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.
SARS-CoV-2 vaccine-induced antibody and T cell response in SARS-CoV-1 survivors

Graphical abstract

Highlights

- Vaccination of SARS-CoV-1 survivors boosts the neutralizing antibody response
- Omicron subvariants resist neutralization by serum more than earlier VOCs
- SARS-CoV-2-specific T cell response is not affected by prior SARS-CoV-1 infection
- SARS-CoV-1-specific T cell response is boosted by prior SARS-CoV-1 infection

Authors

Li-Jun Duan, Xiao-Ming Cui, Ka-Li Zhu, Lin Yao, Guo-Lin Wang, Wu-Chun Cao, Mai-Juan Ma

Correspondence

cawuchun@126.com (W.-C.C.), mjma@163.com (M.-J.M.)

In brief

Duan et al. examine antibody and T cell responses in Ad5-nCoV-vaccinated SARS-CoV-1 survivors 6 months after vaccination. They show that vaccination boosts neutralizing antibodies in SARS-CoV-1 survivors but neutralization against VOCs is limited. SARS-CoV-1 survivors elicit a comparable SARS-CoV-2-specific T cell response but a stronger SARS-CoV-1-specific T cell response than naive healthy individuals.
SARS-CoV-2 vaccine-induced antibody and T cell response in SARS-CoV-1 survivors

Li-Jun Duan,1,3 Xiao-Ming Cui,1,3 Ka-Li Zhu,1,2 Lin Yao,1 Guo-Lin Wang,1 Wu-Chun Cao,1,* and Mai-Juan Ma1,2,4,*
1State Key Laboratory of Pathogen and Biosafety, Beijing Institute of Microbiology and Epidemiology, Beijing 100071, China
2Department of Epidemiology and Biostatistics, School of Public Health, Anhui Medical University, Hefei 230032, China
3These authors contributed equally
4Lead contact
*Correspondence: caowuchun@126.com (W.-C.C.), mjma@163.com (M.-J.M.)
https://doi.org/10.1016/j.celrep.2022.111284

SUMMARY
Preexisting immunity cross-reactive to severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) in SARS-CoV-1 survivors suggests that a coronavirus disease 2019 vaccine may boost such preexisting cross-reactive memory T cells. We measure SARS-CoV-2 and SARS-CoV-1 spike-specific neutralizing antibody and T cell responses in a single dose of Ad5-nCoV-immunized SARS-CoV-1 survivors 6 months after vaccination. Compared with Ad5-nCoV-immunized naive healthy individuals (NHIs), vaccination of Ad5-nCoV in SARS-CoV-1 survivors boosts the antibody response against SARS-CoV-1 but induces a limited neutralizing antibody that is capable of neutralizing SARS-CoV-2 variants of concern, and nearly all serum samples lose neutralization to Omicron subvariants. Immunized SARS-CoV-1 survivors produce a T cell response to SARS-CoV-2 comparable with that of Ad5-nCoV-immunized NHIs. However, a robust cross-reactive T cell response to SARS-CoV-1 is identified in immunized SARS-CoV-1 survivors compared with Ad5-nCoV-immunized NHIs. These findings suggest that vaccination with Ad5-nCoV elicits a stronger neutralizing antibody and cross-reactive T cell responses against SARS-CoV-1 in SARS-CoV-1 survivors.

INTRODUCTION
Since the emergence of severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1), the causative agent of SARS, in Guangdong Province in China in November 2002, 18 years have passed (Sariol and Perlman, 2020). SARS-CoV-1 is a Betacoronavirus that belongs to Coronavirusidae, a family of large single-stranded positive-sense RNA viruses that contains viruses from four genera (Alpha, Beta, Gamma, and Delta coronavirus) (Sariol and Perlman, 2020). SARS-CoV-1, like SARS-CoV-2, induces antibody and cellular responses in patients after infection (Sariol and Perlman, 2020). While the antibody responses have been reported to lack longevity that could persist 2 to 3 years (Cao et al., 2007) and undetectable memory B cell responses by 6 years after infection (Tang et al., 2011), SARS-CoV-1 nucleocapsid (N) protein-specific memory T cells could be detected in SARS-CoV-1 survivors even 17 years after infection (Le Bert et al., 2020). These memory T cells showed substantial cross-reactivity to the N protein of SARS-CoV-2 (Le Bert et al., 2020).

Moreover, there is increasing evidence of memory CD4+ T cells in a significant proportion of SARS-CoV-2-naïve individuals cross-reactive to SARS-CoV-2 and of cross-reactivity between CD4+ T cells specific for SARS-CoV-2 and CD4+ T cells specific for human common cold coronaviruses and animal betacoronaviruses (Ahmed et al., 2020; Braun et al., 2020; Grifoni et al., 2020; Le Bert et al., 2020; Mateus et al., 2020; Sariol and Perlman, 2020), which likely result from previous exposure to human common cold coronaviruses as well as animal coronaviruses. In addition, more than 90% of tested healthy adults also have immunoglobulin (Ig) G antibodies specific for all four human common cold coronaviruses (Sariol and Perlman, 2020). These data indicate that a coronavirus disease 2019 (COVID-19) vaccine may boost such preexisting cross-reactive memory T cells in individuals with a significant presence of cross-reactive immunity and contribute to vaccine-induced protective immunity.

