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RESEARCH ARTICLE

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SARS-CoV-2 reinfection broadens the antibody responses and promotes the phenotypic differentiation of virus-specific memory T cells in adolescents

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Abstract

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Omicron subvariants raises concerns regarding the effectiveness of immunity acquired from previous Omicron subvariants breakthrough infections (BTIs) or re-infections (RIs) against the current circulating Omicron subvariants. In this study, we prospectively investigate the dynamic changes of virus-specific antibody and T cell responses among 77 adolescents following Omicron BA.2.3 BTI with or without subsequent Omicron BA.5 RI. Notably, the neutralizing antibodies (NAbs) titers against various detected SARS-CoV-2 variants, especially the emerging Omicron CH.1.1, XBB.1.5, XBB.1.16, EG.5.1, and JN.1 subvariants, exhibited a significant decrease along the time. A lower level of IgG and NAbs titers post-BTI was found to be closely associated with subsequent RI. Elevated NAbs levels and shortened antigenic distances were observed following Omicron BA.5 RI. Robust T cell responses against both Omicron BA.2- and CH.1.1-spike peptides were observed at each point visited. The exposure to Omicron BA.5 promoted phenotypic differentiation of virus-specific memory T cells, even among the non-seroconversion adolescents. Therefore, updated vaccines are needed to provide effective protection against newly emerging SARS-CoV-2 variants among adolescents.

KEYWORDS

breakthrough infection, differentiation of memory T cells, neutralizing antibody, reinfection, SARS-CoV-2, T cell responses

Xin-Jing Zhao, Xiao-Lin Liu, and Hong-Jing Gu contributed equally to this study.

1 | INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic, which commenced over 3 years ago, has resulted in more than 775 million confirmed cases and a global death toll exceeding seven millions as of March 31, 2024.[1] Currently, with newly emerging Omicron subvariants, particularly CH.1.1, XBB.1.5, XBB.1.16, EG.5.1, and JN.1, the investigation of whether the patients who have recovered from the previous Omicron subvariant breakthrough infections (BTIs) still retain sufficient immunity to effectively prevent reinfections (RIs) against newly emerging Omicron subvariants is of significant importance.

The majority of previous studies on Omicron BA.1 or BA.2 BTI has primarily focused on assessing humoral immunity during the early convalescent stage,[2–6] demonstrating broadly neutralizing activity against previous variants of concern and Omicron subvariants derived from the BA.2. However, there is a limited number of studies that specifically investigate the persistence of immunity beyond 6 months post-Omicron subvariants BTI,[7] revealing diminished levels of neutralizing antibodies (NAbs) against BA.2.75.2, BA.4/5, and BQ.1.1. Given the ongoing evolution of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), various Omicron subvariants have been reported to evade NAbs induced by both vaccination and prior infection, especially for Omicron BQ.1.1, BA.2.75.2, CH.1.1, XBB.1.5, XBB.1.16, EG.5.1, and JN.1.[8–10] Therefore, further studies are needed to evaluate the neutralizing capacity against various emerging Omicron subvariants and to explore the persistence of NAbs in a longer followed-up prospective cohort.

Considering the T cell responses, Tan et al. have found that they may play an important role in virus prevention and clearance,[11] when robust $CD4^+$ and $CD8^+$ T cell responses are elicited post-SARS-CoV-2 vaccination or infection.[12,13] The persistence of memory T cell response becomes particularly critical given the rapid decline of humoral immunity post-infection or vaccination. Several studies have shown that the majority of T cell responses induced by vaccination or infection can cross-recognize both the wild-type (WT) and Omicron variants,[14,15] and Tarke et al. also have demonstrated that T cell cross-reactivity is observed within Alpha and Beta coronaviruses.[16] However, whether T cells respond to distinct peptides from the spike protein in different strains in not tested.

