Ablation of CD8⁺ T-cell recognition of an immunodominant epitope in SARS-CoV-2 Omicron

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Abstract

The emergence of the SARS-CoV-2 Omicron variant has raised concerns of escape from vaccine-induced immunity. A number of studies have demonstrated a reduction in antibody-mediated neutralization of the Omicron variant in vaccinated individuals. Preliminary observations have suggested that T cells are less likely to be affected by changes in Omicron. However, the complexity of human leukocyte antigen genetics and its impact upon immunodominant T-cell epitope selection suggests that the maintenance of T-cell immunity won't be universal. In this study, we describe the impact that changes in Omicron have on recognition by spike-specific T cells. These T cells constitute the immunodominant CD8⁺ T-cell response in HLA-A*29:02⁺ COVID-19 convalescent and vaccinated individuals; however, they fail to recognize the Omicron-encoded sequence. We also failed to detect other immunodominant responses in vaccinated HLA-A*29:02⁺ individuals, suggesting that the Omicron variant may have an increased capacity to escape immune recognition in this population.

Full Text

T-cell immunity plays an important role in augmenting vaccine-mediated protection against SARS-CoV-2, particularly in the face of emerging variants such as Omicron^{1,2}, and is likely to be critically important in individuals who fail to generate efficient neutralizing antibody responses due to underlying disease^{3,4}. Following the emergence of Omicron, studies have demonstrated that most T-cell epitopes are conserved^{5,6}, and the majority of vaccine recipients maintain T-cell immunity.⁷⁻⁹ Nevertheless, evidence in convalescent individuals has demonstrated the capacity for variant strains to evade T cell responses¹⁰. In this study we sought to investigate the impact of changes in Omicron on a uniquely immunodominant spike-encoded CD8⁺ T-cell epitope.

Our group recruited 59 COVID-19 convalescent participants during the initial 2020 wave of the pandemic in Australia. All participants were typed for human leukocyte antigens (HLA, Supplementary Table 1) and SARS-CoV-2-specific T cells were expanded from peripheral blood mononuclear cells (PBMC) using SARS-CoV-2-encoded antigens¹¹. In this analysis, we noted striking immunodominant CD8⁺ T cell responses to both spike (S-1 pool) and ORF3A in HLA-A*29:02⁺ participants (Figure 1A, Supplementary Figure 1). We detected a median S-1 pool-specific CD8⁺ T-cell response of 44.6% in HLA-A*29:02⁺ participants compared to 0.85% in HLA-A*29:02⁻ participants (Figure 1B). The ORF3A-specific CD8⁺ T-cell response was detected at a median of 31.2% in HLA-A*29:02⁺ participants compared to 1.47% in HLA-A*29:02⁻ participants. Using an overlapping peptide matrix, we identified that the spike-specific T-cell response targeted spike matrix pools 5, 6 and 23 (Figure 1C), corresponding to overlapping peptides GFNCYFPLQSYGFQP and YFPLQSYGFQPTNGV (Supplementary Figure 2A). Peptide minimization and HLA restriction (Supplementary Figure 2B) revealed the immunodominant response to be encoded by a HLA-A*29:02-restricted epitope, YFPLQSYGF (Figure 1D). Similar analysis revealed an immunodominant response in matrix pools 7, 8 and 15 from ORF3a (Figure 1E), corresponding to overlapping peptides

VVLHSYFTSDYYQLY and SYFTSDYYQLYSTQL (Supplementary Figure 2C), which contain the previously published HLA-A*29:02-restricted epitope YFTSDYYQLY (Figure 1F) ¹².

Analysis of the amino acid sequences of the HLA-A*29:02 epitopes in SARS-CoV-2 variants revealed that while the ORF3A-encoded epitope was conserved, the Omicron variant contained amino acid substitutions at positions 5 and 8 in the spike-encoded epitope (Figure 1G). Although not anchor residues for A*29:02, the substitutions, particularly the non-conserved glutamine (Q) to arginine (R) change at position 5, have the potential to significantly impact T-cell receptor (TCR) engagement. To address this, we generated T cells specific for the YFPLQSYGF epitope from three COVID-19-convalescent participants and performed intracellular cytokine analysis using 10-fold serial peptide titration with both the cognate epitope and Omicron variant. Despite strong functional avidity directed towards the cognate peptide, we saw poor reactivity against the Omicron variant (Figure 1H, Supplementary Figure 3).

