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Intradermal skin test with mRNA vaccines as a surrogate marker of T cell immunity in immunocompromised patients

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SUMMARY

Objectives: Intradermal skin test (IDT) with mRNA vaccines may represent a simple, reliable, and affordable tool to measure T cell response in immunocompromised patients who failed to mount serological responses following vaccination with mRNA covid-19 vaccines.

Methods: We compared anti-SARS-CoV-2 antibodies and cellular responses in vaccinated immunocompromised patients (n = 58), healthy seronegative naive controls (NC, n = 8), and healthy seropositive vaccinated controls (VC, n = 32) by Luminex, spike-induced IFN-γ Elispot and an IDT. A skin biopsy 24 h after IDT and single-cell RNAseq was performed in three vaccinated volunteers.

Results: Twenty-five percent of seronegative NC had a positive Elispot (2/8) and IDT (1/4), compared to 95% (20/21) and 93% (28/30) in seropositive VC, respectively. Single-cell RNAseq data in the skin of VC showed a predominant mixed population of effector helper and cytotoxic T cells. The TCR repertoire revealed 18/1064 clonotypes with known specificities against SARS-CoV-2, among which six were spike-specific. Seronegative immunocompromised patients with positive Elispot and IDT were in 83% (5/6) treated with B cell-depleting reagents, while those with negative IDT were all transplant recipients.

Conclusions: Our results indicate that delayed local reaction to IDT reflects vaccine-induced T-cell immunity opening new perspectives to monitor seronegative patients and elderly populations with waning immunity.

Introduction

Neutralizing antibodies directed against the spike of SARS-CoV-2 are immune correlates of protection as their absence predicts disease severity.[1,2] T-cell response is another essential component controlling the viral reservoir. Thus, the proportion of multifunctional virus-specific CD4$^+$ and CD8$^+$ T cells is increased in covid-19 patients with mild disease, likely resulting from pre-existing immunity against common cold coronaviruses.[3–6] Additionally, lymphopenic individuals or individuals with dysfunctional T cells commonly develop more severe covid-19 disease, while recovered individuals have an efficient induction of T cells.[3,7] T cells with specificities for multiple SARS-CoV-2 variants have been identified.[8] Thus, adequately assessing SARS-Cov-2 CoV-2-specific T-cell responses may also contribute to stratifying the risk of developing severe covid-19.

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Most previous studies assessing SARS-CoV-2 specific immune response used Elispot or flow cytometry as a readout.[9,10] Yet, those technologies are time-consuming considering the need to extract peripheral blood mononuclear cells (PBMC), expensive and require specific expertize that is not always available. In our center, while investigating patients with a history of Polyethylene glycol allergy (at risk of anaphylaxis) or with a suspected allergic reaction after the primary vaccination,[11,12] we and others commonly observed delayed positive skin tests in immunized but not in naïve individuals.[13,14] The group of Luca Stingeni performed IDT with two COVID-19 vaccine dilutions in six healthcare volunteers who had received the two doses of Pfizer-BioNTech vaccine, in six healthcare volunteers who had received only the first dose of Pfizer-BioNTech and in six volunteers who did not receive Pfizer-BioNTech vaccine. IDT was positive in the 12 vaccinated volunteers and negative in the six unvaccinated volunteers.[13,15] Altogether, this led to the hypothesis that intradermal testing (IDT) with mRNA vaccines recalls the memory response similarly to tuberculosis skin tests.

A large proportion of immunocompromised patients, particularly those receiving depleting B cell therapies, are not able to generate antibody responses following vaccination. The present study aimed to evaluate T cell immunity using IDT in vaccinated (VC), unvaccinated healthy individuals (NC), and in vaccinated immunocompromised patients either transplant recipients or with autoimmune diseases. Those results were compared to Elispot and serological results and showed IDT to be a simple method to assess SARS-CoV-2 specific T cell immunity in seronegative patients.

Results

To validate IDT as a surrogate for T cell immunity, we first included 32 healthy individuals from the ImmunoVax (see in the Methods for the study details) study 3–6 months after receiving 2 doses of mRNA covid-19 vaccines (referred to hereafter as vaccine controls). The median age was 43 (range 25–64), 81% (26/32) were females. Anti-spike IgGs were detected in all individuals (Supplemental Fig. 1). As controls, we included eight females from the AllerVax (see in the Methods for the study details) study who had not received any vaccines nor been exposed to SARS-CoV-2 infection as indicated by a negative serology (Fig. 1A-B). The median age was 53.5. PBMC of naive controls produced significantly less IFNγ upon stimulation with two pools of overlapping peptides of the spike protein (Fig. 1B).

As for Elispot data, IDT was significantly larger in vaccine controls at 24 h (Fig. 1B). Skin tests with mRNA vaccines were performed with the same brand used for vaccination (4/32 with the Moderna vaccine, 28 with the Pfizer vaccine). The levels of anti-spike antibodies did not correlate with IFNγ secretion nor with the size of the IDT. The positive correlation between IFNγ secretion and the papule size was not statistically significant either (Supplemental Fig. 2).

Interestingly, two naïve volunteers with negative serologies and IDT spontaneously reported positive IDT at day 7 (Fig. 1C). Four weeks later, when receiving the second dose of the primary vaccination, they accepted a second mRNA vaccine IDT. At 24 h, IDT was 5 mm large in the first patient, while IDT was 25 mm large and came together with a covid arm in the second patient (Fig. 1E).

