Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
A comparative characterization of SARS-CoV-2-specific T cells induced by mRNA or inactive virus COVID-19 vaccines

Graphical abstract

Highlights

- Inactivated SARS-CoV-2 vaccine induces a multi-protein-specific T cell response

- The total T cell response is quantitatively similar to that induced by mRNA vaccine

- Inactivated vaccine-induced multi-protein-specific T cells are primarily CD4+

- The induced T cell responses tolerate the mutations of the Omicron viral lineage

Authors

Joey Ming Er Lim, Shou Kit Hang, Smrithi Hariharaputran, ..., Nina Le Bert, Antonio Bertoletti, Anthony T. Tan

Correspondence

antonio@duke-nus.edu.sg (A.B.), anthony.tan@duke-nus.edu.sg (A.T.T.)

In brief

Inactivated SARS-CoV-2 vaccine induces a multi-protein-specific T cell response. By comparing healthy individuals following vaccination with inactivated SARS-CoV-2 or mRNA vaccines, Lim et al. investigate the differences in specificity, quantity, and phenotype of the vaccine-induced T cell responses and their ability to tolerate mutations characterizing the Omicron viral lineage.
A comparative characterization of SARS-CoV-2-specific T cells induced by mRNA or inactive virus COVID-19 vaccines

Joey Ming Er Lim,1 Shou Kit Hang,1 Smrithi Hariharaputran,1 Adeline Chia,1 Nicole Tan,1 Eng Sing Lee,2,3 Edwin Chng,4 Poh Lian Lim,3,5,6 Barnaby E. Young,3,5,6 David Chien Lye,3,5,6,7 Nina Le Bert,1,9 Antonio Bertoletti,1,8,10,* and Anthony T. Tan1,*

1Programme in Emerging Infectious Diseases, Duke-NUS Medical School, 8 College Road, Singapore 169857, Singapore
2Clinical Research Unit, National Healthcare Group Polyclinics, Singapore 138543, Singapore
3Lee Kong Chian School of Medicine, Singapore 308232, Singapore
4Parkway Shenton Pte Ltd, Singapore 048583, Singapore
5National Center of Infectious Diseases, Singapore 308442, Singapore
6Department of Infectious Diseases, Tan Tock Seng Hospital, Singapore 308433, Singapore
7Yong Loo Lin School of Medicine, Singapore 119228, Singapore
8Singapore Immunology Network, A*STAR, Singapore 138648, Singapore
9Twitter: @bertoletti_lab
10Lead contact
*Correspondence: antonio@duke-nus.edu.sg (A.B.), anthony.tan@duke-nus.edu.sg (A.T.T.)
https://doi.org/10.1016/j.xcrm.2022.100793

SUMMARY

Unlike mRNA vaccines based only on the spike protein, inactivated severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) vaccines should induce a diversified T cell response recognizing distinct structural proteins. Here, we perform a comparative analysis of SARS-CoV-2-specific T cells in healthy individuals following vaccination with inactivated SARS-CoV-2 or mRNA vaccines. Relative to spike mRNA vaccination, inactivated vaccines elicit a lower magnitude of spike-specific T cells, but the combination of membrane, nucleoprotein, and spike-specific T cell response is quantitatively comparable with the sole spike T cell response induced by mRNA vaccine, and they efficiently tolerate the mutations characterizing the Omicron lineage. However, this multi-protein-specific T cell response is not mediated by a coordinated CD4 and CD8 T cell expansion but by selective priming of CD4 T cells. These findings can help in understanding the role of CD4 and CD8 T cells in the efficacy of the different vaccines to control severe COVID-19 after Omicron infection.

INTRODUCTION

The availability of vaccines against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) have altered the landscape of the COVID-19 pandemic and allowed the virus to persist among vaccinated populations. Globally, numerous SARS-CoV-2 vaccines have been developed, clinically trialed, and administered to date,1 and vaccines based on inactivated virus (CoronaVac-Sinovac and BBIBP-CorV-Sinopharm) have been utilized in almost half of the 7.3 billion doses that have been delivered to people worldwide before the end of 2021.2 Despite this staggering number, a detailed analysis of cellular immune response elicited by inactivated vaccines compared with mRNA or adenoviral-based vaccines are still limited.3 The reasons for the lack of a comparative analysis of the components of the adaptive immunity responsible for killing virus-infected cells (virus-specific CD8 T cells), aiding the production of high-affinity antibodies (T follicular helper T cells), and sustaining CD8 T cell function (T helper 1 [Th1]) are different.4 Inactivated vaccines were utilized largely in China, where Western vaccines based on mRNA or adenoviral vectors were not available for a parallel comparison, or in less wealthy nations that often lack research infrastructure to perform the complex characterization of T cells. Furthermore, the evaluation of the efficacy of different vaccines was performed before the emergence of the antibody-escaping Omicron variant,4 where clinical and virological parameters, such as the protection from infection or from symptomatic disease, were associated with the quantity of neutralizing antibodies.5 These comparative studies showed that the quantity of neutralizing antibodies stimulated by inactivated vaccines were ~10 times lower than those induced by spike mRNA vaccines and that significant waning of the antibodies occurs approximately 3 months post vaccination,6–10 faster than the decline of antibodies observed with mRNA vaccines, which appear to persist at high levels for at least 6 months.11–17 These antibody response comparisons are, however, now obsolete since the evaluation of vaccination efficacy against Omicron is now directed more toward understanding the ability...
of vaccines to protect from disease and not infection.18 Here, T cells are likely to play a more important role in light of their ability to recognize a large number of epitopes that were not affected by the amino acid mutations present in Omicron and their ability to target virus-infected cells.11,19,20 In this regard, real-world vaccine efficacy data showed that individuals vaccinated with inactivated virus developed, in general, milder disease than non-vaccinated individuals.21–24 However, a direct comparative analysis of individuals vaccinated with inactivated SARS-CoV-2 and mRNA vaccines showed lowered protection from infection and severe disease against Delta lineage in the former,17 in line with a meta-analysis comparing the clinical efficacy of multiple COVID-19 vaccines.25 These data suggest that inactivated vaccines might actually induce not only a weaker humoral response toward spike but also an impaired level of T cell response, which was, however, not experimentally supported. Analysis of SARS-CoV-2-specific T cells in individuals vaccinated with inactivated virus demonstrated the induction of T cells specific for spike and other structural proteins (nucleoprotein and membrane), and a magnitude of vaccine-induced CD8 T cells that were superior to the spike-specific CD8 T cells induced by mRNA vaccines.3 Such strong induction of CD8 T cell response was perplexing since the antigen processing and presentation pathway associated with an exogenous protein antigen such as inactivated SARS-CoV-2 vaccine is expected to induce primarily a CD4 T cell response.27 Even though adjuvants can increase cross-presentation of viral antigen to CD8 T cells mediated by a specialized population of dendritic cells,28 such processes should be less efficient than the direct presentation of viral antigen by major histocompatibility complex (MHC) class I occurring in vaccine preparations where antigen is endogenously synthesized within the cells, such as in the case of mRNA-based vaccines.

We therefore longitudinally analyzed the vaccine-specific T cells induced in healthy individuals after SARS-CoV-2 vaccination with inactivated (CoronaVac or BBIBP-CorV) or mRNA vaccine (BNT162b2) and characterized their ability to recognize multiple proteins and the involvement of CD4 and CD8 T cells in such response. In addition, some of the inactivated SARS-CoV-2 vaccinees received a homologous booster within the study period of 6 months. The effects of boosting on the vaccine-induced T cell response were also analyzed together with the evaluation of the effects of mutations present in the Omicron variant of concern (VOC) on the vaccine-induced T cell response against multiple viral proteins.

RESULTS

Magnitude of spike-specific T cell response following vaccination with inactivated SARS-CoV-2 vaccines

Utilizing a pool of peptides covering the major immunodominant regions of the spike protein (SpG pool) in a whole-blood cytokine release assay (CRA) that we have previously developed and validated in mRNA vaccinated or infected individuals,28 we longitudinally analyzed the magnitude of spike-specific T cell response following vaccination with inactivated SARS-CoV-2 vaccines in two cohorts (Figure 1A; Table 1). The first cohort (inactivated cohort) are healthy individuals who received two doses of BBIBP-CorV inactivated vaccine (Sinopharm) given 21 days apart (n = 30). The second cohort (heterologous cohort) are healthy individuals who first received a single dose of spike mRNA vaccine (BNT162b2-Pfizer-BioNTech or mRNA-1273-Moderna) and developed adverse events that precludes the administration of the second dose of mRNA vaccine. After the normalization of the adverse events (1–6 months after occurrence of adverse events), these individuals received two doses of CoronaVac inactivated vaccine (Sinovac) given 21 days apart.
A reference cohort (mRNA cohort) of healthy individuals who received two doses of spike mRNA vaccine (Pfizer-BioNTech) were also analyzed with the whole-blood CRA (n = 76).

