The impact of viral mutations on recognition by SARS-CoV-2 specific T cells

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Highlights
Amino acid variants in dominant SARS-CoV-2 T cell epitopes result in recognition loss

CD8+ clones with diverse T cell receptor repertoires fail to recognize variant epitopes

Ongoing surveillance for SARS-CoV-2 variants resulting in T cell evasion is important

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The impact of viral mutations on recognition by SARS-CoV-2 specific T cells

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SUMMARY
We identify amino acid variants within dominant SARS-CoV-2 T cell epitopes by interrogating global sequence data. Several variants within nucleocapsid and ORF3a epitopes have arisen independently in multiple lineages and result in loss of recognition by epitope-specific T cells assessed by IFN-γ and cytotoxic killing assays. Complete loss of T cell responsiveness was seen due to Q213K in the A*01:01-restricted CD8+ ORF3a epitope FTSDYYQLY207-215; due to P13L, P13S, and P13T in the B*27:05-restricted CD8+ nucleocapsid epitope QRNAP-RITF9-17; and due to T362I and P365S in the A*03:01/A*11:01-restricted CD8+ nucleocapsid epitope KTFPPTEPK361-369. CD8+ T cell lines unable to recognize variant epitopes have diverse T cell receptor repertoires. These data demonstrate the potential for T cell evasion and highlight the need for ongoing surveillance for variants capable of escaping T cell as well as humoral immunity.

INTRODUCTION
Evolution of SARS-CoV-2 can lead to evasion from adaptive immunity generated following infection and vaccination. Much focus has been on humoral immunity and spike protein mutations that impair the effectiveness of neutralizing monoclonal antibodies and polyclonal sera. T cells specific to conserved proteins play a significant protective role in respiratory viral infections such as influenza, particularly in broad heterosubtypic immunity (Hayward et al., 2015). T cell responses following SARS-CoV-2 infection are directed against targets across the genome and may play a role in favorable outcomes during acute infection and in immunosuppressed hosts with deficient B cell immunity (Huang et al., 2021; Peng et al., 2020; Tan et al., 2021). Although CD8+ T cells may not provide sterilizing immunity, they can protect against severe disease and limit risk of transmission, with a potentially more important role in the setting of antibody escape.

Little is known about the potential for SARS-CoV-2 mutations to impact T cell recognition. Escape from antigen-specific CD8+ T cells has been studied extensively in HIV-1 infection, where rapid intra-host evolution renders T cell responses ineffective within weeks of acute infection (Goonetilleke et al., 2009). Although these escape variants play an important role in the dynamics of chronic viral infections, the opportunities for T cell escape in acute respiratory viral infections are fewer and consequences are different. Nevertheless, several cytotoxic T-lymphocyte (CTL) escape variants have been described in influenza, such as the R384G substitution in the HLA B*08:01-restricted nucleoprotein380-388 and B*27:05-restricted nucleoprotein383-391 epitopes (Voeten et al., 2000). Long-term adaptation of influenza A/H3N2 has been demonstrated, with the loss of one CTL epitope every 3 years since its emergence in 1968 (Woolthuis et al., 2016).

RESULTS AND DISCUSSION
Amino acid variants within experimentally proven SARS-CoV-2 T cell epitopes
To explore the potential for viral evasion from SARS-CoV-2-specific T cell responses, we conducted a proof-of-concept study, focusing initially on identifying common amino acid mutations within experimentally proven T cell epitopes and testing the functional implications in selected immunodominant epitopes

Continued
that we and others have described previously. We conducted a literature review in PubMed and Scopus databases (November 29, 2020; Data S4) that identified 14 publications defining 360 experimentally proven CD4+ and CD8+ T cell epitopes (Chour et al., 2020; Ferretti et al., 2020; Gangaev et al., 2020; Habel et al., 2020; Kared et al., 2021; Keller et al., 2020; Le Bert et al., 2020; Nele et al., 2021; Peng et al., 2020; Poran et al., 2020; Schulien et al., 2021; Sekine et al., 2020; Shomuradova et al., 2020; Snyder et al., 2020). Of these, 53 that were described in ≥1 publication were all CD8+ epitopes (Table S1) and distributed across the genome (n = 14 open reading frame [ORF]) in冽, n = 5 ORF1b, n = 18 S, n = 2 M, n = 8 N, n = 5 ORF3a, n = 1 ORF7a). In total, 12,503 amino acid substitutions or deletions were identified within the 360 T cell epitopes by searching the mutation datasets downloaded from CoV-GLUE (http://cov-glue.cvr.gla.ac.uk/#/home) on July 30, 2021 (Figure S1 and Table S2). A total of 1,370 amino acid variants were present within the 53 CD8+ T cell epitopes with responses described across multiple cohorts, with at least one variant in all epitopes (Figure S2 and Table S3).

