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Distinct immune responses in the early phase to natural SARS-CoV-2 infection or vaccination

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Abstract

Immune responses elicited by viral infection or vaccination play key roles in the viral elimination and the prevention of reinfection, as well as the protection of healthy persons. As one of the most widely used Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccines, there have been increasing concerns about the necessity of additional doses of inactivated vaccines, due to the waning immune response several months after vaccination. To further optimize inactivated SARS-CoV-2 vaccines, we compared immune responses to SARS-CoV-2 elicited by natural infection and immunization with inactivated vaccines in the early phase. We observed the lower antibody levels against SARS-CoV-2 spike (S) and nucleocapsid (N) proteins in the early phase of postvaccination with a slow increase, compared to the acute phase of SARS-CoV-2 natural infection. Specifically, IgA antibodies have the most significant differences. Moreover, we further analyzed cytokine expression between these two groups. A wide variety of cytokines presented high expression in the infected individuals, while a few cytokines were elicited by inactivated vaccines. The differences in antibody responses and cytokine levels between natural SARS-CoV-2 infection and vaccination with the inactivated vaccines may provide implications for the optimization of inactivated SARS-CoV-2 vaccines and the additional application of serological tests.

KEYWORDS
antibody response, cytokines, natural infection, SARS-CoV-2, vaccination

1 | INTRODUCTION

The worldwide prevalence of coronavirus disease 2019 (COVID-19), which developed a range of symptoms ranging from mild to severe illness, has lasted more than 2 years. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for this global pandemic, which had caused several waves of pandemics by various SARS-CoV-2 variants.[1–5] Notably, the decreased severity of disease symptoms has been observed in the pandemics caused by some SARS-CoV-2 variants with the viral evolution. Omicron infection, as an example, demonstrates a high rate of asymptomatic carriage or mild symptoms,[6] even though some cases with severe symptoms, hospitalization, or death were reported. This could be due to, in part, the contributions of immune protection established by previous infection or vaccination.[7]

Currently, due to the lack of ideal antiviral drugs, vaccines have been one of the most important strategies to combat COVID-19. There are 65.5% of the world's population who received at least one dose of SARS-CoV-2 vaccines (the statistic was from Our World in Data). SARS-CoV-2 vaccines have been developed by several technological platforms producing different types of vaccines: inactivated vaccines, live attenuated vaccines, recombinant protein vaccines, viral vector vaccines, DNA vaccines, and messenger RNA (mRNA) vaccines.[8] Immune responses elicited by vaccines are the basis of the immune protection provided for healthy persons. As a novel technology of vaccine development, mRNA vaccine eliciting high titers of antibody response is one of the most promising vaccine types due to their high immunogenicity. Several inactivated vaccines also have been approved. Among them, CoronaVac produced by Sinovac Biotech (China) is one of the most extensively used inactivated vaccines around the world. Reports demonstrated that the efficiency of this vaccine at 14 days after two doses was 50.38% (for the prevention of mild cases of the disease) and 78% (for the prevention of mild to severe cases of the disease) in Brazil; 65% in Indonesia; and 91.25% in Turkey.[9] In the two-dose immunization of inactivated vaccines, most of the anti-S IgG antibodies were detected 14 days after the second dose of vaccination (i.e., 42 days after the first dose).[10] However, with time and the appearance of new variants, the decreased antibody response of inactivated vaccines has been reported.[11,12] Furthermore, neutralizing antibody titers elicited by an inactivated SARS-CoV-2 vaccine were observed to be lower than that of COVID-19 recovered individuals at 8 months postsymptom onset.[13] Even though a booster of inactivated vaccine increased antibody levels, the unsatisfactory half-life of neutralizing antibodies was estimated.[14] Hence, except for the additional doses of vaccination, further optimization of vaccines is needed.

In terms of the optimization of inactivated SARS-CoV-2 vaccines, except for the antigens matched with the circulating variants, we can learn from the Immune response elicited by natural SARS-CoV-2 infection, which plays pivotal roles in the elimination of viruses and protection against reinfection. Antibody response elicited by SARS-CoV-2 infection has been reported to be similar to that of other coronaviruses. From the onset of viral infection, B cells elicit antibody responses against viral proteins. At different time points after infection, SARS-CoV-2-specific IgG, IgM, and IgA antibodies were produced with distinct antibody kinetics.[15,16] IgM appears in the early stage of SARS-CoV-2 infection and maintains for a short time, while IgG remains detectable for at least several months, which is associated with the viral-neutralizing activity. Based on the characteristics of SARS-CoV-2 specific IgG and IgM antibody responses, serological antibody tests were used as a supplementary diagnostic tool of molecular tests in the early phase of the SARS-CoV-2 outbreak. However, with the global spread of vaccination, the application of serological tests was limited. Except for the antibody response to viral infection, an aberrant increase of cytokines is another important characteristic of the SARS-CoV-2-specific immune response. Cytokine production not only plays a crucial role in the defence against viruses but it also leads to autoinflammation and consequent organ failure, even death. It has been reported that hyperproduction of cytokines was correlated with the worsened prognosis in severe SARS-CoV-2 cases.[16,17] Thus, in consideration of inactivated whole-virion SARS-CoV-2 vaccines, the cytokine response elicited by them needs to be concerned and characterized. Additionally, the correlation between antibody response with cytokine expression has yet to be depicted.

Although immune responses have been reported in COVID-19-infected patients and vaccinated individuals with inactivated vaccines,[18–20] respectively, comparison between these two groups under the same condition of laboratory study is rarely found, especially in inactivated vaccines.[21,22] To investigate the differences between active and passive immunity, in this study, we compared the distinct early humoral immune response to SARS-CoV-2 infection or vaccination with inactivated vaccines. A better understanding of the advantages of humoral immune response elicited by SARS-CoV-2 natural infection may provide further implications for the optimization of vaccines and the additional application of serological tests.