Previous studies have shown that children had a stronger early innate antiviral response against SARS-CoV-2 infection in their airway immune cells compared to adults,[17] and children are capable of generating robust, cross-reactive and sustained immune responses to SARS-CoV-2 when compared to adults.[18] Although clinical manifestations of children's COVID-19 cases were generally less severe than those of adult patients, young children were vulnerable to SARS-CoV-2 infection.[19] Therefore, considering the virus susceptibility and place aggregation of adolescents, it is of great importance to conduct prospective studies on the characteristics of immune response among them post-SARS-CoV-2 BTI and RI.

Overall, the full comprehension of humoral and cellular immunity durability characteristics among adolescents following Omicron BTI and RI remains incomplete.[20,21] Our previous cross-sectional studies have demonstrated the presence of NAbs responses following Omicron BA.2 BTIs and/or Omicron BA.5 RIs.[8,22] In this study, we conducted a prospective follow-up over a 12-month period on 77 adolescent patients post-Omicron BA.2.3 BTI with or without Omicron BA.5 RI to measure the virus-specific binding IgG antibodies, detect NAbs against various emerging Omicron subvariants, and elucidate the activation and differentiation of T cell responses toward different Omicron peptide pools. These results can expand our understanding of the dynamic changes in NAbs and T cell responses among adolescents.

2 | METHODS

2.1 | Study design and participants

In March 2022, a cohort of 77 participants with Omicron BA.2.3 infections were enrolled in Binzhou City, Shandong Province, China to investigate the characteristics of humoral and cellular immunity (Supporting Information S1: Figure S1). The participants consisted of adolescents aged 13–16 years (median age: 14.0), with 37 (48.1%) being male. All subjects had previously received two doses of inactivated vaccines (CoronaVac or BBIBP-CorV). Among them, 30 (39.0%) subjects were asymptomatic, while 47 (61.0%) subjects were symptomatic after contracting Omicron BA.2.3 infection, with fever (41.6%), cough (31.2%), and headache (11.7%) being the most commonly reported symptoms. Four followed-up visits were conducted at 0.5-month (T1), 3-month (T2), 6-month (T3), and 12-month (T4) post-Omicron BA.2.3 BTI (Table 1). Serum and peripheral blood mononuclear cells (PBMCs) were collected from all participants. The detailed information is given in Supporting Information Methods.

2.2 | Enzyme-linked immunosorbent assay analysis of binding IgG antibody to spike trimer of Omicron BA.2 and BA.5

The serum binding IgG antibody against Omicron BA.2 and BA.5 was assessed using an enzyme-linked immunosorbent assay, as previously reported.[23] The detailed information is given in Supporting Information Methods.

2.3 | Pseudovirus neutralization assay

The serum NAbs responses against the D614G strain and Omicron BA.2, BA.5, BF.7, BQ.1.1, BA.2.75.2, CH.1.1, XBB.1.5, XBB.1.16, EG.5.1, and JN.1 subvariants were measured using a pseudovirus neutralization assay (Supporting Information S1: Figure S2).[8] Detailed information regarding the production of pseudoviruses and the execution of neutralization assay can be found in Supporting Information Methods.

TABLE 1 Characteristics of donor cohorts.

	COVID-19 (n = 77)	Healthy controls (n = 20)
Age (median, range)	14.0 (13.0–16.0)	16.0 (12.0–16.0)
Gender (n, %)		
Male	37 (48.1)	10 (50.0)
Female	40 (51.9)	10 (50.0)
Vaccination (n, %)		
First dose		
CoronaVac	28 (36.4)	6 (30.0)
BBIBP-CorV	49 (63.6)	14 (70.0)
Second dose		
CoronaVac	53 (68.8)	15 (75.0)
BBIBP-CorV	24 (31.2)	5 (25.0)
Clinical symptoms after Omicron BA.2 BTI (n, %)		
Asymptomatic	30 (39.0)	N/A
Symptomatic	47 (61.0)	N/A
Fever	32 (41.6)	N/A
Cough	24 (31.2)	N/A
Headache	9 (11.7)	N/A
Sore throat	8 (10.4)	N/A
Runny nose	4 (5.2)	N/A
Vomiting	1 (1.3)	N/A
Clinical symptoms after Omicron BA.5 reinfection (n, %)		
Asymptomatic	22 (100)	N/A
Symptomatic	0 (0)	N/A
Days between last vaccination and BTI (median, IQR)	202.0 (169.0–207.0)	N/A
Sampling time (median, IQR)		
Days between BTI/ vaccination and first visit	15.0 (14.0–17.0)	15.5 (13.0–18.8)[a]
Days between BTI and second visit	99.0 (98.0–101.0)	N/A
Days between BTI and third visit	199.0 (197.0–200.0)	N/A
Days between BTI and fourth visit	343.0 (341.0–344.0)	N/A
Days between BTI and reinfection	287.5 (278.8–293.3)	N/A
Days between reinfection and fourth visit	55.0 (48.8–64.0)	N/A