We next sought to determine if individuals vaccinated against SARS-CoV-2 would show a similar pattern in their response to YFPLQSYGF. From a cohort of 104 participants, recruited following vaccination with either Vaxzevria or Comirnaty, we identified three participants with HLA-A*29:02 (Supplementary Table 2). PBMC from these individuals were isolated 28 days after their second vaccine dose, then stimulated with a spike peptide pool, the YFPLQSYGF epitope or the Omicron variant. We assessed expression of IFN- γ and TNF by CD8⁺ T cells using intracellular cytokine analysis to identify low-frequency T-cell responses. Controls were unstimulated PBMC (no peptide) and PBMC incubated with a cell stimulation cocktail (CSC). Although low in abundance, IFN- γ ⁺TNF⁺ CD8⁺ T cell responses against the spike overlapping peptide pools (0.006%, 0.009%, and 0.002% above no peptide) and the YFPLQSYGF epitope (0.046%, 0.012%, and 0.006% above no peptide) were detected in PBMC from all three individuals (Figure 2A). Similar observations were evident following analysis of IFN- γ alone (Supplementary Figure 4A). Conversely, CD8⁺ T cells capable of recognizing the Omicron variant were not detected. To validate these observations, we generated T cells from these three participants by stimulating with the spike peptide pools and culturing in the presence of IL-2 for 14 days. In addition, we set up cultures from a fourth individual in our vaccine cohort who is HLA-A*29:11⁺. Spike-specific CD8⁺ T cell responses were observed in all four T cell expansions (Figure 2B, Supplementary Figure 4B). All four cultures were dominated by T cells specific for YFPLQSYGF, but displayed no recognition of the Omicron variant. In the three HLA-A*29:02⁺ cultures, the frequency of the YFPLQSYGF-specific CD8⁺IFN- γ ⁺TNF⁺ response was comparable to the response against whole spike protein, demonstrating similar immunodominance to that seen in convalescent individuals. To determine if we could generate variant-specific T cells, we stimulated PBMC with either YFPLRSYSF or the Omicron variant and assessed functional avidity after 14 days in culture. While the Omicron epitope failed to induce the expansion of T cells in these donors, YFPLRSYSF-specific T cells were detected in cultures from all four vaccine recipients (Figure 2C&D).

Given the differences in the T cell response between YFPLRSYSF and its variant, we wanted to understand the impact of the Omicron mutation on peptide presentation by HLA-A*29:02. YFPLRSYSF peptide was refolded with HLA-A*29:02 and then crystallised to understand the basis of peptide

presentation (Supplementary Table 3). The HLA-A*29:02 molecule adopted the canonical fold of HLA molecules with YFPLRSYSF (10.3390/ijms22010068) (Supplementary Figure 5A). The primary anchors of the YFPLRSYSF peptide at P2 and P9 are both phenylalanine residues that are favored for HLA-A*29:02 binding (Figure 2E, Supplementary Figure 6) and in line with the thermal stability observed for the YFP-HLA-A*29:02 complex (Supplementary Table 4). The P5-Gln acts as a secondary anchor with its side chain buried in the center of the peptide binding cleft (Figure 2E), forming hydrogen bond with the Arg114 as well as hydrophobic interaction with Tyr99, Gln70, Leu156 and Met97 (Supplementary Figure 5B). The P7-Tyr is half buried with its aromatic side chain placed in between the α 2-helix and the backbone of the peptide. The peptide has four residue side chains that are solvent exposed (Figure 2E), namely the P1-Tyr, P4-Leu, P6-Ser and P8-Gly that could potentially be contacted by TCRs.

The two residues of the peptide that are mutated in Omicron are at positions 5 and 8. While the substitution at P8 from a glycine to a serine is unlikely to impact peptide presentation and conformation (Figure 2F), the mutation at position 5 will have an impact on the conformation of the peptide. The P5-Gln is buried in the cleft of HLA-A*29:02, and modeling of a P5-Arg shows a steric clash of the large and charged side chain (Figure 2F) with residues in the binding cleft due to the presence of large amino acid residues Met97, Tyr99 and Arg114 that occupy the β -sheet floor of HLA-A*29:02. The P5 mutation in Omicron is likely to change peptide and could also weaken its binding to HLA-A*29:02.