A single dose of inactivated vaccine induced an increase in spike-induced interferon (IFN-γ) and/or interleukin (IL)-2 in all individuals of the inactivated cohort 21 days post vaccination (Figure 1B). In the heterologous cohort, spike-induced cytokines were already elevated in most of the individuals before receiving inactivated vaccines due to the prior dose of spike mRNA vaccine, and the response remained high after inactivated SARS-CoV-2 vaccination (Figure 1B). The level of cytokines induced by the spike peptide pool remained stable at 2–3 months post vaccination (1–2 months after receiving the second dose) in both cohorts (Figure 1B). Even with the initial priming from mRNA vaccines prior to inactivated SARS-CoV-2 boosting, the peak magnitude of the spike-induced cytokines detected in the heterologous cohort do not differ from the reference mRNA cohort (Figures 1B and 1C).

While the response kinetics appear similar between the inactivated (BBIBP-CorV) and mRNA (BNT162b2) cohorts, with peaks of spike-induced IFN-γ and IL-2 observed 21 days post vaccination, the magnitude of the spike-induced response was lower in individuals who received inactivated vaccines compared with those who had spike mRNA vaccination (Figures 1B and 1C). This difference was particularly significant 2–3 months post vaccination (Figure 1C). Even in the heterologous cohort where individuals were primed with spike mRNA vaccines before boosting with inactivated vaccine (CoronaVac), the magnitude of the spike-induced T cell cytokines was lower but did not reach statistical significance (Figure 1C). Analysis of the quantities of IFN-γ and IL-2 secreted after SpG peptide pool stimulation did not show significant differences between all three cohorts at 21 days or 2–3 months post vaccination (Figure 1D).

These results show that inactivated SARS-CoV-2 vaccines (BBIBP-CorV and CoronaVac) induced a quantitatively lower spike-specific T cell response with minimal differences in the balance of IFN-γ and IL-2 secreted compared with BNT162b2 mRNA vaccine. Consistent with what was observed previously, quantifying the spike T cell response through IL-2 secretion also appears more robust with less variability than by IFN-γ secretion (Figure 1).

### Immunodominance of spike-specific T cells induced by inactivated SARS-CoV-2 vaccines

To understand the breadth of the spike-specific T cell response induced by inactivated SARS-CoV-2 vaccines, we also performed the whole-blood CRA assay using seven overlapping peptide pools that spans the entire spike protein on blood collected 2–3 months after receiving inactivated or mRNA vaccines. A schematic representation of the localization of peptide pools 1 to 7 in relation to the S1 (N-terminal), RBD and S2 (C-terminal) regions of spike is displayed in Figure 2A. Randomly selected individuals from the inactivated (BBIBP-CorV, n = 5) and heterologous (mRNA + CoronaVac, n = 8) cohorts were analyzed and we detected spike-specific T cells in all individuals (Figure 2B). Almost all of the tested individuals had dominant spike-specific T cells targeting the S2 chain of the spike protein (spike pool 5–7) (Figures 2B and 2C), with the strongest response targeting pool 6, which covers amino acid 886–1,085 of the spike protein. There were no observable differences between the cohorts, and this immunodominance pattern is similar to that observed in a previous study on mRNA vaccinated individuals (Figure 2B, mRNA n = 6), where most of the spike-specific T cells were targeting epitopes present in the S2 chain of spike.29

### Inactivated vaccine-induced T cell responses targeting multiple viral structural proteins

The breadth of inactivated SARS-CoV-2 vaccine-induced T cell response should extend beyond the spike protein since the inactivated vaccines (BBIBP-CorV and CoronaVac) contain other structural proteins in addition to spike.30,31 Similar to the analysis of spike-specific T cell response, we used overlapping peptides covering the entire membrane and nucleoprotein in a whole-blood CRA. We observed induction of membrane- (Figure 3A) and nucleoprotein-specific (Figure 3B) T cell response in both cohorts. Around 90% of the individuals in both cohorts had detectable membrane- and nucleoprotein-specific T cells 21 days after vaccination, and the T cells persisted for at least 2–3 months post-vaccination (Figures 3A and 3B). Secretion of IL-2 was again more robust with less variability among the individuals. Importantly, the magnitude of the spike T cell response was positively correlated with both the membrane and nucleoprotein T cell response (Figure 3C), demonstrating that these responses were indeed induced and associated with inactivated SARS-CoV-2 vaccination. Comparative analysis of the cytokine quantity induced by spike, membrane, and nucleoprotein peptide pools 2–3 months post vaccination showed that the majority of the vaccine-induced T cells were targeting the spike protein, followed by nucleoprotein and membrane, a hierarchy proportional to the size of the respective proteins (Figure 3D).

We also measured the global level of T cell response induced by inactivated SARS-CoV-2 and spike mRNA vaccines. The amount of IFN-γ or IL-2 secreted after stimulation with SpG, membrane, and nucleoprotein peptide pools in both inactivated (n = 27) and heterologous (n = 18) vaccinated cohorts were comparable with the one detected by SpG only in mRNA vaccine recipients (n = 76) (Figure 4).

### Phenotype of vaccine-induced T cells against multiple viral proteins

We next sought to determine whether the inactivated vaccine-induced T cells were mediated by helper CD4+ or cytotoxic CD8+ T cells. Two different methods were used: a classical analysis of upregulation of T cell activation markers (AIMs) on the CD4 (CD25+ + OX40 + 41BB+) and CD8 (CD69 + 41BB+) T cell subsets after peripheral blood mononuclear cell (PBMC) stimulation with peptide megapools, or a depletion approach where CD8 and CD4 T cells were removed from the PBMC before stimulation with the peptide indicated megapools in an IFN-γ enzyme-linked immunosorbent spot (ELISPOT) assay (Figure 5A).

The two methods yielded completely different results in terms of the composition of the peptide pools’ responsive T cells. Using the depletion approach, we observed a clear CD4-centric spike T cell response induced by inactivated vaccines (Figure 5B). Spike-specific CD8 T cells were only
observed at very low quantities in two out of 12 individuals vaccinated with inactivated SARS-CoV-2 (only found in the heterologous cohort), in contrast to the spike-specific CD4 T cells, which were present in all individuals tested (Figure 5B). In addition, T cells specific for nucleoprotein and membrane were also CD4 centric with no detectable response in the CD8-enriched samples (Figure S3). On the other hand, both CD4 and CD8 spike-specific T cells were induced by mRNA vaccines (Figure 5B). In contrast, a significant upregulation of AIM on both CD4 and CD8 T cells upon spike stimulation was detected in all tested individuals from both inactivated and mRNA cohorts (Figures 5C and 5D).
A. Membrane-specific response

| Inactivated BBIBP-CorV (n=30) | Heterologous mRNA+CoronaVac (n=20) |
|-------------------------------|-----------------------------------|
| IFN-γ concentration (Log10(pg/ml)) | % pos  |
| Pre-vac D21 2-3 mths | 46 75 |
| Pre-vac D21 2-3 mths | 46 85 |
| Pre-vac D21 2-3 mths | 90 85 |
| Pre-vac D21 2-3 mths | 68 100 |

B. Nucleoprotein-specific response

| Inactivated BBIBP-CorV (n=30) | Heterologous mRNA+CoronaVac (n=20) |
|-------------------------------|-----------------------------------|
| IFN-γ concentration (Log10(pg/ml)) | % pos  |
| Pre-vac D21 2-3 mths | 46 75 |
| Pre-vac D21 2-3 mths | 46 85 |
| Pre-vac D21 2-3 mths | 90 85 |
| Pre-vac D21 2-3 mths | 68 100 |

C. Inactivated BBIBP-CorV (n=30) vs Heterologous mRNA+CoronaVac (n=20)

| Response (Log10 IFN-γ(pg/ml)) | SpG pool response (Log10 IFN-γ(pg/ml)) |
|-------------------------------|-----------------------------------|
| R² = 0.29 / 0.1848 P < 0.0001 / 0.0001 |
| R² = 0.3558 / 0.3895 P < 0.0001 / 0.0001 |

D. 2-3 mths post-vaccination

| Inactivated BBIBP-CorV (n=28) | Heterologous mRNA+CoronaVac (n=20) |
|-------------------------------|-----------------------------------|
| IFN-γ Concentration (Log10(pg/ml)) | |
| Total Spike | M | NP |
| M | NP |
| Sp | M | NP |

(legend on next page)
Effects of inactivated SARS-CoV-2 vaccine booster (third dose) on the T cell response

Studies following individuals who received primary vaccination (two doses) with inactivated SARS-CoV-2 vaccines have reported a progressive waning of neutralizing antibody at 3 months with titers almost reaching pre-vaccination levels at ~6 months. This has prompted policy changes in Singapore to recommend the inclusion of a third dose of inactivated SARS-CoV-2 into the primary vaccination series 3 months after the administration of the second dose. While the additional dose of inactivated SARS-CoV-2 vaccine has been shown to effectively reactivate neutralizing antibody titers that have declined substantially after the second dose, little is known about its effects on the amplification of vaccine-induced T cell responses. As such, we compared the vaccine-induced T cell response before and after the third homologous vaccine dose in the inactivated and heterologous cohorts using the whole-blood CRA with SpG, membrane, and nucleoprotein peptide pools.