2 | MATERIALS AND METHODS

2.1 | Study design

To compare the humoral immune response to SARS-CoV-2 natural infection and vaccination, 239 convalescents who recovered from SARS-CoV-2 infection and 165 vaccinees who were not previously infected with SARS-CoV-2 were enrolled. Peripheral blood samples collected within comparable days after infection or vaccination were separately detected for different types of antibodies against spike (S) and nucleocapsid (N) proteins by magnetic chemiluminescence enzyme immunoassay kits and enzyme-linked immunosorbent assay (ELISA). The difference in antibody levels and antibody dynamic changes in the early phase of infection or vaccination was described between the infected group and the vaccinated group. In addition, the data of participants were grouped by sex, age, and the time interval from infection or vaccination to sample collection for further analysis.

2.2 | Ethics approval

The study has been approved by the Chinese clinical test registration center (the World Health Organization international clinical trials registered organization registered platform, registration number: ChiCTR2100042528) and the Ethics Committees of Chongqing Medical University (approval number: 2021006). All study participants provided informed consent.

2.3 | Sample collection

Peripheral blood of SARS-CoV-2-infected individuals was obtained (interquartile range [IQR]: 13–21 days; median: 17 days) post-symptom onset from Yongchuan Hospital Affiliated to Chongqing Medical University and Wanzhou People's Hospital (Chongqing, China) from February 5 to March 3, 2020. Peripheral blood of vaccinees was collected around 6 weeks after the first-dose vaccination in several designated hospitals (median: 41 days; IQR: 36–45 days), including Chongqing Medical University, the First Affiliated Hospital of Chongqing Medical University, the Second Affiliated Hospital of Chongqing Medical University, and Yongchuan Hospital Affiliated to Chongqing Medical University in 2021. After heat inactivation of blood samples at 56°C for 1 h, sera were collected by centrifuging 3000g for 5min, and then aliquoted and stored at −80°C.

2.4 | Detection of SARS-CoV-2-specific anti-spike antibodies by commercial test kits

Magnetic chemiluminescence enzyme immunoassay kits (developed by Bioscience Co., Ltd.) were used to examine SARS-CoV-2 anti-spike IgG/IgM/IgA antibodies of sera according to the manufacturer's instructions. The ratio between the chemiluminescence signal and the cutoff value (S/CO) was used as antibody levels. When the S/CO value is higher than 1.0, the tests were thought as positive.

2.5 | Expression and purification of SARS-CoV-2 nucleocapsid protein

SARS-CoV-2 nucleocapsid sequence was cloned into the pET28a expression vector and transformed into BL21(DE3) *Escherichia coli* (New England BioLabs), which was grown in a lysogeny broth growth medium. After cultivation, bacteria were induced by 1 mM isopropyl β-D-1-thiogalactopyranoside for 16–20 h at 16°C. *Escherichia coli* were harvested and resuspended in binding buffer (20 mM HEPES, pH 7.5, 150 mM NaCl, 40 mM imidazole), followed by ultrasonication. After centrifugation at 12 000g for 30 min to separate cell debris, the supernatant of lysate through a 0.22-µm filter was added into PurKine™ His-Tag Ni-NTA Packed Column (cat.: BBMC20010; Abbkine, USA), which had been equilibrated in binding buffer. The column was then washed three times with five volumes of wash buffer. Bound proteins were eluted with a linear concentration of 0%–100% elution buffer (20 mM HEPES, pH 7.5, 150 mM NaCl, 500 mM imidazole). When the protein absorbance to ultraviolet light reaches the peak at 280 nm, the eluted protein was collected and concentrated. After measuring the concentration of proteins, they were flash-frozen in liquid nitrogen.

2.6 | ELISA for antibody titers against SARS-CoV-2 nucleocapsid protein

The 96-well microtiter plates coated with the recombinant SARS-CoV-2 nucleocapsid protein (100 ng/well) were incubated at 4°C overnight. After blocking with 5% skim milk powder and 2% bovine serum albumin at 37°C for 2 h, sera of enrolled participants at a dilution of 1:100 were added to the plates, and then incubated at 37°C for 1 h. After washing, wells were incubated with goat anti-human IgG/IgA/IgM-horseradish peroxidase antibody (cat.: ab97225/ab7383/ab97205; Abcam) for 1 h at 37°C. TMB substrate was added for color development with incubation at 37°C for 15 min. Reactions were stopped with 2 M H_2SO_4, and the absorbance was determined at 450 nm using a microplate reader (Biotek).

2.7 | Determination of human serum cytokines

Cytokines of serum samples were determined by Bio-plex Pro™ human cytokine assay according to the instruction manual (Bio-Rad). Briefly, diluted magnetic beads (50 µl/well) were added to the 96-well assay plate. After washing, the plate was added to diluted samples, standards, and controls and incubated on a shaker at 850 ± 50 rpm at room temperature for 30 min. Then, 25 µl diluted detection antibodies were transferred to each well of the assay plate at the same incubation condition with the last step for 30 min. The streptavidin–phycoerythrin incubation was performed under the same condition for 10 min. Finally, after washing, the assay plate was added 125 µl assay buffer into each well to resuspend magnetic beads and placed on the Reader (Bio-Plex 200). Bio-Plex Manager Software was used for data acquisition and analysis. The expression levels of 48 cytokines were measured in this assay: cutaneous T-cell-attracting chemokine, eotaxin, fibroblast growth factor (FGF), granulocyte colony-stimulating factor (G-CSF), granulocyte–macrophage CSF, growth-regulated oncogene-α (GRO-α), hepatocyte growth factor (HGF), interferon α-2 (IFN-α2), interferon-γ (IFN-γ), interleukin-1α (IL-1α), IL-1β, interleukin 1 receptor antagonist (IL-1RA), IL-2, interleukin-2 receptor-α (IL-2Rα), IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12(p40), IL-12(p70), IL-13, IL-15, IL-16, IL-17A, IL-18, interferon-γ-inducible protein (IP-10), leukemia inhibitory factor, monocyte chemoattractant protein-1 (MCP-1, MCAF), MCP-3, macrophage CSF, macrophage migration inhibitory factor (MIF), monokine induced by γ-interferon (MIG), macrophage inflammatory protein-1α (MIP-1α), MIP-1β, β-nerve growth factor (β-NGF), platelet-derived growth factor-BB, regulated upon activation, normal T-cell expressed

and presumably secreted (RANTES), stem cell factor, stem cell growth factor-β, stromal cell-derived factor 1 (SDF-1α), tumor necrosis factor-α (TNF-α), TNF-β, TNF-related apoptosis-inducing ligand (TRAIL), vascular endothelial growth factor-A.

