Cell Host & Microbe

Memory T cells effectively recognize the SARS-CoV-2 hypermutated BA.2.86 variant

Graphical abstract

Cohorts

Stimulation

Spike peptide pools

Response size

Proliferation

Effector cytokines

Cohorts

CLL

HC

Full vaccinated
+/- Omicron infection

Spike peptide pools

Wu-Hu.1

BA.2.86

BA.1

Response size

Wu-Hu.1-reactive

BA.2.86-reactive

Proliferation

Effector cytokines

Highlights

- SARS-CoV-2 spike-specific T cells are profiled in healthy individuals and CLL patients

- T cell cross-recognition of mutated BA.2.86 spike epitopes is largely preserved

- BA.2.86-reactive T cells display features of high functional capacity

Authors

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In brief

T cells are critical in controlling SARS-CoV-2 infection. The hypermutated BA.2.86 variant exhibits a range of mutations in the spike protein, potentially facilitating evasion from cellular immunity. Müller et al. demonstrate preserved and functional T cell cross-recognition of mutated BA.2.86 epitopes in healthy individuals and patients with chronic lymphocytic leukemia.
Memory T cells effectively recognize the SARS-CoV-2 hypermutated BA.2.86 variant

T cells are critical in mediating the early control of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) breakthrough infection. However, it remains unknown whether memory T cells can effectively cross-recognize new SARS-CoV-2 variants with a broad array of mutations, such as the emergent hypermutated BA.2.86 variant. Here, we report in two separate cohorts, including healthy controls and individuals with chronic lymphocytic leukemia, that SARS-CoV-2 spike-specific CD4+ and CD8+ T cells induced by prior infection or vaccination demonstrate resilient immune recognition of BA.2.86. In both cohorts, we found largely preserved SARS-CoV-2 spike-specific CD4+ and CD8+ T cell magnitudes against mutated spike epitopes of BA.2.86. Functional analysis confirmed that both cytokine expression and proliferative capacity of SARS-CoV-2 spike-specific T cells to BA.2.86-mutated spike epitopes are similarly sustained. In summary, our findings indicate that memory CD4+ and CD8+ T cells continue to provide cell-mediated immune recognition to highly mutated emerging variants such as BA.2.86.

The BA.2.86 variant of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first detected in July 2023 in Denmark and soon classified as a variant under monitoring by the World Health Organization. As of December 2023, BA.2.86 and the closely related JN.1 sublineage are rapidly spreading on a global scale, supplanting XBB as the predominant variant of SARS-CoV-2.1 Whole-genome sequencing identified a total of 122 mutations relative to the Wu-Hu.1 reference genome, and 79 of these mutations were present in the spike protein.2 In comparison with the currently circulating BA.2 and XBB subvariants, BA.2.86 harbors more than 30 additional spike mutations.1,3 BA.2.86 poses challenges since the large number of mutations likely supports increased immune evasion, as highlighted by a
case study from the UK reporting a large outbreak in a care home.4 Early studies in September 2023 suggested that, albeit having lower infectivity, BA.2.86 would still have greater fitness compared with the currently circulating XBB subvariants due to an increased resistance to humoral immunity.3,5 Furthermore, it was shown that BA.2.86 is resistant to clinically relevant monoclonal antibodies.1 More recent studies indicate that neutralizing antibody titers against BA.2.86 are comparable to titers against the currently circulating XBB sublineages.3,4 This is problematic due to the important role of neutralizing antibodies in protection from infection and severe disease.5 Yet, compiled evidence, for instance, from B cell-deficient patients or animal models, suggests that memory T cell responses can step in and provide protection from severe disease in the absence of B cell responses.10–12 Whereas the extent of BA.2.86’s ability to evade humoral immunity seems to be still under debate, there is, to date, a complete lack of T cell immunity data.

