Ancestral SARS-CoV-2-specific T cells cross-recognize Omicron (B.1.1.529)

Yu Gao
Karolinska Institutet
Curtis Cai
Karolinska Institutet
Alba Grifoni
La Jolla Institute for Immunology
Thomas Müller
Karolinska Institutet
Julia Niessl
Karolinska Institutet
Anna Olofsson
Karolinska Institutet
Marion Humbert
Karolinska Institutet
Lotta Hansson
Karolinska Institutet
Anders Österborg
Karolinska Institutet
Peter Bergman
Karolinska Institutet
Puran Chen
Karolinska Institutet
Annika Olsson
Karolinska Institutet
Johan K. Sandberg
Karolinska Institutet
Daniela Weiskopf
La Jolla Institute for Immunology
David A. Price
Cardiff University
Hans-Gustaf Ljunggren
Karolinska Institutet
Annika C. Karlsson

Karolinska Institutet

Alessandro Sette

La Jolla Institute for Immunology

Soo Aleman

Karolinska Institutet

Marcus Buggert (marcus.buggert@ki.se)

Karolinska Institutet https://orcid.org/0000-0003-0633-1719

Short Report

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Abstract

The emergence of the SARS-CoV-2 variant-of-concern Omicron (B.1.1.529) has destabilized global efforts to control the impact of COVID-19. Recent data have suggested that B.1.1.529 can readily infect people with naturally acquired or vaccine-induced immunity, facilitated in some cases by viral escape from antibodies that neutralize ancestral SARS-CoV-2. However, severe disease appears to be relatively uncommon in such individuals, highlighting a potential role for other components of the adaptive immune system. We report here that SARS-CoV-2 spike-specific $CD4^+$ and $CD8^+$ T cells induced by prior infection and, more extensively, by mRNA vaccination provide comprehensive heterologous immune reactivity against B.1.1.529. Pairwise comparisons across groups further revealed that SARS-CoV-2 spike-reactive $CD4^+$ and $CD8^+$ T cells exhibited similar functional attributes, memory distributions, and phenotypic traits in response to the ancestral strain or B.1.1.529. Our data indicate that established SARS-CoV-2 spike-specific $CD4^+$ and $CD8^+$ T cell responses, especially after mRNA vaccination, remain largely intact against B.1.1.529.

Main

Natural infection with SARS-CoV-2 and vaccination with mRNA constructs encoding the viral spike protein typically generate effective immunity against COVID-19. However, the current pandemic has been fueled by the continual emergence of variants-of-concern (VOCs), such as Omicron (B.1.1.529). Emerging data indicate that B.1.1.529 is more transmissible than previous VOCs[1]. This phenotype can be explained by key mutations in the receptor-binding domain (RBD), which confer enhanced affinity for the ACE2 receptor[2]. Another major concern is that B.1.1.529 harbors a large number of additional mutations in the spike protein that could feasibly subvert immune recognition[3]. In line with this possibility, emerging reports have shown that neutralizing antibodies elicited against the ancestral Wuhan reference strain, either in the context of infection or vaccination, are less able to combat B.1.1.529[2,4]. These observations likely align with the propensity of B.1.1.529 to cause breakthrough infections, also in persons who have been vaccinated with two doses of mRNA vaccine or experienced previous SARS-CoV-2 infection[5,6].

Preliminary data suggest that breakthrough infections with B.1.1.529 might be associated with a lower risk of hospitalization and less often cause severe disease compared with the Delta VOC (B.1.617.2)[7,8]. One possible inference from the less aggressive clinical spectrum is that additional immune components beyond the antibody response attenuate the course of infection with B.1.1.529. Previous studies have demonstrated that robust $CD4^+$ and $CD8^+$ T cell responses are induced following SARS-CoV-2 infection or vaccination[9–15]. Several lines of evidence further suggest that $CD4^+$ and $CD8^+$ T cell responses can modulate disease severity in humans and suppress viral replication in animal models[16–19]. However, it has remained unclear to what extent ancestral SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T cells cross-recognize B.1.1.529, especially given the unprecedented number of mutations in the spike protein, which likely shift the antigenic landscape more profoundly in relation to antecedent VOCs[20].