In the cohorts studied, a total of 18 individuals in the inactivated cohort and seven in the heterologous cohort were given the third dose of vaccine at the time of blood collection and they were analyzed as controls. None of the vaccinated individuals analyzed had a known history of SARS-CoV-2 infection up until the last blood sample collection at ~6–7 months from the first vaccination dose.

From the whole-blood CRA, we did not observe an appreciable change in the amount of secreted IFN-γ and IL-2 after SpG peptide pool stimulation of whole-blood samples collected before (~1–2 months after the second dose) and after (either 1–3 months or <1 month after the third dose) the third dose of inactivated SARS-CoV-2 vaccine in both inactivated and heterologous cohorts (Figure 6B). The response to membrane and nucleoprotein also remained largely unchanged before and after the third vaccination dose (Figure 6C). Importantly, the magnitude of the whole-blood CRA response against all peptide pools tested did not differ between the individuals in either cohort who received the third inactivated vaccine dose and the controls who did not (Figures 6B and 6C).

Thus, the third dose of inactivated SARS-CoV-2 vaccine did not effectively induce a significant boost of the vaccine-induced T cell response stimulated by the first two inactivated vaccine doses. Unlike the neutralizing antibody titers that wane within 3–6 months...
Figure 5. Inactivated SARS-CoV-2 vaccines stimulate primarily CD4 T cell responses

(A) Schematic illustrates the analysis of SARS-CoV-2-specific CD4 and CD8 T cells by IFN-γ ELISPOT and by the detection of AIMs on CD4 (CD25+ OX40+ 4-1BB+) and CD8 (CD69+ 4-1BB+) T cells. PBMCs collected from the three cohorts of vaccinees 2–3 months after vaccination (inactivated, BBIBP-CorV, n = 6; mRNA, BNT162b2, n = 6; heterologous, mRNA + CoronaVac, n = 6) were either depleted of CD8 or CD4 T cells through negative selection before stimulation with overlapping peptides covering the entire spike for ELISPOT. Total PBMCs were also analyzed as a control. For the AIM assay, total PBMCs were stimulated with overlapping peptides covering the entire spike protein for 24 h before flow cytometry analysis.

(B) Bars denote the IFN-γ SFU quantified for each vaccinee after stimulating CD4-enriched (green), CD8 enriched (blue), or total PBMCs (gray) with spike overlapping peptides. Representative ELISPOT well images of the respective peptide-stimulated cell populations are shown.

C) Inactivated - BBIBP-CorV (n=6) and mRNA – BNT162b2 (n=6)

Legend continued on next page.

Inactivated SARS-CoV-2 vaccines stimulate primarily CD4 T cell responses

(A) Schematic illustrates the analysis of SARS-CoV-2-specific CD4 and CD8 T cells by IFN-γ ELISPOT and by the detection of AIMs on CD4 (CD25+ OX40+ 4-1BB+) and CD8 (CD69+ 4-1BB+) T cells. PBMCs collected from the three cohorts of vaccinees 2–3 months after vaccination (inactivated, BBIBP-CorV, n = 6; mRNA, BNT162b2, n = 6; heterologous, mRNA + CoronaVac, n = 6) were either depleted of CD8 or CD4 T cells through negative selection before stimulation with overlapping peptides covering the entire spike for ELISPOT. Total PBMCs were also analyzed as a control. For the AIM assay, total PBMCs were stimulated with overlapping peptides covering the entire spike protein for 24 h before flow cytometry analysis.

(B) Bars denote the IFN-γ SFU quantified for each vaccinee after stimulating CD4-enriched (green), CD8 enriched (blue), or total PBMCs (gray) with spike overlapping peptides. Representative ELISPOT well images of the respective peptide-stimulated cell populations are shown.

C) Inactivated - BBIBP-CorV (n=6) and mRNA – BNT162b2 (n=6)

Legend continued on next page.
of the second dose, the inactivated vaccine-induced T cell response was maintained for at least 6 months without observable waning (Figures 6B and 6C).

**Inactivated vaccine-induced T cell responses are largely preserved against the Omicron VOC**

Given that the Omicron VOC is the prevailing SARS-CoV-2 lineage circulating globally, we determined whether the inactivated vaccine-induced T cell responses can recognize the Omicron VOC proteins. We designed three sets of peptide pools specific for the spike, membrane, and nucleoprotein respectively (Table S1). Each set consist of a megapool made up of overlapping peptides spanning the entire vaccine-derived protein, a wild-type (WT) pool containing peptides covering the regions affected by mutations present in the Omicron variant of the protein, and a mutant (MT) pool consisting of peptides from the WT pool with the amino acid mutations present in the Omicron variant of the protein (Figure 7A). These peptide pools were used in the whole-blood CRA using samples collected from individuals in the inactivated and heterologous cohorts ~6–7 months after their first inactivated vaccine dose. Only individuals who received the third inactivated vaccine dose were analyzed to ensure that the vaccine-induced T cell response was at its peak.

We observed a wide heterogeneity in the ability of inactivated vaccine-induced T cell response to recognize the spike and membrane proteins from the Omicron VOC, which ranges from the complete abrogation to a gain of function exceeding the response to the corresponding vaccine-derived proteins (Figure 7B). Based on the amount of IFN-γ secreted in the whole-blood CRA, 13 out of 18 individuals from the inactivated cohort and six out of seven individuals from the heterologous cohort have at least 50% of their spike-specific T cell response preserved against the Omicron spike protein (Figure 7B). For the membrane-specific T cell response, at least 50% of the response was maintained in 10 out of 18 individuals from the inactivated cohort and two out of seven individuals in the heterologous cohort (Figure 7B). Interestingly, almost all of the nucleoprotein-specific T cell response was preserved, with no individual exhibiting less than 80% of response against the Omicron nucleoprotein (Figure 7B). This is in stark contrast to the membrane-specific T cell response even though both proteins have similar number of mutations present in the Omicron VOC (Table S1).

Combining the T cell response against multiple proteins, most of the vaccinees still maintained their response to the Omicron VOC (Figure 7C). Importantly, in vaccinees where the singular spike-specific T cell response was significantly inhibited, the combined multi-antigenic T cell response showed better preservation against Omicron (Figure 7C). Similar observations were made when the amount of secreted IL-2 was analyzed (Figures 7B and 7C).

**DISCUSSION**

The virological landscape of the COVID-19 pandemic was radically modified by the emergence of new SARS-CoV-2 lineages able to escape the neutralizing ability of antibodies elicited by vaccines based on the spike protein of the ancestor Wuhan isolates. This necessitates a more comprehensive analysis of vaccine immunogenicity that could not only be based on antibody measurements but also requires an evaluation of cellular immunity. T cells do not prevent infection per se, although their activity can mediate abortive infection at early stages, but they can be extremely important in the control of the viral pathogenesis due to their ability to recognize and lyse virus-infected cells and their association with viral control in acutely infected patients and animal models. For this reason, we performed a detailed characterization of the cellular immunity specific for different SARS-CoV-2 proteins elicited by inactivated virus (BBIBP-CorV and CoronaVac) and mRNA (BNT162b2) vaccines in a population of healthy adult individuals.

Using a whole-blood CRA that measures the ability of T cells to secrete cytokines directly in whole blood after encounter with specific antigens, we observed that the quantity of spike peptide-stimulated T cell cytokines were lower in vaccinees who received inactivated vaccines compared with those who had spike mRNA vaccine. However, both vaccine preparations (inactivated and mRNA) induced a Th1 response with similar IFN-γ and IL-2 secretion profile. Both vaccines also elicited a comparable spike immunodominance hierarchy focused more on the S2 region of spike (Figures 1 and 2). On the other hand, inactivated vaccines did not elicit a response only focused on the spike protein. Significant quantities of membrane- and nucleoprotein-specific T cells were also induced after vaccination that are clearly absent in individuals vaccinated with mRNA spike-only vaccine (Figure 3). A comparison of the multi-antigenic vaccine-induced T cell response calculated by quantifying the levels of IFN-γ and IL-2 elicited by inactivated versus mRNA vaccine showed that inactivated vaccines not only elicited a broader T cell immunity but they also induced a quantitatively comparable T cell response against SARS-CoV-2 (Figure 4).