2.8 | Statistical analysis

Continuous variables were described as the median (IQR) and compared with the Mann–Whitney U-test between groups. Significant differences in cytokine levels between pre- and postvaccination individuals were evaluated by paired t-test. p Value less than 0.05 was considered statistically significant, and p values less than 0.001, 0.01, and 0.05 were marked as ***, ** and * respectively. One-way analysis of variance (ANOVA) was used to estimate the differences in antibody levels at different clinical characterize subgroups. Pearson's correlation test was used to calculate the correlation coefficient of IgA levels to cytokines levels. The cytokines heatmap was generated using the Pheatmap package with default parameters. Statistical analyses were performed using R software, version 3.6.0.

3 | RESULTS

3.1 | Comparison of antibody response to SARS-CoV-2 between natural infection and vaccination

To compare the antibody response to SARS-CoV-2 between natural infection and vaccination, 239 individuals who were previously infected with SARS-CoV-2 in the early phase of 2020 and 165 naïve individuals with one or two doses of inactivated vaccines (CoronaVac or Sinopharm) in 2021 were enrolled (Table 1). Twenty healthy people were included as controls. Sera samples were collected with a median of 17 days (IQR: 13–21 days) and 41 days (IQR: 36–45 days) after infection or vaccination, respectively. At first, we examined anti-spike protein (anti-S) (Figure 1A,B) and anti-nucleocapsid protein (anti-N) (Figure 1C,D) IgG/IgM/IgA antibody levels of sera between three groups by magnetic chemiluminescence enzyme immunoassay

kits and ELISA, respectively. Compared with healthy controls, both SARS-CoV-2 infection and inactivated vaccines can elicit SARS-CoV-2 specific IgG/IgM/IgA antibodies against spike and nucleocapsid proteins. However, the overall levels of three antibody subtypes in infected groups are significantly higher than that of the vaccinated group. Anti-S IgG/IgM/IgA seropositive rates in the infected group were 90.4%, 92.9%, and 85.8%, respectively, while they are 93%, 74.7%, and 14% in the vaccinated group. Moreover, anti-N IgG/IgM/IgA seropositive rate between two groups were 87.3% versus 80.6%, 72.8% versus 64.8%, and 77.2% versus 40.6%, respectively. Both anti-S and anti-N IgA antibodies represent the low seropositive rate in the early phase after vaccination. Results indicated that there is a most significant difference in anti-S and anti-N IgA antibody levels between the infected group and the vaccinated group.

3.2 | Longitudinal observation of SARS-CoV-2-specific antibody levels after natural infection and vaccination

To better understand the humoral immune response elicited by inactivated vaccines, 20 healthy persons who were mentioned as controls above were immunized with two-dose CoronaVac inactivated vaccines at an interval of 21 days. Among these 20 vaccinated persons and 18 SARS-CoV-2-infected individuals chosen from the cross-sectional cohort above, the dynamic antibody responses to SARS-CoV-2 were observed. Individuals within and between groups showed various kinetics of antibody levels (Figure 2A–F). Nevertheless, antibody levels elicited by inactivated vaccines were lower than that elicited by natural infection within 46 days after SARS-CoV-2 infection or vaccination. In particular, significant differences in antibody levels ($p < 0.0001$) were observed at approximately 21 and 35 days after SARS-CoV-2 infection or vaccination (the time was allowed 3-day deviation on both sides in the infected group) (Supporting Information: Table 1). The fitted curves of antibody response were drawn by using the locally weighted regression method (Figure 2G,H). After SARS-CoV-2 infection, increased anti-S IgG/IgM/IgA antibodies were shown following a peak at the median of 34 days (IQR: 29–38), 26 days (IQR: 24–28 days), and 26 days (IQR: 24–32 days) respectively, while

TABLE 1 Clinical characteristics of three tested groups in this study

Characteristic	Infected group ($n = 239$)	Vaccinated group ($n = 165$)	Healthy group ($n = 20$)
Age (mean ± SD)	47.1 ± 16.5	35.1 ± 10.2	38.0 ± 7.4
Sex			
Male (n, %)	134 (56.1%)	39 (23.6%)	10 (50.0%)
Female (n, %)	105 (43.9%)	126 (76.4%)	10 (50.0%)
BMI	-	21.8 (19.6–24.6)	23.7 (21.9–25.6)
Days after infection/ vaccination (median, IQR)	17 (13–21)	41 (36–45)	-