To address this question, we collected peripheral blood mononuclear cells from mRNA-vaccinated individuals 6 months after the second dose of Pfizer/BioNTech BNT162b2 (*n* = 40), individuals in the convalescent phase 9 months after mild or severe COVID-19 (*n* = 48), and seronegative individuals (*n* = 48). Cells were stimulated in parallel with overlapping peptide pools spanning the entire spike protein sequences of the Wuhan reference strain (wildtype) or B.1.1.529. Activation-induced marker assays were used to quantify spike-specific $CD4^+$ T cell responses via the upregulation of CD69 and CD40L (CD154) and spike-specific $CD8^+$ T cell responses via the upregulation of CD69 and 4-1BB (CD137) (Extended Data Fig. 1a).

The overall magnitude of the SARS-CoV-2 spike-specific $CD4^+$ T cell response against B.1.1.529 showed a median reduction of 9% in mRNA-vaccinated individuals and a median reduction of 16% in convalescent individuals relative to the wildtype response (Fig. 1a, b). The corresponding response frequencies were also slightly lower for B.1.1.529 (Fig. 1c). Individual comparisons further revealed maximum reductions in magnitude of 58% in mRNA-vaccinated individuals, 56% in convalescent individuals, and 75% in seronegative individuals, comparing SARS-CoV-2 spike-specific $CD4^+$ T cell responses against B.1.1.529 *versus* wildtype (Fig. 1d). These findings were validated using independently synthesized peptide pools spanning the entire spike protein (Extended Data Fig. 1b).

To extend these findings, we investigated the phenotypic characteristics of SARS-CoV-2 spike-specific $CD4^+$ T cells that cross-recognized B.1.1.529, with a particular focus on markers of T helper polarization (CCR4, CCR6, CXCR3, CXCR5) and memory differentiation (CCR7, CD45RA). No significant differences in T helper polarization were detected across intragroup comparisons of SARS-CoV-2 spike-specific $CD4^+$ T cell responses against B.1.1.529 *versus* wildtype (Fig. 1e). Central memory T (T_{CM}) cells predominated among SARS-CoV-2 spike-specific $CD4^+$ T cells in mRNA-vaccinated, convalescent, and seronegative individuals, but again, the subset composition of SARS-CoV-2 spike-specific $CD4^+$ T cell responses against B.1.1.529 *versus* wildtype were similar across all groups (Fig. 1f). We also assessed the functionality of SARS-CoV-2 spike-specific $CD4^+$ T cells in mRNA-vaccinated individuals, measuring the intracellular expression of IFN-γ, TNF, and IL-2 alongside CD69 and CD154. No significant differences in the ability of SARS-CoV-2 spike-specific $CD4^+$ T cells to deploy multiple functions were apparent in response to stimulation with peptides representing B.1.1.529 *versus* wildtype (Fig. 1g).

The overall magnitude of the SARS-CoV-2 spike-specific $CD8^+$ T cell response against B.1.1.529 showed a median reduction of 8% in mRNA-vaccinated individuals and a median reduction of 30% in convalescent individuals relative to the wildtype response (Fig. 2a, b). These differences were mirrored in the corresponding response frequencies for B.1.1.529 *versus* wildtype (Fig. 2c). Individual comparisons further revealed maximum reductions in magnitude of 55% in mRNA-vaccinated individuals, 63% in convalescent individuals, and 60% in seronegative individuals, comparing SARS-CoV-2 spike-specific $CD8^+$ T cell responses against B.1.1.529 *versus* wildtype (Fig. 2d). These findings were again validated using independently synthesized peptide pools spanning the entire spike protein (Extended Data Fig. 1c).

