Material and Methods

TCR data

This study is based on public data from three previous works (Alter et al., 2021; Nolan et al., 2020; Mayer-Blackwell et al., 2020).

The dataset used for TCR repertoire analysis (Alter et al., 2021) includes samples from 32 individuals: 8 convalescent from COVID-19, 19 who received the Ad26.COV2.S vaccine developed by Janssen Pharmaceutica during a clinical trial, and 5 subjects who received a placebo. Peripheral blood samples were collected post diagnosis or vaccination and immunosequencing of the CDR3 regions of human TCRβ chains was performed with the immunoSEQ Assay (Adaptive Biotechnologies). Data was accessed on July 2021 via Adaptive Biotechnologies immuneACCESS® database (immuneACCESS® DOI: `https://doi.org/10.21417/GA2021N`).

To match the sample size of vaccinated individuals with data generated with the same procedure, 11 TCR repertoire samples from COVID-19-convalescent subjects were randomly selected from the COVID-19-HUniv12Oct dataset on Adaptive Biotechnologies ImmuneCODE™ project (Nolan et al., 2020). The full dataset contains TCRβ repertoires from 193 convalescent patients whose blood sample was collected at the Hospital Univesitario 12 de Octubre (Madrid, Spain). Data was accessed on Aug 2021 via Adaptive Biotechnologies immuneACCESS® database (immuneACCESS® DOI: `https://doi.org/10.21417/ADPT2020COVID`, ImmuneCODE-COVID-Release-002).

SARS-CoV-2-specific CD8$^+$ TCRβ sequences were obtained from Mayer-Blackwell et al. (2020). This sequences are proven to bind SARS-CoV-2 epitopes by Multiplex Identification of Receptor Antigen (MIRA) assays (Nolan et al., 2020) and are also enriched in bulk TCRβ repertoires of convalescent individuals compared to healthy controls. For the present study, only TCRβ sequences with a strong evidence of HLA restriction (N = 1831) were taken into consideration.

SARS-CoV-2-specific CD4$^+$ TCRs discovery

While SARS-CoV-2-specific CD4$^+$ have been used to annotate TCR repertoires in previous studies (Alter et al., 2021; Gittelman et al., 2021), those enriched and high-reliable datasets are not currently public. ImmuneCODE™ project contains an unenriched dataset of 6809 CD4$^+$ TCRs that bind 49 different SARS-CoV-2 epitopes presented by class II MHC molecules in MIRA assays. Data was accessed on Aug 2021 via Adaptive Biotechnologies immuneACCESS® database (immuneACCESS® DOI: `https://doi.org/10.21417/ADPT2020COVID`, ImmuneCODE-COVID-Release-002).

These TCRs were further screened for enrichment compared to a background of healthy individuals repertoires in order to remove TCRs that may be highly public or cross-reactive to common antigens. The enrichment analysis was performed with tcrdist3 Python toolkit (Docker image v0.1.9) (Mayer-Blackwell et al., 2020; Dash et al., 2017), following the same meta-clonotype discovery pipeline employed for SARS-CoV-2 CD8$^+$ TCR discovery as in Mayer-Blackwell et al. (2020).

Measurement of T-cell response to SARS-CoV-2

The 43 TCRβ repertoires were annotated for antigen-specificity with the SARS-CoV-2-specific TCRs (CD4$^+$ and CD8$^+$) by matching CDR3 aminoacid sequence and V gene. The SARS-CoV-2 response of each individual to spike and non-spike proteins was measured in terms of breadth, defined as the proportion of distinct TCRs recognizing certain protein among all the unique sequences in a repertoire, and in terms of depth, which is the proportion of the frequency of those SARS-CoV-2-specific TCRs.

