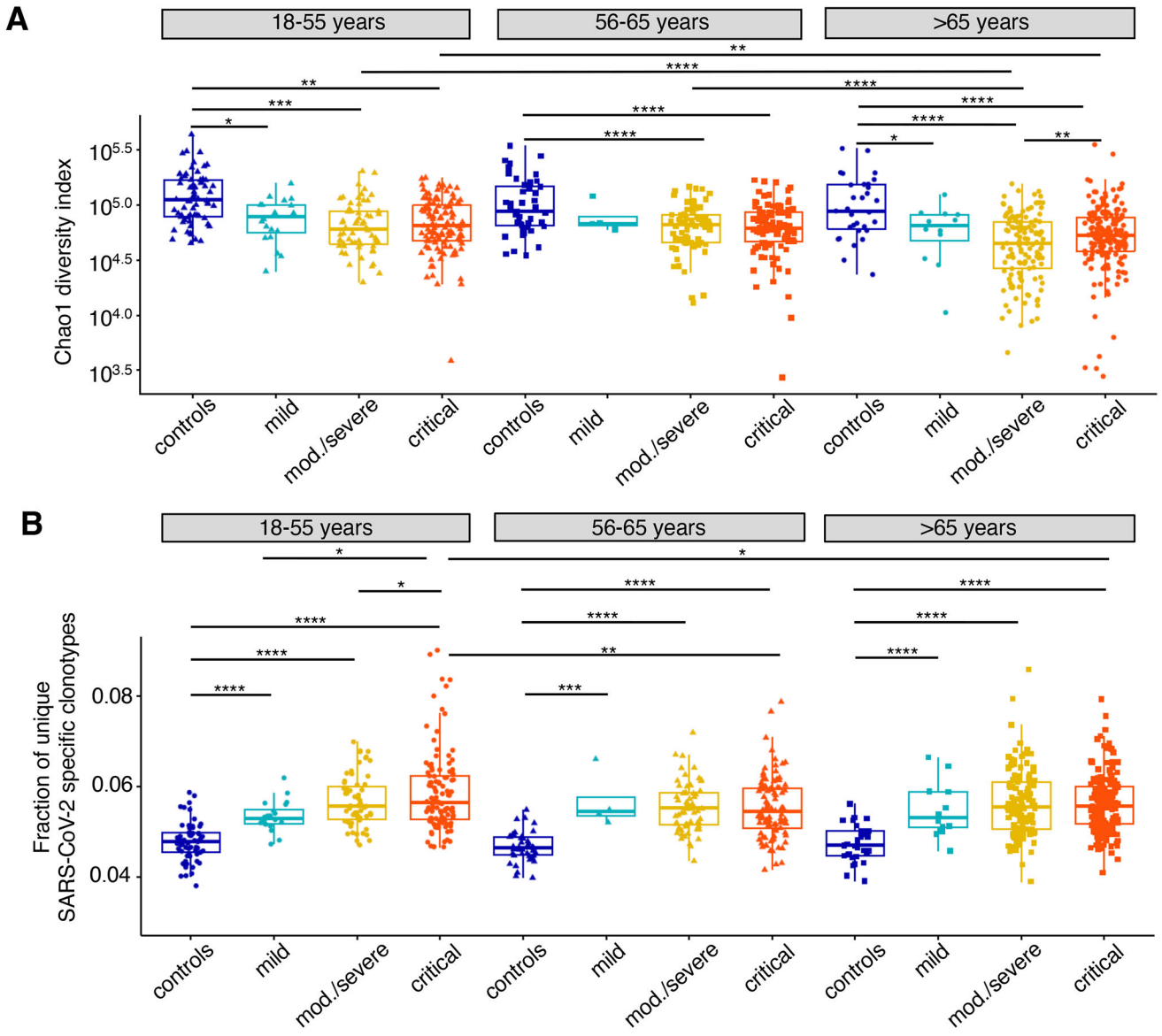

Figure 1. T-cell repertoire diversity and breadth of the SARS-CoV-2 specific immune response.