Functional impact of variants within immunodominant SARS-CoV-2 T cell epitopes

We focused on evaluating the functional impact of variants within seven immunodominant epitopes in nucleocapsid, ORF3a, and spike (five CD8+, two CD4+) described in our study of UK convalescent donors (Peng et al., 2020), along with a further immunodominant ORF1a CD8+ epitope described in several other studies (Table 1). Of these, all six CD8+ epitopes have been described in at least two cohorts. In particular, responses to the A*03:01/A*11:01-restricted nucleocapsid KTFPPTEPK361-369 (Ferretti et al., 2020; Gangaev et al., 2020; Kared et al., 2021; Peng et al., 2020) epitope, A*01:01-restricted ORF3a FTSDYQQLY207-215 (Ferretti et al., 2020; Kared et al., 2021; Peng et al., 2020; Schulien et al., 2021) epitope, and A*01:01-restricted ORF1a TTDPSFLGRY1637-1646 (Ferretti et al., 2020; Gangaev et al., 2020; Nele et al., 2021) epitope are consistently dominant and of high magnitude. We tested the functional avidity of SARS-CoV-2-specific CD4+ and CD8+ polyclonal T cell lines by interferon (IFN)-γ ELISpots using wild-type and variant peptide titrations (Figures 1A–1F). We found that several variants resulted in complete loss of responsiveness to the T cell lines evaluated: the Q213K variant in the A*01:01-restricted CD8+ ORF3a epitope FTSDYQQLY207-215 (Ferretti et al., 2020; Kared et al., 2021; Peng et al., 2020; Schulien et al., 2021); the P13L, P13S, and P13T variants in the B*27:05-restricted CD8+ nucleocapsid epitope QRNAPRITF9-17 (Nele et al., 2021; Peng et al., 2020); and T362I and P365S variants in the A*03:01/A*11:01-restricted nucleocapsid KTFPPTEPK361-369 (Ferretti et al., 2020; Gangaev et al., 2020; Nelde et al., 2021) epitope are at positions 2 and 9, T362I (position 2) may impair any partial impairment of T cell recognition is due to reduced TCR binding to MHC-peptide. Reasons for complete escape are more difficult to predict. As the anchor residues of peptide-MHC binding in the MHC to peptide, or T cell receptor (TCR) recognition of the MHC-peptide complex. Although we did not explicitly establish which of these was responsible in each case, it is likely that any partial impairment of T cell recognition is due to reduced TCR binding to MHC-peptide. Reasons for complete escape are more difficult to predict. As the anchor residues of peptide-MHC binding in the potential mechanisms of loss of T cell recognition

T cell escape can occur via interrupting several mechanisms: antigen processing, binding of major histocompatibility complex (MHC) to peptide, or T cell receptor (TCR) recognition of the MHC-peptide complex. Although we did not explicitly establish which of these was responsible in each case, it is likely that any partial impairment of T cell recognition is due to reduced TCR binding to MHC-peptide. Reasons for complete escape are more difficult to predict. As the anchor residues of peptide-MHC binding in A*03:01/A*11:01-restricted KTFPPTEPK361-369 are at positions 2 and 9, T362I (position 2) may impair
| Epitope       | ORF | CD4/CD8 | HLA       | Variant | Frequency (%) | Countries | Global lineages | Loss of T cell response | References describing epitope |
|--------------|-----|---------|-----------|---------|---------------|-----------|-----------------|--------------------------|-------------------------------|
| FTSDYQLY207-215 | 3a  | CD8     | A*01:01  | Q213K   | 0.059         | 56        | 95              | Yes                      | Ferretti et al. (2020); Kared et al., 2021; Peng et al. (2020) |
| QRNAPRITF9-17  | N   | CD8     | B*27:05  | Q9H     | 0.289         | 74        | 176             | No                       | Nelde et al. (2021); Peng et al. (2020) |
| QRNAPRITF9-17  |     |         |          | P13L    | 0.978         | 97        | 194             | Yes                      | Nelde et al. (2021); Peng et al. (2020) |
| QRNAPRITF9-17  |     |         |          | P13S    | 0.210         | 83        | 132             | Yes                      | Peng et al. (2020); Nelde et al. (2021) |
| QRNAPRITF9-17  |     |         |          | P13T    | 0.102         | 45        | 81              | Yes                      | Nelde et al. (2021); Peng et al. (2020) |
| MEVTPSGLW322-331 | N   | CD8     | B*40:01  | T325I   | 0.069         | 52        | 109             | Partial                  | Nelde et al. (2021); Peng et al. (2020); Schulien et al. (2021) |
| KTFPPTEPK361-369 | N   | CD8     | A*03:01  | A*11:01 | 0.293         | 85        | 165             | Yes                      | Ferretti et al. (2020); Gangaev et al. (2020); Kared et al., 2021; Peng et al. (2020) |
| KTFPPTEPK361-369 |     |         |          | T366I   | 0.237         | 74        | 154             | No                       | Ferretti et al. (2020); Gangaev et al. (2020); Kared et al., 2021; Peng et al. (2020) |
| KTFPPTEPK361-369 |     |         |          | P365S   | 0.794         | 73        | 142             | Yes                      | Ferretti et al. (2020); Gangaev et al. (2020); Kared et al., 2021; Peng et al. (2020) |
| KYGVSPTK378-386 | S   | CD8     | A*03:01  | P384L   | 0.085         | 59        | 116             | No                       | Ferretti et al. (2020); Peng et al. (2020) |
| CTFEVSQPFMLDLE166-180 | S  | CD4     | –        | L176F   | 0.260         | 68        | 158             | No                       | Peng et al. (2020) |
| CTFEVSQPFMLDLE166-180 |     |         |          | M177I   | 0.083         | 60        | 92              | Partial                  | Peng et al. (2020) |
| NLLLQYGSFCQNLKR751-765 | S  | CD4     | DRB1*15:01 | R765L | 0.017         | 31        | 64              | Partial                  | Peng et al. (2020) |
| TTDPSFLGRY1637-1646 | ORF1A | CD8 | A*01:01 | T1637I | 0.189         | 58        | 113             | Partial                  | Ferretti et al. (2020); Gangaev et al. (2020); Nelde et al. (2021) |
| TTDPSFLGRY1637-1646 |     |         |          | T1638I | 0.066         | 52        | 96              | Partial                  | Ferretti et al. (2020); Gangaev et al. (2020); Nelde et al. (2021) |
| TTDPSFLGRY1637-1646 |     |         |          | P1640S | 0.202         | 69        | 165             | Partial                  | Ferretti et al. (2020); Gangaev et al. (2020); Nelde et al. (2021) |
| TTDPSFLGRY1637-1646 |     |         |          | P1640L | 2.540         | 105       | 156             | Partial                  | Ferretti et al. (2020); Gangaev et al. (2020); Nelde et al. (2021) |
| TTDPSFLGRY1637-1646 |     |         |          | P1640H | 0.050         | 27        | 20              | Partial                  | Ferretti et al. (2020); Gangaev et al. (2020); Nelde et al. (2021) |

Mutated positions detailed in red within wild-type epitope sequence. Frequency indicates % of sequences where variant is seen within the SARS-CoV-2 mutation dataset downloaded from CoV-GLUE (http://cov-glue.cvr.gla.ac.uk/#/home) on July 30, 2021. Global Lineages refers to Pango lineage assignment. ORF, open reading frame; HLA, human leukocyte antigen.