[a]Days between the last vaccination and sampling for healthy controls.

2.4 | Flow cytometry-based T cell assays

The activation-induced cell marker (AIM) assay and intracellular staining (ICS) assay were conducted as previously described.[24] The PBMCs were stimulated with Omicron BA.2 or CH.1.1 peptides (Table S1), followed by incubation with selected flow antibodies. All samples were acquired on an ID7000™ Cell Analyzer (Sony Biotechnology) and analyzed using the ID7000 Software (https://www.sonybiotechnology.com/us/instruments/id7000-spectral-cell-analyzer/software/). The detailed information is given in Supporting Information Methods.

2.5 | Statistical analysis

All statistical analyses were performed using GraphPad Prism (version 8.0.2) and RStudio (version 4.2.3). Normality was assessed by performing the Kolmogorov–Smirnov test ($n > 50$) or the Shapiro–Wilk test ($n \leq 50$). For normally distributed data, paired or unpaired t-tests were used for comparison. In the case of non-normal data, differences between paired groups were assessed using the Wilcoxon test or Friedman test, while differences between unpaired groups were assessed using the Mann–Whitney test or Kruskal–Wallis test. The strength of correlations was evaluated using Spearman's test. All statistical tests were two-sided with a significance level of 0.05.

3 | RESULTS

3.1 | Omicron subvariants BTI and RI elicit high spike-specific binding IgG antibodies against both Omicron BA.2 and BA.5

The data revealed that the Omicron BA.2.3 BTI can induce significantly higher spike-specific binding IgG antibodies against Omicron BA.2 and BA.5 compared to healthy controls (HCs) immunized with two-dose inactivated vaccines (Figure 1A). Comparison of the geometric mean titers (GMT) at different time points demonstrated a significant reduction in both Omicron BA.2- and BA.5-specific binding IgG antibodies at T2 compared to that at T1, followed by a gradual decline in T3 and T4 (Figure 1A). Further comparison revealed no significant differences in the levels of Omicron BA.5-specific binding IgG antibodies and Omicron BA.2-specifc binding IgG antibodies at all time points examined. Notably, a majority of the participants showed detectable levels of binding IgG antibodies during the follow-up period (Figure 1B).

More importantly, we observed a significant increase in Omicron BA.2- and BA.5-specific binding IgG antibodies at T4 among adolescents in the RI group compared to that of T3 (Figure 1C), while the non-reinfection (NRI) group exhibited a significant decrease in binding IgG antibodies from T3 to T4 (Figure 1D). Furthermore, upon comparing the RI group with the NRI group, it became apparent that