A) Chao1 diversity index of the T-cell receptor β (TRB) repertoire. **B)** Breadth of SARS-CoV-2-specific clonotypes, measured as the fraction of all unique TCR clonotypes that are SARS-CoV-2 specific. In each panel, data from the following sample set are shown: 677 samples of 540 individuals with COVID-19 in the time window of 1–60 days since onset and 140 uninfected healthy control samples with matching ethnicity whose samples were collected prior to the COVID-19 pandemic. COVID-19 patients are split by the age groups (18–55, 56–65, and >65-year-old) and disease severity levels (mild n=33, light blue; moderate-severe n= 196, yellow; critical n=311, red). Each dot represents one sample, whereas p-values are derived from linear regression models adjusted for days since onset, gender (for all comparisons), in addition to with respect to age for comparisons within the same age group. Box plots show the median, first and third quantiles (lower and upper hinges) and smallest (lower hinge − 1.5× IQR) and largest (upper hinge + 1.5× IQR) values

(lower and upper whiskers). Significance is shown as follows: *, $p<0.05$; **, $p<0.01$; ***, $p<0.005$; ****, $p<0.001$.

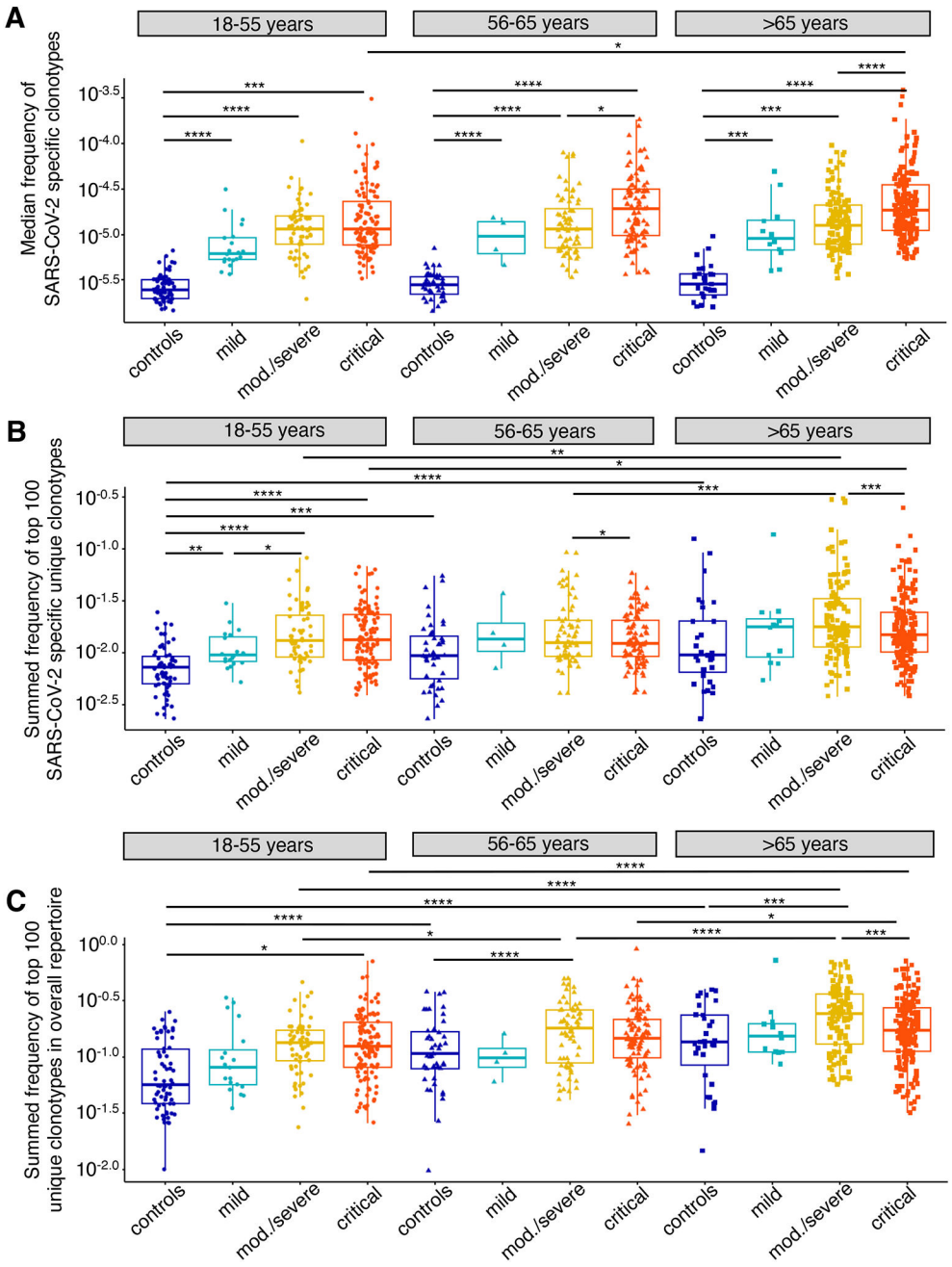

Figure 2. Relative abundance of SARS-CoV-2-specific and total TRB clonotypes in COVID-19 patients and uninfected controls.