CDR3 pairwise distances

Pairwise distances between all CDR3 sequences in a given sample were computed with tcrdist3 (Mayer-Blackwell et al., 2020; Dash et al., 2017), which implements a custom distance metric based on BLOSUM62 substitution matrix to account for similar aminoacid substitutions, and applies different weights depending on the importance of every CDR3 position in antigen binding. Total runtime was □103 hours with parallel processing (40 CPUs, 256 GB of RAM).
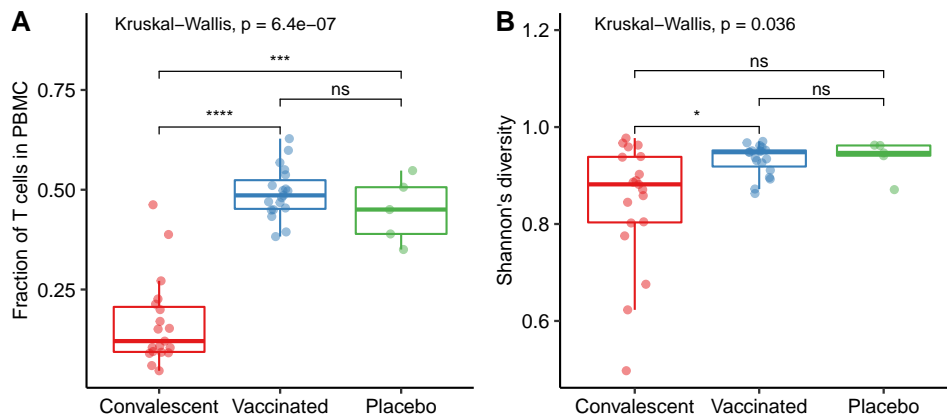
Figure 1: TCR repertoire preliminary analysis. (a) Fraction of T-cells among peripheral blood mononuclear cells (PBMC). **(b)** Shannon diversity index. A value closer to 0 indicates the emergence of a few dominant clones, whereas it reaches its maximum when TCR frequencies are evenly distributed. Statistical significance was determined by two-sided Wilcoxon rank-sum tests. N = 43 independent samples (19 SARS-CoV-2 convalescent individuals, 19 Ad26.COV2.S vaccine recipients, 5 placebo recipients).

Network analysis

In this analysis each unique CDR3 aminoacid sequence were considered as a node. An edge was built between two nodes if their pairwise distance was ≤ 12. The reason behind this threshold is that 12 is the greatest possible distance between two CDR3 with one mismatch according to tcrdist3 algorithm. Networks were built and analyzed with R igraph package v1.2.6 (Csardi and Nepusz, 2006).

Data analysis and visualization

All plots and analyses were carried in R 3.6.1 (R Core Team, 2019). For data analysis, the packages dplyr v1.0.2 (Wickham et al., 2020), tidyr v1.1.2 (Wickham, 2020), rstatix v0.7.0 (Kassambara, 2021) and parallel v3.6.1 (R Core Team, 2019) were used. Networks were plotted with the ggraph v2.0.2 package (Pedersen, 2020). All other plots were generated with ggplot2 v3.3.2 (Wickham, 2016) and ggpubr v0.4.0 (Kassambara, 2020). 3D visualization of the SARS-CoV-2 spike protein (PDB ID: 6XR8) was generated with Protein Imager (Tomasello et al., 2020).

Results

TCR repertoire preliminary analysis

One of the clinical characteristics of SARS-CoV-2-infected patients is lymphopenia or low lymphocyte counts in peripheral blood, specially affecting to T cells. The degree of lymphopenia correlates with disease severity, and it is usually associated with a pro-inflamatory cytokine storm (Liu et al., 2020). It is hypothesized that the underlying causes of lymphopenia can be the pro-inflamatory cytokine levels, exhaustion of T-cells upon COVID-19 infection and direct SARS-CoV-2-infection of T-cells (Tavakolpour et al., 2020). In TCR repertoire sequencing from a peripheral blood sample, both the number of nucleated cells and total T cells can be estimated by the amplification of reference gene primers. The fraction of T-cells is significantly lower in convalescent individuals compared to vaccinated and placebo ($p = 2.5 \cdot 10^{-9}$ and $p = 5.6 \cdot 10^{-4}$, two-sided Wilcoxon rank-sum test) and no significant differences were observed between vaccinated and placebo subjects (Fig. 1a). Some convalescent patients TCRβ repertoires have a low Shannon diversity index (Fig. 1b), indicating that a few clones are expanded and possibly reflecting that these individuals had a recent adaptive immunity response, most likely to SARS-CoV-2 infection.