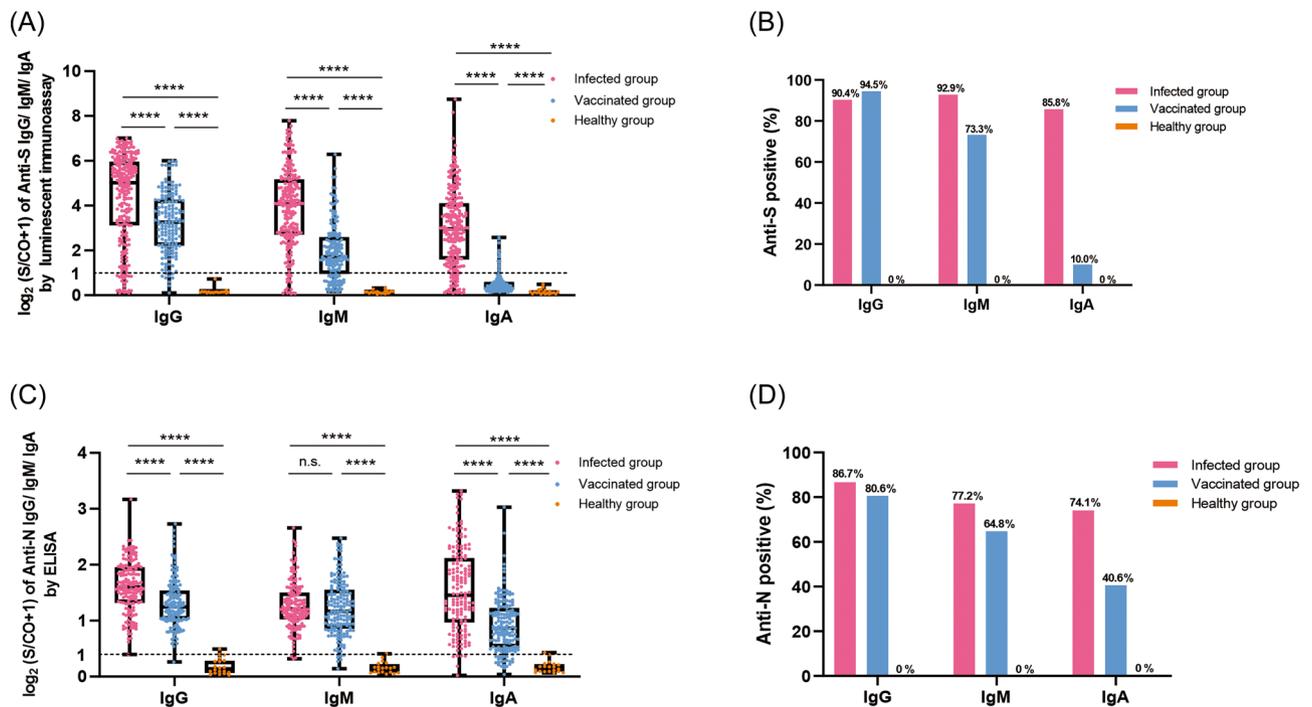Abbreviation: BMI, body mass index; IQR, interquartile range.

FIGURE 1 Humoral immune response to SARS-CoV-2 in the early phase of natural infection and vaccination (A) Anti-spike IgG/IgM/IgA antibody levels in SARS-CoV-2-infected individuals, vaccinated individuals, and naïve persons. (B) Seropositive rate of anti-spike IgG/IgM/IgA antibody levels in three groups. (C) Anti-nucleocapsid IgG/IgM/IgA antibody levels in these three groups. (D) Seropositive rate of anti-nucleocapsid IgG/IgM/IgA antibody levels in three groups. Statistical significance was determined with the Mann–Whitney test. Dotted lines indicate cut-off values of the serological tests. The S/CO of antibody levels above 1 was considered positive. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; S/CO, signal to a cut-off ratio. ****$p < 0.0001$.

anti-N IgG/IgM/IgA antibodies peak at the median of 26 days (IQR: 22–28), 20 days (IQR: 16–22), and 22 days (IQR: 20–25 days), respectively. Thereinto, IgM and IgA antibody binding to S and N proteins declined rapidly after reaching the peak, while IgG antibody levels with higher peak levels presented a slow reduction. However, most detectable anti-S and anti-N IgG/IgM/IgA antibodies appeared later in the vaccinated group than in the infected group within the 46-day longitudinal observation. Moreover, the peak of antibody levels in the vaccinated group was not observed with a slowly increasing rate. Of note, IgG/IgM/IgA antibodies against N protein were detectable earlier than antibodies against S protein.

3.3 | Clinical factors correlated with SARS-CoV-2-specific antibody levels

Based on the various levels of SARS-CoV-2-specific antibodies, we analyzed the correlation between antibody responses and clinical factors, including age, sex, and the time interval from natural infection or vaccination to sample collection (Figure 3A). Time intervals were grouped according to weeks, while ages were divided into six subgroups (<18, 18–30, 30–40, 40–50, 50–60, and ≥60 years). One-way ANOVA test was used to estimate the difference in antibody levels of the subgroups. The variance of anti-S IgG/IgM/IgA levels in different time-from-infection was significant ($p = 3.70e − 6$, $p = 0.002$ and

$p = 0.005$ separately), while that with time-from-vaccination were not significant ($p = 0.119$, $p = 0.767$, and $p = 0.295$, separately) (a separate correlation data were not shown). The variance of anti-S and anti-N IgG levels were significantly different in age subgroups in both the infected and vaccinated individuals, and the anti-N IgA levels were significantly different in age subgroups of the infected and vaccinated individuals ($p = 0.003$ and $p = 0.037$, respectively). Moreover, 30–40 years subgroup individuals had higher antibody levels (Figure 3B and Supporting Information: Figure 1). Furthermore, the variance of anti-S IgA levels was significantly different between sex subgroups in the vaccinated individuals ($p = 0.003$), in which male individuals had a higher positive rate of anti-S IgA levels than female individuals (Figure 3C).

3.4 | Different expression of cytokines between previously infected individuals with SARS-CoV-2 and vaccinated individuals