A) Median frequency of the SARS-CoV-2-specific TRB clonotypes. **B)** Summed frequency of the 100 most abundant SARS-CoV-2-specific TRB clonotypes. **C)** Summed frequency of the 100 most abundant TRB clonotypes in the overall TRB repertoire.

In each panel, data from the following sample set are shown: 677 samples of 540 individuals with COVID-19 in the time window of 1–60 days since onset and 140 uninfected healthy control samples with matching ethnicity whose samples were collected prior to the COVID-19 pandemic. COVID-19 patients are split by the age groups (18–55, 56–65, and >65-year-old) and disease severity levels (mild n=33, light blue; moderate-severe n= 196,

yellow; critical n=311, red). Each dot represents one sample, whereas p-values are derived from linear regression models adjusted for days since onset, gender (for all comparisons), in addition to with respect to age for comparisons within the same age group. Box plots show the median, first and third quantiles (lower and upper hinges) and smallest (lower hinge − 1.5× IQR) and largest (upper hinge + 1.5× IQR) values (lower and upper whiskers). Significance is shown as follows: *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.005$; ****, $p \leq 0.001$.

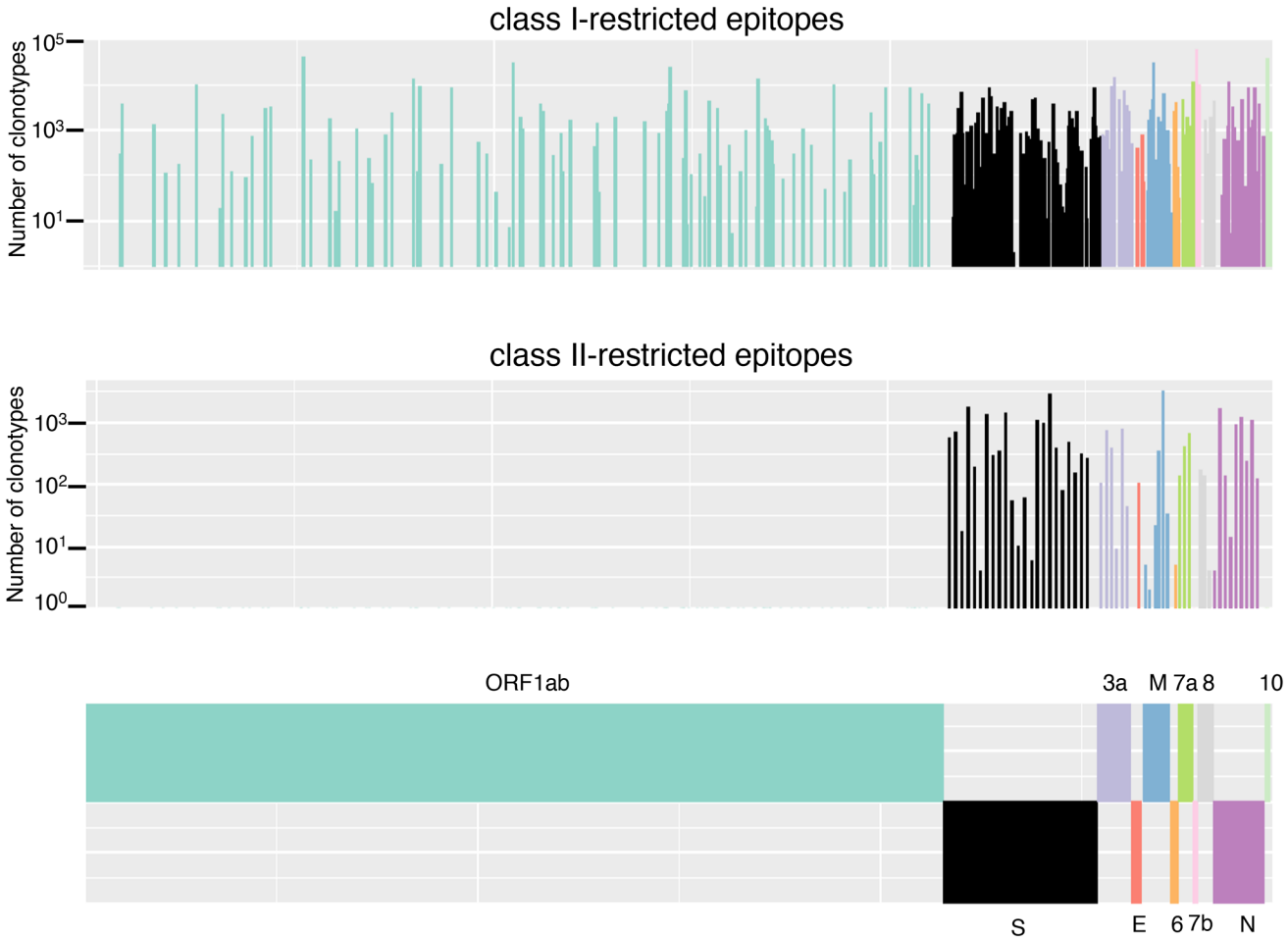

Figure 3. Numbers of HLA class I- and HLA class II-restricted SARS-CoV-2 specific clonotypes recognizing distinct epitope sets within amino acid intervals along the viral proteome.

SARS-CoV-2-specific hits in the TCR repertoire data of 677 samples, which were collected within 1–60 days from the onset of symptoms, from 540 COVID-19 infected individuals, are shown. Bar length denotes the number of clonotypes. Each bar represents a MIRA interval of amino acid residues containing one or more contiguous epitopes characterized in the ImmuneCODE database. Bar colors represent theSARS-CoV-2-specific antigens recognized by the clonotypes with at least two copies (minimum abundance). A scheme of the viral open reading frame (ORF) across the SARS-CoV-2 antigens is shown at the bottom.