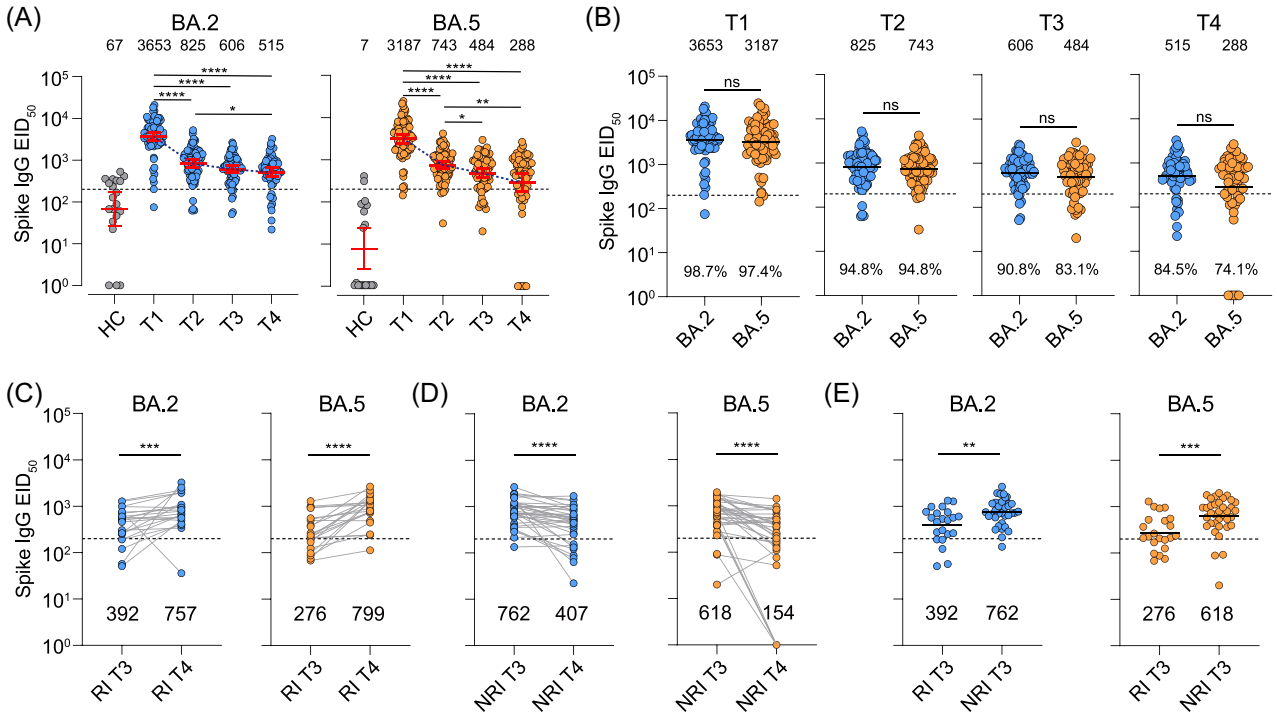

FIGURE 1 Spike-specific binding IgG antibodies responses against Omicron BA.2 and BA.5 in adolescents. (A) Dynamic changes of spike-specific binding IgG antibodies against Omicron BA.2 and BA.5 at the four investigated time points. (B) Comparison of spike-specific binding IgG antibodies titers between Omicron BA.2 and BA.5 by different visited time points. (C) Comparison of spike-specific binding IgG antibodies titers against Omicron BA.2 or BA.5 at 6- and 12-months of adolescents with Omicron BA.5 reinfection (RI). (D) Comparison of spike-specific binding IgG antibodies titers against Omicron BA.2 or BA.5 between 6- and 12-months of adolescents without Omicron BA.5 RI. (E) Comparison of spike-specific binding IgG antibodies titers against Omicron BA.2 or BA.5 between Omicron RI group and non-reinfection (NRI) group at 6 months. Sera were collected from the adolescents at 0.5-, 3-, 6-, and 12-month post-Omicron BA.2.3 breakthrough infection (BTI) with or without subsequent Omicron BA.5 RI. Sera of healthy controls (HC) in panel (A) were collected from 20 adolescents with only two-dose inactivated vaccination. The geometric mean with a 95% confidence interval (CI) or geometric mean alone is shown in the above panels. The black dashed line indicates the threshold for initial dilution (1:200). The Kruskal–Wallis test adjusted with the false discovery rate (FDR) method for multiple comparisons was performed in panel (A), Wilcoxon matched-pairs signed rank test was performed in panels (B–D). Mann–Whitney test was performed in panel (E). $p < 0.05$ was considered statistically significant. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

individuals who experienced subsequent RI exhibited significantly lower levels of binding IgG antibodies against both Omicron BA.2 and BA.5 at T3, thereby indicating a close association between reduced levels of binding IgG antibodies and subsequent RI (Figure 1E).