However, whether a COVID-19 vaccine will boost preexisting cross-reactivity of the memory T cell response is unknown. Therefore, we examined SARS-CoV-2-specific antibody and T cell responses in SARS-CoV-1 survivors after a single dose of the Ad5-nCoV vaccine (a one-dose primary series is recommended) and its cross-reactivity to SARS-CoV-1 compared with naive healthy individuals (NHIs) who also received a single dose of the Ad5-nCoV vaccine.

RESULTS
Characteristics of the study subjects
Twenty-five SARS-CoV-1 survivors were enrolled between June and July 2020 prior to receiving one dose of Ad5-nCoV. In July 2021, 20 of these 25 survivors who received one dose of Ad5-nCoV were followed up, and an additional three SARS-CoV-1 survivors who also received one dose of Ad5-nCoV were included approximately 6 months after vaccination (median day of 175, interquartile range [IQR], 175–175). Meanwhile, 18
NHIs who received one dose of Ad5-nCoV were enrolled, with a median day of 175 (IQR, 175–175) after vaccination. Ten samples of serum and peripheral blood mononuclear cells (PBMCs) from biobanked normal healthy donors cryopreserved before September 2019 were included as healthy controls (HCs). The demographic information (age and sex) of the participants is shown in Table S1.

**Ad5-nCoV vaccination boosted neutralizing antibodies against SARS-CoV-1**

Using serum samples from SARS-CoV-1 survivors before vaccination, Ad5-nCoV-immunized SARS-CoV-1 survivors or NHIs, and HCs, we measured the serum neutralizing antibodies against Wuhan-Hu-1 (WA1), Alpha, Gamma, Delta, BA.1, BA.2, BA.2.12.1, and BA.4/BA.5. Of the 25 SARS-CoV-1 survivors prior to vaccination, none had neutralizing antibody titers (< 30) against WA1 and variants, whereas most (23 out of 25) had detectable neutralizing antibodies against SARS-CoV-1 (Figure 1A). For the 23 SARS-CoV-1 survivors 6 months after vaccination, 13 (56.5%), 14 (60.9%), five (17.4%), 12 (52.6%), nine (39.1%), two (8.7%), three (13.0%), one (4.2%), and two (8.7%) of 23 neutralized WA1, Alpha, Gamma, Beta, Delta, BA.1, BA.2, BA.2.12.1, and BA.4/BA.5, respectively, and Omicron subvariants with the greatest loss of neutralizing activity compared with WA1 and only one to three serum samples had detectable neutralizing antibody titers above 30. However, the geometric mean titer (GMT) of neutralizing antibodies against SARS-CoV-1 was significantly higher than that of antibodies against WA1 (Figure 1A). For the 18 NHIs vaccinated with Ad5-nCoV, we observed that only 33.3% had detectable neutralizing antibodies against WA1, with a GMT of 27.96 (95% confidence interval [CI], 12.98–60.22). Although no significant differences were observed in GMT between WA1 and variants or SARS-CoV-1 for vaccinated NHIs, most of them had lost the capacity to neutralize Beta, Gamma, Delta, and Omicron subvariants (Figure 1A). Further comparisons of the GMT to each tested virus between the four groups of study participants showed that vaccinated SARS-CoV-1 survivors were the only ones showing neutralizing antibodies against all viruses assessed in this study (Figure 1B). Collectively, these results indicate that one dose of Ad5-nCoV boosted neutralizing antibody against SARS-CoV-1 in SARS-CoV-1 survivors but not for WA1 and variants.

