Impaired CD4+ T cell recognition of SARS-CoV-2 variants of concern

We isolated CD4+ T cell clones from healthcare workers infected with SARS-CoV-2 during the first COVID-19 wave and identified 21 epitopes across three viral proteins: spike, membrane and nucleoprotein. Focusing on spike protein, for seven of ten epitopes mutated in variants of concern, we found that T cell recognition was impaired.

Publisher's note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

The problem

Evolution of the coronavirus SARS-CoV-2 has led to the emergence of variants of concern (VOCs) that evade antibody responses. T cells restrict SARS-CoV-2 infection and limit COVID-19 severity[1], although the impact of VOC mutations on T cell recognition is unknown. CD4+ T cells orchestrate antiviral immunity, but a lack of defined CD4+ T cell epitopes and the imprecision of in silico prediction of human leukocyte antigen (HLA) class II–restricted epitopes has limited understanding of the impact of SARS-CoV-2 mutations at the epitope level. Ex vivo assays using peptide mixtures that span whole antigens can be used to measure the overall frequency of CD4+ T cell responses to a particular antigen but do not reveal the number and identity of the constituent epitopes and their HLA class II restriction. The use of high peptide concentrations in such assays may also mask the effects of mutations on T cell recognition[2]. More knowledge of SARS-CoV-2-specific CD4+ T cell immunity at the level of defined epitopes is needed to understand the impact of viral mutations on CD4+ T cell surveillance.

The solution

We recruited healthcare workers who were infected with SARS-Cov2 during the first wave of the COVID-19 pandemic in the United Kingdom. Recruited participants had memory responses to the main CD4+ T cell targets: spike protein, membrane protein and nucleoprotein[3]. In peripheral blood mononuclear cell samples that had been depleted of CD8+ T cells, screening with 186 individual 20–amino acid peptides, covering the entire sequence of these proteins, revealed that every donor had CD4+ T cell responses to multiple epitopes. We generated 159 CD4+ T cell clones and used these to define the optimal peptide and HLA class II restriction of 21 epitopes within spike protein, membrane protein and nucleoprotein. Responses to the 17 epitopes identified within spike protein were detected in multiple donors with the appropriate HLA class II restriction allele.

This work used donors infected with the ancestral wild-type (D614G) strain of SARS-CoV-2 and peptides based on this virus sequence. To explore whether viral evolution impaired T cell recognition, we exposed clones to varying concentrations of wild-type epitope peptide and the same peptide containing mutations found in VOCs (Fig. 1). Strikingly, we found that T cell recognition was impaired for seven of ten spike protein epitopes containing mutations in VOCs. A single mutation in some epitopes was sufficient to abolish recognition by CD4+ T cells, whereas for other epitopes, multiple mutations had no impact. Notably, for several epitopes, T cell stimulation was equivalent at the high concentration used in ex vivo screening assays but was impaired at the lower levels of peptide more likely to be present in vivo. Ex vivo testing of peripheral blood mononuclear cell samples that had been depleted of CD8+ T cells confirmed that the effects of epitope mutations were not limited to a single T cell antigen receptor but also affected the circulating polyclonal response comprising multiple different T cell antigen receptors.

The implications

The fact that each person convalescing from SARS-CoV-2 infection or vaccinated against SARS-CoV-2 had memory CD4+ T cell responses to multiple epitopes probably allowed CD4+ T cell recognition of the current VOCs. However, our data clearly show that certain spike protein epitopes in wild-type SARS-CoV-2 have already been lost in VOCs.

Understanding the impact of the evolution of SARS-CoV-2 on T cell surveillance is important because further epitope loss is likely as the virus continues to evolve. Our work shows that a complete understanding will require a detailed map of experimentally verified T cell epitopes and the amino acids essential for their recognition. We highlight the fact that the number of amino acid mutations in an epitope is, by itself, an unreliable guide to epitope loss; furthermore, we tested the NetMHCIIpan-4.0 binding-prediction algorithm[4] and found it did not predict several of the epitopes we identified.

Whether the mutations we observed in the spike protein epitopes were driven by antibody-mediated immune pressure, selection to increase viral fitness or T cell surveillance is unknown. We focused on spike protein on the basis of its use in vaccination against COVID-19 and its high mutation rate. Other viral proteins targeted by T cells[5] are less prone to mutation, given SARS-CoV-2 sequencing data and their high level of conservation with other human β-coronaviruses. Extending our T cell epitope mapping across the SARS-CoV-2 proteome will allow us to better understand the risks presented by emerging SARS-CoV-2 variants and the mechanisms that drive viral evolution. It will also provide a valuable resource for the rational design of future vaccines against COVID-19 and for assessing the risk of emerging SARS-CoV-2 isolates.