In further experiments, we investigated the phenotypic characteristics of SARS-CoV-2 spike-specific $CD8^+$ T cells that cross-recognized B.1.1.529, focusing on classic markers of memory differentiation (CCR7, CD45RA). Late effector memory T (T_{EMRA}) cells predominated among SARS-CoV-2 spike-specific $CD8^+$ T cells in mRNA-vaccinated, convalescent, and seronegative individuals, but no significant differences in subset composition were detected across intragroup comparisons of SARS-CoV-2 spike-specific $CD8^+$ T cell responses against B.1.1.529 *versus* wildtype (Fig. 2e). We also assessed the functionality of SARS-CoV-2 spike-specific $CD8^+$ T cells in mRNA-vaccinated individuals, measuring the intracellular expression of granzyme B, IFN-γ, TNF, and IL-2 alongside CD69 and CD137. Akin to the corresponding analyses of SARS-CoV-2 spike-specific $CD4^+$ T cells, no significant differences in the ability of SARS-CoV-2 spike-specific $CD8^+$ T cells to deploy multiple functions were apparent in response to stimulation with peptides representing B.1.1.529 *versus* wildtype (Fig. 2f). In a final set of analyses, we merged all SARS-CoV-2 spike-specific $CD4^+$ and $CD8^+$ T cell data points together and found a significantly lower total T cell response against B.1.1.529 *versus* wildtype in convalescent, but not vaccinated subjects (Extended Data Fig. 1d). These analyses suggest that SARS-CoV-2 spike-specific T cell responses remain less intact against B.1.1.529 in convalescent individuals.

The current global pandemic has been destabilized by the recent emergence of B.1.1.529, which continues to spread rapidly and supersede other VOCs. Our collective data indicate that SARS-CoV-2 spike-specific $CD4^+$ and $CD8^+$ T cells elicited by mRNA vaccination or prior infection remain largely intact against B.1.1.529. Alongside intrinsic viral factors, such as altered tropism and decreased replication in the lower respiratory tract[21], such heterologous immune reactivity may explain why severe disease appears to be relatively uncommon after infection with this particular VOC. Moreover, the degree of cross-reactivity varied to some extent among individuals, most likely as a consequence of genetically encoded differences in antigen presentation, which could further modulate clinical outcomes associated with B.1.1.529. It should be noted that our evaluations were confined to peripheral blood samples, which do not necessarily reflect the entirety of the cellular immune response against SARS-CoV-2[22]. In addition, SARS-CoV-2 spike-specific $CD4^+$ and $CD8^+$ T cells cross-recognized B.1.1.529 less comprehensively in convalescent *versus* mRNA-vaccinated individuals, suggesting that booster immunization may provide benefits that extend beyond the induction of broadly neutralizing antibodies to enhance natural protection against recurrent episodes of COVID-19[2].

Declarations

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AUTHOR CONTRIBUTIONS

Conceptualization: Y.G., C.C., J.K.S., D.A.P., H.G.L., A.C.K., A.S., S.A., M.B.

Sample collection: Y.G., C.C., T.R.M., J.N., L.H., A.Ö., P.B., P.C., A.OLS., H.G.L., S.A., M.B.

Investigation: Y.G., C.C., A.G., A.OLO., M.H., M.B.

Formal analysis: Y.G., C.C.

Visualization: Y.G., C.C., M.B.

Resources: A.G., J.K.S., D.W., H.G.L, A.C.K., A.S., S.A., M.B.

Funding acquisition: D.A.P., H.G.L., A.S., S.A., M.B.

Supervision: J.K.S., H.G.L., S.A., M.B.

Writing—original draft: Y.G., C.C., D.A.P., M.B.

Writing—review and editing: Y.G., C.C., A.G., T.R.M., J.N., A.Ö., P.B., J.K.S., D.A.P., H.G.L., A.C.K., S.A., M.B.

DECLARATION OF INTERESTS

A.S. is a consultant for Gritstone Bio, Flow Pharma, Arcturus Therapeutics, ImmunoScape, CellCarta, Avalia, Moderna, Fortress and Repertoire., and the La Jolla Institute has filed patents to protect various aspects of the T cell epitope and vaccine design work. SA has received honoraria for lectures from Gilead, AbbVie, MSD, Biogen, and has received research grants from Gilead and AbbVie. M.B. is a consultant for Oxford Immunotec. The other authors declare no conflicts of interest.