**Ad5-nCoV vaccination induced a comparable SARS-CoV-2-specific T cell response in SARS-CoV-1 survivors and NHIs**

Because we observed an equal antibody response to SARS-CoV-2 between Ad5-nCoV-vaccinated SARS-CoV-1 survivors and NHIs, we assessed whether Ad5-nCoV vaccination would induce an equal or even stronger T cell response in SARS-CoV-1 survivors and NHIs. We utilized three complementary methodologies, enzyme-linked immune absorbent spot (ELISpot), activation-induced marker (AIM), and intracellular cytokine staining (ICS) assays, to quantify the SARS-CoV-2 spike (S)-specific T cell response (Figure 2). We first measured SARS-CoV-2-S-specific interferon-gamma (IFN-γ) secreting T cells using an ELISpot assay and observed that SARS-CoV-1 survivors (276; IQR, 200–406) and NHIs (206; IQR, 75–455) had a comparable median number of IFN-γ-secreting T cells after vaccination but were significantly higher than HCs (0; IQR, 0–5) (Figure 2A). Furthermore, 100% (23 of 23) of SARS-CoV-1 survivors and HCs had detectable AIM+ CD4+ T cells, with comparable median frequencies of 0.24% (IQR, 0.09%–0.35%) and 0.13% (IQR, 0.09%–0.24%), respectively (Figure 2B), which were significantly higher than those of HCs (0.01; IQR, 0–0.02) (Figure 2B). A similar pattern was observed with SARS-CoV-2-S-specific AIM+ CD8+ T cell responses, which were significantly increased in vaccinated groups compared with HCs (Figure 2C). We found that 95% (19 of 20) and 88.9% (16 of 18) of SARS-CoV-1 survivors and NHIs had detectable AIM+ CD8+ T cells, with median frequencies of 0.36% (IQR, 0.23%–0.64%) and 0.32% (IQR, 0.11%–0.61%), respectively (Figure 2C).

We further evaluated the functionality of the SARS-CoV-2-S-specific T cell responses by measuring the IFN-γ secreted CD4+ and CD8+ T cells using the ICS assay (Figures 2D and 2E). The results showed that IFN-γ+ CD4+ T cells were detected in 75% (15 of 20) of SARS-CoV-1 survivors and 66.7% (12 of 18) of HCs, with median frequencies of 0.102% (IQR, 0.06%–0.16%) and 0.02% (IQR, 0.007%–0.11%), respectively, which were significantly higher than those in HCs (0; IQR, 0%–0.001%) (Figure 2D). In line with SARS-CoV-2-S-specific IFN-γ+ CD4+ T cell responses, we observed that 80% (16 of 20) of SARS-CoV-1 survivors had detect SARS-CoV-2-S-specific IFN-γ+ CD8+ T cells and had significantly higher frequencies of IFN-γ+ CD8+ T cells than HCs, whereas only 38.9% (7 of 18) of HCs had detect SARS-CoV-2-S-specific IFN-γ+ CD8+ T cells and had a comparable frequency of IFN-γ+ CD8+ T cells with HCs (Figure 2E). We observed that a few of the HCs had few cytokine-producing SARS-CoV-2-specific T cell responses overall, although HCs were all sampled before exposure, suggesting a possible preexisting cross-reactive T cell memory in HCs. These data suggest that Ad5-nCoV vaccination in SARS-CoV-1 survivors and NHIs induced a comparable cross-reactive T cell response against SARS-CoV-2.

**Ad5-nCoV vaccination boosted the SARS-CoV-1-specific T cell response in SARS-CoV-1 survivors**

Because Ad5-nCoV vaccination boosted neutralizing antibody titers against SARS-CoV-1 in SARS-CoV-1 survivors, we next tested whether Ad5-nCoV vaccination would include a stronger SARS-CoV-1-specific T cell response in SARS-CoV-1 survivors. We first measured SARS-CoV-2-S-specific IFN-γ secreting T cells using the ELISpot assay (Figure 3A). PBMC samples from two of 23 SARS-CoV-1 survivors and one of 18 NHIs that had >30 IFN-γ-secreting T cells in the blank control wells were excluded from further analyses. We observed that 81% (17 of 21) of SARS-CoV-1 survivors had detectable IFN-γ-secreting T cells, whereas only 40% of NHIs had detectable IFN-γ-secreting T cells (Figure 3A). Moreover, SARS-CoV-1 survivors had a higher median number of IFN-γ-secreting T cells (156; IQR, 77–341) than NHIs (20; IQR, 1–52) and HCs (15; IQR, 4–22.5). In line with the
results from the ELISpot assay, further analysis of SARS-CoV-2-S-specific CD4+ and CD8+ T cells showed that >80% of SARS-CoV-1 survivors had detectable SARS-CoV-2-S-specific AIM+ CD4+ and CD8+ T cells as well as IFN-γ+ CD4+ T cells, whereas only ~60% of them had detectable IFN-γ+ CD8+ T cells. In contrast, fewer NHIs had detectable SARS-CoV-2-S-specific CD4+ and CD8+ T cells and had significantly lower median frequencies than SARS-CoV-1 survivors (Figures 3B–3E). These data indicate that Ad5-nCoV vaccination elicited a stronger T cell response against SARS-CoV-1 in SARS-CoV-1 survivors than NHIs.

