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CORRESPONDENCE

T cell immune responses to SARS-CoV-2 and variants of concern (Alpha and Delta) in infected and vaccinated individuals

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Our understanding of immune responses to SARS-CoV-2 and variants of concern (VOCs) has been primarily acquired through analysis of Spike-specific IgG responses. However, a more comprehensive understanding of the breadth and longevity of immune responses after infection and vaccination requires analysis of cellular immunity. Herein, we report on T cell immunity in infected and vaccinated individuals, identifying CD4+/CD8+ T cell cytokine responses to SARS-CoV-2 and variant peptides (Alpha, B.1.1.7 and Delta, B.1.617.2). Our results demonstrate that T cells in infected or vaccinated individuals can elicit robust and cross-reactive immune responses against VOCs. This information could be helpful in understanding the composition and durability of human immunity to SARS-CoV-2 and VOCs.

The limited efficacy of most treatments and high death rates associated with SARS-CoV-2 infection have led to a focus on vaccines as the last best hope for stemming the pandemic. However, the persistence of SARS-CoV-2, driven primarily by VOCs, has raised concerns regarding our ability to induce durable immunity through vaccination [1].

Early observations have raised concerns regarding the rapid and unpredictable dissipation of IgG responses to SARS-CoV-2 peptides and the potential effect on long-term immunity [2, 3]. In addition, reports have shown that IgG spike protein-specific immune responses induced by the BNT162b2 (Pfizer) vaccine may have reduced activity against the Alpha variant [4]. The recent emergence of the Delta variant has reinvigorated concerns about the protective immunity provided by current SARS-CoV-2 vaccines since disturbing reports of vaccine breakthrough infections have been published [5].

Spike-specific IgG receptor-binding domain (RBD) antibodies are evanescent and do not reflect important memory components. Thus, additional analysis of CD4+/CD8+ T cell responses to SARS-CoV-2 spike peptides and VOCs [6] could broaden our understanding of SARS-CoV-2-specific T cell immunity.

The emerging Delta variant is characterized by multiple mutations in the spike protein including T19R, Δ157–158, L452R, T478K, D614G, P681R, and D950N. It is likely that these mutations affect immune responses to important antigenic regions of the receptor-binding domain. In addition, strains with the P681R mutation have accelerated replication, increasing infectivity. Data on the effectiveness of SARS-CoV-2vaccines against the Delta variant are limited [7].

We developed a whole-blood assay to detect SARS-CoV-2-specific T cells. Analysis of cytokine production in stimulated T cells confirmed that IL-2 and TNF-α are consistent markers for activated CD4+ T cells, while activated CD8+ T cells mainly produce TNF-α and IFN-γ. After incubating whole blood with a SARS-CoV-2 Spike peptide pool, we were able to identify Spike-reactive T cells by dual-cytokine gating (Supplementary Fig. S1). In this assay, healthy individuals with no history of SARS-CoV-2 infection demonstrated no significant T cell response to the SARS-CoV-2 spike peptide. However, T cells from SARS-CoV-2-infected or vaccinated individuals showed substantial spike-specific CD4+ and CD8+ T cell populations.

Next, *we* examined memory T cell immunity against SARS-CoV-2 in 134 patients with documented SARS-CoV-2 infection (Supplementary Table S1 and Supplementary Fig. S2). T cell immune responses to peptide pools of 5 major SARS-CoV-2 proteins (Spike, VME, NCAP, AP3A, and NS7A) were analyzed. For healthy control individuals, no significant CD4+ T cell responses to the 5 SARS-CoV-2 proteins were seen (Fig. 1A, IL-2+/TNF-α+ (%) in CD4+ <0.05%, mean = 0.01%). However, 20% of healthy individuals showed heterogeneous TNF-α+/IFN-γ+CD8+ T cell (>0.05%) responses to the 5 SARS-CoV-2 proteins, which could represent cross-reactivity among CD8+ T cells generated during previous endemic coronavirus infection (Fig. 1B) [8]. Based on the background level of the CD4+ T cell response in healthy controls, we set 0.05% dual-positive CD4+ and CD8+ T cells as the cutoff level for identifying positive T cell immunity against SARS-CoV-2. Overall, we observed that 88% (30 of 34) of infected patients had either positive CD4+ or CD8+ T cell immunity to one or more of the 5 SARS-CoV-2 proteins. Most patients showed positive CD4+ T cell immunity (85%, 29 of 34), and CD4+ T cells demonstrated immunodominant responses to Spike peptides, as previously described [8] (Fig. 1A). CD8+ T cells showed similar responses to the 5 proteins.