To investigate the impact of amino acid changes on another spike-encoded epitope, we assessed T cell responses to the HLA-B*07:02-restricted epitope SPRRARSVA¹³ in five HLA-B*07:02-positive vaccine recipients (Supplementary Table 2). This epitope contains mutations detectable in multiple variants that alter the HLA-B*07:02 anchor residue at P2 (Supplementary Table 5). While these changes ablated the ability of T cells from vaccinated individuals to recognize the variant peptides, other spike-specific T cell responses were detected, including those against the conserved HLA-A*02:01-restricted epitope YLQPRTFLL¹² (Figure 2G, Supplementary Figure 4C), demonstrating the complex nature of T-cell immunodominance in HLA-distinct individuals.

Despite the potentially large number of antigenic targets in the spike protein¹²⁻¹⁴, our observations suggest that the immunodominance profile in HLA-A29-positive individuals is associated with a skewed response towards a single dominant epitope. While it remains to be determined if this could impact susceptibility in vaccinated individuals, it was clear that changes in the amino acid sequence ablated T cell activation. Although HLA-A29 was present in only 6.8% and 3.8% of our convalescent and vaccinated cohort respectively, HLA-A29 frequencies have been reported as high as 24% in some populations in Africa (allele frequencies.net, Supplementary Table 6), which could render a high proportion of these populations susceptible to evasion of CD8⁺ T-cell immunity following exposure to Omicron. These analyses highlight the potential impact of genetic variation in newly emerging SARS-CoV-2 variants. Demonstrating that ongoing genetic variation may lead to escape from cellular immune control, especially in ethnic groups in which HLA alleles impacted by epitope mutations are dominant.

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Online Methods

Study participants

This study was approved by the QIMR Berghofer Medical Research Institute Human Research Ethics Committee and was performed according to the principles of the Declaration of Helsinki. All participants were over the age of 18 and provided informed consent to participate in this study. We recruited two cohorts: COVID-19-convalescent participants who had been clinically diagnosed by PCR with SARS-CoV-2 infection, and individuals who had not been exposed to COVID-19 but had received two doses of a COVID-19 vaccine. COVID-19-convalescent individuals donated blood for PBMC isolation following resolution of symptoms, while vaccinated individuals donated blood 28 days after their second vaccine dose. HLA typing was performed on each donated sample. The HLA types of the COVID-19 convalescent participants are provided in Supplementary Table 1, and the HLA types of the vaccinated participants are provided in Supplementary Table 2.

Expansion of SARS-CoV-2-specific T cells

The strategy used for the expansion of SARS-CoV-2-specific T cells has been described previously¹¹. For the expansion of T cells from convalescent participants, pools of 15-mer peptides with an 11-amino-acid overlap were supplied by JPT technologies and are listed in Supplementary Table 7. SARS-CoV-2-specific peptide matrix pools were also supplied by JPT Technologies. The overlapping peptide pools used to expand T cells from vaccinated participants were supplied by Mimotopes. Individual peptide epitopes used to stimulate T cells were provided by GenScript.

Intracellular cytokine assay

Cultured T cells (5×10^5 per test) or PBMC (2×10^6 per test) were stimulated separately with the SARS-CoV-2 overlapping peptide pools (1 μ g/mL of each peptide), individual defined epitopes (1 μ g/mL) or with a cytokine stimulation cocktail (eBioscience), and incubated for 4 hours (T cells) or 6 hours (PBMC) at 37°C in the presence of GolgiPlug, GolgiStop and anti-CD107a-FITC (BD Biosciences). Following stimulation, cells were washed and stained with anti-CD8-PerCP-Cy5.5 (eBioscience), anti-CD4-Pacific Blue (BD Biosciences) and live/dead fixable near-IR dead cell stain (Life Technologies) for 30 minutes at

4°C before being fixed and permeabilized with Fixation/Permeabilization solution (BD Biosciences). After 20 minutes of fixation, cells were washed in BD Perm/Wash buffer (BD Biosciences) and stained with anti-IFN- γ -Alexa Fluor 700, anti-IL-2-PE and anti-TNF-APC (all from BD Biosciences) for a further 30 minutes at 4°C. Finally, cells were washed again and acquired using a BD LSRFortessa with FACSDiva software. Post-acquisition analysis was performed using FlowJo software (TreeStar). Cytokine levels detected in the no-peptide control condition were subtracted from the corresponding test conditions to account for non-specific, spontaneous cytokine production.