**DISCUSSION**

Several safe and effective COVID-19 vaccines have been developed and made available in most countries worldwide, including mRNA, inactivated, viral-vector-based vaccines (Li et al., 2021). However, there is a continuing risk that SARS-CoV-2 will mutate in ways that cause existing COVID-19 vaccines to be less effective, as we have already seen for the Beta, Gamma, Delta, and Omicron variants, or even ineffective (Andrews et al., 2022; Bar-On et al., 2022; Collie et al., 2022; Koff and Berkley, 2021; Li et al., 2021).
Figure 2. SARS-CoV-2-specific T cell response after one dose of Ad5-nCoV
(A) Measurement of SARS-CoV-2-spike (S)-specific IFN-γ-producing T cells using the ELISpot assay in Ad5-nCoV-immunized SARS-CoV-1 survivors (n = 23), Ad5-nCoV-immunized NHIs (n = 18), and HCs (n = 10) after stimulation with SARS-CoV-2 S peptide pools. (Left) The representative ELISpot of IFN-γ-producing T cells, and (right) the summary of IFN-γ-producing T cells against SARS-CoV-2. (B and C) Assessment of SARS-CoV-2-S-specific CD4+ (B) and CD8+ (C) T cell responses using AIm markers in individuals corresponding to (A) after stimulation with SARS-CoV-2 S peptide pools. (Left) The representative flow cytometric plots of CD4+ T cells and CD8+ T cells, and (right) the comparison of SARS-CoV-2-S-specific CD4+ and CD8+ T cells.
(D and E) Analysis of SARS-CoV-2-S-specific CD4+ (D) and CD8+ (E) T cell responses using intracellular cytokine staining in individuals corresponding to (A) after stimulation with SARS-CoV-2 S peptide pools. (Left) The representative flow cytometric plots of CD4+ (D) and CD8+ (E) T cells expressing IFN-γ, and (right) the comparison of SARS-CoV-2-specific CD4+ (D) and CD8+ (E) T cells expressing IFN-γ. Box plots show all data points and indicate the median and IQR; the whiskers represent 1.5 times the IQR. Statistical significance was determined by the two-tailed Friedman test with a false discovery rate for multiple comparisons. A p value of less than 0.05 was considered to indicate statistical significance. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. ns, no significance.
Figure 3. SARS-CoV-1-specific T cell response after one dose of Ad5-nCoV

(A) Measurement of SARS-CoV-1-spike (S)-specific IFN-γ-producing T cells using the ELISpot assay in Ad5-nCoV-immunized SARS-CoV-1 survivors (n = 23), Ad5-nCoV-immunized NHIs (n = 18), and HCs (n = 10) after stimulation with SARS-CoV-1 spike (S) peptide pools. (Left) The representative ELISpot of IFN-γ-producing T cells, and (right) the summary of IFN-γ-producing T cells against SARS-CoV-1.

(B and C) Assessment of SARS-CoV-1-S-specific CD4+ (B) and CD8+ (C) T cell responses using AIM markers in individuals corresponding to (A) after stimulation with SARS-CoV-1 S peptide pools. (Left) The representative flow cytometric plots of CD4+ (B) and CD8+ (C) T cells, and (right) the comparison of SARS-CoV-1-S-specific CD4+ (B) and CD8+ (C) T cells.

(D and E) Analysis of SARS-CoV-1-S-specific CD4+ (D) and CD8+ (E) T cell responses using intracellular cytokine staining in individuals corresponding to (A) after stimulation with SARS-CoV-1 S peptide pools. (Left) The representative flow cytometric plots of CD4+ (D) T cells and CD8+ (E) T cells expressing IFN-γ, and (right) the comparison of SARS-CoV-1-S-specific CD4+ (D) and CD8+ (E) T cells expressing IFN-γ among the three subject groups.

Box plots show all data points and indicate the median and IQR; the whiskers represent 1.5 times the IQR. The two-tailed Friedman test with a false discovery rate determined statistical significance. A p value of less than 0.05 was considered to indicate statistical significance. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. ns, no significance.
neutralizing variants of concern (VOCs) and sarbecoviruses that SARS-CoV-1 survivors immunized with BNT162b2 capable of recent study showed a pansarbecovirus neutralizing antibody in tent with the study by Tan et al. (2021), which showed that all and BA.4/BA.5 with low antibody titers. Our results are inconsis-tivity of neutralizing antibodies to WA1 and early VOCs. More-over, we observed that only one to three serum samples from vaccinated individuals could neutralize BA.1, BA.2, BA.2.12.1, and BA.4/BA.5 with low antibody titers. Our results are inconsis-tent with the study by Tan et al. (2021), which showed that all serum samples from SARS-CoV-1 survivors after immunization with BNT162b2 neutralized VOC. Two possible reasons may explain such a difference. First, SARS-CoV-1 survivors immunized with Ad5-nCoV were included in this study rather than survi-vorized with mRNA vaccines (e.g., BNT162b2 or mRNA-1273), and BNT162b2 induced higher antibody titers than Ad5-nCoV (Guzmán-Martínez et al., 2021). Second, we as-sessed the neutralizing antibodies in immunized SARS-CoV-1 survivors approximately 6 months after vaccination, whereas Tan et al. measured neutralizing antibodies approximately 1–2 months after vaccination. Thus, the waning of neutralizing antibodies over time may also contribute to the reduced neutral-izing activity of VOCs.