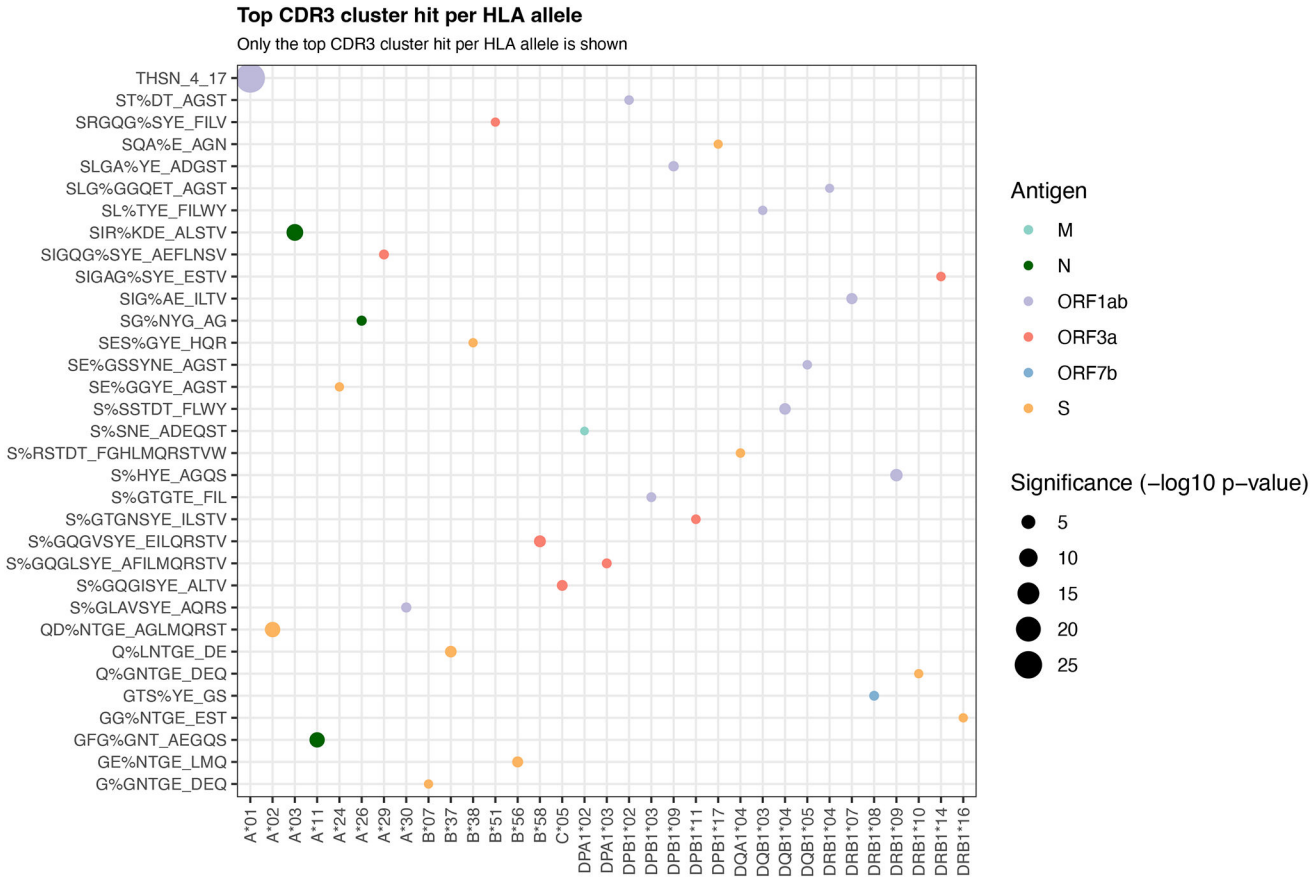

Figure 4. HLA restriction patterns identified by GLIPH2 across the 33 HLA alleles.

GLIPH2 clustering analysis of the TCR CDR3 sequences in the samples collected within 1–60 days from onset of symptoms (samples, n=677) identify enriched sequence motifs and patterns present in a SARS-CoV-2 antigen-specific manner. The dot plots show the most significant CDR3 motif per HLA allele, where each shown motif (or cluster) meets the following criteria: a unique SARS-CoV-2 antigen association, contribution of CDR3 sequences from at least four samples and the HLA association p-value< 0.05 by Fisher's exact test. The HLA alleles are listed at the bottom of the columns of the dot plot, whereas each row corresponds to a CDR3 amino acid sequence motif annotated by a color-coded SARS-CoV-2 antigen (top-right legend). The dot size is representative of the significance level (bottom-right legend).

Clusters are defined in one of the two formats: a) SYEA_4_17: Composed of the motif, the first and the last N-terminal starting position of the motif for local similarities; b) S%THSNQP_DGKQRST: For global similarities, it is composed of the global structure (% indicates the variable portion) and all unique amino acids at the variable position. These two pieces of information are separated by underscores.

