T cell responses

T cells in COVID-19 - United in Diversity

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Comprehensive mapping reveals functional $CD4^+$ and $CD8^+$ T cells targeting multiple regions of SARS-CoV-2 that are maintained in the resolution phase of both mild and severe COVID-19 and correlate in magnitude with the antibody response.

$CD4^+$ and $CD8^+$ T cells work together with other constituents of a coordinated immune response to first resolve acute viral infections, and to then provide protection against re-infection. Careful delineation of the frequency, specificity, functionality and durability of T cells in COVID-19 is vital to understanding how to use them as biomarkers and targets for immunotherapies or vaccines. In this issue of Nature Immunology, Peng *et al* take a comprehensive approach to characterising circulating SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T cells following resolution of COVID-19[1]. They report a robust and diverse T cell response targeting multiple structural and non-structural regions of SARS-CoV-2 in most resolved cases, whether they had mild or severe infection. While the most frequent responses were against peptides spanning spike, membrane, and nucleoprotein antigens, all eight regions tested were recognised by multiple individuals, with a maximum of 23 reactive pools in two individuals. Such multispecific T cell responses are well-suited to providing a failsafe form of multi-layered protection, mitigating against viral escape by mutation or variable antigen presentation.

Peng *et al* carefully map which parts of the virus are recognised by T cells using overlapping peptides spanning the whole viral proteome, with the exception of the large ORF-1 region. They use the IFN-γ-ELISpot for initial broad screening of antiviral effector responses, followed by intracellular cytokine staining to show that detected responses are comprised of polyfunctional $CD4^+$ and $CD8^+$ T cells[1]. Such comprehensive studies are an important first step to identify the targets of SARS-CoV-2-specific T cells so that the heterogeneity of the response can be unpicked, and future targeted studies can be carried out. Many factors determine the immunodominance hierarchy of viral antigens, including: frequency of naïve precursors; level,

timing, and location of antigen expression; efficiency of antigen processing and presentation by professional or non-professional antigen presenting cells. The immune response directed against SARS-CoV-2 turns out to be broader than that seen after infection with SARS-CoV-1, where T cells are highly focused on spike [2]. A key finding by Peng *et al* is that the breadth and magnitude of the T cell response is greater in those that suffered more severe COVID-19 (**Figure 1**). However, the proportion of the T cell response that is attributable to $CD8^+$ (rather than $CD4^+$) T cells is increased in mild infection, consistent findings in another study, which showed a higher percentage of activated and proliferating $CD8^+$ T cells in mild compared to severe COVID-19[1,3]. These findings hint at a protective role for SARS-CoV-2-specific $CD8^+$ T cells, further supported by the greater proportion of clonally expanded $CD8^+$ T cells in the infected lung in mild disease[4].

The relatively high frequency SARS-CoV-2-specific T cell responses seen after severe COVID-19 are dominated by $CD4^+$ T cells, with responses against spike particularly abundant (**Figure 1**). The correlation Peng *et al* observed between spike-specific T cells and antibodies to spike (and the receptor binding domain within it) is, therefore, likely attributable to $CD4^+$ T cells, as observed by Grifoni *et al* [5]. $CD4^+$ T cells come in many flavours, with T follicular helper cells (Tfh) being crucial for a successful germinal centre response generating long-lived plasma cells and broadly neutralising high affinity antibody responses and studies are now starting to identify the components of a successful T-B cell collaboration in the context of COVID-19[3,6,7]. Although Peng *et al* did not present viral load data, many other studies have shown this is higher in cases with worse COVID-19. The increase in $CD4^+$ T cells in those with a severe outcome, as also noted for antibodies in a number of studies, is likely a reflection of the increased antigenic burden, characteristic of these cases, driving stronger immune responses. However, the possibility that stronger $CD4^+$ T cells and/or antibody responses contribute to disease severity, rather than just reflecting it, cannot yet be dismissed.