To further characterize differences in humoral immune responses between natural infection and vaccination, the expression of 48 cytokines induced in sera was determined by a Bio-Plex assay. Sera samples from 20 infected individuals were collected at an average of 32 days after infection (range: 28–40 days) and sera samples from 20 vaccinated individuals collected on Days 0 and 35 after vaccination
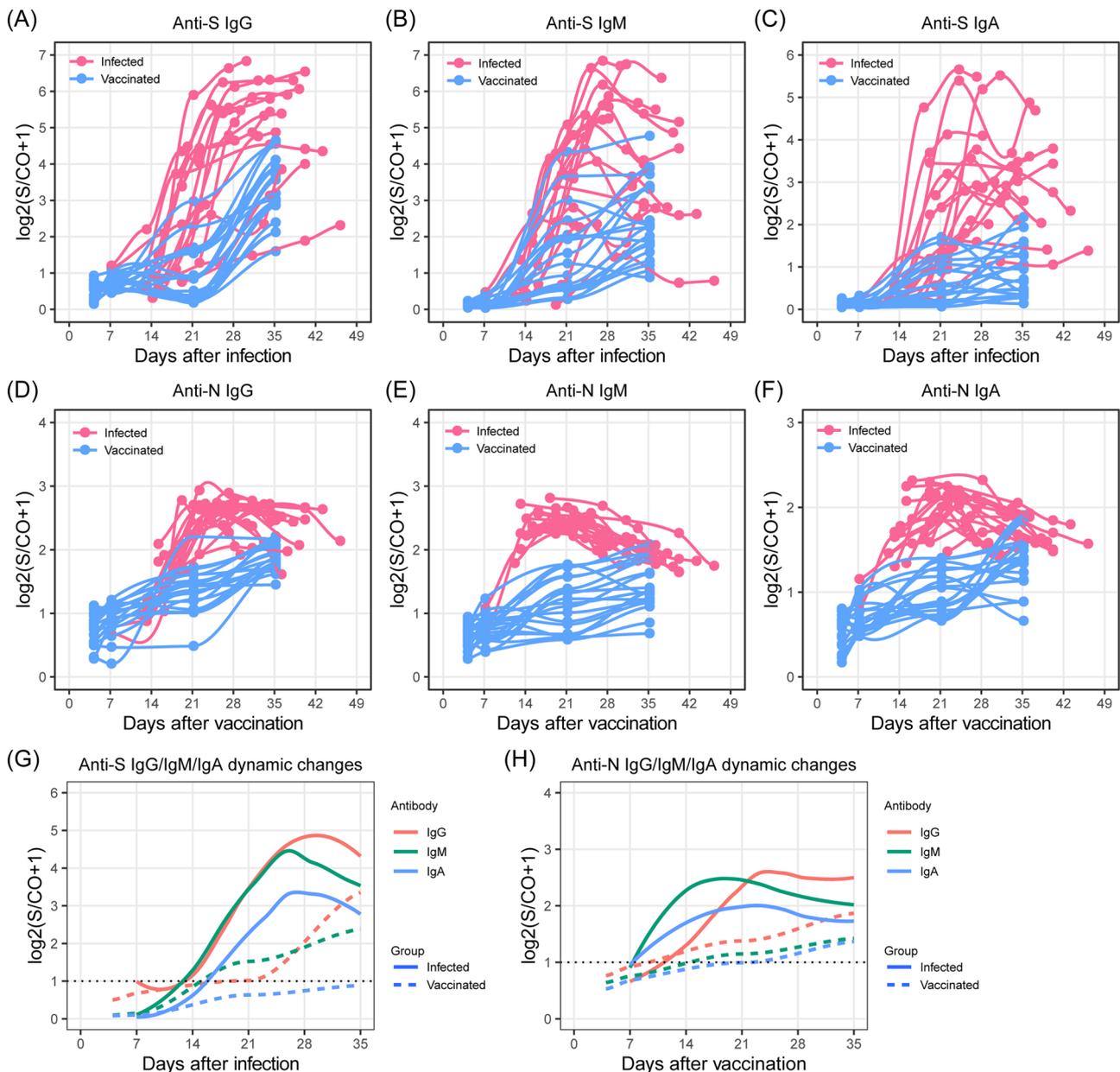
FIGURE 2 Kinetics of antibody responses in SARS-CoV-2-infected patients and vaccinated individuals. (A–F) Dynamic changes of anti-spike (anti-S) and anti-nucleocapsid (anti-N) IgG/IgM/IgA antibody levels 7–46 days after infection (red) or vaccination (blue). Each line represents one individual (it included 18 infected individuals and 20 vaccinated individuals). (G, H) Fitted curves of dynamic antibody responses in the early phase of SARS-CoV-2 infection (full lines) or vaccination (dotted lines) drawn by the LOESS method. The S/CO of antibody levels above 1 was considered positive. LOESS, locally weighted regression; S/CO, signal to a cut-off ratio; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

were chosen. The expression profile of cytokines was summarized in a heat map (Figure 4A). There is a special group with significantly high levels of IL-8 ($p < 0.001$), IFN-γ ($p < 0.001$), IL-17 ($p < 0.001$), TNF-α ($p < 0.001$), MCP-3 ($p < 0.001$), IL-6 ($p < 0.001$), IL-2Rα ($p < 0.001$), IL-3 ($p < 0.001$), IL-12 ($p < 0.001$), RGO-α ($p < 0.001$), G-CSF ($p < 0.01$), MIP-1α ($p < 0.001$), IL-7 ($p < 0.001$), β-NGF ($p < 0.001$), TRAIL ($p < 0.001$), IL-10 ($p < 0.001$), IL-2 ($p < 0.001$), IL-15 ($p < 0.001$), and HGF ($p < 0.1$) in the infected individuals, compared to the vaccinated individuals. By contrast, MIF ($p < 0.001$), TNF-β ($p < 0.001$), IL-9 ($p < 0.001$), SDF-1α ($p < 0.1$), IL-16 ($p < 0.001$), and IL-4 ($p < 0.1$) were

elicited by SARS-CoV-2 inactivated vaccines, while the expression of these cytokines showed low levels in the acute phase of SARS-CoV-2 infection. Results displayed that 31 of 48 cytokines have no significant changes before and after vaccination. However, 70.8% (34/48) of cytokines showed significant differences between natural infection and vaccination (Figure 4B,C and Supporting Information: Figure 2).