Despite the escape of VOCs to antibodies elicited by Ad5-nCoV vaccination in SARS-CoV-1 survivors and NHIs, we observed that most immunized SARS-CoV-1 survivors and NHIs had detectable SARS-CoV-2-specific T cell responses 6 months after vaccination. Recent studies have shown that the majority of T cell responses elicited by infection or vaccina-tion remain capable of recognizing the emerged variants (Keeton et al., 2022; Tarke et al., 2022). Whether T cell immunity will be effective as a second-level defense in preventing severe disease after infection in the absence of a potent neutralizing antibody response remains to be determined (Goel et al., 2021; Sette and Crotty, 2021). These data indicate that single-dose Ad5-nCoV vaccination in SARS-CoV-1 survivors seems insufficient to drive the breadth and persistence of the antibody response to SARS-CoV-2 but leads to a persistent T cell response. In addi-tion, despite a robust T cell response, low levels of neutralizing antibodies 6 months after vaccination suggested that a homolo-gous or heterologous prime-boost vaccination regimen will be required to sustain protection. We also observed that most SARS-CoV-1 survivors had detectable neutralizing antibodies 17 years after the initial infec-tion, and neutralizing antibodies were significantly elevated after vaccination with Ad5-nCoV, which is in line with previous studies (Anderson et al., 2020; Tan et al., 2021). Moreover, a significant cross-reactive T cell response to SARS-CoV-1 was identified in immunized SARS-CoV-1 survivors. However, the potential role of SARS-CoV-1 neutralizing antibodies and T cell response on infection or the severity of SARS-CoV-2 infection remains un-known, which requires further investigation considering the limited cross-reactivity between SARS-CoV-1 and SARS-CoV-2 observed in this study and other studies (Anderson et al., 2020; Tan et al., 2021). These data are important for understanding the longevity of SARS-CoV-1 protective immunity in general, and how preexisting SARS-CoV-1 immunity affects antibody and T cell responses to SARS-CoV-1 after vaccination. Further studies are needed to determine whether priming from the SARS-CoV-2 vaccine followed by boosting from the SARS-CoV-1 vaccine will enhance the level of pansarbecovirus neutralizing antibodies.

In summary, our data show that Ad5-nCoV vaccination in SARS-CoV-1 survivors induced a comparable antibody and T cell response to SARS-CoV-2 with NHIs. Despite low antibody levels to SARS-CoV-2, SARS-CoV-2-specific CD4+ and CD8+ T cell responses were observed in most SARS-CoV-1 survivors and NHIs 6 months after vaccination. However, SARS-CoV-1 survivors had significantly enhanced neutralizing antibodies and T cell responses to SARS-CoV-1 compared with NHIs. These findings provide important evidence for understanding preexisting cross-reactive immunity in vaccine design and cross-neutralization by cross-clade boosting.

**Limitations of the study**

Natural limitations of our study arise from the number of the SARS-CoV-1 survivors and the fact that limited survivors could be contacted. In addition, these SARS-CoV-1 survivors were not followed up after immunization with one dose of the Ad5-nCoV vaccine. We used IFN-γ ELISpot, AIM, and ICS assays to detect virus-specific T cell responses, but we could not iden-tify the cross-reactive epitopes of T cells specific to SARS-CoV-1 S protein in SARS-CoV-1 survivors due to the limited blood samples collected from the subjects. Full epitope-specific mapping of T cell responses in the future will add the important detailed resolution of SARS-CoV-1-specific T cell responses.

**STAR METHODS**

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

- **KEY RESOURCES TABLE**
- **RESOURCE AVAILABILITY**
  - Lead contact
  - Materials availability
  - Data and code availability
- **EXPERIMENTAL MODEL AND SUBJECT DETAILS**
  - Human subjects and samples
  - Cell lines
- **METHOD DETAILS**
  - Serum and PBMC isolation
  - Pseudovirus production and neutralization assay
  - Enzyme-linked immunospot (ELISpot) assays
  - Activation-induced marker (AIM) and intercellular cyto-kine staining (ICS) T-cell assays
- **QUANTIFICATION AND STATISTICAL ANALYSIS**
Roederer, M., Nozzi, J.L., and Nason, M.C. (2011). SPICE: exploration and analysis of post-cytometric complex multivariate datasets. Cytometry A. 79, 167–174. https://doi.org/10.1002/cyto.a.21015.

Sariol, A., and Perlman, S. (2020). Lessons for COVID-19 immunity from other coronavirus infections. Immunity 53, 248–263. https://doi.org/10.1016/j.immuni.2020.07.005.

Saunders, K.O., Lee, E., Parks, R., Martinez, D.R., Li, D., Chen, H., Edwards, R.J., Gobeil, S., Barr, M., Mansouri, K., et al. (2021). Neutralizing antibody vaccine for pandemic and pre-emergent coronaviruses. Nature 594, 553–559. https://doi.org/10.1038/s41586-021-03594-0.