Breadth and depth of SARS-CoV-2-specific T-cell response

To evaluate the magnitude of the T-cell response to SARS-CoV-2 after disease and vaccination, TCR repertoires of convalescent, vaccinated and placebo recipients individuals were annotated with CD8^{+} and CD4^{+} TCR datasets that had previously been determined to be SARS-CoV-2-specific and screened for enrichment compared to a background of healthy individuals repertoires in order to remove TCRs that may be unspecific (i.e. cross-reactive to common antigens) (See Material and Methods). Response to SARS-CoV-2 spike and non-spike was measured in terms of breadth (unique TCR sequences) and
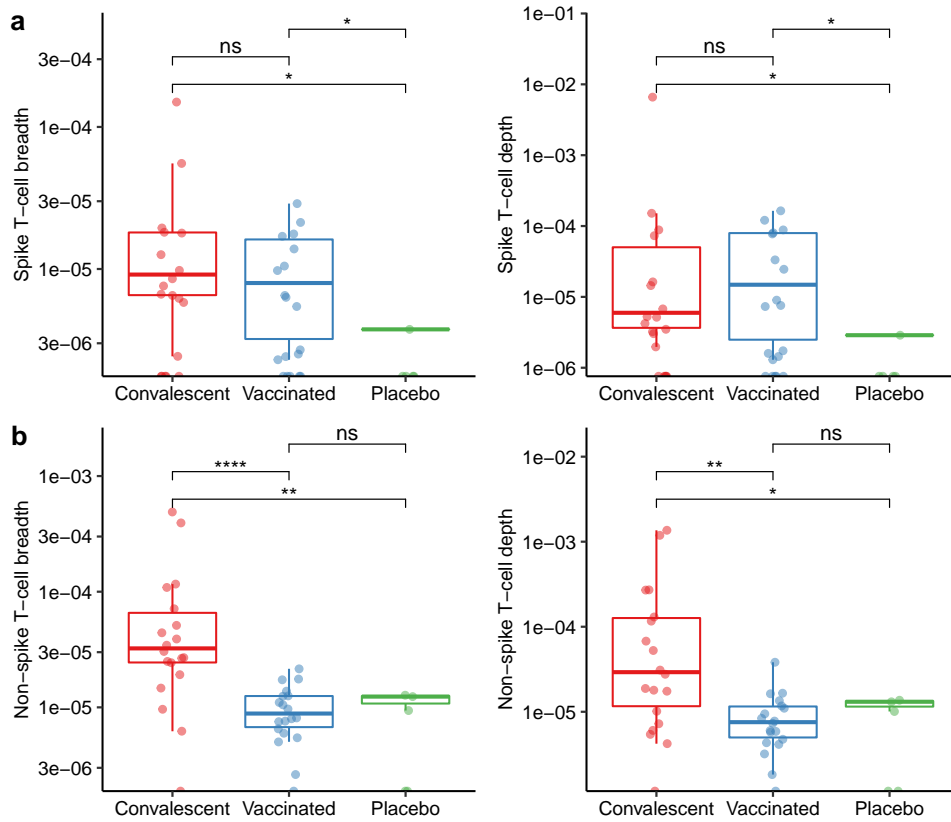
Figure 2: SARS-CoV-2-specific TCRβ repertoire analysis. (a) Spike-specific T-cell breadth and depth. **(b)** Non-spike-specific T-cell breadth and depth. Breadth is calculated as the fraction of unique TCR sequences specific to spike / non-spike proteins; depth is the relative frequency of those specific TCRs in the repertoire. Statistical significance was determined by two-sided Wilcoxon rank-sum tests. N = 43 independent samples (19 SARS-CoV-2 convalescent individuals, 19 Ad26.COV2.S vaccine recipients, 5 placebo recipients)

depth (frequency of those TCRs). Both convalescent and vaccinated subjects have a higher spike-specific response compared to placebos in termns of breadth and depth (Fig. 2a). By contrast, breadth and depth of non-spike TCRs were significantly higher in convalescent individuals versus vaccinated and placebos, and there were no significant differences between the latter two (Fig. 2b), as expected because Ad26.COV2.S vaccine only encodes the spike antigen.