To further explore the nature of the infiltrating immune cells after an IDT, we enrolled three vaccinated volunteers 4–6 months after the primary vaccination series. Twenty-four hours after an IDT with the Moderna vaccine, we performed a skin biopsy/digestion and single-cell RNAseq of the skin-infiltrating $CD45^+$ cells (Fig. 2A). In total, 2609 cells were analyzed (1293 donor 1, 916 donor 2, 400 donor 3), of which the majority were T cells (n = 2009, 77%) and $CD16^+$ NK cells (n = 493, 19%) (Fig. 2B-C). A few numbers of B cells (n = 25, < 1%), monocytes (n = 49, 1.9%), and dendritic cells (n = 33, 1,2%) were identified (Fig. 2B-D). Among T cells, the memory profile was dominant in the infiltrating helper, cytotoxic resident (rm), and cytotoxic effector re-expressing CD45 (1746/2009, 87%). The ratio of cytotoxic to helper T cells was 0.69 (804/1159). Data were remarkably similar across the three donors (Fig. 2D).

To better address the specificity of the infiltrating T cells, we first looked at the number of clonotypes. Most of them were cytotoxic effectors re-expressing CD45 (Fig. 3A). We then matched the 1064 TCRB sequences with the MIRA database (regrouping 162′652 SARS-CoV-2 related clonotypes, TCRB sequence only) and with the VDJdb database (regrouping 2706 paired TCRs SARS-CoV-2 related clonotypes). We found 18 SARS-CoV-2 specific (one from the VDJb database) TCRs from 36 individuals, among which six were spike-specific (Table 1). In 66% (4/6), the spike-specific TCRs came from resident memory cytotoxic effector T cells. Interestingly, SARS-CoV-2 specific TCRs were not clonally expanded except for 2 clones (Fig. 3B, Table 1). As controls, we analyzed two donors' single-cell TCR sequencing data publicly available before COVID-19. Both datasets were analyzed using the same pipeline as the original vaccinated donors. No paired alpha/beta spike-specific TCRs were present in either of the two healthy donors. Overall, we found significantly more SARS-Cov2 and spike-specific TCR in the vaccinated donors ($p < 0.007$ Fisher exact test, Supplemental Table 1).

To further interpret the relevance of the SARS-CoV-2 specific TCRs, we next compared the HLA profiles between the three vaccinated controls and the 36 individuals identified with an identical TCRB within both databases. We hypothesize some degree of HLA-A/B-DR matching for a given TCR. In 35/36 of cases, we confirmed the presence of at least one HLA (two-digit resolution) matching. Impressively, a single TCR (TRBV19/TRBJ2–7) was detected in 32 individuals (Fig. 3C-D).

We next determined the value of IDT in a cohort of 58 immunocompromised individuals. Seventeen (29%) were transplant recipients (referred to as the TX group), 33 had autoimmune diseases, and eight suffered from primary immunodeficiencies (referred to as the A-ID group, n = 41) (Table 1). Except for one, all patients were under immunosuppressive treatment, which included corticosteroids in 23, calcineurin inhibitors in 16, B cell depleting drugs in 8, and anti-proliferative drugs (methotrexate/mycophenolate/azathioprine/mTOR inhibitor) in 29 (Table 1). The mean daily dose of corticosteroids was 9 mg.

We arbitrarily subdivided this cohort into vaccine-responder (defined as positive serology ≥1000UI/ml at visit 2 (week 5), 3 (week 8) or 4 (week 16), n = 23), low-responder (defined as positive serology but < 1000UI/ml at visit 2, 3 and 4, n = 18) and non-responder (negative serologies < 50UI/ml at visit 2, 3 and 4, n = 17) (Fig. 4A). Only 18% (3/17) of the TX group responded (> 1000UI) to vaccination compared to 51% (21/41) in the A-ID group. The Elispot response was positive in 79%, 69% and 43% of the responder, low-responder, and non-responder groups, respectively. The IFNγ response was significantly better in non-responders with positive IDT (papule ≥5 mm) (Fig. 4B). IDT showed 91% (20/22) responders in the sero-positive group, 76% (13/17) of positivity in the low-responders and 69% (11/16) positive in the sero-negative group (Fig. 4C). In three individuals, the IDT results were not recorded. Interestingly, all patients with negative IDTs were transplant recipients. When further stratifying the non-responder group into positive and negative Elispot, we found significantly larger IDT responses in the Elispot positive group at 72 h (Fig. 4D). Eighty percent of non-responders with positive Elispot were treated with rituximab, whereas Elispot-negative patients were mostly treated with CNI, MMF, or steroids (Fig. 4D). These results confirmed that the vaccine could induce a selective T-cell response in seronegative patients treated with anti-CD20 monoclonal antibodies.