Next, we analyzed Spike-specific CD4+/CD8+ immune responses induced by the Pfizer BNT162b2 vaccine. We compared Spike-specific

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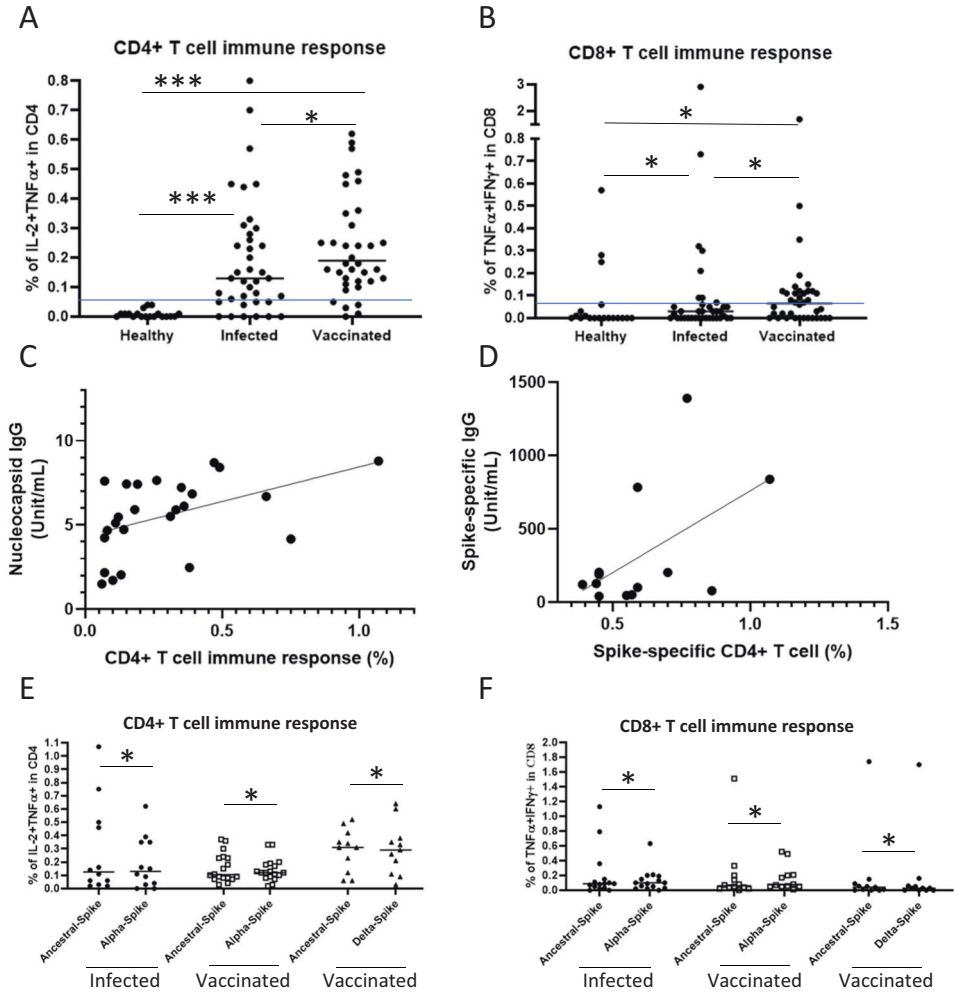

Fig. 1 Immune responses in SARS-CoV-2-infected patients and vaccinated individuals. A, **B** SARS-CoV-2 Spike-specific CD4+ and CD8+ T cells in healthy, infected and vaccinated individuals. Whole blood was stimulated with Spike peptides, and T cells with dual-cytokine staining were gated. The blue line shows the 0.05% cutoff. **C** The correlation of Nucleocapsid-specific IgG titers with CD4+ T cell immune responses to one or more of 5 major SARS-CoV-2 proteins in 25 patients. **D** The correlation between Spike-specific CD4+ T cell immune responses and Spike-specific IgG levels in 13 patients with elevated CD4+ Spike-specific T cell immune responses (IL-2+/TNF-α+ cell% in CD4+ >0.3%). **E**, **F** T cell immune responses to ancestral and variant spike peptides are shown. CD4+/CD8+ Spike-specific T cells from infected and vaccinated healthy individuals were assessed for reactivity to ancestral, Alpha variant and Delta variant Spike peptides. Each dot represents one individual. *P = NS; ***$p < 0.001$.

T cell immunity among 19 healthy controls, 38 infected patients, and 38 vaccinated individuals 1 month after the 2nd vaccine dose (Fig. 1A, B). No healthy unvaccinated individuals showed positive CD4+ T cell immunity against SARS-CoV-2, but infected patients and vaccinated individuals demonstrated substantial spike-specific CD4+ T cell immunity, with rates of 87% (33 of 38) and 89% (34 of 38), respectively. CD8+ T cells from healthy controls, infected patients, or vaccinated individuals showed 21% (4 of 19), 34% (13 of 38), and 58% (22 of 38) positivity for immune responses against SARS-CoV-2 spike peptides, respectively. Therefore, the Pfizer BNT162b2 vaccine induced T cell reactivity to Spike-specific peptides that was equivalent to that seen in infected patients after recovery.