3.2 | Omicron RI broadens the neutralizing ability against emerging Omicron subvariants

Firstly, we conducted a comparative analysis of NAbs titers against the same strain at different time points during followed-up (Figure 2A). At T2, significantly lower NAbs titers were observed against D614G and all Omicron subvariants compared to those at T1. At T3, there was a slight or significant decrease in NAb titers against all strains compared to those at T1. Overall, within 6 months post-Omicron BA.2.3 BTI, the NAbs titers against all detected variants continuously decreased, while there was a significant or slight increase in NAbs titers against all Omicron subvariants at T4 compared to those at T3, following the Omicron BA.5 wave. After excluding participants with BA.5 RI from the cohorts, we observed a continuous decrease in NAbs titers against all detected variants from T1 to T4 (Supporting Information S1: Figure S3A). Then, we compared the NAbs titers against various stains at the same time points of followed-up (Figure 2B). Overall, compared to the D614G, the serum NAbs titers were comparable against Omicron BA.2 and slightly decreased against both Omicron BA.5 and BF.7, whereas there was a significant decrease in NAbs titers against all other Omicron subvariants.

The NAbs titers against Omicron BA.5 at T2 and T3 were 1.1 to 2.5 times lower than those at T1. For Omicron BF.7, the NAbs titer at T2 and T3 were 1.6 to 2.2 times lower than those at T1 (Figure 2A). Consistent with the change in binding IgG antibodies, adolescents with RI exhibited significantly higher NAbs titers at T4 than those at T3 (Supporting Information S1: Figure S3B). Importantly, the RI group of adolescents showed slightly or significantly lower NAbs titers against all detected variants than the NRI group at T3, revealing that lower levels of NAbs were also associated with subsequent RI (Supporting Information S1: Figure S3C). The antigenic map analysis