*aResponses to longer peptide also seen in Snyder et al. (2020).

*bResponses to longer peptide also seen in Snyder et al. (2020) and Kared et al. (2021).
peptide-MHC binding, while P365S (position 5) may affect a T cell binding residue (Rammensee et al., 1999). The proline changes (P13L, P13S, P13T) in the B*27:05-restricted QRNAPRITF9-17 (position 5) again may be at a key T cell contact residue, as peptide-MHC binding anchor residues are at positions 2 and 9 (Rammensee et al., 1999). The anchor residues for the A*01:01-restricted FTSDYYQLY207-215 are predicted to be at positions 3 and 9, with auxiliary anchors at positions 2 and 7 (Rammensee et al., 1999), which may

Figure 1. Functional impact of mutations in key SARS-CoV-2 dominant epitopes

(A–F) Recognition of wild-type (black) and mutant (red) peptide titrations by polyclonal epitope-specific T cell lines in IFN-γ ELISpot assays. SFU, spot forming units. Shown are mean values from three or more replicates +/− standard deviation.

(G–J) Ability of CD8+ T cell lines to kill autologous B cells loaded with wild-type (black) or mutant (red) peptides in carboxyfluorescein succinimidyl ester (CFSE) assays. The effector:target ratio denotes the proportion of CD8+ T cells:B cells in each assay.

(K–N) Recognition of wild-type (black) and mutant (red) peptide titrations by a polyclonal CD8+ T cell line specific for the HLA*A01:01-restricted ORF1a epitope TTDPSFLGRY1637-1646, using intra-cellular cytokine staining for interferon-gamma (IFNγ, K), tumor necrosis factor (TNFα, L), and the degranulation factor CD107a (M), and a killing assay (N). Similar findings were seen with a T cell line generated from another donor (Figure S4).
explain the impact of the Q213K (position 7) variant. In keeping with this, we see no significant impact of these mutations on the predicted binding affinities of epitope to MHC (Table S4). Despite a modest 4-fold decrease in predicted IC50 for Q213K compared with wild type, FTSDYYKLY207-215 is still a strong binder to A*01:01.

Ex vivo IFN-γ ELISpots in two A*03:01 and two B*27:05 convalescent donors confirmed loss of responses to variant peptides seen with T cell lines specific to KTFPPTEPK361-369 and QRNAPRITF9-17 (Figure S5). Thus, our findings using T cell lines are representative of the circulating T cell response to these epitopes and of physiological relevance. Of interest, one A*03:01 donor had low-level responses to P365S and T362I, suggesting that subdominant responses via alternative TCR are possible.

**T cell receptor diversity in CD8⁺ T cell lines with loss of epitope recognition due to amino acid variants**

TCR sequencing of polyclonal CD8⁺ T cell lines specific for the FTSDYYKLY207-215 epitope and B*27:05-restricted QRNAPRITF9-17 epitope was undertaken to explore whether the complete loss of T cell recognition observed was dependent on specific TCRs. A diverse range of TCRs was found in FTSDYYKLY207-215 T cell lines from four donors (Figure 2), demonstrating that Q213K results in escape from several TCRs. Similar findings were seen in TCR data from a B*27:05-restricted QRNAPRITF9-17 T cell line from one donor (Figure S6). It is worth noting that our data are biased by using T cell lines generated from donors recruited early in the pandemic and therefore likely infected with “wild-type” viruses (i.e., lineage B or B.1 viruses) (Peng et al., 2020). Although variants that impair antigen processing or MHC-peptide binding result in irreversible loss of T cell recognition, CTLs with new TCR repertoires can overcome TCR-mediated escape variants, as has been described in HIV-1 infection (Ladell et al., 2013).

**Frequency of epitope variants over time and appearance in global SARS-CoV-2 phylogeny**

Many variants examined in our study were at relatively low frequency and stable prevalence at the time of writing, other than P365S in KTFPPTEPK361-369, P1640L in TTDPFLGKRY1637-1646, and variants affecting the proline at position 13 in QRNAPRITF9-17 (Table 1 and Figure 3A). We explored whether variants that result in loss of T cell recognition appeared as homoplasies in the phylogeny of SARS-CoV-2 suggestive of repeated independent selection, or whether global frequency is due mainly to the expansion of lineages after initial acquisition. Although in some cases variant frequency was dependent on a few successful lineages, P365S, Q213K, T362I, P13L, P13S, and P13T had arisen independently on several occasions including within the B.1.1.7 lineage (Figures 3B, 3C, 3D, 3E, S7A, and S7B). It is important to emphasize that this homoplasy and our functional data do not prove selection due to T cell escape, which would require demonstration of intra-host evolution. The positions we find important for T cell recognition may be under selective pressure for reasons other than T cell immunity. A recent study has documented intra-host evolution of minority variants within A*02:01 and B*40:01 CD8⁺ epitopes that impair T cell recognition, although not all epitopes are dominant and very few of the variants studied were represented among the global circulating viruses (Agerer et al., 2021).