Sette, A., and Crotty, S. (2021). Adaptive immunity to SARS-CoV-2 and COVID-19. Cell 184, 861–880. https://doi.org/10.1016/j.cell.2021.01.007.

Tan, C.W., Chia, W.N., Young, B.E., Zhu, F., Lim, B.L., Sia, W.R., Thein, T.L., Chen, M.I.C., Leo, Y.S., Lye, D.C., and Wang, L.F. (2021). Pan-Sarbecovirus neutralizing antibodies in BNT162b2-immunized SARS-CoV-1 survivors. N. Engl. J. Med. 385, 1401–1406. https://doi.org/10.1056/NEJMoa2108453.

Tang, F., Quan, Y., Xin, Z.T., Wrammert, J., Ma, M.J., Lv, H., Wang, T.B., Yang, H., Richardus, J.H., Liu, W., and Cao, W.C. (2011). Lack of peripheral memory B cell responses in recovered patients with severe acute respiratory syndrome: a six-year follow-up study. J. Immunol. 186, 7264–7268. https://doi.org/10.4049/jimmunol.0903490.

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.
STAR METHODS

KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| Anti-human CD28 antibody | Biolegend | Cat# 302902; RRID: AB_314304 |
| Anti-human CD49d antibody | Biolegend | Cat# 304334; RRID: AB_2749896 |
| Anti-human CD3 antibody | Biolegend | Cat# 300468; RRID: AB_2629574 |
| Anti-human CD4 antibody | BD Biosciences | Cat# 562970; RRID: AB_2744424 |
| Anti-human CD134 antibody | Biolegend | Cat# 301046; RRID: AB_2563264 |
| Anti-human CD69 antibody | Biolegend | Cat# 350020; RRID: AB_2571940 |
| Anti-human CD137 antibody | Biolegend | Cat# 310910; RRID: AB_314845 |
| Anti-human IFN-\(\gamma\) antibody | Biolegend | Cat# 502506; RRID: AB_315231 |
| **Bacterial and virus strains** |        |            |
| SARS-CoV-2 pseudovirus for WA1 | This study | N/A |
| SARS-CoV-2 pseudovirus for Alpha | This study | N/A |
| SARS-CoV-2 pseudovirus for Beta | This study | N/A |
| SARS-CoV-2 pseudovirus for Gamma | This study | N/A |
| SARS-CoV-2 pseudovirus for Delta | This study | N/A |
| SARS-CoV-2 pseudovirus for BA.1 | This study | N/A |
| SARS-CoV-2 pseudovirus for BA.2 | This study | N/A |
| SARS-CoV-2 pseudovirus for BA.2.12.1 | This study | N/A |
| SARS-CoV-2 pseudovirus for BA.4/BA.5 | This study | N/A |
| SARS-CoV-1 pseudovirus | This study | N/A |
| *E.coli* DH5\(\alpha\) Competent Cells | TaKaRa | Cat# 9057 |
| **Sera samples from SARS-CoV-1 survivors** | This study | N/A |
| **Blood samples from SARS-CoV-1 survivors with vaccination of Ad5-nCoV** | This study | N/A |
| **Blood samples from na"ive healthy individuals with vaccination of Ad5-nCoV** | This study | NA |
| **Blood samples from healthy controls** | This study | NA |
| **Chemicals, peptides, and recombinant proteins** |        |            |
| Phosphate buffered saline (PBS) | Gibco | Cat# C10010500BT |
| Dulbecco’s modified eagle medium (DMEM) | Gibco | Cat# C111959500BT |
| Trypsin-EDTA (0.25%) | Solarbio | Cat# T1300 |
| HEPES | Gibco | Cat# 15630-080 |
| Fetal bovine serum (FBS) | Gibco | Cat# 10099-141C |
| Penicillin/streptomycin | Gibco | Cat# 15140-122 |
| PEI MAX (MW 40000) | Polysciences | Cat# 24765-1 |
| Luciferase Assay Reagent | Vazyme | Cat# DD1201-01 |
| BD Horizon™ Fixable Viability Stain 780 | BD Biosciences | Cat# 565388 |
| PMA | Sigma | Cat# P8139 |
| Ionomycin | Sigma | Cat# I3909 |
| Brefeldin A | eBiocience | Cat# 00-4506-51 |
| Monensin | Biolegend | Cat# 420701 |
| S protein peptide pool of SARS-CoV-2 | Genscript | Cat# RP30020 |
| S protein peptide pool of SARS-CoV-1 | JPT | Cat# PM-CVHSA-S-1 |

(Continued on next page)
RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Mai-Juan Ma (mjma@163.com).

Materials availability
All unique reagents generated during this study are available from the lead contact with a completed Materials Transfer Agreement.