Hence, we set out to investigate T cell responses to BA.2.86 in both immunocompetent healthy controls (HCs) and individuals with chronic lymphocytic leukemia (CLL), a patient group that responded poorly to initial vaccine doses and displayed particularly high mortality rates early in the pandemic along with low vaccine-induced T cell responses.13,14 All individuals were vaccinated with three to five mRNA vaccine doses, with some also having experienced a natural Omicron infection as verified by PCR or rapid antigen test. To this end, our cohort reflects the current real-life situation of highly individual vaccination schemes and infection histories throughout the population. Furthermore, this setup facilitates the cross-comparison of T cell responses directed against the two variants, which made the most considerable mutational leaps in the evolutionary course of SARS-CoV-2, Omicron BA.1, and Omicron BA.2.86, both in infection-naive individuals and individuals with hybrid immunity upon Omicron infection. Peripheral blood mononuclear cells (PBMCs) were collected from both HC and CLL patients about 7 months after the Omicron wave peak in Europe. Additionally, we gathered a second CLL cohort with a similar vaccination scheme and infection histories throughout the population. The baseline cohort characteristics, along with clinical and sampling parameters, are provided in Table S1. T cell responses were measured via activation-induced marker (AIM) staining after short-term in vitro stimulation of PBMCs with overlapping peptide pools spanning the whole spike region of the ancestral Wuhan strain or smaller variant pools containing only the BA.2.86- or BA.1-mutated spike peptides and their homologous Wu-Hu.1 counterparts (Figure 1B). Reactive memory CD4+ T cells were defined by a Boolean OR gate on all combinations of CD40L, 4-1BB, CD69, and OX40 co-expression (as described recently15) and reactive memory CD8+ T cells were defined based on CD69 and 4-1BB co-expression (Figure 1C; see Figure S1 for gating strategy). T cell responses to ancestral peptide pools correlated with pool size, where we detected larger CD4+ T cell responses than CD8+ T cell responses (Figure 1D). We did not observe a significantly reduced T cell response to the variant BA.1 peptide pool but did observe a tendency of slightly increased responses in recently Omicron-infected individuals. These findings align with previous studies investigating T cell cross-recognition of the Omicron variant.16–25 Notably, despite the increased number of spike mutations in BA.2.86, we did not observe significantly impaired CD4+ or CD8+ T cell responses in both cohorts, neither in infection-naive individuals nor in Omicron-infected individuals. Furthermore, we used our dataset to mathematically calculate CD4+ and CD8+ T cell response sizes to the full spike peptidome of BA.2.86 and BA.1 and observed no significant differences (Figure 1E). Together, our data indicate preserved CD4+ and CD8+ T cell cross-recognition of BA.2.86-mutated spike epitopes.

Despite the robust cross-recognition of BA.2.86 spike, potentially lower binding avidities between vaccination-induced, ancestral spike-specific T cell clones and BA.2.86-mutated spike epitopes might result in impaired functional responses. To address this question, we used the PBMCs of healthy donors (with unknown vaccination and infection histories) collected before the onset of the BA.2.86 variant and determined the proliferative capacity upon in vitro stimulation with the same five peptide pools as in Figure 1. We measured cell proliferation on day 3 by staining for Ki67. Specifically, we identified cells that were actively proliferating in response to antigen-specific stimulation by gating on Ki67 and CD69 double-positive cells, as this strategy helped exclude cells that proliferated in the absence of antigen-specific stimulation (Figure 2A). Proliferative capacity was significantly higher in response to the Wu-Hu.1 full spike peptide pool, and we observed a slight tendency of reduced CD4+ and CD8+ T cell proliferation in response to both BA.2.86 and BA.1 variant pools (Figure 2B). Overall, proliferation capacity was not significantly changed between ancestral and variant pools (Figure 2B), resulting in highly similar responses toward calculated full spike pools (Figure 2C).

Using the same donors, we also determined intracellular cytokine expression in spike-reactive CD4+ T cells upon short-term in vitro stimulation (Figure 2D). Again, T cell responses correlated with peptide pool size, and we did not detect significantly different response sizes between the smaller BA.2.86 variant and homologous ancestral spike peptide pool (Figure 2E). Accordingly, mathematically calculated full spike responses were not significantly changed between Wu-Hu.1 and both variants (Figure 2F), reflecting data presented in Figure 1. Cytokine expression patterns followed a similar profile, with the highest expression observed in response to Wu-Hu.1 full spike peptide pool. Cytokine responses stimulated by BA.2.86 mutated peptides or their ancestral counterparts were highly similar. A slight tendency of reduced cytokine expression was observed in the BA.1 variant pool (Figure 2G). Overall, polyfunctionality was marginally reduced in the smaller peptide pools but not significantly different between both BA.2.86 and BA.1 variant and homologous ancestral peptide pools (Figure 2H). The lowest polyfunctionality was observed in response to the BA.1 variant pool, which was also significantly reduced in comparison with the Wu-Hu.1 full spike pool. However, differences between stimulations with differently large peptide pools should be interpreted with caution, as a higher number of responsive T cells per well might influence the amount of cytokine expression. Collectively, we observed largely preserved functional T cell responses to BA.2.86-mutated spike peptides, both in terms of cytokine expression and proliferation.
appears to be relatively uncommon after breakthrough infection, particularly in individuals with hybrid immunity. In this regard, memory T cells most likely play a key role in limiting viral replication and generating early control after breakthrough infection. With the recent evolution of heavily mutated SARS-CoV-2 variants, marked by the appearance of BA.2.86, it remains, however, possible that the virus might escape cell-mediated immunity. Our collective data indicate that SARS-CoV-2 spike-specific CD4+ and CD8+ T cells elicited by vaccination or prior infection remain largely intact against BA.2.86 in both healthy individuals and vulnerable CLL patients. Aligning with previous findings, our data suggest that a considerable...
Figure 2. Preserved functional capacity of BA.2.86-cross-recognizing T cells
(A) Representative plots depicting the upregulation of Ki67 and CD69 after 3 days of in vitro stimulation with the indicated peptide pools or DMSO negative control.
(B) Quantification of Ki67 and CD69 co-expression as a proxy for proliferative capacity in response to Wu-Hu.1 full spike and variant peptide pools.
(C) Mathematically calculated full spike proliferative responses using data from (B).
(D) Representative plots depicting interleukin (IL)-2, interferon (IFN) γ, and tumor necrosis factor (TNF) cytokine expression in spike-reactive (CD69+CD40L+) memory CD4+ T cells.
(E) Net frequencies of CD4+ T cell responses to Wu-Hu.1 full spike and variant peptide pools.
(F) Mathematically calculated full spike responses using data from (E).
majority of spike major histocompatibility complex (MHC)-restricted peptides remain conserved across variants. Notably, although our study sheds light on the immune response against the spike protein of BA.2.86 in peripheral blood, it is critical to acknowledge its limitation of not encompassing non-spike protein regions. However, given that BA.2.86 exhibits fewer mutations in non-spike proteins, it is conceivable that T cell recognition is even broader and more robust than our findings suggest in previously infected individuals. As such, population-wide immunity against BA.2.86 is presumably robust and may be an underlying reason why BA.2.86 accounts for only a minority of circulating variants worldwide. Collectively, we provide proof-of-principle data that SARS-CoV-2 spike-specific CD4+ and CD8+ T cell responses remain largely intact against highly mutated emerging variants such as BA.2.86.