Functional avidity assay

T cells (5×10^5 per test) were stimulated in duplicate with 10-fold serial dilutions of each peptide epitope, starting at 1 $\mu\text{g/mL}$, then incubated for 4 hours in the presence of GolgiPlug. Cells were then washed and stained with anti-CD8-PerCP-Cy5.5, anti-CD4-FITC (BD Biosciences) and live/dead fixable near-IR dead cell stain for 30 minutes at 4°C before being fixed and permeabilized with Fixation/Permeabilization solution for 20 minutes. Cells were washed with BD Perm/Wash and stained for 30 minutes at 4°C with anti-PE-IFN- γ . (BD Biosciences). Cells were washed and acquired using a BD LSRFortessa with FACSDiva software. Post-acquisition analysis was performed using FlowJo software (TreeStar). Cytokine levels detected in the no-peptide control condition were subtracted from the corresponding test conditions to account for non-specific, spontaneous cytokine production.

HLA Restriction assay

T cells (5×10^5 per test) were stimulated with HLA-A*29:02⁺ and HLA-A*29:02⁻ PHA blasts pulsed with 0.1 $\mu\text{g/mL}$ of peptide, then incubated for 4 hours in the presence of GolgiPlug. Cells were then washed and stained with anti-CD8-PerCP-Cy5.5, anti-CD4-PE-Cy7 (BD Biosciences) and live/dead fixable near-IR dead cell stain for 30 minutes at 4°C before being fixed and permeabilized with Fixation/Permeabilization solution for 20 minutes. Cells were washed with BD Perm/Wash and stained for 30 minutes at 4°C with anti-PE-IFN- γ . (BD Biosciences). Cells were washed and acquired using a BD LSRFortessa with FACSDiva software. Post-acquisition analysis was performed using FlowJo software (TreeStar).

Protein expression, refold and purification of pHLA complexes

pET-30a(+) DNA plasmids encoding HLA-A*29:02 a-chain and human β -2-microglobulin were transformed separately into BL21 E. coli and expressed. Inclusion bodies containing the individually expressed recombinant proteins were extracted, purified and quantified as per previously described (31). Soluble pHLA-A*29:02 complexes were produced by refolding 30 mg of HLA-A*29:02 a-chain with 10 mg of β -2-microglobulin and 5 mg of peptide (Genscript) into a buffer of 3M Urea, 0.5 M L-Arginine, 0.1 M Tris-HCl pH 8.0, 2.5 mM Ethylenediaminetetraacetic acid (EDTA) pH 8.0, 5 mM glutathione (reduced), 1.25 mM glutathione (oxidised), 0.2mM Phenylmethylsulfonyl fluoride (PMSF). The refold mixture was dialysed at 4° C over 36 hours in 10 mM Tris-HCl pH 8.0 and soluble pHLA complexes were purified via anion exchange chromatography. First using a Diethylaminoethyl cellulose resin, then a HiTrapQ column (GE Healthcare).

Differential scanning fluorimetry

Thermal stability assay was performed by Differential Scanning Fluorimetry carried out in ViiA 7 real-time PCR system (Thermofisher), where HLA-A*29:02-YFP complex was heated from 25 to 95°C at a rate of 1°C/min in 0.5°C steps. The excitation and emission channels were set to the TAMRA reporter (x3m3 filter) with excitation of ~550 nm and detection at ~587 nm. The experiment was performed at two concentrations of pHLA (5 µM and 10 µM) in duplicates. Each sample was dialysed in 10mM Tris-HCl pH 8.0, 150mM NaCl and contained a final concentration of 10X SYPRO Orange Dye. Fluorescence intensity data was normalised and plotted using GraphPad Prism 9 (version 9.3). The T_m value for a pHLA is equal to its the temperature when 50% of maximum fluorescence intensity is reached, which approximately equals to 50% of unfolded protein and summarized in Supplementary Table 4.