Data and code availability
- All data reported in this paper are available within the main manuscript and the supplemental information.
- 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.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Human subjects and samples
In June 2020, over 17 years after infection, 25 individuals (22 females/3 males with a median age of 45 years; interquartile range [IQR], 39–53) who experienced but recovered from SARS-CoV-1 infection in 2003 were recruited to measure the persistence of the T-cell response and cross-reactivity with SARS-CoV-2. Upon enrollment, blood samples from 25 SARS-CoV-1 survivors were collected. At the end of January and beginning of February 2021, healthcare workers in the hospital, including 25 SARS-CoV-1 survivors, were immunized with one dose of the recombinant viral-vectored vaccine Ad5-nCoV. In late July 2021, approximately 6 months after vaccination, 20 of these 25 immunized SARS-CoV-1 survivors were followed up, an additional three immunized SARS-CoV-1 survivors were enrolled to participate in this study, and blood samples were collected for analysis. Meanwhile, 18 naïve healthy individuals (13 females/5 males with a median age of 38 years; IQR, 46–47) working in the same hospital as SARS-CoV-1 survivors and receiving a single dose of Ad5-nCoV were recruited. In addition, blood samples from 10 naïve healthy individuals (8 females/2 males with a median age of 45 years; IQR, 32.8–45.3) whose samples were collected before September 2019 were included as controls. This study...
was conducted following the Declaration of Helsinki and approved by the Institutional Review Board of the Beijing Institute of Microbiology and Epidemiology (IRB number: AF/SC-08/02.124). All participants provided written consent.

**Cell lines**

Human embryonic kidney HEK-293T cells were cultured at 37°C and 5% CO₂ in Dulbecco’s modified Eagle’s medium (DMEM, Gibco) containing 10% (v/v) heat-inactivated fetal bovine serum (FBS, Gibco) and supplemented with 1% penicillin–streptomycin (Gibco). Cells were disrupted at confluence with 0.25% trypsin in 1 mM EDTA (Solarbio) every 48–72 h. HeLa-hACE2 cells were provided by Prof. Lin-Qi Zhang from Tsinghua University and were cultured under the same conditions.

**METHOD DETAILS**

**Serum and PBMC isolation**

Sera were separated by centrifugation at 2000 rpm for 10 min, aliquoted into three cryovials, and preserved at –80°C until testing. PBMCs were isolated by density gradient centrifugation with Lymphoprep in SepMate tubes (Stemcell Technologies) according to the manufacturer’s instructions. Briefly, the blood was placed on top of Lymphoprep in SepMate tubes and centrifuged at 1,200 × g for 10 min. PBMCs from the top layer were harvested and washed twice with fetal bovine serum (PBS) at 400 × g for 10 min. Isolated PBMCs were frozen in cell recovery media containing 10% DMSO (GIBCO) supplemented with 90% heat-inactivated fetal bovine serum and stored in liquid nitrogen before assay analyses.

**Pseudovirus production and neutralization assay**

Pseudoviruses were generated as previously described (Li et al., 2020; Nie et al., 2020b) by cotransfecting HEK-293T cells (ATCC, CRL-3216) with human immunodeficiency virus backbone expressing firefly luciferase (pNL4-3-3-E-luciferase) and pcDNA3.1 vector encoding either SARS-CoV-2 Wuhan-Hu-1 (WA1) or mutated S proteins (Alpha, Beta, Gamma, Delta, BA.1, BA.2, BA.2.12.1, and BA.4/BA.5) and SARS-CoV-1 S protein plasmid (Table S2). The medium was replaced with fresh medium at 24 h, and the supernatants were harvested at 48 h post-transfection and clarified by centrifugation at 300 × g for 10 min before being aliquoted and stored at –80°C until use. A SARS-CoV-2 pseudovirus neutralization assay (NT) was performed as described (Nie et al., 2020a) with the target cell line HeLa-hACE2. Threefold serially diluted serum (starting at 1:30) from COVID-19-convalescent individuals and vaccines was incubated with 500–1000 TCID₅₀ of SARS-CoV-2 pseudotyped virus for 1 h at 37°C. HeLa-hACE2 cells (20,000 cells/well) were subsequently added to the mixture and incubated for approximately 48 h at 37°C with 5% CO₂. Luciferase activity was then measured using a GloMax 96 Microplate Luminometer (Promega). The half-maximal neutralization titers for serum were determined by luciferase activity 48 h after exposure to the virus-serum mixture with a four-parameter nonlinear regression inhibitor curve in GraphPad Prism 8.4.1 (GraphPad Software). Titers are reported as the serum dilution with a 50% inhibitory dilution (NT₅₀). Samples with NT₅₀ values no more than 30 (the detectable limit) were considered negative for neutralizing antibodies and were assigned a value of 10 for calculation.