Serum from SARS-CoV-2 patients was submitted for clinical nucleoprotein IgG titering. In 25 patients with nucleocapsid IgG positivity, we analyzed the association with CD4+ T cell immunity to the 5 SARS-CoV-2 peptides (the highest response was compared). Although T cell immunity was not nucleocapsid-specific, there was a significant association between CD4+ T cell immunity and nucleocapsid IgG titers ($p = 0.022$; $R = 0.457$; Fig. 1C).

We then examined Spike-specific IgG levels in 80 SARS-CoV-2-infected patients and compared them to Spike-specific T cell immunity results. In total, 72.58% of patients with detectable Spike-specific CD4+ T cells were positive for Spike-specific IgG. Among patients without detectable Spike-specific T cell immunity, 61.11% also were negative for Spike-specific IgG by serology. In 13 patients with high Spike-specific CD4+ T cell immunity (IL-2+/TNF-α+ (%) in CD4+ ≥0.3%), we also observed a strong correlation between T cell immunity and the level of Spike-specific IgG ($p = 0.0316$; $R = 0.5288$; Fig. 1D).

The Alpha (B.1.1.7) variant contains the E484K mutation, which establishes resistance to serologic responses in infected individuals [4]. To determine whether this VOC evades T cell immunity, we analyzed 19 infected/recovered patients and 18 healthy vaccinated individuals for CD4+/CD8+ T cell responses against the Alpha variant Spike protein. As shown in Fig. 1E, F, there were no significant reductions in CD4+/CD8+ T cell responses to the Alpha variant Spike peptides compared to those to the ancestral Spike peptides (mean of infected patients: 0.23% ancestral vs. 0.18% Alpha variant; mean of vaccinated individuals: 0.16% ancestral vs. 0.14% Alpha variant). In addition, nearly identical CD4+ and CD8+ T cell responses to the Delta variant peptides and SARS-CoV-2 spike peptides were detected in 11 healthy BNT162b2-vaccinated individuals (Fig. 1E, F). In

summary, T cell memory induced by SARS-CoV-2 infection or vaccination produces similar immune responses against the Alpha and Delta variants. This suggests protective immunity against Alpha and Delta variant infection and possibly infection by other VOCs [9].

Herein, we present data identifying memory T cells with specificity and accuracy for the detection of CD4+/CD8+ T cell responses to SARS-CoV-2 peptides that differentiate infected and vaccinated individuals from those not exposed to SARS-CoV-2. In addition, analysis of T cell responses to VOCs (Alpha and Delta) showed that SARS-CoV-2 infection and vaccination with BNT162b2 elicited equivalent T cell responses.

The development of dormancy in memory T cells, B cells, and plasma cells is a natural temporal evolution after infection and/or vaccination that produces populations that can rapidly be activated upon re-exposure to SARS-CoV-2 and are likely to have an important role in preventing or modifying infection by SARS-CoV-2 VOCs [9, 10]. An important consideration in this regard is the dissipation of humoral immunity over time. IgG responses are critical for sterilizing immunity. However, T cell immunity does require an infection to reactivate memory responses. This may result in mild or asymptomatic infections that would be considered "breakthrough" infection. Thus, the level and robustness of T cell memory responses would likely affect the clinical manifestations of the disease.

DATA AVAILABILITY

Request for data relevant to the manuscript will be considered after assessment and review.

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AUTHOR CONTRIBUTIONS

SCJ: Involved in the design and execution of the research and primarily responsible for writing and editing the manuscript. B-HS, PhD: Primarily involved in designing the T cell assay and generating data for the manuscript. T-AMG: Involved in the performance of the T cell and antibody assays. MC: Involved in performance of the T cell and antibody assays. AP: Involved in the performance of the antibody assays. CNL: Involved in gathering and analyzing data and writing the manuscript. RZ: Involved in gathering and analyzing data for the manuscript. JO: Involved in gathering and analyzing data for the manuscript. IP: Involved in gathering and analyzing data for the manuscript. SC: Provided technical and editing support for the project. AV: Provided support for developing the protocol and regulatory documents. NA: Provided regulatory support for the development of the project. JP: Provided laboratory support and editing for the project. SG: Helped in assay development and implementation. MF: Aided in the design and performance of assays. AB: Aided in the development and implementation of antibody assays and the interpretation of data. MT: Helped in the development and implementation of the T cell assay. RZ: Primarily involved in the development of the assays and project and contributed to writing and editing the manuscript

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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