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Online Methods

Samples

Healthy individuals (n=40) were sampled 6 months after the second dose of the BNT162b2 vaccine (Comirnaty®, Pfizer/BioNTech) as part of a clinical trial registered at EudraCT (2021-000175-37) and https://clinicaltrials.gov (2021-000175-37) at Karolinska University Hospital, Sweden[23]. The two doses of the vaccine were given 21 days apart in standard doses. The vaccinated cohort of healthy individuals consisted of 23 women (58%)/17 men (43%), and the median age (range) was 53 (22-79) years. The study was approved by the Swedish Medical Product Agency. Additional samples from 15 of the vaccinated cohort participants were collected 3 months after the second dose for validation purposes (Extended Data Figure 1). Convalescent individuals after previous COVID-19 were sampled 9 months after RT-PCR verified SARS-CoV-2 infection leading to mild (i.e. non-hospitalized, n=26) or severe (i.e. hospitalized, n=22) disease during the first wave of the pandemic in March-April of 2020, before the emergence of the Alpha, Beta and Delta variants. The cohort of convalescent individuals after mild COVID-19 consisted of 8 women (31%)/18 men (69%), and the median age (range) was 54 (44-68) years. The cohort of convalescent individuals after severe COVID-19 consisted of 3 women (14%)/19 men (86%), and the median age (range) was 58 (33-66) years. Fourteen of the patients with severe COVID-19 (64%) had received intensive care at the time of COVID-19. None of the convalescent individuals had received any COVID-19 vaccination at the time of sample collections. Seronegative samples were

acquired from healthy blood donors in late 2020. The absence of spike-specific antibodies was confirmed using an Anti-SARS-CoV-2 S Immunoassay (Roche) for vaccinated healthy individuals and seronegative blood donors. All participants provided written informed consent in accordance with the principles of the Declaration of Helsinki. The study was approved by the Regional Ethics Committee in Stockholm, Sweden. Peripheral blood mononuclear cells (PBMCs) were isolated via standard density gradient centrifugation and cryopreserved in fetal bovine serum (FBS) containing 10% dimethyl sulfoxide (DMSO).

Peptides

Overlapping peptides were designed to span the entire spike protein sequence of SARS-CoV-2 corresponding to the ancestral Wuhan strain (wildtype) or B.1.1.529. Test peptides comprising 15mers overlapping by 10 amino acids were synthesized as crude material for functional screens (TC Peptide Lab). Validation peptides comprising 20mers overlapping by 10 amino acids were synthesized to an equivalent specification (Sigma-Aldrich). All peptides were reconstituted in DMSO, diluted to stock concentrations of 100 µg/ml in phosphate-buffered saline (PBS), and stored at −20 ºC.

Activation-induced marker assays

PBMCs were thawed quickly, resuspended in RPMI 1640 containing 10% FBS, 1% L-glutamine, and 1% penicillin/streptomycin (complete medium) in the presence of DNase I (10 U/ml; Sigma-Aldrich), and rested at 1×10^6 cells per well in 96-well U-bottom plates (Corning) for 4 h at 37 ºC. The medium was then supplemented with anti-CXCR5–BB515 and anti-CD40 (unconjugated), followed 15 min later by the relevant peptide pools (1 µg/ml/peptide). Negative control wells contained equivalent DMSO. After 12 h, cells were washed in PBS supplemented with 2% FBS and 2 mM EDTA (FACS buffer) and stained with anti-CCR4/CD194–BB700, anti-CCR6/CD196–BUV737, anti-CCR7–APC-Cy7, and anti-CXCR3–AF647 for 10 min at 37 ºC. Additional surface stains were performed for 30 min at room temperature in the presence of Brilliant Stain Buffer Plus (BD Biosciences). Viable cells were identified by exclusion using a LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (Thermo Fisher Scientific). Stained cells were then washed in FACS buffer, fixed in PBS containing 1% paraformaldehyde (PFA; Biotium), and acquired using a FACSymphony A5 (BD Biosciences). The gating strategy is shown in Extended Data Figure 1. All flow cytometry reagents are detailed in Supplementary Table 1.