The importance of eliciting a multi-antigenic T cell response against different SARS-CoV-2 proteins should not be underestimated since T cell response in individuals who control SARS-CoV-2 with limited or no symptoms possess T cell responses against different epitopes present in different structural and non-structural proteins. In addition, in experimental SARS-CoV-2 challenge of non-human primates and mice vaccinated against nucleoprotein or envelope and membrane, the vaccinated animals had less severe pathology and lower viral loads. This protective effect was associated with the rapid recall of antigen-specific T cell responses without robust humoral immune response, showing the protective capacity of T cells specific for other structural proteins of SARS-CoV-2. The broad
specificity is also potentially beneficial when considering the effects of amino acid mutations present in the different SARS-CoV-2 proteins of known VOCs. While the inactivated SARS-CoV-2 vaccine-induced T cell response was largely preserved against the Omicron VOC, there were differences in the responses against different structural proteins (Figure 7). In all vaccinees tested, nucleoprotein-specific T cell responses were not affected by the mutations present in Omicron, while both spike-and membrane-specific T cell responses were inhibited to various levels (Figure 7). This is likely explained by the density...
of amino acid mutations present in each protein (spike, one mutation in 40 amino acids, 35 mutations in the whole protein; membrane, one in 67, three mutations in the whole protein; nucleoprotein, one in 80, five mutations in the whole protein) (Table S1). A protein with a low density of amino acid mutation is more likely to contain T cell epitopes that are fully conserved between the vaccine strain and Omicron. Importantly, in vaccinates where their spike-specific T cell response was significantly inhibited by the mutations present in Omicron, their combined vaccine-induced T cell response (spike, membrane, and nucleoprotein) is shown in Figure 7. The inactivated SARS-CoV-2 vaccine-induced T cell response against the Omicron variant of concern (A) Schematic showing the concept behind the design of the peptide pools to assess the inactivated SARS-CoV-2 vaccine-induced T cell response against the spike, membrane, and nucleoprotein from the Omicron VOC. Orange regions refer to amino acid mutations present in the Omicron variant relative to the vaccine-derived SARS-CoV-2. The respective megapool contains peptides covering the whole wild-type (WT) SARS-CoV-2 protein (spike, membrane, or nucleoprotein). The respective WT pool contains peptides, with the WT amino acid sequence, covering the regions affected by mutations present in the Omicron variant of the protein. The respective mutant (MT) pool contains peptides from the WT pool with the amino acid mutations present in the Omicron variant. The equation shows how the percentage preservation of the vaccine-induced T cell response against the spike, membrane, or nucleoprotein from the Omicron VOC was calculated. (B) Inactivated SARS-CoV-2 vaccine-induced T cell response of individuals from the inactivated (BBIBP-CorV n = 18, dark blue) and heterologous (mRNA + CoronaVac n = 7, light blue) cohorts were evaluated by whole-blood CRA using the peptide pools described in (A). The percentage preservation of the vaccine-induced T cell response against the spike, membrane, and nucleoprotein from the Omicron VOC were calculated using IFN-γ (top) or IL-2 (bottom) concentrations from whole-blood CRA and are displayed as bars. Bars that enter the green shaded areas refer to a gain-of-function response against the Omicron VOC. (C) The percentage preservation of the spike (blue) and total inactivated vaccine-induced T cell response (red; spike, membrane, and nucleoprotein) against the Omicron VOC in vaccinates from both inactivated and heterologous cohorts were calculated using IFN-γ (left) or IL-2 (right) concentrations from whole-blood CRA and are displayed as bars.
nucleoprotein) was better preserved against Omicron (Figure 7C). The presence of multi-protein-specific T cells in individuals vaccinated with inactivated virus can provide them with a population of memory T cells more likely to tolerate the frequently found amino acid substitutions in spike that can partially suppress the spike-specific T cell response elicited by current spike mRNA vaccines. While the ability of Omicron to escape the full repertoire of spike-specific T cells induced by mRNA vaccines occurs only in a minority of individuals (10%–15%), immune escape can be substantial, in particular for spike-specific CD8 T cells.

However, the potential advantages of the heterogeneous specificity of T cells generated by inactivated SARS-CoV-2 vaccination needs to be balanced by our observation that inactivated vaccines do not elicit any CD6 T cell response against any viral proteins. By performing T cell assays utilizing CD4 and CD8 T cell enrichment methods, we showed that the robust inactivated vaccine-induced T cell response was exclusively mediated by CD4 T cells. In contrast, our data and others showed, using multiple different assays, that individuals vaccinated with mRNA and non-replicating adenoviral vectored spike vaccines are capable of inducing both CD4 and CD8 T cell responses. Thus, our results are in contrast with previous observations by other groups that showed the induction of both CD4 and CD8 T cell responses by inactivated SARS-CoV-2 vaccines, but are, however, in line with historical data of T cell response induced by other non-SARS-CoV-2-inactivated viral vaccines. The differences observed might be due to different causes, such as the presence of cross-reactive SARS-CoV-2-specific CD8 T cells induced by seasonal coronaviruses in a proportion of healthy individuals that can clearly confound the determination of the real SARS-CoV-2-specific vaccine-induced T cells. In addition, some of the vaccinated individuals might not be naive but could have been infected asymptomatically by SARS-CoV-2 and, as such, harbor SARS-CoV-2-specific CD8 T cells. However, we think that the major factor that could explain the discrepancy between our data and the ones published in the SARS-CoV-2 literature lies in the different method of T cell characterization. Differently than us, other groups utilized exclusively the AIM assay that characterizes responsive T cells based on the upregulation of T cell activation markers after stimulation with large pool of peptides. The AIM assay utilized by many groups worldwide (including us) is a powerful cellular technique that can define the phenotype of peptide responsive T cells. However, it is equally well known that the robust cytokine response of peptide responsive T cells can also activate other T cells in a bystander fashion without T cell receptor/epitope engagement. From our comparative analysis of results obtained by AIM and by depleting CD4 and CD8 T cells, it was clear that a robust activation of CD8 T cells was observed in inactivated vaccine recipients only with the AIM assay, showing that the results derived from the AIM assay can be confounded by robust bystander activation at least in PBMCs activated by large peptide pools. This possibility has also been discussed by others, but this should not be taken as a proof that the AIM assay is inherently unreliable. One has only to be aware of its potential limitations and consider the use of orthogonal assays (i.e., human leukocyte antigen [HLA]-tetramers staining, intracellular cytokine staining, utilization of single-peptide stimulation) to confirm the real antigen specificity of the T cells upregulating AIMs. It will certainly be necessary for other groups to try to better define such controversies and confirm or negate our data that, despite their clarity, have been obtained in a limited number of individuals vaccinated with inactivated vaccines due to the fact that performing a CD4 and CD8 T cell enrichment requires large volumes of blood.

The combined impact of the multi-protein-specific T cell response and its association with a deficiency of CD8 T cell induction on the protection from disease development is difficult to measure. In SARS-CoV-2-convalescent rhesus macaques with sub-protective antibody titers, depletion of CD8 T cells partially abrogated the protective efficacy of natural immunity against viral challenge, showing the anti-SARS-CoV-2 effects mediated directly by CD8 T cells. Recent data in vaccinated macaques have extended the importance of the vaccine-induced CD8 T cells in SARS-CoV-2 control. At the same time, the induction of CD4 T cells specific for the nucleoprotein of SARS-CoV in the nasal cavity of mice protected the animal from lethal disease after infection with different coronaviruses. This protective effect was mediated through the secretion of IFN-γ and, importantly, through the subsequent recruitment of virus-specific CD8 T cells. Similarly, memory CD4 T cells also directly and indirectly mediated protective effects in influenza A virus-infected mice. Clinical analysis of the efficacy of inactivated vaccines in different populations before the emergence of Omicron has shown that inactivated virus vaccines provided protection against the development of severe COVID-19 but with lower rates than the one provided by mRNA vaccines. On the contrary, recent data from Hong Kong measuring the efficacy against mild and severe COVID-19 development in healthy adults infected with Omicron showed similar efficacy of the two vaccine preparations after three doses. Perhaps the lack of the coordinated activation of CD4 and CD8 T cells observed in inactivated virus vaccine recipients infected with Wuhan or Delta strains of SARS-CoV-2 was then compensated by the protein multi-specificity that better tolerates the mutations present in Omicron.