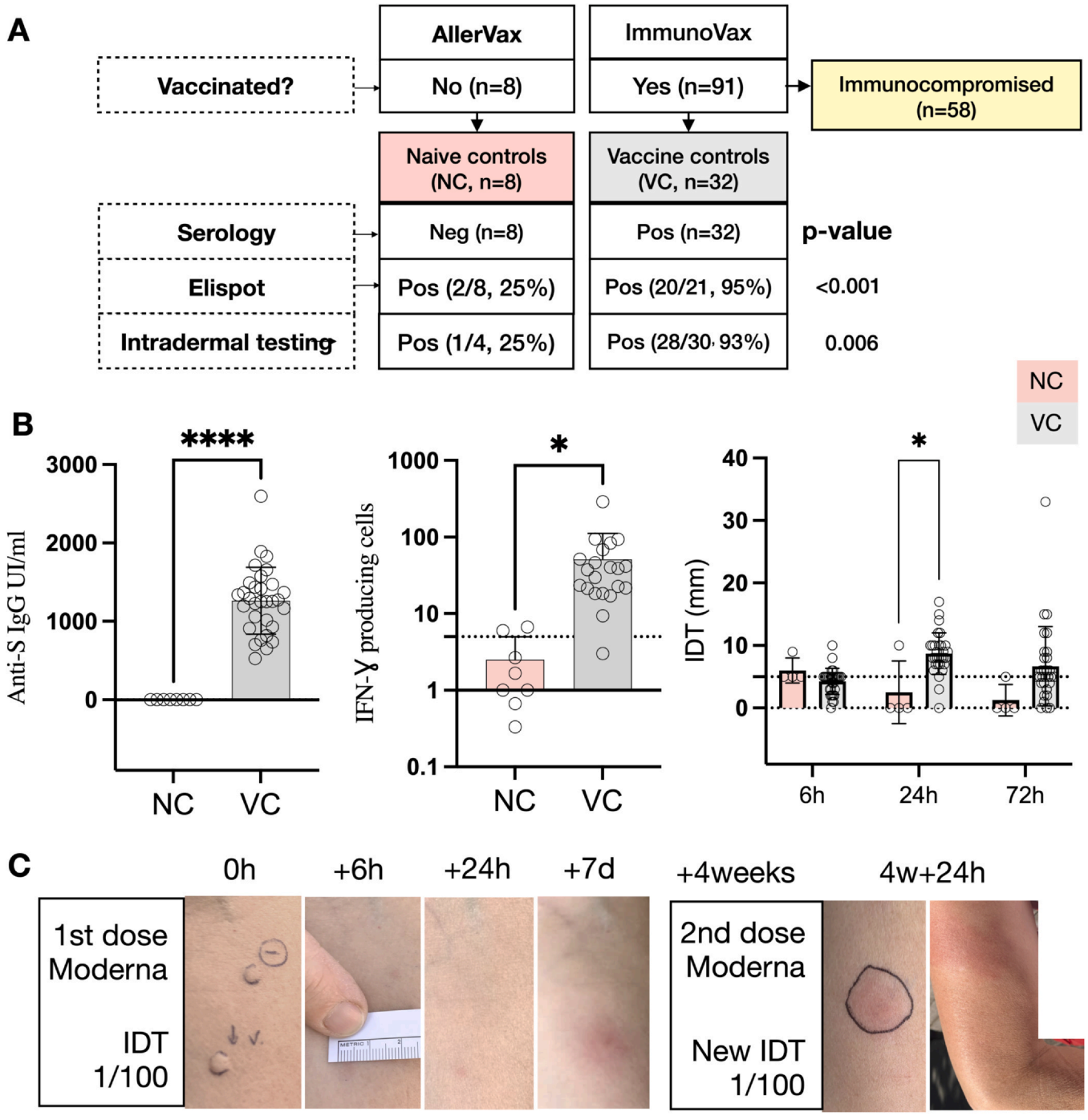

Fig. 1. T cell immunity in healthy seropositive and seronegative individuals from two prospective cohorts. A. Flow diagram of patients included in this study. Pearson's Chi-squared tests was used for statistical analysis. B. Anti-spike Ig, Elispot and IDT results. Unpaired T-test and Two-way ANOVA were used for statistical analysis. C. Representative skin test erythema over time in a seronegative healthy volunteer after the first and second immunization with mRNA vaccines. COVID-Arm 24 h after receiving the second mRNA vaccine dose. * $P < 0.05$, **** $P < 0.0001$. *Abbreviation* NC: naïve controls, VC: vaccinated controls, IDT: intradermal testing, h: hours, d: day, w-week.

Discussion

This is a comprehensive study on the value of IDT with mRNA vaccines comparing healthy seronegative, healthy immunized and vaccinated immunocompromised patients. Our single-cell RNAseq data demonstrated skin infiltration mainly with memory effector and cytotoxic T cells. While their TCR repertoire remains largely unknown, we were able to detect some publicly available SARS-CoV-2-specific TCRs. This finding, together with the clinical IDT results, validate this test as a surrogate marker for assessing T cell immunity following vaccination.

Performing IDT is clinically relevant for individuals with immune dysfunction, particularly for solid organ transplant recipients who are more susceptible to covid-19-related hospitalization and