Then, we analyzed the association between cytokine expressions and antibody responses between the two groups tested above. In the infected individuals, 46 cytokines presented efficacy data and 6
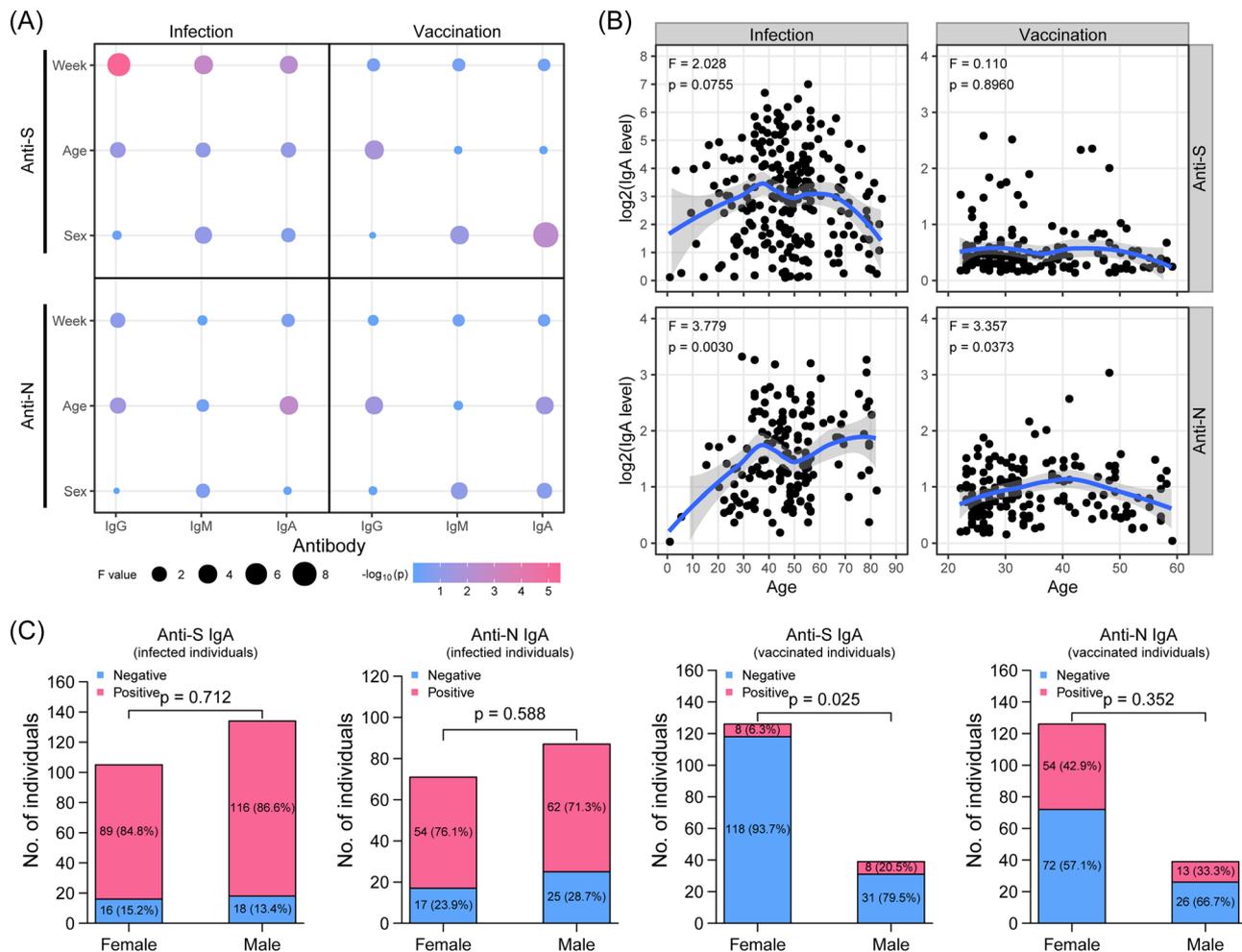
FIGURE 3 Demographic factors associated with antibody responses elicited by SARS-CoV-2 infection or vaccination. (A) Correlation analysis of anti-S and anti-N IgG/IgM/IgA antibody responses with age, sex, and time interval from SARS-CoV-2 infection or vaccination to sample collection. (B) Correlation between age and SARS-CoV-2-specific IgA antibodies in the infected and vaccinated groups. (C) Correlation between sex and SARS-CoV-2-specific IgA antibodies in the infected and vaccinated groups. One-way ANOVA tests were used to estimate the differences among different subgroups. ANOVA, analysis of variance; S/CO, signal to a cut-off ratio; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2. p values are depicted in the plots, and the p value cutoff was set as significant at 0.05.

cytokines (TNF-β, MIP-1β, GRO-α, IL-9, RANTES, and IL-13) were clustered with anti-S IgG/IgM/IgA with a positive correlation (Figure 5A). We found four cytokines (IL-3, IL-15, IL-1RA, and IFN-α2) were significantly negatively associated with anti-S IgG levels with moderate correlation coefficient ($R^2 > 0.5$ or $R^2 < -0.5$), and two cytokines (MIP-1β and TNF-β) were significantly positively associated with anti-S IgA level (Figure 5B). Furthermore, nine cytokines had a low correlation ($0.4 < R^2 < 0.5$ or $-0.5 < R^2 < -0.4$) with antibody levels in the infected group (Supporting Information: Figure 3). By contrast, there were only 26 cytokines that had efficacy data in the vaccinated group (Figure 5C). None of the cytokines was clustered with IgG/IgM/IgA antibody subtypes. In the vaccinated group, FGF basic was negatively associated with anti-S IgG level with a lower correlation coefficient ($R^2 = -0.438$, $p = 0.0534$), and SDF-1α was negatively associated with anti-S IgM level ($R^2 = -0.478$, $p = 0.0330$) (Figure 5D).

4 | DISCUSSION

In this study, we compared immune responses to SARS-CoV-2 after natural infection and vaccination with inactivated vaccines to obtain implications for the optimization of SARS-CoV-2-inactivated vaccines and the additional application of serological antibody tests. At first, in the comparison of antibody responses, significantly lower antibody levels than that of infected individuals, especially the IgA subtype, were observed in the vaccinated group. It suggested that IgA may be a potential auxiliary indicator to distinguish between SARS-CoV-2 natural infection and vaccination in the early phase. It may provide the possibility of extending the application of serological diagnostic tests under the situation of the presence of SARS-CoV-2 specific IgG and IgM antibodies in both the infected group and the vaccinated group.