Intracellular cytokine staining

PBMCs were thawed quickly, resuspended in complete medium in the presence of DNase I (10 U/ml; Sigma-Aldrich), and rested at 1×10^6 cells/well in 96-well U-bottom plates (Corning) for 4 h at 37 ºC. The medium was then supplemented with anti-CXCR5–BB515, followed 15 min later by the relevant peptide pools (1 µg/ml/peptide), and a further 1 h later by brefeldin A (1 µg/ml; Sigma-Aldrich) and monensin (0.7 µg/ml; BD Biosciences). Negative control wells contained equivalent DMSO. After 9 h, cells were washed in FACS buffer and stained with anti-CCR4/CD194–BB700, anti-CCR6/CD196–BUV737, anti-CCR7–APC-Cy7, and anti-CXCR3–BV750 for 10 min at 37 ºC. Additional surface stains were performed for 30 min at room temperature in the presence of Brilliant Stain Buffer Plus (BD Biosciences). Viable cells were

identified by exclusion using a LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (Thermo Fisher Scientific). Cells were then washed in FACS buffer and fixed/permeabilized using a FoxP3 Transcription Factor Staining Buffer Set (Thermo Fisher Scientific). Intracellular stains were performed for 30 min at room temperature. Stained cells were washed in FACS buffer, fixed in PBS containing 1% PFA (Biotium), and acquired using a FACSymphony A5 (BD Biosciences). All flow cytometry reagents are detailed in Supplementary Table 1.

Data analysis and statistics

Flow cytometry data were analyzed using FlowJo version 10.8.0 (FlowJo LLC). Stimulation indices were calculated as fold change in frequency relative to the negative control (equivalent DMSO). Positive responses were identified using a threshold stimulation index >2. Statistical analyses were performed using Prism version 9 (GraphPad). Significance between two paired groups was assessed using the Wilcoxon signed rank test. Functional profiles were deconvoluted using Boolean gating in FlowJo version 10.8.0 (FlowJo LLC) followed by downstream analyses in SPICE version 6.1 (https://niaid.github.io/spice/).

Data availability

Requests for data should be addressed to the corresponding author and will be made available within the bounds of confidentiality and data protection obligations.