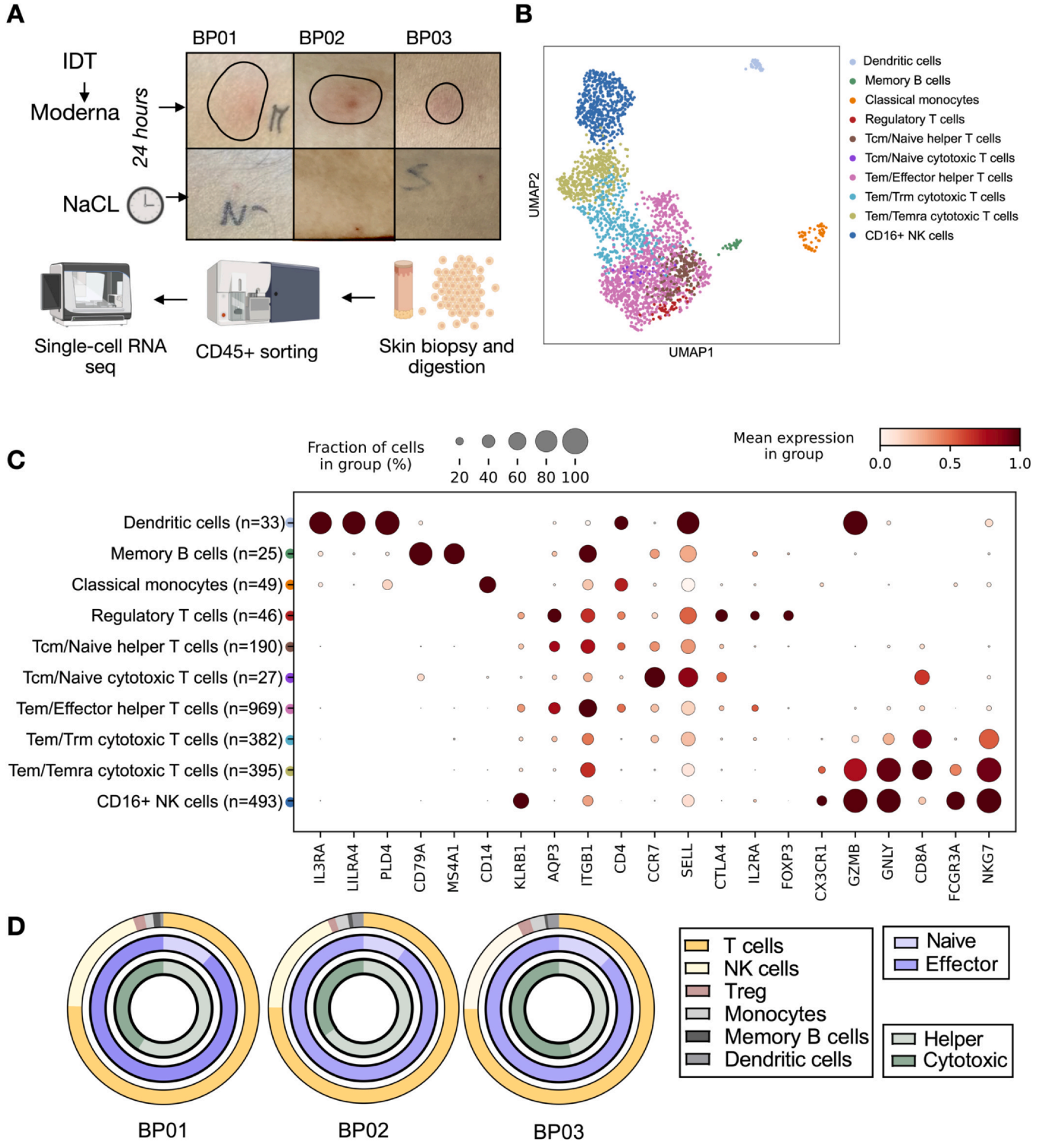

Fig. 2. T cell infiltrate after intradermal skin testing with mRNA vaccines. A. Experimental design. Cells from BP01, 02 and 03 were processed independently. B. Two-dimensional UMAP projection of single-cell gene expression data of CD45⁺ cells coming from three different donors. Annotation was performed using 'Immune_All_Low' CellTypist model (https://www.celltypist.org/). C. Dotplot showing the expression of typical marker genes used to identify the different cell types. D. Pie charts for each healthy vaccinated volunteer (BP01, BP02, BP03) showing cell repartition, naïve versus effector in the T compartment and the proportion of helper versus cytotoxic T cells.

mortality.[16,17] Thus, immunosuppressed patients have been excluded from pivotal efficacy trials as they frequently develop suboptimal humoral responses.[18] Since many immunosuppressive drugs, including B cell-depleting therapies, mycophenolate mofetil, and glucocorticoids impair humoral immunity,[19] additional markers are needed to stratify risk better and predict clinical outcomes. Thus, our results align with recent studies confirming that it is possible to develop robust T-cell immunity without antibody detection after vaccination.[10,20] Those results may be extended to the elderly population at higher risk for covid-19 breakthrough infection because of immunosenescence.[21,22]

The observation that IDT becomes positive seven days after the first vaccine dose and the association between covid arms and strong IDT positivity reinforce the hypothesis of a link between