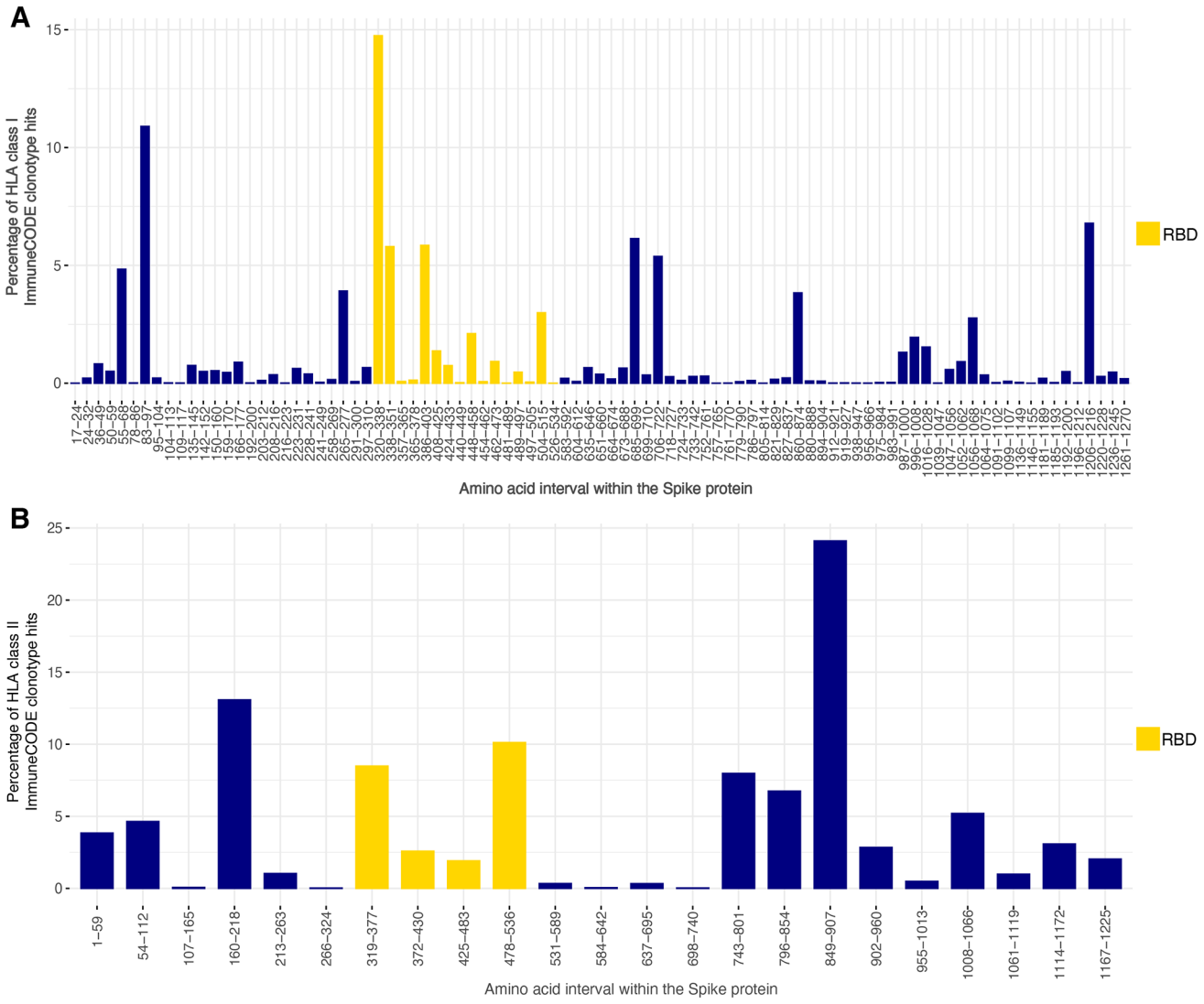

Figure 5. Percentage of HLA class I- and HLA class II-restricted SARS-CoV-2 specific clonotypes recognizing distinct Spike protein epitope intervals.

HLA class I- and HLA class-II-restricted clonotype hits are shown in panels **A)** and **B)**, respectively. Ranges on the x-axis represent the amino acid intervals that contain Spike protein epitopes characterized in the ImmuneCODE database. The bar height represents the percentage of the ImmuneCODE clonotype hits in 677 samples from 540 COVID-19 infected patients, whereas the yellow bars correspond to the amino acid intervals within the RBD of the Spike protein.