**Conclusions**

There is unlikely to be adequate population immunity at present to see global changes due to T cell selection akin to what has been seen in adaptation of H3N2 influenza over time (Woolthuis et al., 2016). Furthermore, polymorphism in HLA genes restricts the selective advantage of escape within one particular epitope to a relatively small proportion of the population, given the breadth in T cell responses we and others have shown. The polyclonal T cell response in a given individual is therefore unlikely to be diminished significantly by mutations present in any one circulating variant, unlike the potential impact on neutralizing antibody responses seen with mutations in the spike protein. Nevertheless, responses to many of the CTL epitopes we have studied are dominant within HLA-matched individuals across many cohorts (Peng et al., 2020). As A*03:01, A*11:01, and A*01:01 are common HLA alleles globally, loss of T cell responses to dominant epitopes such as KTFPPTEPK361-369 and FTSDYYQLY207-215 may be significant. Substitution of three different amino acid variants at nucleocapsid position 13 within the B*27:05-restricted QRNAPRITF9-17 epitope is also striking and suggests significant positive selective pressure at this site. Successful maintenance of these substitutions within some lineages also suggest that this is a position where such amino acid changes are tolerated with limited impact on the virus life cycle. A single dominant, protective B*27:05-restricted epitope has been described in HIV-1 infection, with T cell escape associated with progression to AIDS. T cell escape from a B*27:05-restricted influenza A epitope (nucleoprotein383-391) has also been observed (Voeten et al., 2000).
Figure 2. T cell receptor (TCR) repertoire of polyclonal CD8+ T cell lines specific for A*01:01-restricted ORF3a epitope FTSDYYKLY207-215

(A, C, E, and G) Data are shown from T cell lines generated using peripheral blood mononuclear cells from four donors. The Q213K variant (red) showed complete loss of recognition in each case using peptide titrations in IFN-γ ELISpot assays compared with recognition of the wild-type (black) peptide. Shown are mean values from three or more replicates +/- standard deviation. SFU, spot forming units.

(B, D, F, and H) A diverse range of TCRs was observed.
Figure 3. **Global presence of variants in key dominant SARS-CoV-2 epitopes**

(A) Weekly frequency over time since beginning of SARS-CoV-2 pandemic of all variants studied in functional experiments. Mutation counts were obtained from COG-UK global metadata (dated August 4, 2021). Variants named with
A significant increase in sites under diversifying positive selective pressure was observed around November 2020, most notably in ORF3a, N, and S (Martin et al., 2021). As vaccine and naturally acquired population immunity increase further, the frequency of variants we have described should be monitored globally, as well as further changes arising within all immunodominant T cell epitopes. We have recently incorporated the ability to identify spike T cell epitope variants in real-time sequence data into the COG-UK mutation explorer dashboard (http://sars2.cvr.gla.ac.uk/cog-uk/). Non-spike T cell immune responses will also become increasingly important to vaccine-induced immunity as inactivated whole-virus vaccines are rolled out. Our findings demonstrate the potential for T cell evasion and highlight the need for ongoing surveillance for variants capable of escaping T cell as well as humoral immunity.

Limitations of the study

We have chosen to focus on key SARS-CoV-2 immunodominant epitopes characterized early in the pandemic, and further epitopes have been identified since. It would be important to assess mutations of increasing prevalence within all immunodominant epitopes in the future to provide a comprehensive overview of potential SARS-CoV-2 T cell escape. Although our findings suggest that reduced T cell receptor binding to MHC-epitope complex is likely responsible for the most striking impact of mutations on T cell responses we describe, this needs to be demonstrated experimentally. Finally, further studies are required to demonstrate the occurrence of T cell escape within individuals and establish how frequently this occurs. Given the potential for immune escape in prolonged or chronic SARS-CoV-2 infections that could give rise to new variants of concern, a focus on infections in immunocompromised individuals would be important.

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2021.103353.
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AUTHOR CONTRIBUTIONS

T.I.d.S. and T.D. conceptualized the project; T.D., T.I.d.S., L.T., and Y.P. designed and supervised T cell experiments; B.B.L., N.S.H., and M.D.P. conducted the viral sequence analyses; D.S. conducted the literature review and collated T cell epitope information; G.L., D.D., and S.C.M. performed experiments and analyzed the data; X.Y., Z.Y., A.A., and R.B. provided critical reagents and technical assistance; J.C.K. and A.J.M. established clinical cohorts; T.I.d.S. and T.D. wrote and edited the original draft; all co-authors reviewed and edited the manuscript.

DECLARATION OF INTERESTS

The authors have no competing interests to declare.

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REFERENCES

Agerer, B., Koblischke, M., Gudipati, V., Montano-Gutierrez, L.F., Smyth, M., Popa, A., Genger, J.W., Endler, L., Florian, D.M., Muhlgrabner, V., et al. (2021). SARS-CoV-2 mutations in MHC-I-restricted epitopes evade CD8(+) T cell responses. Sci. Immunol. 6, eabg6461. https://doi.org/10.1126/sciimmunol. eabg6461.

Chour, W., Xu, A.M., Ng, A.H.C., Choi, J., Xie, J., Yuan, D.; DeLucia, D.C., Edmark, R.A., Jones, L.C., Schmidt, T.M., et al. (2020). Shared antigen-specific CD8+ T cell responses against the SARS-CoV-2 spike protein in HLA-A*02:01 COVID-19 patients. Res. Sq. https://doi.org/10.21203/rs.3.r35197/v1.

Goonetilleke, N., Liu, M.K., Salazar-Gonzalez, J.F., Ferrari, G., Giorgi, E., Ganusov, V.V., Kleele, B.F., Lear, G.H., Turnbull, E.L., Salazar, M.G., et al. (2009). The first T cell response to transmitted/founder virus contributes to the control of acute viremia in HIV-1 infection. J. Exp. Med. 206, 1253–1272. https://doi.org/10.1084/jem.20090365.

Habel, J.R., Nguyen, T.H.O., van de Sandt, C.E., Juno, J.A., Chaurasia, P., Wragg, K., Koutsakos, M., Hensen, L., Jia, X., Chua, B., et al. (2020). Suboptimal SARS-CoV-2-specific CD8(+) T cell response associated with the prominent HLA-A*02:01 phenotype. Proc. Natl. Acad. Sci. U S A 117, 24384–24391. https://doi.org/10.1073/pnas. 2015486117.

Habermeyer, A., Dunlap, G.S., Xu, Q., Nabilsi, N., Ferretti, A.P., Kula, T., Wang, Y., Nguyen, D.M.V., Yuan, D., DeLucia, D.C., Edmark, R.A., Jones, L.C., Schmitt, T.M., et al. (2020). Shared antigen-specific CD8(+) T cell responses. Sci. Immunol. 6, eabg6461. https://doi.org/10.1126/sciimmunol. eabg6461.