While T cells help to coordinate antiviral immune responses, support the humoral response, limit viral replication, and remove infected cells, in doing so they can directly and indirectly contribute to immunopathology. To get insights into the antiviral versus pathogenic potential of SARS-CoV-2-specific T cells, studies should look earlier than the convalescent phase studied here[8]. Longitudinal studies starting at the first stage of acute infection are needed to evaluate

if the timing, magnitude, and composition of the early T cell response is predictive of disease outcome. The temporal evolution of initial immune responses, and how they coincide with the exponential viral growth phase, can be critical determinants of their efficacy; a delayed or insufficient T cell response could allow uncontrolled viraemia to drive stronger subsequent T cell responses capable of exacerbating tissue damage. Alternatively, the large viral burden triggering strong antibody and $CD4^+$ T cells responses in those with more severe disease may predominantly result from a higher viral inoculum or insufficient dampening down of early viraemia by a failed innate immune response. Dissecting the acute phase response in individuals who clear SARS-CoV-2 infection whilst remaining asymptomatic has also started to be tackled[3] and will help define which combination of the myriad of immune effectors are best at fighting SARS-CoV-2 without damaging the host.

One reason why some individuals may have a head start in the race against the virus is the presence of pre-existing T cell responses (generated by a prior coronavirus or other infection) that are able to recognise SARS-CoV-2 and immediately spring into action. Such cross-reactive T cells have been observed in 20-50% of individuals in some COVID-19 cohorts[3,5,9] and are being intensively studied to see if they can mediate any cross-protection, which might contribute to the observed variation in infection severity between individuals, age groups, and geographic regions. Peng *et al* did not observe SARS-CoV-2-reactive T cells in the small cohort of healthy controls they studied [1]; this could relate to differences in assay sensitivity, omission of ORF-1 peptides, or differences in their cohort's previous exposure to related coronaviruses.

The final pressing set of questions arising from this study concern the protective potential of the identified multispecific T cells beyond the convalescent phase. Will particular specificities prove to be more protective or more durable, or will the principle of "united in diversity" continue to apply? Will some memory cells become "tissue-resident" populations in the respiratory tract, with the features of longevity and rapid frontline immunosurveillance characteristic of these locally compartmentalised responses? In particular, T cells localised to the airways are critical for protective immunity against related coronaviruses in animal models[10]. There is some controversy from recent studies on the degree and speed of antibody waning and its relevance in the face of memory B cells, which if persistent and functional

should replenish the humoral response on virus re-encounter[6]. As with antibodies, those who mounted the strongest and broadest T cell responses would be predicted to sustain them longer (**Figure 1**), but even small persistent memory populations can rapidly expand on re-challenge. Unlike antibodies, T cells cannot block *de novo* infection, because they only recognise the virus once infected cells present viral peptides, and so are unlikely to provide sterilising immunity. But memory T cells can mop up any infected cells slipping through a first layer of defence normally provided by antibodies. Thus, T cell memory at the time of re-exposure may stop severe disease from developing, or it may even lead to aborted subclinical infection. Promisingly, natural SARS-CoV-2 infection in macaques, generating both antibodies and T cells, does result in protection from re-challenge[11].

Follow-up studies will be vital to assess the persistence of T cells directed against different regions of SARS-CoV-2 and how they correlate with memory B cells, neutralising and non-neutralising antibodies. T cells generated in response to some human and other animal coronaviruses have proved to be exceptionally long-lasting, with responses against SARS-CoV-1, for example remaining detectable 17 years later[9,12]. The fine epitope mapping carried out by Peng *et al* allows the design of HLA/peptide multimers for detailed direct *ex vivo* T cell characterisation; initial phenotypic studies have shown a mixed effector and central memory phenotype, as expected from an acute resolving infection[1,3]. Another useful output of this study is the identification of several parts of the virus that are targeted by up to half of the patients tested, despite divergent MHC allele expression. These so-called 'promiscuous' epitopes should be useful for future immunology studies and for consideration in vaccine design. Thus, the data provided by Peng *et al* and other recent T cell studies support the use of vaccine modalities designed to induce both cellular and humoral immunity and raise the possibility of including additional regions beyond spike that have been found to be immunogenic in natural infection.

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Figure Legend

Peng *et al* study the resolution phase of COVID-19 (blue box), showing a broadly targeted $CD4^+$ and $CD8^+$-T cell response (cell colours and numbers represent relative frequencies of marked protein specificities). The total T cell response (solid blue line) is stronger and broader in severe cases (assumed to have had higher viral burden, red curve), correlating with stronger antibody responses (solid grey line). However, there are proportionally more $CD8^+$-T cells in mild disease. Central questions arising from this study (listed in red) concern the unknown hierarchy and kinetics of T cells (dashed blue lines) and antibodies (dashed grey lines) in the acute and memory phases.

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