STAR METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.chom.2023.12.010.

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AUTHOR CONTRIBUTIONS


DECLARATION OF INTERESTS

A.S. is a consultant for AstraZeneca Pharmaceuticals; Calyptus Pharmaceuticals, Inc; Darwin Health; EmerVax; EUROIMMUN; F. Hoffman-La Roche Ltd; Fortress Biotech; Gilead Sciences; Granite Bio; Gritstone Oncology; Guggeheim Securities; Moderna; Pfizer; RiverVest Venture Partners; and Turnstone Biologics. A.G. is a consultant for Pfizer. A.G. has filed for patent protection for various aspects of T cell epitope and vaccine design work. M.B. is a consultant and has received honoraria from Oxford Immunotec, MSD, BMS, Pfizer, and Mabtech. S.A. has received honoraria for lectures and educational events, not related to this work, from Gilead, AbbVie, MSD, and Biogen and reports grants from Gilead and AbbVie.

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REFERENCES

STAR METHODS

KEY RESOURCES TABLE

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RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Marcus Buggert (marcus.buggert@ki.se).

Brief Report
Cell Host & Microbe 32, 156–161.e1–e3, February 14, 2024
Materials availability
BA.2.86 peptide pools generated in this study can be made available under appropriate materials transfer agreement. No other unique reagents were generated.

Data and code availability
Data reported in this paper will be shared by the lead contact upon request.
Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Healthy individuals and CLL patients of Cohort I were part of the prospective longitudinal clinical trial study COVAXID. The trial was registered at EudraCT (no. 2021-000175-37) and clinicaltrials.gov (NCT04780659). The Swedish Medical Product Agency (ID 5.1-2021-5881) and the Swedish Ethical Review Authority (ID 2021-00451) approved the study. The original clinical trial protocol included two vaccine doses and immunogenicity measurements until six months after the second dose and was subsequently extended with permission from the Swedish Ethical Review Board and the Swedish Medical Products Agency (no. 2021-06046-02 and no. 5.1-2021-92151, respectively). Through this extension, continuation of blood sampling for analysis of cellular responses could be performed and participants were offered additional booster vaccine doses following recommendations by the Public Health Agency of Sweden. For this study, we investigated samples that were donated in September and October of 2022, about 18 months after receipt of the second dose, 7 months after the Omicron wave peak in Sweden and before the upcoming of the BA.2.86 variant. CLL patients of Cohort II received booster vaccination shortly before the onset of the Omicron wave during which all of them were infected with the Omicron variant. Blood samples were taken between February and June 2022, approximately one to two months after Omicron infection and about 12 months after receipt of the second vaccine dose. Cohort characteristics and sampling information are summarized in Table S1. Additional blood samples from random healthy donors were used for the performance of functional assays. These samples were selected based on collection time before the onset of the BA.2.86 variant. PBMCs were isolated from whole blood via standard density gradient centrifugation and cryopreserved in fetal bovine serum (FBS) containing 10% dimethyl sulfoxide (DMSO). All participants have given their written informed consent to the study.