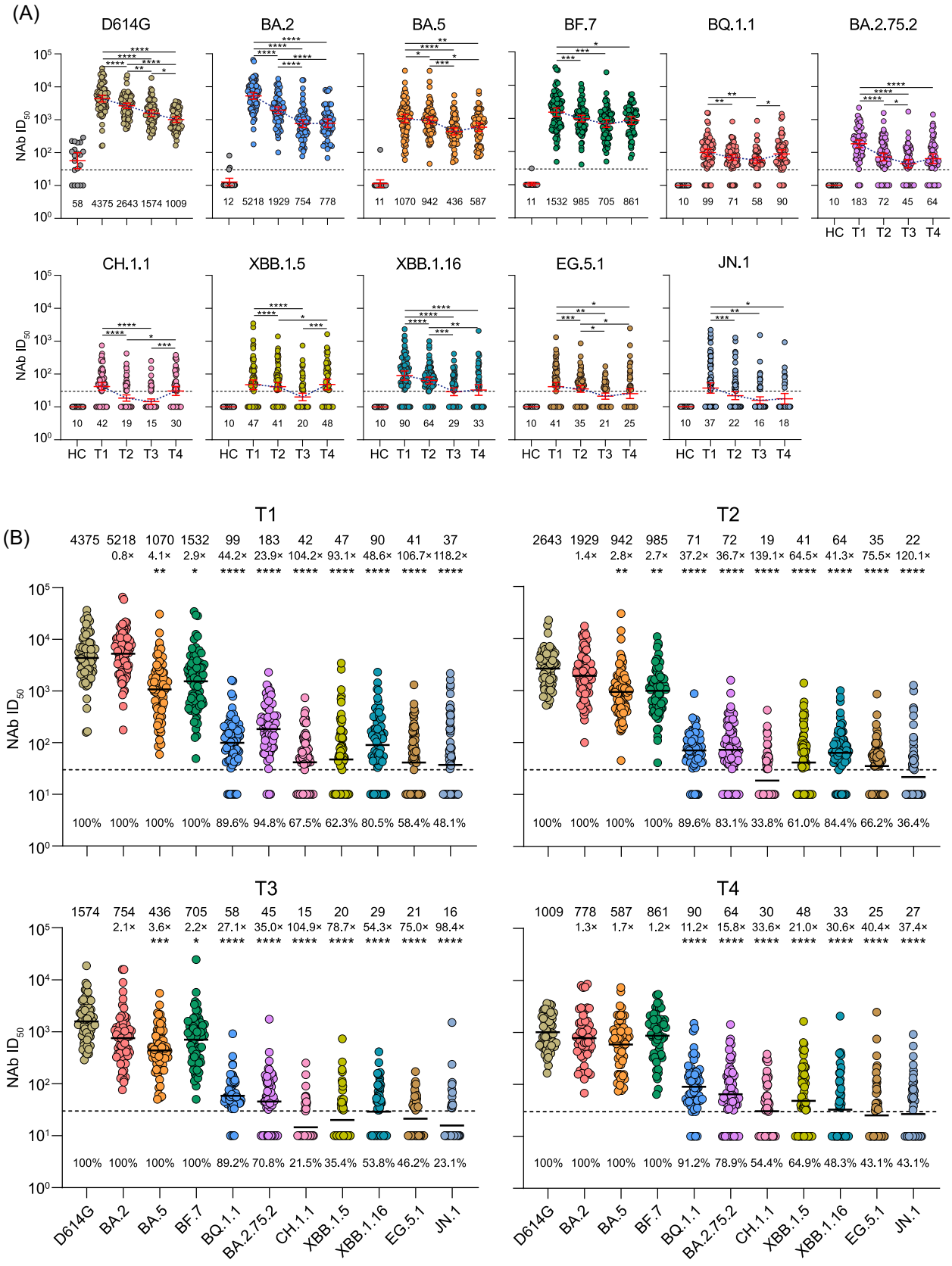

FIGURE 2 (See caption on next page).

further revealed that the SARS-CoV-2 variants used in this study can be divided into two distinct antigenic groups (Group 1 and Group 2) (Supporting Information S1: Figure S3D). By calculating the relative distances between D614G and each of the Omicron subvariants, we observed significant differences between Group 1 and Group 2 at all examined time points (Supporting Information S1: Figure S3E). Considering the different time points during the followed-up, we demonstrated that within a 6-month period post-Omicron BA.2.3 BTI, the relative distances in Group 2 were comparable across all visited time points. However, Omicron BA.5 RI at T4 exhibited a significant reduction in antigenic relative distances compared to others (Supporting Information S1: Figure S3F).

3.3 | Robust T cell responses are observed against both Omicron BA.2 and CH.1.1 peptides

We then combined AIM assay with cytokine ICS assay to evaluate T cell responses (Supporting Information S1: Figure S4). Spike-specific $CD4^+$ and $CD8^+$ T cell responses against each of Omicron BA.2 and CH.1.1 mega peptide pools (MPs) were measured by AIM $OX40^+CD137^+$ ($CD4^+$ T cells) or $CD69^+CD137^+$ ($CD8^+$ T cells) (Figure 3A,C). For AIM^+CD4^+ T cells, comparable percentages against Omicron BA.2 and CH.1.1 MPs were observed at T3 ([median 0.27%, IQR 0.19%−0.45%] and [median 0.27%, IQR 0.19%−0.39%]) and T4 ([median, 0.27%, IQR 0.17%−0.42%] and [median 0.26%, IQR 0.18% −0.37%]), respectively, which were significantly higher than those in HCs. What's more, no significant differences in AIM^+CD4^+ T cell responses were observed between T3 and T4 (Figure 3B). Similarly, the responses of AIM^+CD8^+ T cells against Omicron BA.2 and CH.1.1 were comparable at T3 ([median 0.49%, IQR 0.27%−0.67%] and [median 0.44%, 0.28%−0.65%]) and T4 ([median 0.31%, IQR 0.19% −0.45%] and [median 0.29%, IQR 0.19%−0.47%]), respectively. And the AIM^+CD8^+ responses also significantly higher in infected adolescents than that in vaccinated HCs. However, the AIM^+CD8^+ T cell responses were significantly reduced at T4 compared to those at T3 (Figure 3D). Further comparison showed that there were no significant differences of AIM^+ $CD4^+$ or $CD8^+$ T cell responses between the RI group and the NRI group by different Omicron spike MPs or different RI statuses (Supporting Information S1: Figure S5A, 5B). Notably, regardless of RI or not, AIM^+ $CD8^+$ T cell responses were significantly lower at T4 than that at T3 (Supporting Information S1: Figure S5B).