An in-depth analysis of the genomic localization of SARS-CoV-2 epitopes recognized by TCRs (Fig. 3a) revealed that most of the T-cell immune response in convalescent individuals is directed towards structural proteins S and N. Although vaccinated subjects show some response to non-spike proteins, for most epitopes this signal is residual compared to the convalescent group and most likely due to unspecific annotations, since placebo recipients also show a minimal level of response. The localization of the 5 spike protein epitopes is shown in Fig. 3b. As opposed to B-cell epitopes, T-cell epitopes localization is not restricted to the protein surface since TCRs recognize antigens processed and presented in MHC molecules by human cells, and it shows in the spike protein 3D representation, where most of the epitopes are partially (S1, S3, S5) or completely (S2, S4) buried in the structue. S2 epitope is in fact the most widely recognized by convalescent (9/19) and vaccinated (12/19) individuals, and S4, the most hidden in the protein, is unrecognized by convalescent subjects and exclusively recognized by TCRs of 2/19 vaccine recipients.

Network analysis

In an attempt to capture the SARS-CoV-2-specific TCR repertoire architecture, a graph representing a network of similar TCRs was generated for one convalescent individual and one vaccinated individual (Fig. 4).

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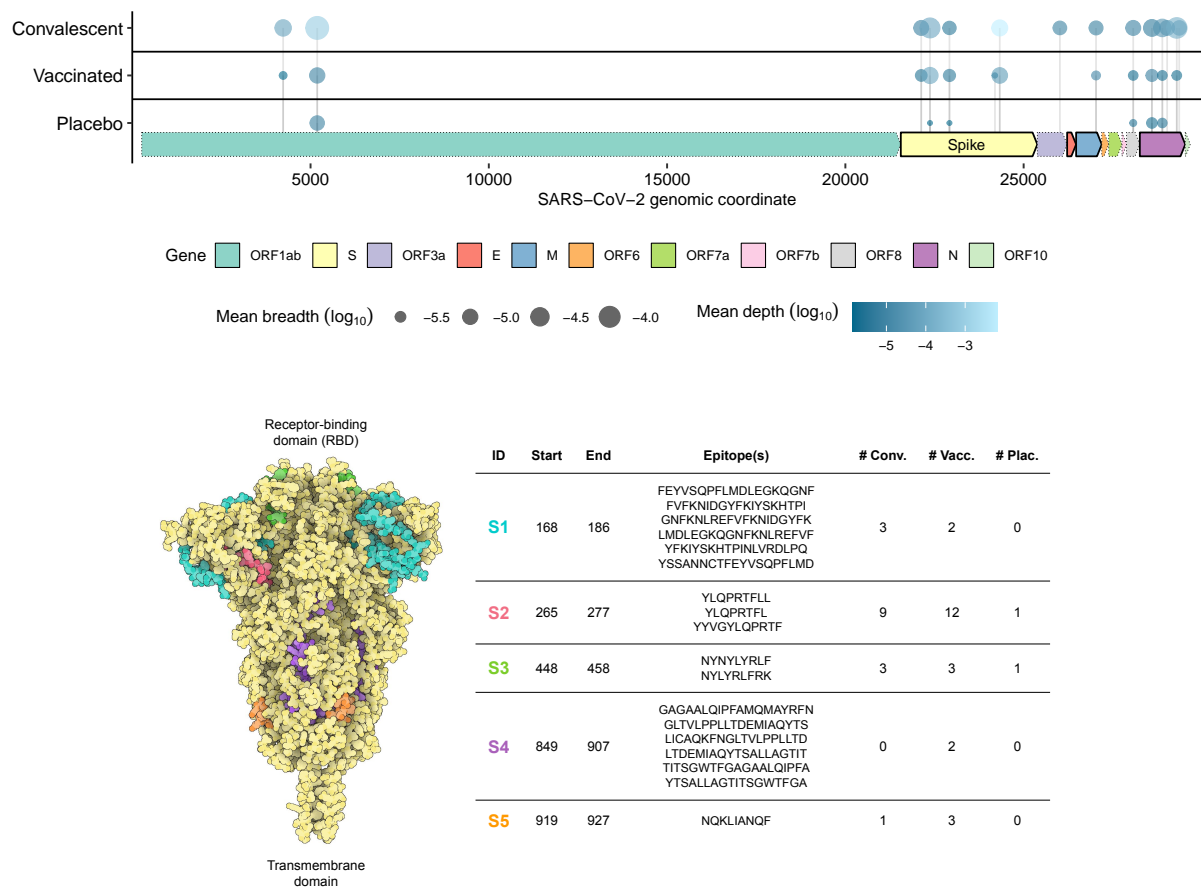
ID	Start	End	Epitope(s)	# Conv.	# Vacc.	# Plac.
S1	168	186	FEYVSQPFLMDLEGKQGNF FVFKNIDGYFKIYSKHTPI GNFKNLREFVFKNIDGYFK LMDLEGKQGNFKNLREFVF YFKIYSKHTPINLVRDLPQ YSSANNCTFEYVSQPFLMD	3	2	0
S2	265	277	YLQPRTFLL YLQPRTFL YYVGYLQPRTF	9	12	1
S3	448	458	NYNYLYRLF NYLYRLFRK	3	3	1
S4	849	907	GAGAALQIPFAMQMAYRFN GLTVLPPLLTDEMIAQYTS LICAQKFNGLTVLPPLLTD LTDEMIAQYTSALLAGTIT TITSGWTFGAGAALQIPFA YTSALLAGTITSGWTFGA	0	2	0
S5	919	927	NQKLIANQF	1	3	0