Figures

Figure 1

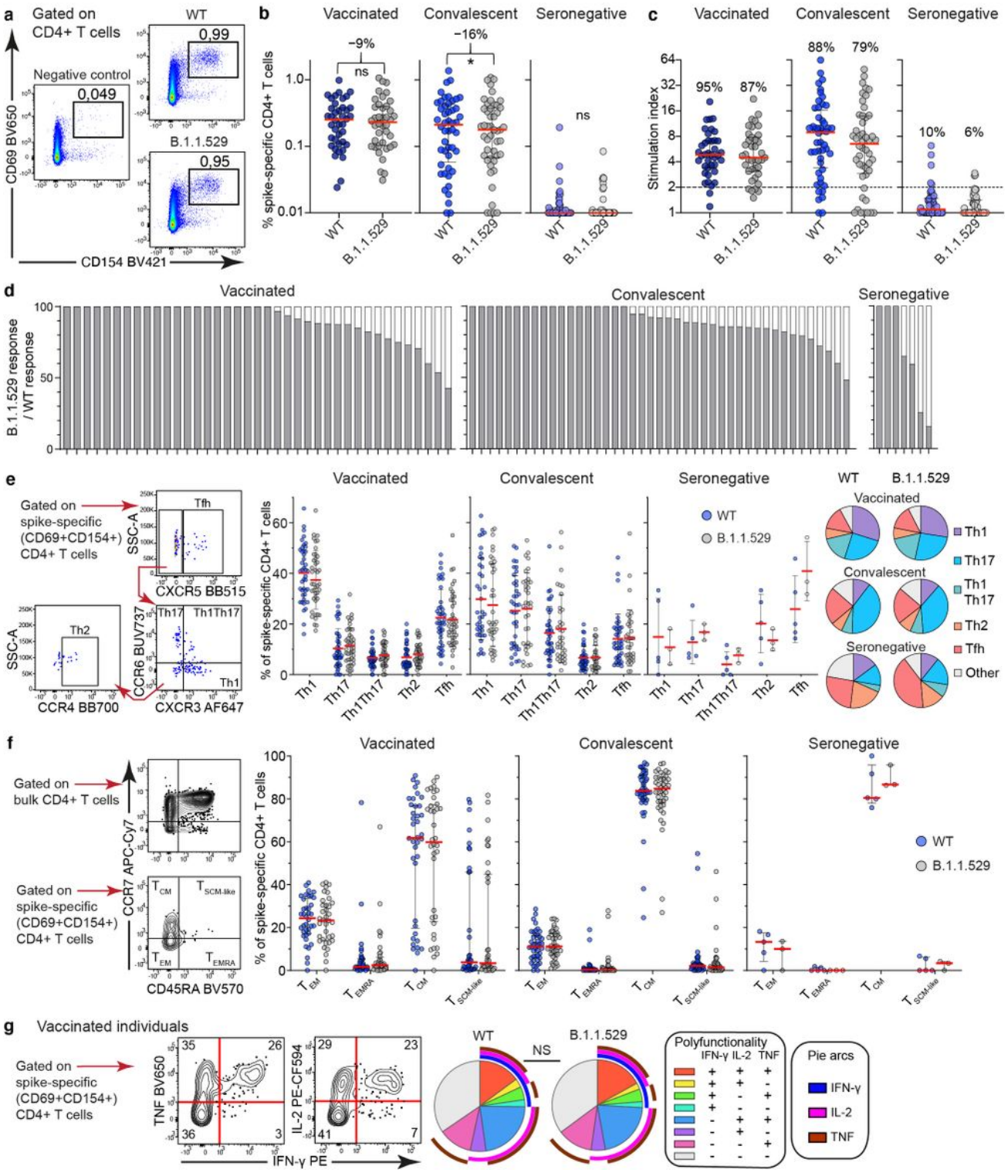

Figure 1

Cross-reactive CD4+ T cell responses against B.1.1.529.

a, Representative flow cytometry plots showing spike-specific CD4+ T cell responses (CD69+CD154+) to peptide pools representing wildtype SARS-CoV-2 (WT) or B.1.1.529. **b**, Frequencies of spike-specific CD4+ T cells from mRNA-vaccinated, convalescent, and seronegative individuals. Each dot represents

one donor. Numbers indicate median reduction in the frequency of detected responses. **c**, Stimulation indices calculated as fold change in frequency relative to the negative control. Numbers indicate the percentage of individuals with a detectable response. **d**, Cross-reactive responses depicted on an individual basis as percent B.1.1.529/WT. **e**, Helper polarization of spike-specific CD4+ T cells with representative gating and dot plots showing the distribution of subsets across individuals with detectable responses. Pie charts show the mean frequency of each subset across all individuals in each group. **f**, Canonical memory differentiation profiles of spike-specific CD4+ T cells with representative gating and dot plots showing the distribution of subsets across individuals with detectable responses. **g**, Functional profiles of spike-specific CD4+ T cell responses from mRNA-vaccinated individuals with representative gating and pie charts showing the mean frequency for each combination. Polyfunctional responses were compared using a permutation test. Data in dot plots are shown as median ± IQR.