Kim, C., Guaraldi, G., Girardis, M., Ormeno, C.M.P.T., Gangaev, A., Genger, J.W., Endler, L., Florian, D.M., Smyth, M., Popa, A., Genger, J.W., Endler, L., Florian, D.M., Muhlgrabner, V., et al. (2021). SARS-CoV-2 mutations in MHC-I-restricted epitopes evade CD8(+) T cell responses. Sci. Immunol. 6, eabg6461. https://doi.org/10.1126/sciimmunol. eabg6461.

Chour, W., Xu, A.M., Ng, A.H.C., Choi, J., Xie, J., Yuan, D.; DeLucia, D.C., Edmark, R.A., Jones, L.C., Schmidt, T.M., et al. (2020). Shared antigen-specific CD8+ T cell responses against the SARS-CoV-2 spike protein in HLA-A*02:01 COVID-19 patients. Res. Sq. https://doi.org/10.21203/rs.3.r35197/v1.

Goonetilleke, N., Liu, M.K., Salazar-Gonzalez, J.F., Ferrari, G., Giorgi, E., Ganusov, V.V., Kleele, B.F., Lear, G.H., Turnbull, E.L., Salazar, M.G., et al. (2009). The first T cell response to transmitted/founder virus contributes to the control of acute viremia in HIV-1 infection. J. Exp. Med. 206, 1253–1272. https://doi.org/10.1084/jem.20090365.

Habel, J.R., Nguyen, T.H.O., van de Sandt, C.E., Juno, J.A., Chaurasia, P., Wragg, K., Koutsakos, M., Hensen, L., Jia, X., Chua, B., et al. (2020). Suboptimal SARS-CoV-2-specific CD8(+) T cell response associated with the prominent HLA-A*02:01 phenotype. Proc. Natl. Acad. Sci. U S A 117, 24384–24391. https://doi.org/10.1073/pnas. 2015486117.

Hayward, A.C., Wang, L., Goonetilleke, N., Fragaszy, E.B., Bermingham, A., Copas, A., Dukes, O., Millett, E.R., Nazareth, I., Nguyen-Van-Tam, J.S., et al. (2015). Natural T cell-mediated protection against seasonal and pandemic influenza. Results of the flu watch cohort study. Am. J. Respir. Crit. Care Med. 191, 1422–1431. https://doi.org/10.1164/rrc. 201411-1988OC.

Huang, A., Banga, E., Han, N., Wileyto, E.P., Kim, J., Gouma, S., Robinson, J., Greenplate, A., Porterfield, F., Owoyemi, O., et al. (2021). CD8 T cells compensate for impaired humoral immunity in COVID-19 patients with hematologic cancer. Res. Sq. https://doi.org/10.21203/rs.3.rs-162289/v1.

Kared, H., Redd, A.D., Bloch, E.M., Bonny, T.S., Sumatoh, H.R., Kairi, F., Carbajo, D., Abel, B., Newell, E.W., Bettinotti, M., et al. (2021). SARS-CoV-2-specific CD8+ T cell responses in convalescent COVID-19 individuals. J. Clin. Invest. https://doi.org/10.1172/JCI145476.
COVID-19. Nat. Immunol. 21, 1336–1345. https://doi.org/10.1038/s41590-020-0782-2.

Peng, Y., Wang, B., Talaat, K., Karron, R., Powell, T.J., Zeng, H., Dong, D., Luke, C.J., McMichael, A., Subbarao, K., and Dong, T. (2013). Boosted influenza-specific T cell responses after H5N1 pandemic live attenuated influenza virus vaccination. Front. Immunol. 6, 287. https://doi.org/10.3389/fimmu.2015.00287.

Poran, A., Harjanto, D., Malloy, M., Arieta, C.M., Rothenberg, D.A., Lenkala, D., van Buuren, M.M., Addiona, T.A., Rooney, M.S., Srivivasan, L., and Gaynor, R.B. (2020). Sequence-based prediction of SARS-CoV-2 vaccine targets using a mass spectrometry-based bioinformatics predictor identifies immunogenic T cell epitopes. Genome Med. 12, 70. https://doi.org/10.1186/s13073-020-00767-w.

Rammensee, H., Bachmann, J., Emmerich, N.P., Bachor, O.A., and Stevanovic, S. (1999). SYFPEITHI: database for MHC ligands and peptide motifs. Immunogenetics 50, 213–219. https://doi.org/10.1007/s002510050595.

Reynisson, B., Alvarez, B., Paul, S., and Peters, B. (2020). NetMHCpan-4.1 and NetMHCIIpan-4.0: improved predictions of MHC antigen presentation by concurrent motif deconvolution and integration of MS MHC eluted ligand data. Nucleic Acids Res. 48, W449–W454.

Schulien, I., Kemming, J., Oberhardt, V., Wild, K., Seidel, L.M., Killmer, S., Sagar, Daul, F., Salvat Lago, M., Decker, A., et al. (2021). Characterization of pre-existing and induced SARS-CoV-2-specific CD8(+T) cell responses. Nat. Med. 27, 78–85. https://doi.org/10.1038/s41591-020-01143-2.

Sekine, T., Perez-Potti, A., Rivera-Ballesteros, O., Stralin, K., Gorin, J.B., Olsson, A., Llewellyn-Lacey, S., Kamal, H., Bogdanovic, G., Muschiol, S., et al. (2020). Robust T cell immunity in convalescent individuals with asymptomatic or mild COVID-19. Cell 183, 158–168.e14. https://doi.org/10.1016/j.cell.2020.08.017.

Shomuradova, A.S., Vagida, M.S., Sheetsik, S.A., Zornikova, K.V., Kuryukhin, D., Titov, A., Peshкова, I.O., Khnelevskaia, A., Dianov, D.V., Malasheva, M., et al. (2020). SARS-CoV-2 epitopes are recognized by a public and diverse repertoire of human T cell receptors. Immunity 53, 1245–1257.e5. https://doi.org/10.1016/j.immuni.2020.11.004.