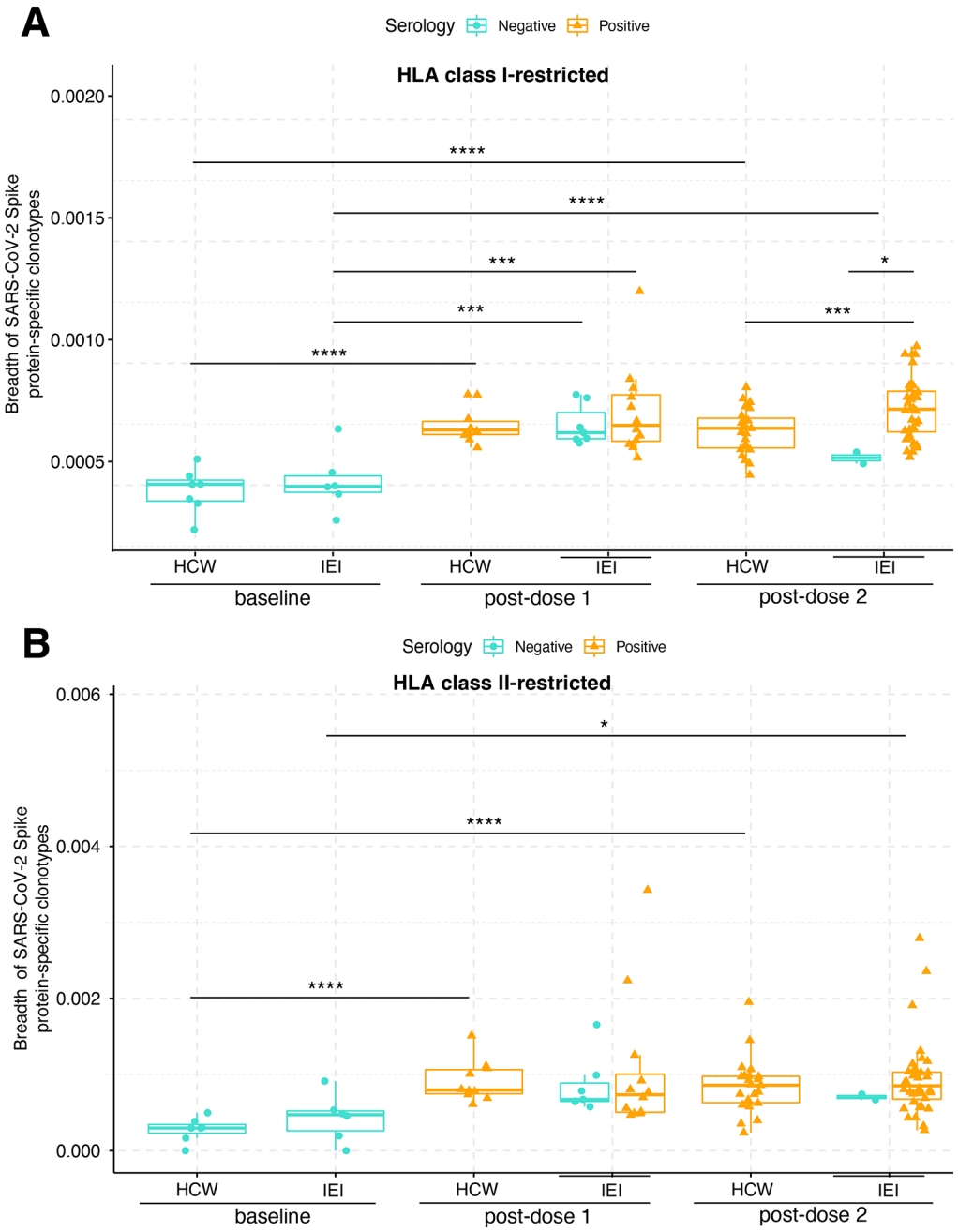

Figure 6. Breadth of the HLA class I- and HLA class II-restricted SARS-CoV-2 specific clonotypes pre- and post-mRNA vaccine in healthcare workers (HCW) and patients with inborn errors of immunity (IEI).

Breadth of HLA class-I-restricted **(A)** and class II-restricted **(B)** SARS-CoV-2-specific clonotypes in HCW (n=13, samples n=38) and IEI patients (n= 31, samples n=63) grouped with respect to their negative (blue) and positive (yellow) anti-Spike serology statuses prior to mRNA vaccine (baseline), 4 weeks after the first vaccine dose (post-dose 1), and > 4 weeks after the second dose of mRNA vaccine (post-dose 2). Each dot represents one sample, whereas p-values are derived from linear regression models adjusted for age and gender. Box plots show the median, first and third quantiles (lower and upper hinges) and

smallest (lower hinge − 1.5× IQR) and largest (upper hinge + 1.5× IQR) values (lower and upper whiskers). Significance value is expressed as follows: *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; ****, $p \leq 0.0001$.

Table 1 –

Demographic characteristics of the COVID-19 cohort and healthy controls

				Age (years)			Sex	
Age group (years)	**Subject group**	**Severity**	**Number of subjects**	**Median**	**IQR**	**s.d.**	**Females**	**Males**
18–55	COVID-19 patients	mild	20	45.25	11.10	9.30	15	5
		moderate/severe	63	49.10	6.95	8.18	23	40
		critical	113	50.00	7.00	7.70	23	90
	Healthy controls		66	34.00	22.50	11.60	41	25
56–65	COVID-19 patients	mild	4	57.50	1.50	1.29	2	2
		moderate/severe	68	60.00	3.72	2.86	27	41
		critical	97	60.00	5.00	2.80	23	74
	Healthy controls		43	63.00	3.00	1.98	30	13
>65	COVID-19 patients	mild	12	75.00	14.45	9.41	9	3
		moderate/severe	128	78.00	12.00	8.04	70	58
		critical	172	73.00	8.17	6.34	42	130
	Healthy controls		31	70.00	7.00	4.43	19	12