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EXPERT OPINION

"This is a very timely report on CD4+ T cell responses against SARS-CoV-2 in convalescent healthcare workers. Strengths of the study are the high clinical and biological relevance, as well as the deep characterization of CD4+ T cell clones." **Marcus Buggert, Karolinska Institute, Stockholm, Sweden.**
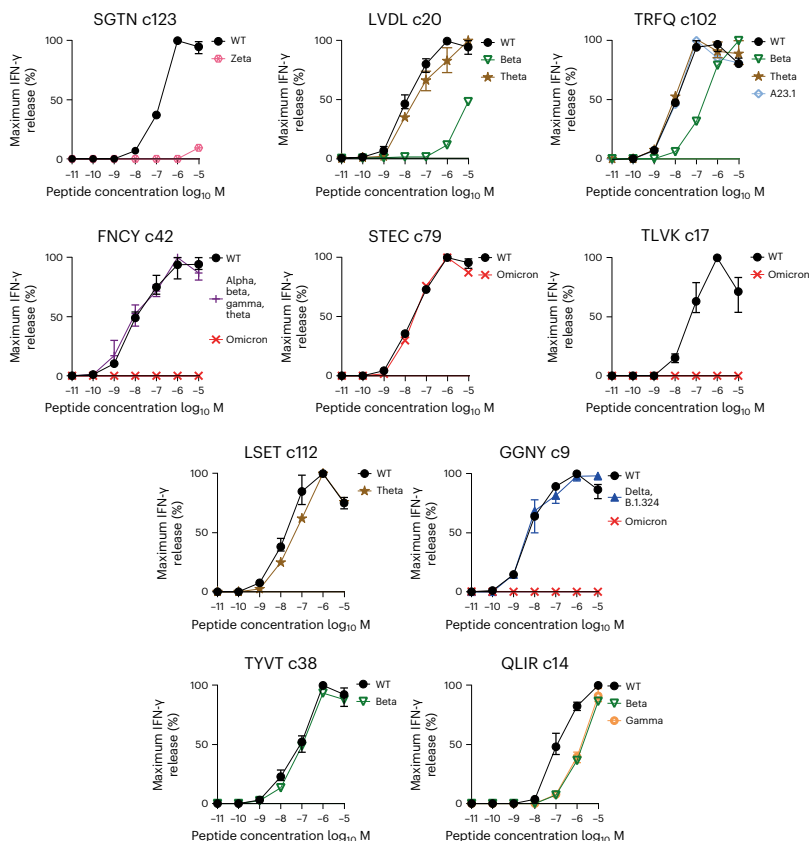
FIGURE

Fig. 1 | Spike protein–specific recognition of wild-type and variant epitope peptides by CD4+ T cell clones. ELISAs measuring interferon-γ (IFN-γ) production by SARS-CoV-2 spike protein–specific CD4+ T cell clones co-cultured with autologous lymphoblastoid cell lines exposed to 20–amino acid peptides. T cell clones, identified (above plots) by the first four amino acids of the epitope, were tested in parallel with wild-type (WT) peptide and corresponding peptides from various SARS-CoV-2 VOCs (keys). Results are presented as a percentage of the maximal interferon-γ produced against the wild-type peptide. © 2022, Tye, E. X. C. et al.

BEHIND THE PAPER

Our research groups have a longstanding interest in T cell control of Epstein–Barr virus and CD4+ T cell immunity. Working under pandemic conditions, we applied our expertise and established assays to SARS-CoV-2. Although they are difficult to isolate, T cell clones have proven to be valuable tools for the study of anti-viral immunity. Using CD4+ T cell clones, we were able to identify and carefully delineate the T cell response to SARS-CoV-2 and the impact of VOC mutations. Going forward, we will use our clones as tools for studying the processing of SARS-CoV-2 antigens and T cell recognition of infected cells. For Epstein–Barr virus, our groups have successfully used T cell clones to generate novel insights into how the immune system controls viral infection and the relative importance of different viral proteins and epitopes as T cell targets. We anticipate the same will be true for SARS-CoV-2. **G.S.T. and H.M.L.**

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FROM THE EDITOR

"CD4+ T cells specific for SARS-CoV-2 proteins have been reported in people with no history of SARS-CoV-2 infection. By identifying CD4+ T cell clones specific for distinct epitopes on the SARS-CoV-2 proteins, the authors can provide a focused characterization of CD4+ T cell cross-reactivity to spike proteins in various human β-coronaviruses, and also analyze how mutations in these epitopes in VOCs lead to loss of recognition by the CD4+ T cells elicited by natural infection or vaccination." **Ioana Visan, Senior Editor,** *Nature Immunology.*