Snyder, T.M., Gittelman, R.M., Klinger, M., May, D.H., Osborne, E.J., Taniguchi, R., Zahid, H.J., Kaplan, I.M., Dines, J.N., Noakes, M.N., et al. (2020). Magnitude and dynamics of the T-cell response to SARS-CoV-2 infection at both individual and population levels. medRxiv. https://doi.org/10.1101/2020.07.31.20165647.

Tan, A.T., Linster, M., Tan, C.W., Le Bert, N., Chia, W.N., Kunasegaran, K., Zhuang, Y., Tham, C.Y.L., Chia, A., Smith, G.J.D., et al. (2021). Early induction of functional SARS-CoV-2-specific T cells associates with rapid viral clearance and mild disease in COVID-19 patients. Cell Rep. 34, 108728. https://doi.org/10.1016/j.celrep.2021.108728.

Voeten, J.T., Bestebroer, T.M., Nieuwkoop, N.J., Foucher, R.A., Osterhaus, A.D., and Rimmelzaaen, G.F. (2000). Antigenic drift in the influenza A virus (H3N2) nucleoprotein and escape from recognition by cytotoxic T lymphocytes. J. Virol. 74, 6800–6807. https://doi.org/10.1128/jvi.74.15.6800-6807.2000.

Woolthuis, R.G., van Dorp, C.H., Kesmir, C., de Boer, R.J., and van Boven, M. (2016). Long-term adaptation of the influenza A virus by escaping cytotoxic T-cell recognition. Sci. Rep. 6, 33334. https://doi.org/10.1038/srep33334.
## STAR METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies** |
| PE-Cy7 anti-human CD107a (H4A3) | BD Biosciences, UK | Cat#561348, RRID:AB_10644018 |
| BV510 anti-human CD3 (UCHT1) | BD Biosciences, UK | Cat#563109, RRID:AB_2732053 |
| BV421 anti-human CD8 (RPA-T8) | BD Biosciences, UK | Cat#562428, RRID:AB_11154035 |
| PE anti-human TNFa (MAb11) | Thermo Fisher Scientific | Cat#12-7349-82, RRID:AB_466208 |
| FITC anti-human IFNg (45-15) | Miltenyi Biotec Ltd | Cat# 130-091-641, RRID:AB_244194 |
| FITC anti-human CD4 (SK3&SK4) | BD Bioscience, UK | Cat#347413, RRID:AB_400297 |
| FITC anti-human CD8 (SK1) | BD Bioscience, UK | Cat#347313, RRID:AB_400279 |
| BV421 anti-human CD19 (HIB19) | Biolegend, UK | Cat#302234, RRID: AB_10897802 |

| **Chemicals, peptides, and recombinant proteins** |
| Synthesized peptides | GenScript Biotech, Netherlands | NA |
| QRNAPRITF-B*27:05 pentamer | Proimmune | Cat#4354 |
| KTFPPTEPK-A*03:01 pentamer | Proimmune | Cat#4356A |
| MEVTPSGTWL-B*40:01 pentamer | Proimmune | Cat#4328 |
| FTSDYYQLY-A*0101 pentamer | Proimmune | Cat#4355 |
| KTFFPTEPK-A*11:01 pentamer | Proimmune | Cat#4356B |
| NLLLQYGSFCTQLNR-DRB1*0101 tetramer | Proimmune | NA (Custom) |
| IL-2/TCGF | Helvetica healthcare | Cat#0801017 |
| IL-7 | Biotechnie, UK | Cat#207-IL |

| **Critical commercial assays** |
| Human IFN-γ ELISpot BASIC kit | Mabtech | Cat#3420-2A |
| SMARTer RACE 5’/3’ kit | TaKaRa | Cat#634858 |
| Monarch DNA gel extraction kit | New England BioLabs | Cat#10205 |
| TOPO™ TA Cloning™ kit for sequencing | Thermo Fisher Scientific | Cat#K457501 |
| RNeasy plus mini kit | Qiagen | Cat#74134 |
| Advantage2 PCR kit | TaKaRa | Cat#639207 |
| LIVE/DEAD fixable near-IR dead cell stain kit | Thermo Fisher Scientific | Cat#L34975 |
| CellTrace™ CFSE cell proliferation kit | Thermo Fisher Scientific | Cat#C34554 |
| CellTrace™ violet cell proliferation kit | Thermo Fisher Scientific | Cat#C34557 |
| eBioscience™ 7-AAD viability staining solution | Thermo Fisher Scientific | Cat#00-6993-50 |
| QIAprep spin miniprep kit | Qiagen | Cat#27106 |

| **Deposited data** |
| GLUE mutation dataset – replacement (In supplementary information Mutation_identification, input_data) | CoV-GLUE | http://cov-glue.cvr.gla.ac.uk/#/home |
| GLUE mutation dataset – deletion (In supplementary information Mutation_identification, input_data) | CoV-GLUE | http://cov-glue.cvr.gla.ac.uk/#/home |

(Continued on next page)
**RESOURCE AVAILABILITY**

**Lead contact**
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Thushan de Silva (t.desilva@sheffield.ac.uk).

**Materials availability**
This study did not generate new unique reagents.

**Data and code availability**
- Additional Supplemental Items are available from Mendeley Data at https://data.mendeley.com/datasets/8gyvpj4wsc/draft?a=b1ce80de-e208-443e-a839-6b1852dabf63
- Code and data used for identifying mutations within T cell epitopes are provided in Data S1 Mutation identification, related to all figures. The analysis folder contains an R code used for data manipulation
and two sub-folders: input_data and output. Mutation datasets downloaded from CoV-GLUE are provided in the input_data sub-folder.

- Code and data used for plotting the variant prevalence over time are provided in Data S2 Variant prevalence, related to Figure 3. The analysis folder contains an R code and two sub-folders: input_data and output. Mutation counts obtained from COG-UK global metadata are provided in the input_data folder.