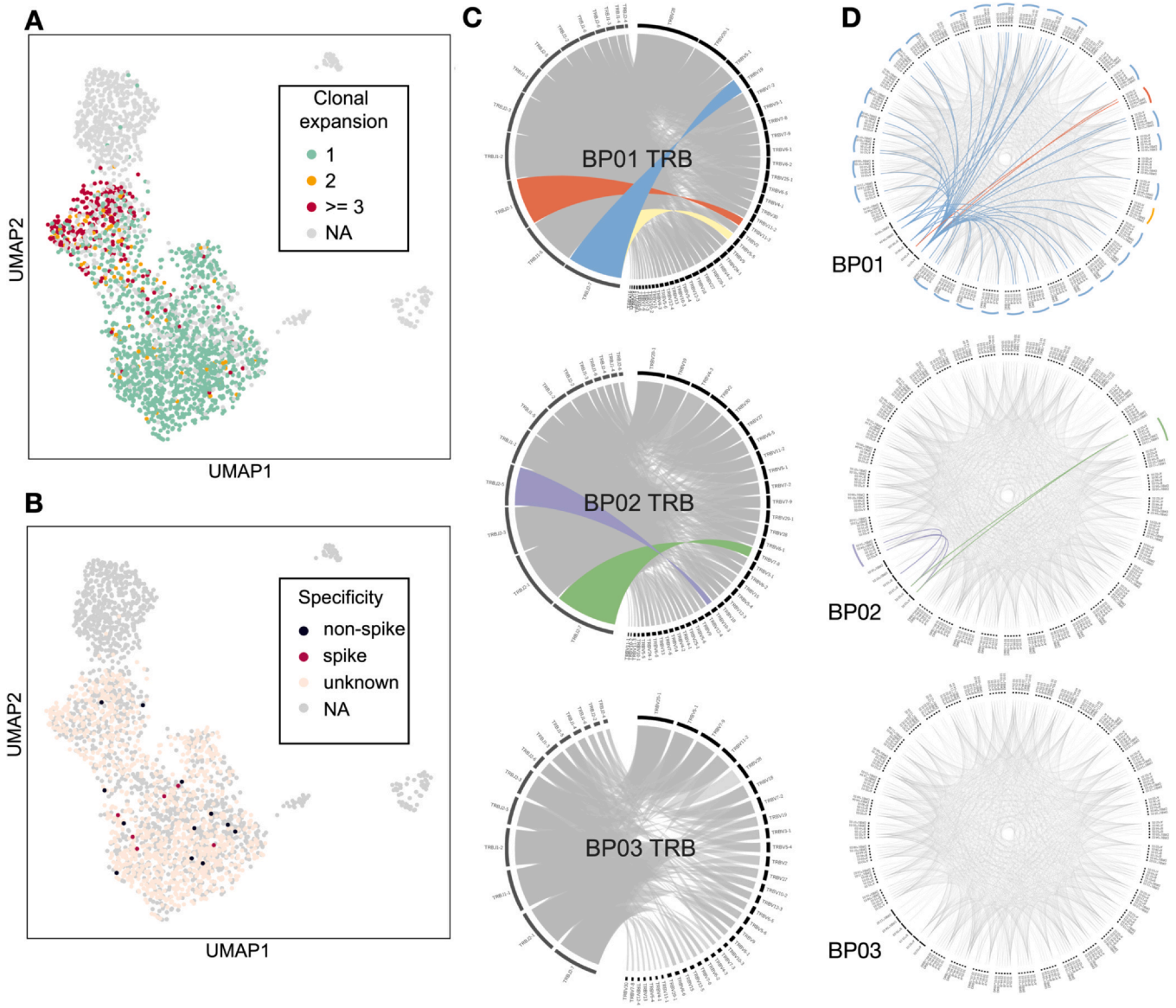

Fig. 3. SARS-CoV-2 specific T cells and T cells clonotypes in the skin. A. Two-dimensional UMAP projection of the clonal expansion of single-cell CD45$^+$ TCR$^+$ T cells coming from three different donors. B. Two-dimensional UMAP projection of single-cell CD45$^+$ TCR$^+$ SARS-CoV-2 (dark blue dots) and spike-specific (red dots) T cells coming from three different donors. C. VJ combinations for each donor (BP01/02/03) in the TRB locus. Highlighted in color are the VJ combinations that were also found in the matched clonotypes with SARS-CoV-2 spike specific TCRs. D. Shown in light gray are the matched HLA genotypes between each donor coming from literature with BP01, BP02 and BP03. Shown in color are the matched HLA where a SARS-CoV-2 spike specific TCR matched on top. Each TCR is linked to one color. The border-colored circle line represents an individual with a specific TCR. HLA-A, HLA-B, and HLA-DRB1 genotypes are presented.

immunization, positive IDT, and covid arm. The covid-arm is a common cutaneous manifestation after covid-19 mRNA immunization, particularly in females who develop stronger vaccine responses.[18,23,24] The mean onset after the first vaccine exposure is 6.9 days which is reduced to 1–2 days after the second dose.[23] Interestingly, the immunohistochemical findings in skin biopsies from covid arms consistently showed T-cell infiltrates.[25,26] While none of those studies has performed single-cell sequencing, these results are consistent with the histological finding in skin biopsies from IDT.[27] Our results, albeit limited by the number of patients, suggest that covid arm also represents a surrogate marker of T cell immunity similar to IDTs. Future studies should compare the immunogenicity and clinical outcomes of patients with/without a covid arm.

We intentionally used a low concentration (1/100) of mRNA vaccines for IDT to avoid immediate irritative reaction[13] and to prevent delayed local adverse reactions.[28] Interestingly, the 1/100 dilution was sufficient to recruit a substantial fraction of granulysin-positive cells composed of cytotoxic memory T cells re-expressing CD45RA (15%) and NK cells (19%). The critical role of granulysin-positive cytotoxic T and NK cells in the pathogenesis of disseminated keratinocyte death in Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis is well-known.[29,30] Therefore, it is tempting to speculate that those two populations are the main ones responsible for local inflammation and possibly the blistering lesions reported by others with undiluted mRNA vaccines IDT.[28] Nevertheless, the specificity of granulysin-positive cells and mechanisms driving their recruitment into the skin warrant further studies.