Finally, our work showed that boosting with the third dose of inactivated SARS-CoV-2 vaccine 3 months after the second dose did not modify the spike, membrane, or nucleoprotein T cell response (Figure 5). The magnitudes of the T cell responses against the three different proteins were even comparable with individuals who completed their primary vaccinations 6 months before and did not receive their booster vaccines within the study period. Our data are in line with what was observed in a phase 2 placebo-controlled trial of inactivated SARS-CoV-2 vaccine: the third dose of inactivated vaccine 2 months after completion of the primary vaccination course (two doses) only modestly increased the neutralizing antibody levels. It is plausible that the ineffective boosting is due to the neutralization of the administered inactivated SARS-CoV-2 by the antibodies generated from the primary vaccination course leading to a decline in antigen availability to stimulate the immune system. This hypothesis was supported by the observation in the same phase 2 clinical trial where the neutralizing antibody titers were significantly boosted and were 3- to 4-fold
higher when the administration of the third vaccine dose was delayed until 8 months (instead of 2 months) after completion of the primary vaccination. It was also observed that humoral and T cell response were both robustly induced when the booster inactivated SARS-CoV-2 vaccine was given 5 months after the primary vaccination. These results certainly call for the future evaluation of the immunogenicity of inactivated SARS-CoV-2 vaccine in a setting where interference from existing virus-specific immune responses can occur.

In conclusion, we present here a detailed functional and quantitative evaluation of the T cell response induced by inactivated SARS-CoV-2 vaccines (BBIBP-CorV and CoronaVac) compared with an mRNA vaccine (BNT162b2). We show that, in sharp contrast to the clear inferiority of the humoral immunogenicity, inactivated vaccines elicited a T cell response of comparable magnitude and superior breadth, relative to the mRNA vaccine, that persisted for at least 6 months without need of further boosting. The ability to recognize different SARS-CoV-2 proteins, in particular membrane and nucleoprotein, allowed the T cell response induced by inactivated vaccines to better tolerate the mutations present in Omicron compared with the spike-focused mRNA vaccine-induced T cells. However, inactivated vaccines induced a SARS-CoV-2 T cell response exclusively mediated by CD4 T cells. Such findings have important clinical implications and should be evaluated in future large clinical studies with sample sizes beyond what we have described, which will also help to clarify the impact of virus-specific CD4 or CD8 T cells in SARS-CoV-2 pathogenesis.

Limitations of the study
Even though the data presented here have clearly shown the preferential induction of SARS-CoV-2-specific CD4 T cells by inactivated SARS-CoV-2 vaccines in our cohort of vaccinees, this observation would have to be confirmed in future large clinical studies with sample sizes beyond what we have described. More importantly, the ability of a multi-protein-specific CD4-centric T cell response to ameliorate the severity of COVID-19 compared with a single-protein-specific coordinated CD4 and CD8 T cell response will have to be evaluated to clarify the impact of virus-specific CD4 or CD8 T cells in SARS-CoV-2 pathogenesis.

STAR METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Material availability
  - Data and code availability
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
- METHOD DETAILS
  - Peptides
  - Whole blood cytokine release assay
  - PBMC isolation
  - Depletion of CD4+ or CD8+ T cells
- IFN-γ ELISPOT assay
- Activation-induced marker (AIM) assay
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xcrm.2022.100793.

ACKNOWLEDGMENTS

We would like to thank all clinical and nursing staff who provided care for the patients and all clinical trial coordinators and staff for their invaluable assistance in coordinating patient recruitment. This study is supported by the Singapore Ministry of Health’s National Medical Research Council under its COVID-19 Research Fund (COVID19RF3-0063, COVID19RF-001, and COVID19RF-008).

AUTHOR CONTRIBUTIONS

A.T.T., N.L.B., and A.B. designed the experiments. J.M.E.L., S.K.H., S.H., A.C., and N.T. performed the experiments. A.T.T., N.L.B., and A.B. analyzed and interpreted all the data. A.T.T. prepared the figures and wrote the paper. A.T.T., N.L.B., P.L.L., B.Y., D.C.L., and A.B. reviewed and edited the manuscript. E.S.L., E.C., P.L.L., B.Y., and D.C.L. designed the clinical trial, recruited all the patients, and provided the clinical samples. A.T.T., N.L.B., and A.B. designed and coordinated the study.

DECLARATION OF INTERESTS

A.T.T., N.L.B., and A.B. reported a patent for a method to monitor SARS-CoV-2-specific T cells in biological samples, which is pending. The other authors declare no competing interests.

Received: May 24, 2022
Revised: August 17, 2022
Accepted: September 30, 2022
Published: November 15, 2022