- Code and data used for plotting the global phylogenies representation are provided in Data S3 Tree visualisation, related to Figure 3. The analysis folder contains an R code and two sub-folders: input_data and output. COG-UK metadata and lists of sequences with our mutations of interest are provided in the input_data sub-folder.

- Any additional information required to re-analyze the data reported in this paper is available from the lead contact upon request.

The graphical abstract was created with Biorender.

**EXPERIMENTAL MODEL AND PARTICIPANT DETAILS**

**Participants**

SARS-CoV-2 recovered donors were recruited in Oxford into the Sepsis Immunomics study (Ref 13/SC/0296) and in Liverpool into the ISARIC study (Ref 13/SC/0149). Both studies were granted ethical approval from the South Central - Oxford C Research Ethics Committee in England. The age and sex of donors used in this study are detailed below.

| Participant | Sex | Age |
|-------------|-----|-----|
| Donor1      | M   | 73  |
| Donor2      | F   | 45  |
| Donor3      | M   | 56  |
| Donor4      | F   | 57  |
| Donor5      | M   | 69  |
| Donor6      | M   | 53  |
| Donor7      | M   | 61  |
| Donor8      | M   | 50  |
| Donor9      | F   | 44  |
| Donor10     | M   | 46  |
| Donor11     | F   | 58  |

**Isolation of peripheral blood mononuclear cells**

Blood from participants was collected in EDTA anticoagulant tubes, layered onto an equal volume of lymphoprep (Stemcell) in a falcon tube at room temperature, then centrifuged at 800 × g for 20 min at room temperature with the brake off. Peripheral Blood Mononuclear Cells (PBMCs) were aspirated at the plasma:lymphoprep interface and washed twice with RPMI medium.

Isolated PBMCs were cultured in RPMI (GIBCO) with 10% (v/v) fetal bovine serum (FBS), 100 units/mL penicillin, 0.1 mg/mL streptomycin at 37°C in 5% carbon dioxide (CO₂) and used to generate polyclonal T cell lines and lymphoblastoid cell lines.

**Generation of polyclonal T cell lines**

1–2 million PBMCs were seeded per well in a 24-well plate in RPMI (GIBCO) with 10% (v/v) human serum, 10% (v/v) IL-2/TCGF (Helvética healthcare), 5 ng/mL IL-7 (Biotechnne). Peptides were added at 10 µg/mL. Cells were fed on day 4 or earlier if media the turned yellow and then every 4 days. On day 14, antigen-specific CD8+ T-cells were sorted with pentamer staining and CD4+ T-cells were sorted using tetramer staining. Subsequently, sorted cells were plated in a 96-well U-bottom plate with 100–1000 cells/well and fed with 200,000 irradiated allogeneic PBMCs with 50µg/mL phytohemagglutinin (PHA).
Generation of lymphoblastoid cell lines

2–2.5 million PBMC were resuspended in 1 mL supernatant from B95-8 cells and added to 24 well plate, then incubated 4–5 h at 37°C in 5% CO₂. Following incubation 1 mL of RPMI (GIBCO) with 20% (v/v) FBS, 100 units/mL penicillin, 0.1 mg/mL streptomycin was added to each well and ciclosporin A (CSA) added to a final concentration of 100 ng/mL. Cells were fed every 4–6 days and lines expanded when required.

Identification of amino acid variants within T cell epitopes

Variants within the 360 experimentally proven T cell epitopes were identified using mutation datasets downloaded from CoV-GLUE (http://cov-glue.cvr.gla.ac.uk/#/home) on the 30th July 2021. Both amino acid substitutions and deletions were considered in this study. Sequences were excluded if they did not contain a start and/stop codon at the beginning and end of each ORF. COG-UK global metadata downloaded on 04th August 2021 was used to plot the variant over time (Figure 2A). Sequence positions mentioned in this study are relative to Wuhan-Hu-1 (GenBank accession MN908947.3) and were compared using custom R scripts (R version 3.5.3).

Peptide titrations using T cell lines and IFN-γ ELISpot assays

Polyclonal CD4+ and CD8+ T cell lines specific for seven previously described immunodominant epitopes (Peng et al., 2020) were generated after MHC class I Pentamer or MHC class II tetramer sorting from cultured short-term cultures of SARS-CoV-2 recovered donor PBMCs. Antigen-specific T-cells were confirmed by corresponding Pentamer or tetramer staining. T-cells were stained with Live/Dead dye (Thermo Fisher Scientific, UK), then stained with pentamer or tetramer, followed by CD8-FITC (BD Bioscience, UK) or CD4-FITC (BD Bioscience, UK) staining. The functional avidity of T cell lines was assessed by IFN-γ ELISpot assays (Peng et al., 2015). T cell lines were stimulated with wild-type and variant peptide-pulsed autologous B-cells, starting at 10 μg/mL and serial 1:5 dilutions using 400 cells T-cells and 20,000 B-cells per condition at 37°C for 6 h. Peptides were synthesised by GenScript Biotech (Netherlands) B.V. To quantify antigen-specific responses, spots of the control wells, containing no peptide, were subtracted from test wells and results expressed as spot forming units (SFU) per 400 T-cells. If negative control wells had >30 SFU/T-cells or positive control (PHA) were negative, results were considered invalid. Duplicate wells were used for each test and results are from three to seven independent experiments.

Ex vivo IFN-γ ELISpots in SARS-CoV-2 recovered donors

Cryopreserved PBMCs were used from SARS-CoV-2 recovered donors for ex vivo IFN-γ ELISpots with wild-type and variant peptides. Peptides were added to 200,000 PBMCs at a final concentration of 2 μg/mL for 16–18 h (two replicates per condition). Results were interpreted as detailed above. PBMCs used were from samples taken when patients were between 35 and 53 days from symptom onset.