Figure 3: Epitopes recognized by T-cells accross SARS-CoV-2 genome. (a) Lollipop plot of the TCRβ SARS-CoV-2 specificity in convalescent, vaccinated and placebo individuals accross the coronavirus genome. Size of the dots indicate the mean breadth accross all samples in a group and color scale indicates the mean depth of the response. Genes outlined with solid lines are structural (S, E, M and N), whereas dotted ones encode non-structural proteins. **(b)** Localization and characteristics of the 5 SARS-CoV-2 spike epitopes recognized by TCRs in this study. Epitopes appear colored in the three monomers of a spike protein 3D representation (PDB: 6XR8, side view). Start, End: epitope protein coordinates (1-based); # Conv., # Vacc., # Plac.: number of individuals in a group with TCRs specific to that epitope.

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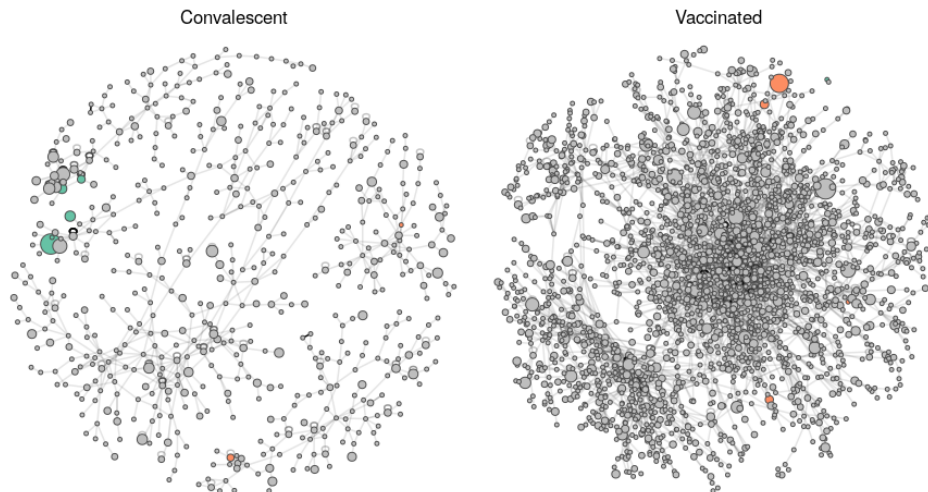
Figure 4: SARS-CoV-2-specific TCRβ similarity networks. (a) TCRβ similarity networks where each node represents a unique V gene + CDR3 aminoacid sequence combination. Only SARS-CoV-2-specific nodes (colored) and the nodes in their components (gray) are shown for convalescent subject 18 and vaccinated subject 12. Two nodes are connected by and edge if their tcrdist3 distance is $\leq$ 12. Loops (self-edges that start and end at the same node) represent additional unique nucleotide sequences encoding the same V gene + amino acid sequence (i.e., convergence).

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