**Enzyme-linked immunospot (ELISpot) assays**

T-cell responses were measured using a Human IFN-γ ELISpotPRO kit (ALP, 3420-2AST-10, Mabtech AB) according to the manufacturer’s protocol. Briefly, plates were washed with filtered PBS (Sigma Aldrich, Missouri, USA) and blocked with RPMI-1640 culture media containing 10% fetal bovine serum (FBS, Gibco, Thermo Fisher Scientific, Massachusetts, US). The plates precoated with monoclonal anti-IFN-γ were incubated for 18 h in RPMI-1640 medium containing 10% FBS supplemented with a mixture containing the S protein peptide pool of SARS-CoV-2 (Genscript, Nanjing, China) or SARS-CoV-1 (JPT, Berlin, Germany). The peptide pool of SARS-CoV-2 S protein has 316 peptides (delivered in two peptide pools of 158 & 158 peptides) made up of 15 mer with 11 amino acid overlaps, and the peptide pool of SARS-CoV-1 S protein has 311 peptides (delivered in two peptide pools of 156 & 155 peptides) made up of 15 mer with 11 amino acid overlaps at a concentration of 2 μg/mL of each peptide, anti-CD28 (0.1 μg/mL) and 250,000 cells per well in a humidified incubator (5% CO₂, 37°C). Negative controls comprising DMSO and positive controls containing anti-CD3 were also included. Spot numbers were analyzed by the CTL ImmunoSpot S6 universal analyzer (Cellular Technology Ltd., USA). SARS-CoV-2- or SARS-CoV-1-specific spots were determined as the mean spots of the control wells subtracted from the positive wells, and the results were expressed as spot-forming cells (SFC) per 10⁶ PBMCs. We defined threefold higher SARS-CoV-2- or SARS-CoV-1-specific spots versus background together with at least three spots above background as a positive response. This cutoff was set based on negative control values as described previously. If negative control wells had SFC per 10⁶ PBMCs ≥30 or positive control wells (anti-CD3 and anti-CD28 stimulation) were negative, the results were excluded from further analysis.

**Activation-induced marker (AIM) and intercellular cytokine staining (ICS) T-cell assays**

Antigen-specific T cells were further measured by AIM and ICS assays after stimulation of PBMCs with overlapping peptides spanning the entire SARS-CoV-2 or SARS-CoV-1 S. In brief, cryopreserved PBMCs were thawed and resuspended in RPMI 1640 medium supplemented with 10% FBS and 1% penicillin–streptomycin (Gibco). Cells were cultured at 1 × 10⁶ per well in 96-well U-bottom plates (Corning) with the relevant peptides (1 μg/mL each) in the presence of anti-CD28/CD49d (0.5 μg/mL, Biolegend) at 37°C and 5% CO₂ for 19 h. Then, brefeldin A (BFA, eBioscience) and monensin (Biolegend) were added and incubated at 37°C and 5%
CO2 for 5 h. Following 5 h of incubation, PBMCs were washed and stained with a LIVE/DEAD fixable dead cell stain kit (Invitrogen) and antibodies against surface markers for 1 h at 4°C. For intracellular staining, cells were subsequently fixed with IC fixation buffer (eBioscience) for 1 h at 4°C and permeabilized with 1× permeabilization buffer (eBioscience). Permeabilized cells were stained with antibodies against cytokines for 1 h at 4°C. Stimulation with an equal volume of DMSO was included as the negative control. For intracellular cytokines, PMA (50 ng/mL)/ionomycin (1 µg/mL) stimulation with the addition of BFA and monensin for 5 h was performed as the positive control. After incubation, the cells were surface and intracellularly stained and subjected to flow cytometry. Data were acquired using BD LSR FortessaTM X-20 Flow Cytometry and analyzed by FlowJo V10 software (Tree Star).

Antigen-specific activated CD4+ cells were defined as activation of OX40+ and CD137+ cells, while the expression of CD69+ and CD137+ identified activated CD8+ cells, as previously described (Dan et al., 2021). Antigen-specific functional CD4+ and CD8+ T cells were defined as the expression of IFN-γ. Antigen-specific T cells were measured as background (DMSO) subtracted data. Following background subtraction of background DMSO cultures, negative values were set to zero. The threshold for positivity for antigen-specific CD4+ T-cell responses (0.018% for AIM+ and 0 for IFN-γ+) and antigen-specific CD8+ T-cell responses (0.01% for AIM+ and 0.031% for IFN-γ+) was calculated using the median 75th percentile of values obtained in all negative controls (Roederer et al., 2011). The antibody panel utilized in the AIM and ICS assays is shown in the key resources table, and the gating strategies are shown in Figure S1.

QUANTIFICATION AND STATISTICAL ANALYSIS

The Friedman and Kruskal–Wallis test with the false discovery rate method was used for multiple comparisons where it appreciates. All statistical analyses were performed using GraphPad Prism (version 8.4.2, La Jolla, California, USA), and all statistical tests were 2-sided with a significance level of 0.05. Details are additionally provided in the Figure legends.