METHOD DETAILS

Peptides
Overlapping peptides were designed to span the entire spike protein sequence of SARS-CoV-2 corresponding to the ancestral Wuhan strain (Wu-Hu.1, 253 peptides) or mutated spike regions of the variants BA.2.86 (88 peptides) or BA.1 (68 peptides) and their corresponding homologous Wu-Hu.1 counterparts. Peptides were 15-mers overlapping by 10 amino acids and were synthesized as crude material (TC Peptide Lab, San Diego, CA). The spike peptide pools were reconstituted in DMSO, diluted to 100 µg/ml in phosphate-buffered saline (PBS), aliquoted, and stored at −20°C.

Activation-Induced Marker (AIM) assay
Cryopreserved PBMCs were thawed quickly, resuspended in complete medium (RPMI 1640 containing 10% FBS, 1% L-glutamine, and 1% penicillin/streptomycin) supplemented with DNase I (10 U/ml; Sigma-Aldrich), and rested at 1°C for 3 hours at 37°C. The medium was then supplemented with anti-CD40 (unconjugated, clone HB14, Miltenyi) followed 15 min later by the respective spike peptide pool (0.5 µg/ml) for stimulation or an equivalent amount of DMSO as a negative control. After 12 hours, cells were washed in PBS supplemented with 2% FBS and 2 mM EDTA (FACS buffer) and stained with other chemokine receptors (CCR7, CX3CR1) for 10 min at 37°C. Additional staining of surface molecules (CD8, CD45RA, CD14, CD19, CD127, CD3, CD27, CD95, CD4), including activation markers CD69, CD40L, OX40, and 4-1BB, was 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 washed in FACS buffer, fixed in PBS containing 1% paraformaldehyde (PFA; Biotium), and acquired using a FACSymphony A5 (BD Biosciences).

Net frequencies of spike-reactive T cells were calculated by subtracting the frequency of AIM marker+ T cells in the negative control from the frequency of AIM marker+ T cells detected after stimulation with each peptide pool. Values were excluded from analysis, if less than ten cells were detected in the AIM marker+ gate as described previously, and net frequency values smaller than 0.01% were set to 0.01%. Full spike responses to BA.2.86 and BA.1 were calculated by: %net response to Wu-Hu.1 full spike - (%net response to BA.2.86/BA.1-Ancestral - %net response to BA.2.86/BA.1-Variant) under exclusion of net frequency values smaller than 0.01%. Individual net responses to Wu-Hu.1 are only included if corresponding BA.2.86 or BA.1 full spike responses could be calculated.

AIM assay with intracellular staining
Stimulation and staining procedure as described for the AIM assay. Chemokine receptor CCR7 was stained for 10 min at 37°C followed by staining of surface markers (CD8, CD45RA, CD14, CD19, CD95, CD4) for 30 min at room temperature in the presence of Brilliant Stain Buffer Plus (BD Biosciences). Cells were then washed in FACS buffer and fixed/permeabilized using a FoxP3
Transcription Factor Staining Buffer Set (Thermo Fisher Scientific). Intracellular stains (CD40L, IL-2, IFNγ, TNF, CD3, CD69) were performed for 30 min at room temperature. Stained cells were washed in FACS buffer and acquired using a FACSymphony A5 (BD Biosciences).

**Proliferation assay**
Peptide pool stimulation as described for the AIM assay. Cells were harvested after three days of stimulation and subsequently stained. Chemokine receptor CCR7 was stained for 10 min at 37°C followed by staining of surface markers (CD8, CD45RA, CD14, CD19, CD95, CD4) for 30 min at room temperature in the presence of Brilliant Stain Buffer Plus (BD Biosciences). Cells were then washed in FACS buffer and fixed/permeabilized using a FoxP3 Transcription Factor Staining Buffer Set (Thermo Fisher Scientific). Intracellular stains (CD3, CD69, Ki67) were performed for 30 min at room temperature. Stained cells were washed in FACS buffer and acquired using a FACSymphony A5 (BD Biosciences).

**QUANTIFICATION AND STATISTICAL ANALYSIS**
All samples from each cohort were randomly assigned and analyzed with all peptide pools and controls in the same experiment. Flow cytometry data were analyzed using FlowJo version 10.8.1 (FlowJo). Data exclusion criteria were established before conduction of experiments. The investigators were not blinded to allocation during experiments and outcome assessment. Statistical analyses were performed using Prism version 10 (GraphPad Software Inc.) using significance tests as indicated in respective figure legends. A P value of < 0.05 was considered statistically significant. Functional profiles were deconvoluted using Boolean gating in FlowJo version 10.8.1 (FlowJo) followed by downstream analyses in SPICE version 6.1 (https://niaid.github.io/spice/).