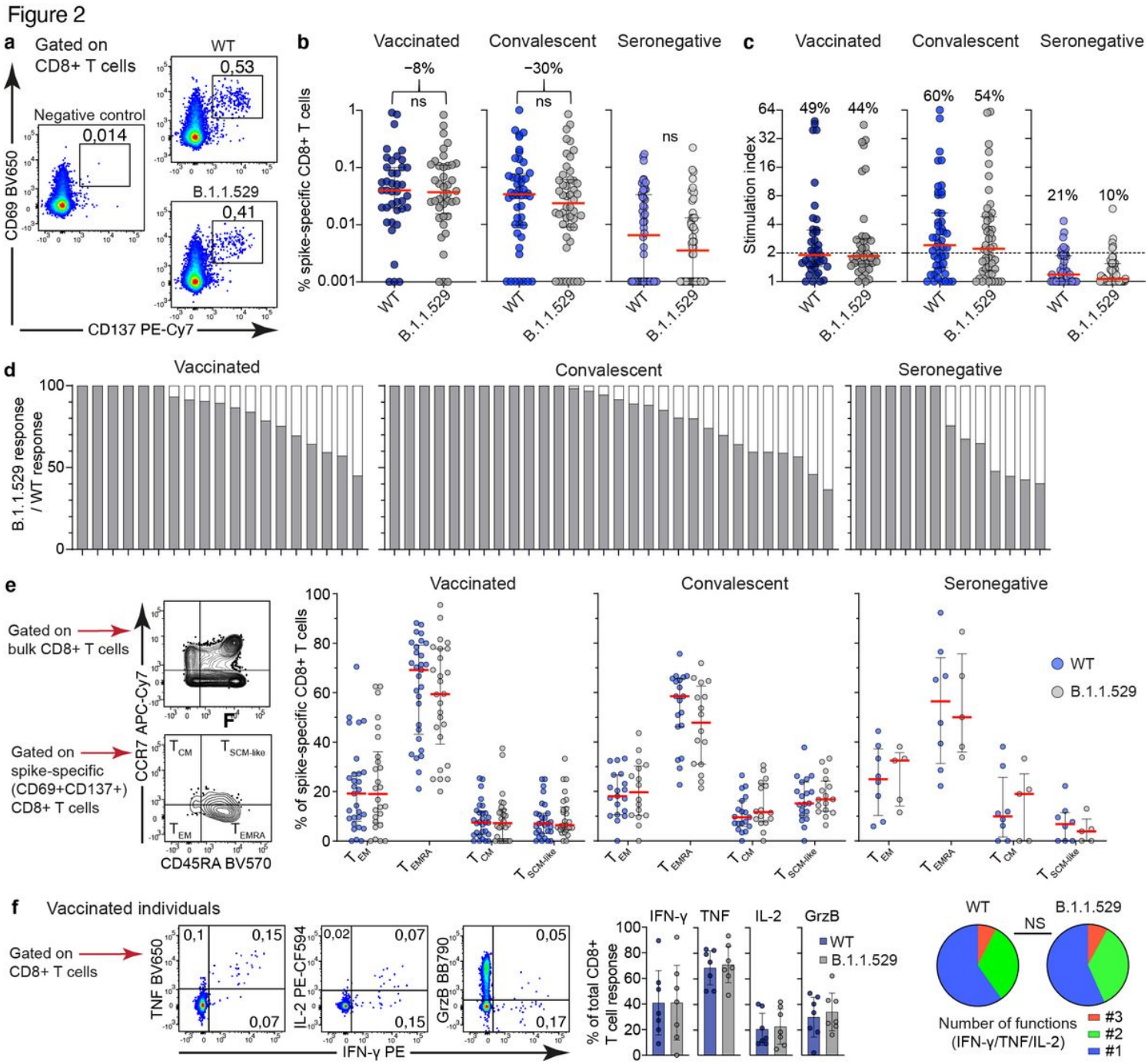

Figure 2

Cross-reactive CD8+ T cell responses against B.1.1.529.

a, Representative flow cytometry plots showing spike-specific CD8+ T cell responses (CD69+CD137+) to peptide pools representing wildtype SARS-CoV-2 (WT) or B.1.1.529. **b**, Frequencies of spike-specific CD8+ T cells from mRNA-vaccinated, convalescent, and seronegative individuals. Each dot represents one donor. Numbers indicate median reduction in the frequency of detected responses. **c**, Stimulation indices calculated as fold change in frequency relative to the negative control. Numbers indicate the percentage of individuals with a detectable response. **d**, Cross-reactive responses depicted on an individual basis as percent B.1.1.529/WT. **e**, Canonical memory differentiation profiles of spike-specific

CD8+ T cells with representative gating and dot plots showing the distribution of subsets across individuals with detectable responses. **f**, Functional profiles of spike-specific CD8+ T cells from mRNA-vaccinated individuals with representative gating and pie charts showing the mean frequency for each combination. Polyfunctional responses were compared using a permutation test. GrzB, granzyme B. Data in bar charts are shown as mean ± 95% confidence intervals, and data in dot plots are shown as median ± IQR.

Supplementary Files

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- rssupplementarytable1.docx
- FigS1.png