Crystallization and structural determination

Crystallisation of HLA-A*29:02-peptide complex was grown via hanging-drop, vapour diffusion at 20°C. The protein: reservoir drop ratio was 1:1, at a concentration of 3 mg/mL in 10 mM Tris-HCl pH 8, 150 mM NaCl; and crystals were grown in 1.9M Ammonium sulphate, 20mM Magnesium Chloride, 0.1M Bis-Tris propane, 2% Ethylene glycol, 2% 2-methyl-2,4-pentanediol Protein crystals were soaked in a cryoprotectant solution containing mother liquor solution with 20% (v/v) Ethylene glycol and then flash-frozen in liquid nitrogen. The data were collected on the MX2 beamline at the Australian Synchrotron, part of ANSTO, Australia ¹⁵. The data were processed using XDS ¹⁶ and the structures were determined by molecular replacement using the PHASER program ¹⁷ from the CCP4 suite ¹⁸ with a model of HLA-A*24:02 without the peptide (derived from PDB ID: 7JYV ¹⁹). Manual model building was conducted using COOT ²⁰ followed by refinement with PHENIX, followed by BUSTER ²¹. The final models have been validated and deposited using the wwPDB OneDep System and the final refinement statistics, PDB codes are summarized in Supplementary Table 3. All molecular graphics representations were created using PyMOL.

Declarations

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Conflicts of interest

The authors report no conflict of interest.

Author contribution

SS, KEL, GRA, MAN, KS, SG, RK and CSm contributed to the design of the study. SS, KEL, AP, JR, LDM, CSz PC, LLT and CSm performed experiments. MAN and SR managed the recruitment of participants. All authors contributed to drafting of the manuscript.

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Figures

Figure 1

Immunodominant HLA-A*29:02-restricted T-cell immunity in COVID-19 convalescent participants. (A) Heatmap of the magnitude of SARS-CoV-2 antigen-specific T-cell responses in COVID-19-convalescent individuals (HLA-A*29:02⁺, n=4; HLA-A*29:02⁻, n=55). **(B)** Frequency of S-1- and ORF3A-specific IFN-g-

producing CD8⁺ T cells in HLA-A*29:02⁺ and HLA-A*29:02⁻ participants. **(C)** Frequency of IFN-g-producing CD8⁺ T cells in response to S-1 peptide matrix pools in HLA-A*29:02⁺ participants (n=2). **(D)** Frequency of IFN-g-producing CD8⁺ T cells in response to the YFPLQSYGF epitope in HLA-A*29:02⁺ participants **(E)** Frequency of IFN-g-producing CD8⁺ T cells in response to the ORF3A peptide matrix pools in HLA-A*29:02⁺ participants (n=2). **(F)** Frequency of IFN-g-producing CD8⁺ T cells in response to the YFTSDYYQLY epitope in HLA-A*29:02⁺ participants. **(G)** Prevalent HLA-A*29:02 epitope sequences in SARS-CoV-2 variants of concern. **(H)** Frequency of IFN-g-producing CD8⁺ T cells in response to serial dilutions of YFPLQSYGF and YFPLRSYSF in HLA-A*29:02⁺ participants.

Figure 2

Figure 2

Recognition of the immunodominant HLA-A*29:02-restricted epitope in vaccinated participants. (A) Flow cytometry plots displaying the frequency of IFN-g-producing CD8⁺ T cells from PBMC of vaccinated HLA-A*29:02⁺ individuals. **(B)** Frequency of IFN-g-producing CD8⁺ T cells following in vitro culture of PBMC from four vaccinated individuals (A*29:02 n=3; A*29:11 n=1) with spike overlapping peptide pools. **(C)** Frequency of IFN-g-producing CD8⁺ T cells following in vitro stimulation with either YFPLQSYGF or YFPLRSYSF and recall with cognate peptide. **(D)** Frequency of IFN-g-producing CD8⁺ T cells in response to serial dilutions of YFPLQSYGF. **(E)** Crystal structure of the HLA-A*29:02-YFPLRSYSF complex with the HLA heavy chain in white cartoon and the peptide in red cartoon and stick. The P8-Gly Ca atom is

represented by a sphere, and the hydrogen bond by a red dashed line. **(F)** Model of the YFP variant peptide (blue cartoon and stick) based on the YFP peptide conformation showing the P5-Arg and P8-Ser substitutions in yellow stick. The HLA Met97, Tyr99, and Arg114 are represented as stick and transparent surface to show the steric clashes with the P5-Arg occurring if the variant adopted the same conformation as the cognate peptide. **(G)** Frequency of IFN-g-producing CD8⁺ T cells following in vitro culture of PBMC from five HLA-B*07:02 vaccinated individuals with spike overlapping peptide pools.

Supplementary Files

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