REFERENCES

1. Krause, P.R., Fleming, T.R., Peto, R., Longini, I.M., Figueroa, J.P., Sterne, J.A.C., Cravioto, A., Rees, H., Higgins, J.P.T., Bouton, I., et al. (2021). Considerations in boosting COVID-19 vaccine immune responses. Lancet 398, 1377–1380. https://doi.org/10.1016/S0140-6736(21)02046-8.
2. Mallapaty, S. (2021). China’s COVID vaccines have been crucial - now immunity is waning. Nature 598, 398–399. https://doi.org/10.1038/d41586-021-02796-w.
3. Rosa Duque, J.S., Wang, X., Leung, D., Cheng, S.M.S., Cohen, C.A., Mu, X., Hachim, A., Zhang, Y., Chan, S.M., Chaathai, S., et al. (2022). Immunogenicity and reactogenicity of SARS-CoV-2 vaccines BNT162b2 and CoronaVac in healthy adolescents. Nat. Commun. 13, 3700. https://doi.org/10.1038/s41467-022-31485-z.
4. McKinstry, K.K., Strutt, T.M., Kuang, Y., Brown, D.M., Sell, S., Dutton, R.W., and Swain, S.L. (2012). Memory CD4+ T cells protect against influenza through multiple synergizing mechanisms. J. Clin. Invest. 122, 2847–2856. https://doi.org/10.1172/JCI63869.
5. Cromer, D., Steain, M., Reynaldi, A., Schlub, T.E., Wheatley, A.K., Juno, J.A., Kent, S.J., Triccas, J.A., Khoury, D.S., and Davenport, M.P. (2022). Neutralising antibody titres as predictors of protection against SARS-CoV-2 variants and the impact of boosting: a meta-analysis. Lancet. Microbe 3, e52–e61. https://doi.org/10.1016/S2666-5247(21)00267-6.
6. Jeewandara, C., Aherathna, I.S., Pushpakumara, P.D., Kamaladasa, A., Guruge, D., Wijesinghe, A., Gunesekera, B., Ramu, S.T., Kuruppu, H., Ranasinghe, T., et al. (2021). Persistence of immune responses to the
Sinopharm/BIBP-CoV vaccine. Immun. Inflamm. Dis. 10, 2021.10.14. 21265030. https://doi.org/10.1101/2021.10.14.21265030.
7. Peng, Q., Zhou, R., Wang, Y., Zhao, M., Liu, N., Li, S., Huang, H., Yang, D., Au, K.K., Wang, H., et al. (2022). Waning immune responses against SARS-CoV-2 variants of concern among vaccinees in Hong Kong. EBioMedicine 77, 103904. https://doi.org/10.1016/j.ebiom.2022.103904.
8. Zeng, G., Wu, Q., Pan, H., Li, M., Yang, J., Wang, L., Wu, Z., Jiang, D., Xu, C., et al. (2022). Immunogenicity and safety of a third dose of CoronaVac, and immune persistence of a two-dose schedule, in healthy adults: interim results from two single-centre, double-blind, randomised, placebo-controlled phase 2 clinical trials. Lancet Infect. Dis. 22, 483–495. https://doi.org/10.1016/S1473-3099(21)00681-2.
9. Zhang, H., Jia, Y., Ji, Y., Cong, X., Liu, Y., Yang, R., Kong, X., Shi, Y., Zhu, L., Wang, Z., et al. (2022). Inactivated vaccines against SARS-CoV-2: neutralizing antibody titers in vaccine recipients. Front. Microbiol. 13, 816778. https://doi.org/10.3389/fmicb.2022.816778.
10. Lim, W.W., Mak, L., Leung, G.M., Cowling, B.J., and Peiris, M. (2021). Comparative immunogenicity of mRNA and inactivated vaccines against COVID-19. Lancet. Microbe 2, e423. https://doi.org/10.1016/S2666-5247(21)00177-4.
11. Tarke, A., Coelho, C.H., Zhang, Z., Dan, J.M., Yu, E.D., Methot, N., Bloom, N.I., Goodwin, B., Phillips, E., Mallal, S., et al. (2022). SARS-CoV-2 vaccination induces immunological T cell memory able to cross-recognize variants from Alpha to Omicron. Cell 185, 847–859.e11. https://doi.org/10.1016/j.cell.2022.01.015.
12. Goel, R.R., Painter, M.M., Apostolidis, S.A., Mathew, D., Meng, W., Rosenfeldt, A.M., Lundgreen, K.A., Reidali, A., Khoury, D.S., Pattekar, A., et al. (2021). mRNA vaccines induce durable immune memory to SARS-CoV-2 and variants of concern. Science 374, abm0829. https://doi.org/10.1126/science.abm0829.
13. Guererra, G., Piccozza, M., D’Orso, S., Placido, R., Gommers, L., Nieuwkoop, N.N., Schmitz, K.S., Rijsbergen, L.C., van Osch, J.A.T., Dijkstra, E., et al. (2021). SARS-CoV-2 variants of concern partially escape humoral but not T-cell responses in COVID-19 convalescent donors and vaccinees. Sci. Immunol. 6, eabj1750. https://doi.org/10.1126/sciimmunol.abj1750.
14. Tarke, A., Sidney, J., Methot, N., Yu, E.D., Zhang, Y., Dan, J.M., Goodwin, B., Rubio, P., Sutherland, A., Wang, E., et al. (2021). Impact of SARS-CoV-2 variants on the total CD4(+) and CD8(+) T cell reactivity in infected or vaccinated individuals. Cell Rep. Med. 2, 100355. https://doi.org/10.1016/j.xcrm.2021.100355.
15. Jara, A., Undurraga, E.A., Gonzalez, C., Paredes, F., Fontecilla, T., Jara, G., Pizarro, A., Acevedo, J., Leo, K., Leon, F., et al. (2021). Effectiveness of an inactivated SARS-CoV-2 vaccine in Chile. N. Engl. J. Med. 385, 875–884. https://doi.org/10.1056/NEJMc2107715.
16. Ranzani, O.T., Hitchings, M.D.T., Dorion, M., D’Agostini, T.L., de Paula, R.C., de Paula, O.F.P., Villela, E.F.d.M., Torres, M.S.S., de Oliveira, S.B., Schulz, W., et al. (2021). Effectiveness of the CoroNaVac vaccine in older adults during a gamma variant associated epidemic of covid-19 in Brazil: test negative case-control study. BMJ 374, n2015. https://doi.org/10.1136/bmj.n2015.
17. Kang, M., Li, Y., Li, Y., Sun, L., Deng, A., Hu, T., Zhang, J., Liu, J., Cheng, M., Xie, S., et al. (2022). Effectiveness of inactivated COVID-19 vaccines against illness caused by the B.1.617.2 (Delta) variant during an outbreak in guangdong, China: a cohort study. Ann. Intern. Med. 175, 533–540. https://doi.org/10.7326/M21-3509.
18. Hu, Z., Tao, B., Li, Z., Song, Y., Li, C., Li, J., Zhu, M., Yu, Y., Huang, P., and Wang, J. (2022). Effectiveness of inactivated COVID-19 vaccines against severe illness in B.1.617.2 (Delta) variant-infected patients in Jiangsu, China. Int. J. Infect. Dis. 116, 204–209. https://doi.org/10.1016/j.ijid.2022.01.030.
19. Rotshild, V., Hirsh-Raccah, B., Miskin, I., Muszkat, M., and Matok, I. (2021). Comparing the clinical efficacy of COVID-19 vaccines: a systematic review and network meta-analysis. Sci. Rep. 11, 22777. https://doi.org/10.1038/s41598-021-02321-z.
20. Deng, Y., Li, Y., Yang, R., and Tan, W. (2021). SARS-CoV-2-specific T cell immunity to structural proteins in inactivated COVID-19 vaccine recipients. Cell. Mol. Immunol. 18, 2040–2041. https://doi.org/10.1038/s41423-021-00730-8.
21. Pisheshna, N., Harmand, T.J., and Ploegh, H.L. (2022). A guide to antigen processing and presentation. Nat. Rev. Immunol. https://doi.org/10.1038/s41577-022-00707-2.
22. Ho, N.I., Huis in, ‘t Veld, L.G.M., Raaijmakers, T.K., and Adema, G.J. (2018). Adjuvants enhancing cross-presentation by dendritic cells: the key to more effective vaccines? Front. Immunol. 9, 2874. https://doi.org/10.3389/fimmu.2018.02874.
23. Tan, A.T., Lim, J.M., Le Bert, N., Kunasegaran, K., Chia, A., Qui, M.D., Tan, N., Chia, W.N., de Alwis, R., Ying, D., et al. (2021). Rapid measurement of SARS-CoV-2 spike T cells in whole blood from vaccinated and naturally infected individuals. J. Clin. Invest. 137, e152379. https://doi.org/10.1172/JCI152379.
24. Lü, C., Mendionda, L., Yang, Y., Gao, Y., Shen, C., Liu, J., Ni, T., Ju, B., Liu, C., Tang, X., et al. (2020). The architecture of inactivated SARS-CoV-2 with postfusion spikes revealed by cryo-EM and cryo-ET. Structure 28, 1218–1224.e4. https://doi.org/10.1016/j.str.2020.10.001.
25. Liu, C., Fang, Y., Yang, Y., Gao, Y., Shen, C., Liu, J., Li, M., Yang, D., Wang, N., Lv, Z., et al. (2020). Development of an inactivated vaccine candidate for SARS-CoV-2. Science 369, 77–81. https://doi.org/10.1126/science.abc1932.
26. Vadrevu, K.M., Ganneru, B., Reddy, S., Joggand, H., Raju, D., Sapkal, G., Yadav, P., Reddy, P., Verma, S., Singh, C., et al. (2022). Persistence of immunity and impact of third dose of inactivated COVID-19 vaccine against emerging variants. Sci. Rep. 12, 12038. https://doi.org/10.1038/s41598-022-16097-3.
27. Andrews, N., Stowe, J., KARSEBOM, T., Tofta, S., Rickard, T., Gallacher, E., Gower, C., Kall, M., Groves, N., O’Connell, A.M., et al. (2022). Covid-19 vaccine effectiveness against the omicron (B.1.1.529) variant. N. Engl. J. Med. 386, 1532–1546. https://doi.org/10.1056/NEJMc2119451.
Cell Reports Medicine

Article

34. Planas, D., Saunders, N., Maes, P., Guivel-Benhassine, F., Planchais, C., Buchrieser, J., Bolland, W.H., Porrot, F., Staropoli, I., Lemoine, F., et al. (2022). Considerable escape of SARS-CoV-2 Omicron to antibody neutralization. Nature 602, 671–675. https://doi.org/10.1038/s41586-021-04389-z.

35. Swadling, L., Diniz, M.O., Schmidt, N.M., Amin, O.E., Chandran, A., Shaw, E., Pade, C., Gibbons, J.M., Le Bert, N., Tan, A.T., et al. (2022). Pre-existing polymerase-specific T cells expand in abortive seronegative SARS-CoV-2. Nature 601, 110–117. https://doi.org/10.1038/s41586-021-04186-8.

36. 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.

37. Liu, J., Yu, J., McMahan, K., Jacob-Dolan, C., He, X., Giffin, V., Wu, C., Sciaccia, M., Powers, O., Nampanya, F., et al. (2022). CD8 T cells contribute to vaccine protection against SARS-CoV-2 in macaques. Sci. Immunol. 9, eabq7647. https://doi.org/10.1126/sciimmunol.abq7647.

38. McMahan, K., Yu, J., Mercado, N.B., Loos, C., Tostanoski, L.H., Chandra-shekar, A., Liu, J., Peter, L., Atyeo, C., Zhu, A., et al. (2021). Correlates of protection against SARS-CoV-2 in rhesus macaques. Nature 590, 630–634. https://doi.org/10.1038/s41586-020-03041-6.

39. Le Bert, N., Clapham, H.E., Tan, A.T., Chia, W.N., Tham, C.Y.L., Lim, J.M., Kunasegaran, K., Tan, L.W.L., Duterte, C.A., Shankar, N., et al. (2021). Highly functional virus-specific cellular immune response in asymptomatic SARS-CoV-2 infection. J. Exp. Med. 218, e20202617. https://doi.org/10.1084/jem.20202617.

40. Sekine, T., Perez-Potti, A., Rivera-Ballesteros, O., Strälin, 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.

41. Sette, A., and Crotty, S. (2021). Adaptive immunity to SARS-CoV-2 and COVID-19. Cell 181, 861–880. https://doi.org/10.1016/j.cell.2021.01.007.

42. Ferretti, A.P., Kula, T., Wang, Y., Nguyen, D.M.V., Weinerheimer, A., Dunlap, G.S., Xu, Q., Nabilis, N., Perullo, C.R., Cristofaro, A.W., et al. (2020). Unbiased screens show CD8(+) T cells of COVID-19 patients recognize shared epitopes in SARS-CoV-2 that largely reside outside the spike protein. Immunity 53, 1095–1107.e3. https://doi.org/10.1016/j.immuni.2020.10.006.