On the other hand, IgA is the most abundant immunoglobulin in the human body with 66 mg/kg each day.[23] It is also the predominant
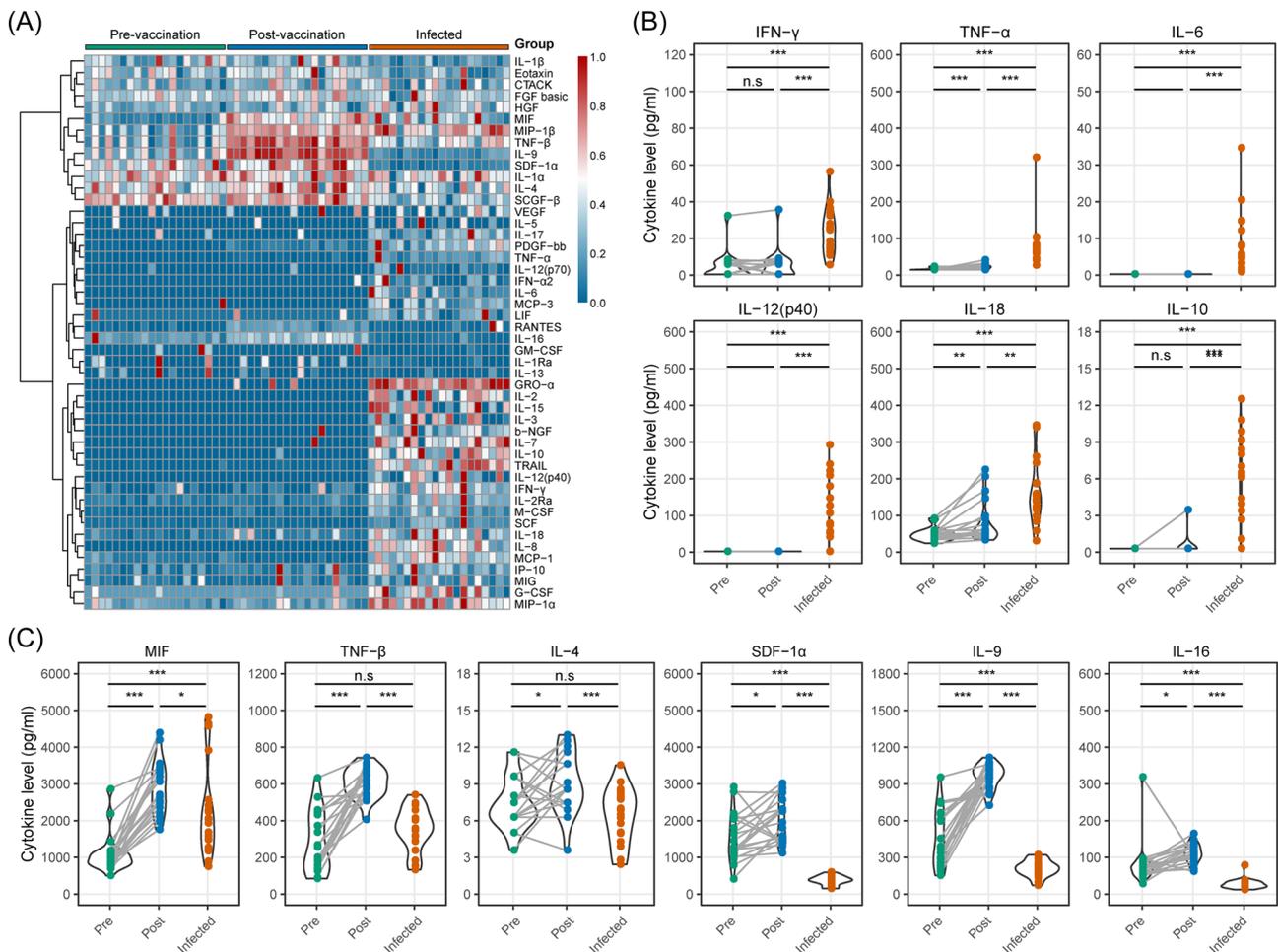
FIGURE 4 Distinct cytokine levels elicited by natural SARS-CoV-2 infection and vaccination. (A) Heat map of the expression of 48 cytokines after SARS-CoV-2 infection (the blue group, *n* = 20), pre- (the green group, *n* = 20) and post- (the orange group, *n* = 20) vaccination. (B, C) Specific hyperexpression of cytokines in the infected or vaccinated groups. In (A), color presents low (blue) to high (red) levels of cytokine expression. In (B, C), *p* values were determined with a two-tailed Mann–Whitney *U*-test between infected and vaccinated individuals and were evaluated by paired *t*-test between pre- and postvaccinated individuals. n.s., no significant differences; S/CO, signal to a cut-off ratio; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

antibody isotype in the mucosal system. IgA production on the mucosal surface plays a pivotal role at the first barrier of entry of SARS-CoV-2.[24] IgA is the main source of the early neutralizing antibody response to SARS-CoV-2.[25] In addition, IgA shows a higher neutralizing potency than IgG and IgM.[25] However, an inactivated vaccine cannot elicit anti-S IgA in the nasal mucosa.[26] Therefore, the optimized strategies of inactivated vaccines with the consideration of mucosal immunity are necessary. Intranasal inactivated vaccines have been recommended because it has been verified to improve the efficacy of influenza vaccines.[27] In the development of the novel SARS-CoV-2 vaccines, intranasal applicable inactivated vaccines can be considered.