This study has several limitations. First, the TCR repertoire has not been specifically tested nor evaluated, and the number of spike-reactive TCR remains uncertain. Thus, our study has not studied the trafficking dynamic of antigen-specific versus bystander T cells. Early T cell recruitment can happen independently of specificity, although the disappearance of antigen-specific T cells from the circulation and accumulation in the challenge sites is a well-

Table 1
Characteristics of immunocompromised individuals.

n	58
Female, n (%)	34 (59)
Age, median (SD)	53.5 (15)
Immunosuppressive treatment	
Corticosteroids	23 (40)
mean prednisone dose (mg/d)	9,28
max prednisone dose (mg/d)	50
IMDHIs	17 (29)
CNIs	16 (28)
MTX	7 (12)
AZA	3 (5)
mTOR inhibitor	2 (3)
RTX	8 (14)
anti-TNF	7 (12)
anti-IL6	6 (10)
Belimumab	1 (2)
ABA	1 (2)
JAKI	1 (2)
HCQ	7 (12)
Transplant recipients, n (%)	17 (29)
Kidney, n (%)	11 (65)
Liver, n (%)	3 (17)
Lung, n (%)	1 (6)
Multiorgan, n (%)	2 (12)
I-AD, n (%)	41 (71)
PID, n (%)	8 (20)
Connectivitis, n (%)	9 (22)
Sarcoidosis, n (%)	5 (12)
Vasculitis, n (%)	3 (7)
Behçet's disease, n (%)	3 (7)
Refractory uveitis, n (%)	3 (7)
Inflammatory cardiomyopathy, n (%)	2 (5)
MS, n (%)	1 (2)
Other, n (%)	7 (17)

IMDHIs: inosine monophosphate dehydrogenase inhibitors; CNI: calcineurin inhibitors; MTX: methotrexate; AZA: azathioprine; RTX: rituximab; ABA: abatacept; JAKI: JAK-inhibitors; HCQ: hydroxychloroquine; PID: primary immunodeficiency; MS: multiple sclerosis.

reported phenomenon.[31] Even if minimally present in the skin, the BCR repertoire of infiltrating B cells also needs further characterization. Future studies are also warranted to address the contribution of other chemicals in mRNA vaccines, such as polyethylene glycols, dimyristoyl glycerol, tromethamine, and the mRNA itself. Those could be haptens, be involved in non-covalent pharmacological interactions, or trigger inflammasome activation pathways.[32–34] Another weakness of our study is related to the fact that patients were instructed to measure and photograph the skin reaction. Ideally, a trained professional should evaluate the skin reaction for increasing reliability, although in our study, returning to the hospital could have dissuaded patients from participating. Thus, patients from the Allervax substudy returned their skin test results as per protocol only in 50% (4/8) of the cases compared to 88% (51/58) and 94% (30/32) in the immunocompromised and vaccine control groups, respectively. At the start of this substudy, the recruitment of patients from Allervax was already complicated, given the widespread vaccination and a high contamination rate. Additionally, those individuals were generally less interested in the study than immunocompromised patients, and the vaccine control group regrouping motivated collaborators from our hospital. Finally, IDT may be limited in clinical practice because of vaccine accessibility. Comparing IDT performed with cryopreserved and freshly prepared vaccines could help scale up this test.

Despite the limitations mentioned above, our study is clinically relevant as it demonstrates that IDT can be used as a surrogate marker of vaccine-induced T-cell immunity with mRNA vaccines. These findings are extremely valuable and indicate that IDT represents a reliable and affordable strategy to measure vaccine-induced T cell immunity in immunocompromised seronegative individuals and in the elderly population with waning immunity that are both at increased risk of severe covid-19 disease.

Material and methods

Study design and population

The current study is a substudy of ImmunoVax, a single-center, prospective, longitudinal comparative study investigating the effectiveness of mRNA covid-19 vaccines in immunocompromised patients as compared to healthy controls.[18] Between July and October 2021, we recruited 91 subjects from the ImmunoVax study population. Participants were included during their 4th or 5th visit according to the ImmunoVax protocol, taking place three respectively six months after complete vaccination with two doses of BNT162b2 (Comirnaty, Pfizer) or mRNA-1273 (Spikevax, Moderna). Eleven healthy naive controls were identified within the AllerVax study population, a single-center, real-life cohort study including a pre-vaccination cohort of 187 individuals.[12] Three of the eleven healthy naive controls were excluded because of a positive serology at the time of inclusion. Patients were included by convenience sampling between August and October 2021. There were no other specific inclusion/exclusion criteria. All participants gave written informed consent. The study was approved by the local ethics institutional review board (BASEC number 2021-00041 and 2021-00735).

Serological assays

Blood samples were collected at baseline before vaccination (visit 1) and one week (visit 2), one month (visit 3), three months (visit 4) and six months (visit 5) after the second vaccine dose, as described for the ImmunoVax study.[18] Anti-spike IgG antibodies were measured by Luminex (Luminex Corp)-based assays as previously described.[2]

IFNγ-Elispot

Peripheral blood mononuclear cells (PBMCs) were collected at the time of skin tests, 3 or 6 months after vaccination, isolated using density gradient centrifugation, and frozen according to standard procedures, and stored in liquid nitrogen. For IFNγ-Elispot PBMCs were thawed and rested for at least 4 h. For each sample triplicates of 200′000 cells were then stimulated overnight in 96-w Elispot plates (MabTech, Stockholm, Sweden) with 2 peptide pools (15-mers pool of 11 amino-acids overlapping peptides covering the entire SARS-CoV-2-spike protein, at a concentration of 1 µg/ml, a kind gift by Yves Levy, Inserm, France), staphylococcus enterotoxin B (SEB, 0.2 µg/ml) as positive control or left unstimulated as a negative control. The following day plates were washed and stained according to the manufacturer's instructions. Spots were counted on an EliSpot reader (AID GmbH). For each sample, the mean of triplicates was calculated and the number of spots obtained from the two peptide pools summed up. The Elispot was arbitrarily considered positive if the number of IFNγ-producing cells was > 5/400′000 cells and > 3x the unstimulated background. Material availability for the participants from the ImmunoVax study limited the number of ELISPOT that could be performed.