43. Harris, P.E., Brasel, T., Massey, C., Herst, C.V., Burkholz, S., Lloyd, P., Blankenberg, T., Bey, T.M., Carback, R., Hodge, T., et al. (2021). A synthetic peptide CTL vaccine targeting nucleocapsid confers protection from SARS-CoV-2 challenge in rhesus macaques. Vaccines (Basel) 9. https://doi.org/10.3390/vaccines9050520.

44. Matchett, W.E., Joag, V., Stolley, J.M., Shepherd, F.K., Angeli, C.H., Cloherty, W., Hall, K., Bertoletti, A., and Low, J.G. (2022). Humoral and cellular immune memory to four COVID-19 vaccines. Cell 185, 2434–2451.e17. https://doi.org/10.1016/j.cell.2022.05.022.

45. Munro, A.P.S., Janani, L., Cornelius, V., Aley, P.K., Babbage, G., Baxter, D., Bula, M., Cathie, C., Chatterjee, K., Dodd, K., et al. (2021). Safety and immunogenicity of seven COVID-19 vaccines as a third dose (booster) following two doses of ChAdOx1 nCoV-19 or BNT162b2 in the UK (COV-BOOST): a blinded, multicentre, randomised, controlled, phase 2 trial. Lancet 398, 2258–2276. https://doi.org/10.1016/S0140-6736(21)02717-3.

46. Khoo, N.K.H., Lim, J.M.E., Gill, U.S., de Alwis, R., Tan, N., Toh, J.Z.N., Abbott, J.E., Usai, C., Ooi, E.E., Low, J.G.H., et al. (2022). Differential immunogenicity of homologous versus heterologous boost in Ad26.COV2.S vaccine recipients. Med (N Y) 3, 104–118.e4. https://doi.org/10.1016/j.med.2021.12.004.

47. Chen, Y., Yin, S., Tong, X., Tao, Y., Ni, J., Pan, J., Li, M., Wan, Y., Mao, M., Xiong, Y., et al. (2022). Dynamic SARS-CoV-2-specific B-cell and T-cell responses following immunization with an inactivated COVID-19 vaccine. Clin. Microbiol. Infect. 28, 410–418. https://doi.org/10.1016/j.cmi.2021.10.005.

48. Skibinski, D.A.G., Jones, L.A., Zhu, Y.O., Xue, L.W., Au, B., Lee, B., Naim, A.R.M., Lee, A., Kaliaperumal, N., Low, J.G.H., et al. (2018). Induction of human T-cell and cytokine responses following vaccination with a novel rabies vaccine. J. Immunol. 200, 1175–1183. https://doi.org/10.4049/jimmunol.2016.12.031.

49. Wijaya, L., Tham, C.Y.L., Chan, Y.F.Z., Wong, A.W.L., Li, L.T., Wang, L.F., Bertolletti, A., and Low, J.G. (2017). An accelerated rabies vaccine schedule based on toll-like receptor 3 (TLR3) agonist PIKA adjuvant augments rabies virus specific antibody and T cell response in healthy adult volunteers. Vaccine 35, 1175–1183. https://doi.org/10.1016/j.vaccine.2016.12.031.

50. Lineburg, K.E., Grant, E.J., Swaminathan, S., Chatzileontiadou, D.S.M., Szeto, C., Sloane, H., Panikkar, A., Raju, J., Crooks, P., Rehan, S., et al. (2021). CD8(+) T cells specific for an immunodominant SARS-CoV-2 nucleocapsid epitope cross-react with selective seasonal coronaviruses. Immunity 54, 1055–1065.e5. https://doi.org/10.1016/j.immuni.2021.04.006.

51. Low, J.S., Vaqueirinho, D., Mele, F., Foglierini, M., Jerak, J., Perotti, M., Jarrosayd, J., Jovic, S., Perez, L., Cacciatorre, R., et al. (2021). Clonal analysis of immunodominance and cross-reactivity of the CD4 T cell response
to SARS-CoV-2. Science 372, 1336–1341. https://doi.org/10.1126/science.abg8985.

62. Loyal, L., Braun, J., Henze, L., Kruse, B., Dingeldey, M., Reimer, U., Kern, F., Schwarz, T., Mangold, M., Unger, C., et al. (2021). Cross-reactive CD4(+) T cells enhance SARS-CoV-2 immune responses upon infection and vaccination. Science 374, eabh1823. https://doi.org/10.1126/science.abh1823.

63. Mateus, J., Dan, J.M., Zhang, Z., Rydzynski Moderbacher, C., Lammers, M., Goodwin, B., Sette, A., Crotty, S., and Weiskopf, D. (2021). Low-dose mRNA-1273 COVID-19 vaccine generates durable memory enhanced by cross-reactive T cells. Science 374, eabj9853. https://doi.org/10.1126/science.abj9853.

64. Sagar, M., Reifler, K., Rossi, M., Miller, N.S., Sinha, P., White, L.F., and Mizgerd, J.P. (2021). Recent endemic coronavirus infection is associated with less-severe COVID-19. J. Clin. Invest. 131, 143380. https://doi.org/10.1172/JCI143380.

65. Kalimuddin, S., Tham, C.Y.L., Qui, M., de Alwis, R., Sim, J.X.Y., Lim, J.M.E., Tan, H.C., Syenina, A., Zhang, S.L., Le Bert, N., et al. (2021). Early T cell and binding antibody responses are associated with COVID-19 RNA vaccine efficacy onset. Med (N Y) 2, 682–688.e4. https://doi.org/10.1038/s41421-021-00403-0.

66. Seder, R.A., and Ahmed, R. (2003). Similarities and differences in CD4+ and CD8+ effector and memory T cell generation. Nat. Immunol. 4, 835–842. https://doi.org/10.1038/ni969.

67. Kim, T.S., and Shin, E.C. (2019). The activation of bystander CD8(+) T cells and their roles in viral infection. Exp. Mol. Med. 51, 1–9. https://doi.org/10.1038/s12276-019-0316-1.

68. Lee, H.G., Cho, M.Z., and Choi, J.M. (2020). Bystander CD4(+) T cells: crossroads between innate and adaptive immunity. Exp. Mol. Med. 52, 1255–1263. https://doi.org/10.1038/s12276-020-00486-7.

69. Sette, A., and Crotty, S. (2022). Immunological memory to SARS-CoV-2 infection and COVID-19 vaccines. Immunol. Rev. 310, 27–46. https://doi.org/10.1111/imr.13089.

70. Zhao, J., Zhao, J., Mangalam, A.K., Channappanavar, R., Fett, C., Meyerholz, D.K., Agnihotram, S., Baric, R.S., David, C.S., and Perlman, S. (2016). Airway memory CD4(+) T cells mediate protective immunity against emerging respiratory coronaviruses. Immunity 44, 1379–1391. https://doi.org/10.1016/j.immuni.2016.05.006.

71. McMenamin, M.E., Nealon, J., Lin, Y., Wong, J.Y., Cheung, J.K., Lau, E.H.Y., Wu, P., Leung, G.M., and Cowling, B.J. (2022). Vaccine effectiveness of one, two, and three doses of BNT162b2 and CoronaVac against COVID-19 in Hong Kong: a population-based observational study. Lancet Infect. Dis. 22, 1435–1443. https://doi.org/10.1016/S1473-3099(22)00345-0.

72. Liu, Y., Zeng, Q., Deng, C., Li, M., Li, L., Liu, M., Ruan, X., Mei, J., Mo, R., et al. (2022). Robust induction of B cell and T cell responses by a third dose of inactivated SARS-CoV-2 vaccine. Cell Discov. 8, 10. https://doi.org/10.1038/s41421-022-00373-7.
STAR METHODS

KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|

**Antibodies**

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Anti-human IFN-γ coating antibody | Mabtech | Cat# 3420-3-1000; RRID: AB_907282 |
| Anti-human IFN-γ biotin | Mabtech | Cat# 3420-6-1000; RRID: AB_907272 |
| Brilliant Violet 605 anti-human CD3 Antibody | Biolegend | Cat# 317322; RRID: AB_2561911 |
| Brilliant Violet 650 Mouse anti-human CD4 Antibody | BD Horizon™ | Cat# 563875; RRID: AB_2744425 |
| PE/Cyanine7 Mouse anti-human CD8 Antibody | BD Pharmingen™ | Cat# 557746; RRID: AB_396852 |
| V450 Mouse anti-human CD25 Antibody | BD Horizon™ | Cat# 560355; RRID: AB_1645565 |
| Alexa Fluor® 700 anti-human CD69 Antibody | Biolegend | Cat# 310922; RRID: AB_493775 |
| PE anti-human CD134 (OX40) Antibody | Biolegend | Cat# 350004; RRID: AB_10645478 |
| APC Mouse anti-human CD137 Antibody | BD Pharmingen™ | Cat# 550890; RRID: AB_398477 |