In the longitudinal observation, even though antibody response presented variety among participants, the characteristics with earlier production of anti-N IgG/IgM/IgA antibodies than anti-S IgG/IgM/IgA antibodies were observed in the fitted curves. In light of the fast production and the high conservation of SARS-CoV-2 N protein, a

serological test targeting N protein can be used as a complement to the anti-S antibody test. On the other hand, in our results, lower antibody levels with a slow increase rate in the vaccinated group suggested the relatively low immunogenicity of inactivated vaccines. Our results were consistent with previous studies,[28] in which low antibody titers after the first dose with a moderate rise of antibody concentrations after the second dose were observed in persons who received inactivated vaccines. To enhance the immunogenicity of inactivated vaccines, increased vaccine dose, adjuvant types, and downstream processes for viral inactivation can be optimized, such as irradiation conditions, concentration, and purification of vaccines.[29–31]

Clinical factors were also verified to influence antibody levels in both infected and vaccinated groups in our study. The significant variance of anti-S IgG/IgM/IgA levels in different time-from-infection and no significant variance in different time-from-vaccination suggested the slow seroconversion elicited by inactivated SARS-CoV-2-inactivated vaccines. It is consistent with the results

FIGURE 5 Correlation between cytokines and antibody response. Pearson's correlation coefficient between cytokines and SARS-CoV-2 anti-S IgG/IgM/IgA antibodies was analyzed in SARS-CoV-2 infected group (sera samples were collected at an average of 32 days postsymptom onest; IQR: 28–40 days). (B) Moderate correlation ($R^2 < -0.5$ or $R^2 > 0.5$, $p < 0.05$) of cytokines with anti-S IgG and IgA levels in the infected individuals. (C) Pearson's correlation coefficient between cytokines and SARS-CoV-2 anti-S IgG/IgM/IgA antibodies was analyzed in SARS-CoV-2-vaccinated group (sera samples were collected on Days 0 and 35 after vaccination). (D) Low correlation ($R^2 > -0.4$ or $R^2 < 0.4$, $p < 0.05$) of cytokines with anti-S IgG and IgM levels in the vaccinated individuals. The size of circles indicates the degree of correlation. A normalized scale is indicated, with a positive correlation shown in red and a negative correlation shown in blue. IQR, interquartile range; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

we observed above. By the comparison of the effect of the age factor on antibody response between infected and vaccinated groups, inactivated vaccines elicited consistently low levels of SARS-CoV-2-specific antibodies with no significant differences in the different age subgroups. This might be due to a weak antibody response elicited by inactivated vaccines. However, even so, individuals in the 30–50 years subgroups have higher antibody titers in both infected and vaccinated groups. Furthermore, we observed a higher positive rate of anti-S IgA levels in vaccinated male individuals, which might be speculated from one study suggesting the positive association of

male sex with IgA antibody due to alcohol consumption, smoking, and common metabolic abnormalities.

Cytokines play an important role in innate and adapted immune responses after viral infection. However, the excessive expression of cytokines has been reported to lead to exaggerated inflammatory reaction and poor prognosis of COVID-19.[32–34] TNF-α and IFN-γ, both of which are associated with tissue damage and mortality of SARS-CoV-2 infection and cytokine shock syndromes,[35] were not found in the vaccinated group. It indicated the safety of inactivated vaccines, in some ways. By comparing different expressions of

cytokines elicited by SARS-CoV-2 infection or vaccination, adverse events of vaccination can be speculated by proinflammatory cytokine responses in the vaccinated group.[36] Among all 48 tested cytokines, 64.6% of cytokines present high levels in the infected individuals, while 29.2% of cytokines were elicited by vaccination. It indicates that vaccination by inactivated vaccines elicits a moderate cytokine response. For instance, IL-18, IL-6, and IL-10, which have been corroborated to play a role in fatal COVID-19[37,38] showed lower serum expression in the vaccinated group. On the other side, the appropriate expression of some cytokines in the infected individuals can inform the optimization of inactivated vaccines, as cytokines have the potential used as the adjuvant. An inactivated influenza virus vaccine with intranasal coadministration of IL-33 has been reported to increase the cross-protective efficacy of the vaccine against influenza virus infection.[39] Moreover, further verification of the respective features of cytokine expression after SARS-CoV-2 infection or vaccination in large-scale cohorts may also provide the value of combined cytokines for SARS-CoV-2 diagnosis in the future.

In our study, we further investigated the correlation between cytokines and antibody response. There were six cytokines that were clustered with anti-S IgG/IgM/IgA elicited by SARS-CoV-2 infection with positive correlation, while none of the cytokines was clustered with anti-S IgG/IgM/IgA elicited by SARS-CoV-2-inactivated vaccines. Among these six cytokines, the incorporation of RANTES into DNA vaccines against influenza has been proved to improve their immunogenicity by the recruitment and activation of dendritic cells.[40] Therefore, the comparison of correlation between cytokines and antibody response in the infected group and the vaccinated group can imply the future role of cytokine supplements on vaccine development. In another study, increased IL-6 in COVID-19 patients with severe diseases has been reported to be associated with SARS-CoV-2 N protein.[41] It may be correlated with the stronger immune response in the infected individuals. Accordingly, the influence of cytokines on vaccine outcomes should be considered in the future of vaccine development.

There are two limitations in the present study. First, the limited follow-up visit hindered us to observe the kinetics of antibody response within a longer interval in the vaccinated group. Our data focused on the early phase after natural infection or vaccination. Furthermore, we did not obtain the saliva samples for the mucosal immunity of IgA.

5 | CONCLUSIONS

We showed significant differences in antibody and cytokine responses after SARS-CoV-2 natural infection and vaccination with inactivated vaccines. Low-level antibody response elicited by inactivated vaccines is correlated with the immunogenicity of antigens. Cytokines associated with the inflammatory response in severely infected individuals were not found in vaccinated persons. Our findings may provide implications for the improvement of inactivated vaccine optimization and the application of serological tests.

AUTHOR CONTRIBUTIONS

Ai-long Huang, Ni Tang, Kai Wang, Juan Chen, and Pai Peng developed the conceptual ideas and designed the study. Pai Peng and Haijun Deng performed the experiments, analysis, and interpretation of data. Zhihong Li was responsible for data collection. All authors provided scientific expertise and the interpretation of data for the work. Pai Peng drafted the manuscript. All authors contributed to the critical revision of the manuscript for important intellectual content. All authors reviewed and approved the final version of the report.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable for this article as no data sets were generated or analyzed during the current study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.