Intradermal skin test (IDT)

IDT were performed during the 4th or 5th visit for the participants from the ImmunoVax study population corresponding to three, respectively six months after complete vaccination, and during the first visit, prior to vaccination for participants from the AllerVax population. Skin tests were repeated four weeks after the

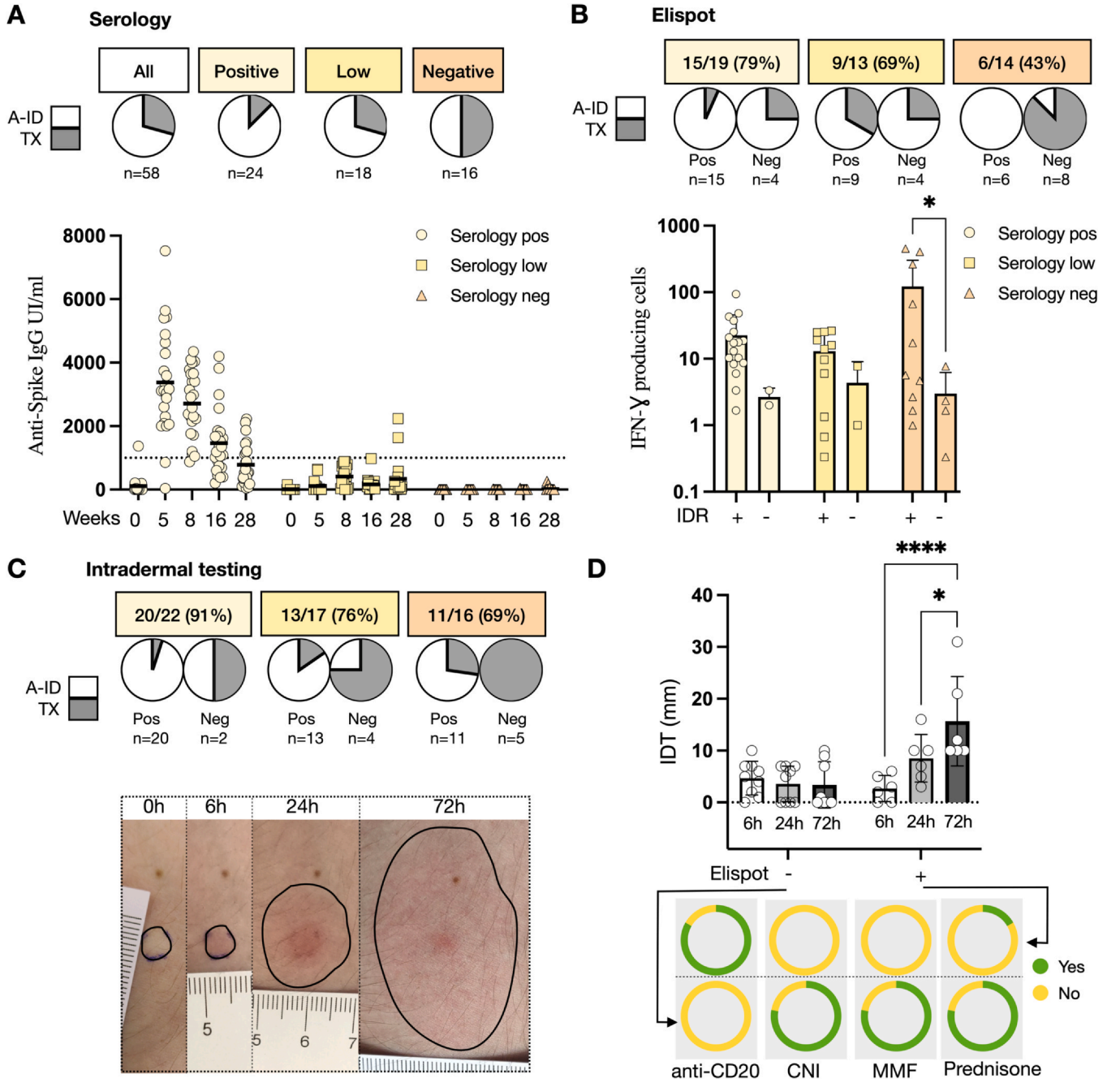

Fig. 4. T cell immunity in immunocompromised individuals. A. Anti-spike IgG level over time. Blood draw was performed 5, 8, 16 and 28 weeks after primary vaccination. Mean is shown. Pie charts represent the proportion of A-ID and TX among the whole immunocompromised group and then those with positive, low and negative serologies. B. PBMC were stimulated with 11 amino-acids overlapping peptides covering the full spike protein. Number and percentage of positive Elispot among individuals with positive, low and negative serologies. Pie charts represent the proportion of A-ID and TX among individuals with positive, low and negative serologies. Cumulative data showing the mean and standard deviations. C. Intradermal testing results in seronegative, low and high patients. Number and percentage of positive IDT among individuals with positive, low and negative serologies. Pie charts represent the proportion of A-ID and TX among individuals with positive, low and negative serologies. Representative image of a seronegative patient with strong IDT positivity. D. Size of IDT (in mm) in patients with positive and negative Elispot results. Pie charts representing the type of immunosuppression in A-ID and TX. Green represents the proportion of individuals who received the drug (anti-CD20 monoclonal antibodies, CNI, MMF and prednisone). Two-way ANOVA test was performed. Mean and standard deviation are shown. * $P < 0.05$, *** $P < 0.001$. Abbreviation A-ID Autoimmune-Immunodeficient group, TX transplant group, IDT intradermal testing. CNI Calcineurin inhibitor, MMF Mycophenolate Mofetil.