Peptide titrations using T cell lines and intra-cellular cytokine staining

The functional avidity of polyclonal CD8+ T cell lines specific for the ORF1a epitope TTDPSFLGRY1637-1646 (Ferretti et al., 2020; Gangav et al., 2020; Nelde et al., 2021) was assessed using stimulation with wild-type and variant peptides starting at 1000 nM and serial 1:10 dilutions, followed by intra-cellular cytokine staining (ICS). 1–1.5 × 10⁶ cells were plated in R10 in a 196 well U-bottom plate and peptide added. DMSO was used as the negative control at the equivalent concentration to the peptides. Degranulation of T cells (a functional marker of cytotoxicity) was measured by the addition of an anti-CD107a-PE-Cy7 antibody (clone H4A3, BD Biosciences) during the culture. Cells were then incubated at 37°C, 5% CO₂ for 1 h before adding Brefeldin A (10 μg/mL). Samples were incubated at 37°C, 5% CO₂ for a further 5 h before proceeding with staining for flow cytometry. Cells were stained with a cell viability dye (near infrared, Thermo Fisher Scientific, UK) at 1:500 then fixed in 2% formaldehyde for 20 min, followed by permeabilization with 1× Perm/Wash buffer (BD Biosciences). Staining was performed with the following antibodies: anti-CD3-BV510 (clone UCHT1, BD Biosciences), anti-CD8-BV421 (clone RPA-T8, BD), TNF-PE (clone MAb11, Thermo Fisher Scientific) and anti-IFN-γ FITC (clone 45-15, Miltenyi Biotec Ltd, UK). Samples were run on a FacsCanto II cytometer and the data were analyzed using FlowJo software version 10 (BD Biosciences). During analysis, exclusion of doublet cells was performed, followed by gating on live peripheral blood mononuclear cells and estimation of the % of CD3+CD8+ T-cells expressing cytokines at each peptide concentration.
Cytotoxic T-lymphocyte (CTL) killing assays

Killing assays were performed in one of two ways. (1) For T cell lines characterised using IFN-γ ELISpot assays, autologous B-cells were stained with 0.5 μmol/L carboxyfluorescein succinimidyl ester (CFSE, Thermo Fisher Scientific) before wild-type or variant peptide loading at 1 μg/mL for one hour. Peptide-loaded B-cells were co-cultured with CTLs at a range of effector:target (E:T) ratios from 1:4 to 8:1 at 37°C for 6 h and cells stained with 7-AAD (eBioscience, UK) and CD19-BV421 (clone HIB19, Biolegend, UK). Assessment of cell death in each condition was based on the CFSE/7-AAD population present. (2) For the ORF1a epitope TTDPSFLG (Ferretti et al., 2020; Gangaev et al., 2020; Nelde et al., 2021) characterised using ICS, appropriately HLA-matched peptide-loaded B-cells were used as target cells and labeled with CFSE according to the manufacturer’s protocol. Briefly, cells were pulsed with varying concentrations of peptide (1000 nM followed by 1:10 serial dilutions) for one hour at 37°C. Unpulsed cells were labeled with Cell Trace violet (CTV; Molecular Probes). Pulsed and unpulsed cells were mixed in a 1:1 ratio and 50 ul added to 50 ul of peptide-specific short-term T cell lines and incubated in a 96 well plate for 10 h at an effector/target ratio of 10:1 in duplicates. After incubation, cells were stained with near IR viability marker, CD3 (clone UCHT1; eBioscience), CD8 (clone RPA-T8; eBioscience), and CD19 (clone LT19; Mitenyi Biotec). The mean percent survival of CFSE-labelled cells in wells containing no effector cells was used to calculate the expected frequency of target cells in each well: expected ratio (ER) was calculated as %CFSE+/%CTV-. The specific killing was then calculated as: % Specific killing = [(ER x %CTV+ cells) - %CFSE + cells]/(ER x %CTV+ cells).

Predictions of binding strength of peptides to MHC

NetMHCpan 4.1 (http://www.cbs.dtu.dk/services/NetMHCpan/, Reynisson et al., 2020) was used to predict the binding strength of wild type and variant epitopes under standard settings (strong binder % rank 0.5, weak binder % rank 2). The predicted affinity (IC50 nM) for variant epitopes was compared with wild type.

T cell receptor (TCR) sequencing

One million cells from each epitope-specific polyclonal CD8+ T-cell line were harvested and washed three times with Phosphate Buffered Saline. Total RNA was extracted using the RNeasy Plus Mini kit (Qiagen, Germany), and cDNA was then synthesized from 300 ng RNA using the SMARTer RACE cDNA amplification kit (Takara Bio, Japan) following the manufacturer’s instruction. Subsequently, cDNA was amplified for variable regions of the TCR-β chain using the PCR Advantage kit (Takara Bio), with the primer 5'-TGCT TCGATGACCTCAACACAGCGACCT-3' and run on a 1.2% agarose gel for PCR band confirmation (at 500 bp). PCR products were purified using the Monarch DNA Gel Extraction kit (New England BioLabs, USA) and then transformed into TOP10 competent cells (ThermoFisher). Plasmid DNA was extract using the Spin Miniprep kit (Qiagen) followed by Sanger sequencing.

Phylogenetic tree generation

Phylogenies were generated using the grapevine pipeline (https://github.com/COG-UK/grapevine) based on all data available on GISAID and COG-UK up until 8th August 2021. To visualise all sequences with a specific amino acid variant of interest in a global context, a representative sample of global sequences was obtained in two steps. First, one sequence per country per epi week was selected randomly, followed by random sampling of the remaining sequences to generate a sample of 6000 down-sampled sequences. The global tree was then pruned using code adapted from the tree-manip package (https://github.com/josephhughes/tree-manip). The tips of sequences with amino acid variants impacting T cell recognition were colour-coded. Visualisations were produced using R/ape, R/ggplot2, R/ggtree, R/treeio, R/phangorn, R/stringr, R/dplyr, R/aplot.

QUANTIFICATION AND STATISTICAL ANALYSIS

Mean and standard deviation for replicates used in T cell experiments were calculated and plotted using GraphPad Prism version 9. No quantitative statistical analysis was undertaken in this manuscript.