**Biological samples**

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Blood from individuals who received SARS-CoV-2 vaccine | National Centre for Infectious Diseases | N/A |

**Chemicals, peptides, and recombinant proteins**

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Ficoll-paque plus | Cytivia | Cat# 17144003 |
| Zombie NIR Fixable Viability Kit | Biolegend | Cat# 423105 |
| Streptavidin-ALP | Mabtech | Cat# 3310-10-1000 |
| KPL BCIP/NBT Phosphatase substrate | SeraCare | Cat# 5420-0038 |
| 15-mer SARS-CoV-2 overlapping Spike, Nucleoprotein and Membrane peptides | Genscript | N/A |

**Critical commercial kit**

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Ella multianalyte immunoassay | Protein simple | N/A |
| EasySep Human CD4 Positive Selection Kits II | Stemcell Technologies | Cat# 17852 |
| EasySep Human CD8 Positive Selection Kits II | Stemcell Technologies | Cat# 17853 |

**Deposited data**

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| No unique dataset was generated | N/A | N/A |

**Software and algorithms**

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Graphpad Prism 9 | Graphpad | https://www.graphpad.com/scientific-software/prism/ |
| Immunospot software | Cellular Technology Limited | http://www.immunospot.com/ImmunoSpot-analyzers-software |
| Kaluza Analysis Software | Beckman Coulter | https://www.beckman.com/flow-cytometry/software/kaluza |

RESOURCE AVAILABILITY

**Lead contact**

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contacts, Anthony T Tan (anthony.tan@duke-nus.edu.sg) and Antonio Bertoletti (antonio@duke-nus.edu.sg).

**Material availability**

This study did not generate new unique reagents.

**Data and code availability**

- All data reported in this paper will be shared by the lead contact upon request.
This paper does not report original code.

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

Additional Supplemental Items are available from Mendeley Data at https://doi.org/10.17632/5vhym6h3cy.1.

**EXPERIMENTAL MODEL AND SUBJECT DETAILS**

The study design and protocol for the COVID-19 PROTECT study group were assessed by National Healthcare Group (NHG) Domain Specific Review Board (DSRB) and approved under study number 2012/00917. Written informed consent was obtained from all study participants in accordance with the Declaration of Helsinki for Human Research.

3 cohorts of vaccinated healthy individuals were studied (Table 1). Vaccinated individuals were between 22–69 years of age, healthy (do not report any health issue, they were not hospitalized in the last year and they were not under treatment with immunosuppressive drugs) and with no history of SARS-CoV-2 infection as evaluated by antigen rapid tests and SARS-CoV-2 surrogate virus neutralization assay (GenScript). The first cohort (Inactivated cohort) are healthy individuals who received two doses of BBIBP COVID-19 inactivated vaccine (BBIBP-CoV-Sinopharm) given 21 days apart (n = 30). The second cohort (Heterologous cohort) are healthy individuals who developed adverse events after receiving a single dose of mRNA vaccine (BNT162b2-Pfizer-BioNTech or mRNA-1273-Moderna) and proceeded to switch to vaccination with two doses of CoronaVac inactivated vaccine (Sinovac) given 21 days apart (n = 19). A reference cohort (mRNA cohort) of healthy individuals who received two doses of Spike mRNA vaccine (BNT162b2-Pfizer-BioNTech) were also analysed (n = 76). Blood samples were collected before receiving inactivated vaccines (Inactivated and Heterologous cohort) or mRNA vaccines (mRNA cohort), 21 days and 2–3 months post-vaccination (Figure 1A).

Local policy changes recommended the inclusion of a third homologous dose of inactivated SARS-CoV-2 vaccines into the primary vaccination series 3 months after the administration of the second dose. To assess the effects of this booster dose of inactivated vaccines, blood samples of individuals from the inactivated and heterologous cohort were also collected 6 months post-vaccination. A total of 18 individuals in the Inactivated cohort and 7 in the Heterologous cohort were given the booster dose 1–3 months and <1 month before blood collection respectively. Individuals with a recorded SARS-CoV-2 infection during the booster dose study period (within 6–7 months of the first inactivated vaccine dose) were excluded from the analysis.

**METHOD DETAILS**

**Peptides**

15-mer peptides that overlapped by 10 amino acids spanning the entire protein sequences of Nucleoprotein, Membrane and Spike protein of SARS-CoV-2 (GISAID: EPI_ISL_410713) were synthesized and pooled into mega-pools. In addition, 55 Spike peptides covering the immunogenic regions of the SARS-CoV-2 spike protein representing 40.5% of the whole Spike protein (SpG peptide pool) were also synthesized, pooled and used to stimulate whole blood as described previously. To measure the T cell response against the Omicron variant, two additional peptide pools were designed for each viral structural protein (Membrane, Nucleoprotein and Spike). The two pools include peptides impacted by the mutations in Omicron (identified using https://outbreak.info), with one consisting of ancestral-derived peptides and another consisting of Omicron-derived peptides covering the same region. Details of all the peptides used are found in Table S1 (Mendeley Data: https://doi.org/10.17632/5vhym6h3cy.1).

**Whole blood cytokine release assay**

The whole blood cytokine release assay was performed as described previously. In brief, 320 μL of freshly drawn blood (drawn within 6 h of venepuncture) were mixed with 80 μL RPMI and stimulated with the indicated SARS-CoV-2 Spike peptide pools (Table S1) at 2 μg/mL or with DMSO as a control. After 16 h of incubation, the culture supernatant (plasma) was collected and stored at −30°C. Cytokine concentrations in the plasma were quantified using an ELLA machine with microfluidic multiplex cartridges that measured IFN-γ and IL-2 according to the manufacturer’s protocol (Protein Simple). The levels of cytokines present in the plasma of DMSO controls were subtracted from the corresponding peptide pool-stimulated samples. The positivity threshold was set at 10 x times the lower limit of quantification of each cytokine (IFN-γ = 1.7 pg/mL; IL-2 = 5.4 pg/mL) after DMSO background subtraction.

**PBMC isolation**

Peripheral blood was collected from all individuals in heparin-containing tubes, and peripheral blood mononuclear cells (PBMCs) from all collected blood samples were isolated by Ficoll-Paque density gradient centrifugation. Isolated PBMCs were either analysed directly or cryopreserved in liquid nitrogen until required.

**Depletion of CD4+ or CD8+ T cells**

CD4+ or CD8+ T cells were positively selected from freshly isolated PBMCs with EasySep Positive Selection Kits II (Stemcell Technologies). After magnetic affinity cell sorting, flow through consisting of CD4-enriched and CD8-enriched PBMCs were collected and used immediately for IFN-γ ELISPOT assay.
**IFN-γ ELISPOT assay**

The frequency of SARS-CoV-2 specific T cells was quantified as described previously. Briefly, ELISPOT plates (Millipore Sigma) were coated with human IFN-γ antibody overnight at 4°C. 250,000 to 400,000 total, CD4-enriched or CD8-enriched PBMCs were seeded per well and stimulated for 18 h with the indicated peptide pools at 1 μg/mL. The plates were then incubated with a human biotinylated IFN-γ detection antibody, followed by streptavidin–alkaline phosphatase (streptavidin-AP) and developed using the KPL BCIP/NBT phosphatase substrate (Seracare Life Sciences). To quantify the peptide-specific responses, spots of the unstimulated wells were subtracted from the peptide-stimulated wells, and the results were expressed as spot-forming cells (SFC) per 10⁶ PBMCs. Results were excluded if negative control wells had more than 30 SFC/10⁶ PBMCs or if positive control wells stimulated with PMA/ionomycin were negative.

**Activation-induced marker (AIM) assay**

Cryopreserved PBMCs were thawed and stimulated with 1 μg/mL mega-pool Spike peptides or equivalent amount of DMSO for 24 h at 37°C. Cells were then washed in phosphate buffered saline and stained with Zombie NIR Fixable Viability Kit (Biolegend) to exclude dead cells in the analysis. The cells were next washed in FACS buffer with 2mM EDTA and surface markers were stained with the surface markers, anti-CD3, anti-CD4, anti-CD8, anti-CD25, anti-CD69, anti-CD134 (OX40) and anti-CD137 (4-1BB), diluted in FACS buffer for 30 min on ice. After two more washes in FACS buffer, cells were resuspended in PBS prior to acquisition with Beckman Coulter CytoFLEX S analyser.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

All statistical analyses were performed using GraphPad Prism, version 9 (GraphPad Software). Where applicable, the statistical tests used and the definition of centre were indicated in the figure legends. Statistical significance was defined as having a p-value of less than 0.05. In all instances, “n” refers to the number of patients analysed.