initial immunization on two patients. IDT was performed on the anterior face of the forearm, using 1 ml syringe and 25 or 27 G intradermal needles. About 20 µl of mRNA vaccine diluted 1:100 in 0.9% saline solution was injected intradermally to form a 3–6 mm papule. The 1:100 dilution is recommended to avoid false positive irritative skin tests.[35,36] Spikevax (Moderna) and Comirnaty (Pfizer) vaccines were used. IDT was done with the same vaccine used for vaccination for each patient. All patients received a self-reporting sheet and a ruler and were instructed to measure and photograph the skin reaction at 6, 24, and 72 h. IDT was considered positive if a skin infiltration/erythema of ≥ 5 mm was present at 24 h. All skin test solutions were prepared by the pharmacology center of the University Hospital of Lausanne according to the European Network of Drug Allergy (ENDA) guidelines. Patients who failed to return those documents despite two written reminders were excluded from the analysis.

Skin biopsies and cell sorting

4 mm punch biopsies were harvested from the IDT site 24 h after skin test from 3 vaccinated controls. Samples were immediately provided to the lab following biopsy procedure. Fat lobules were removed using surgical clamp. Samples were mechanically cut and chopped using surgical scissors followed by an incubation in RPMI (30 U/ml DNASE1 (Sigma) 1 mg/ml Collagenase D (Sigma) 1 mg/ml) for 30 min at 37 degrees 5% CO2. After digestion, enzymes were blocked using EDTA at a concentration of 10 mM. Samples were thereafter filtered through a 40 µm cell strainer, washed in PBS, centrifuged (1500 g, 1900 rpm, 5 min). Single-cell suspensions were stained with APC conjugated anti-CD45 antibodies. Fluorescence-activated cell sorting (FACS) of CD45-labeled cells was performed on a BD aria sorter for 10x RNA sequencing.

10X single-cell RNA and TCR sequencing analysis

Fastq files were aligned to the human transcriptome (GRCh38–2020-A) using CellRanger (count and vdj) v6.0.0. Each sample was processed with scanpy[37] standard workflow. Filtering was performed with the parameters min_genes = 200, min_cells = 3, min_counts = 500, n_genes_by_counts > 300, percent_mito = 10, max_counts = 10,000. All filtered cells were further merged across all samples. In brief, RNA counts were normalized per 10,000, the top most highly variable genes were selected, PCA was performed and Batch balanced kNN was used for nearest-neighbor calculations and Leiden clustering, as well as for UMAP-based visualization. Annotation was performed using 'Immune_All_Low.pkl' CellTypist model.[38] TCR analysis was performed in Python with the toolkit scirpy.[39] Only 'single pair' clonotypes were selected, and clonotypes clustering was determined on the basis of CDR3 sequence identity, with the parameters receptor_arms ="all", dual_ir ="primary_only". SARS-CoV-2 specific clonotypes were searched against MIRA dataset[40] and VDJdb database.[41] Clonotypes sharing the same CDR3 from the beta chain and the same V beta gene were considered as SARS-CoV-2 specific. Two datasets from 10X Genomics database of donors (pre-COVID) were added as control, including[1] PBMCs from one healthy donor (NextGEM v1.1), obtained using Single Cell Immune Profiling Dataset analysis by Cell Ranger 3.1.0, 10x Genomics, (2019) and[2] PBMCs from another healthy donor (v1), obtained using Single Cell Immune Profiling Dataset by Cell Ranger 3.1.0, 10x Genomics, (2018). Both datasets were analyzed using the same pipeline as for our original vaccinated donors. Briefly, we applied transcriptome filtering followed by single-paired TCR filtering using Scanpy and ScirPy packages. Next, single paired TCRs (4433 for dataset 1 and 2743 for dataset 2) were processed to match with either the same TRB V-gene and TRB-CDR3 from the MIRA database, or the same TRB V-gene, TRB-CDR3, TRA V-gene and TRA-CDR3 with VDJdb database. To illustrate the V and J genes pairs of the SARS-CoV-2 spike-specific clonotypes among the non-specific clonotypes, circos plots were generated for each donor.[42] Jupyter notebooks and Java scripts are available for data preprocessing, clustering, visualization, and cell annotation, as well as for TCR analysis, at https://github.com/MathildeFogPerez/manuscript-tcell-fallet.

HLA genotyping

The HLA genotyping of each donor was determined with arcasHLA[43] using the bam files of the transcriptomics data from the three 10X genomics runs performed for each donor. Two out three HLA genotyping were confirmed by deep-sequencing of the complete HLA genes (BP01 not done). A circos plot, showing the HLA matching between the three donors and the donors with whom they share SARS-CoV-2 spike specific TCR clonotypes, was generated (HLA alleles A, B and DRB1 are shown).

CRediT authorship contribution statement

YDM, BF and MF designed the study. YDM, BF, MF, wrote of the manuscript. BF, MF, RP, AA, CS, RJ, TC, RG performed the experiments and analyzed the results. MG, LP, GF, AH, SB and RG, and GP supervised laboratory testing. All authors have revised and approved the final version.

Data availability

Raw and processed data are available on the gene expression omnibus (GEO) in GSE224028 https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE224028.

Declaration of Competing Interest

Dr Pantaleo and Dr Fenwick report having a patent pending (application No. EP20205298.1) for a SARS-Cov2 neutralization assay. Dr Gottardo has received consulting income from Takeda, Ozette Technologies and declares ownership in Ozette Technologies. The research was conducted without any other commercial or financial relationships that could be construed as a potential conflict of interest to this study.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2023.06.005.

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