Then the spike-specific $CD4^+$ or $CD8^+$ T cells secreting TNF-α, IL-2, and IFN-γ were measured by ICS assay (Figure 4A,B). At the same time point (T3 or T4), the percentage of ICS^+ $CD4^+$ or $CD8^+$ T cells stimulated by Omicron BA.2 or CH.1.1 MPs was comparable and significantly higher than that in HCs (Figure 4C,D). When comparing different followed-up time points, both Omicron BA.2 and CH.1.1 spike-specific ICS^+ $CD4^+$ or $CD8^+$ T cell percentages were significantly reduced at T4 compared to those at T3 (Figure 4C,D). When the adolescents were divided into RI and NRI groups, we observed comparable ICS^+ $CD4^+$ or $CD8^+$ T cell responses induced by Omicron BA.2 or CH.1.1 MPs in each group at the same time points (Supporting Information S1: Figure S6A, 6B), and within the same group, the ICS^+ responses were significantly reduced at T4 compared to those at T3 (Supporting Information S1: Figure S6A, 6B). By comparing the T4/T3 ratio values for ICS^+ $CD4^+$ or $CD8^+$ T cell responses among different groups, we observed a significantly lower $TNF\text{-}\alpha^+$ $CD4^+$ T cells ratio value in the NRI group compared to the RI group, however, no significant differences were observed in other ICS^+ $CD4^+$ or $CD8^+$ T cell responses between the groups (Supporting Information S1: Figure S6A, 6B). We further analyzed the polyfunctional profiles of T cells that produce cytokines in multiple secreting patterns and demonstrate similar capacities for cytokines co-expression for both Omicron BA.2 and CH.1.1-spike specific T cells at T3 or T4, indicating the normal function of T cell responses in recognizing different Omicron subvariants (Figure 4E–H). Notably, there were also no significant differences observed in the polyfunctional profiles for either ICS^+ $CD4^+$ or $CD8^+$ T cells between T3 and T4, suggesting that the ICS^+ responses maintained stable following Omicron BA.2.3 BTI (Figure 4E–H). Considering the RI status, no significant differences were found in the polyfunctional profiles among groups of RI T3, NRI T3, RI T4, and NRI T4 for both $CD4^+$ and $CD8^+$ T cells (Supporting Information S1: Figure S6C, 6D). Overall, our findings demonstrated a significant increase in AIM^+ or ICS^+ T cell responses following Omicron BA.2.3 BTI compared to those observed in solely vaccinated HCs. Furthermore, robust T cell responses were observed against various Omicron subvariants, including Omicron BA.2 and CH.1.1.

FIGURE 2 Neutralizing antibodies (NAbs) responses against D614G and emerging Omicron subvariants in adolescents. (A) Dynamic changes of NAbs titers against D614G and Omicron BA.2, BA.5, BF.7, BQ.1.1, BA.2.75.2, CH.1.1, XBB.1.5, XBB.1.16, EG.5.1, and JN.1 at the four investigated time points. (B) Comparison of virus-specific neutralizing antibody titers among D614G and various emerging Omicron subvariants by different followed-up time points. Sera were collected from the adolescents at 0.5-, 3-, 6-, and 12-month post-Omicron BA.2.3 breakthrough infection (BTI) with or without subsequent Omicron BA.5 reinfection (RI). Sera of healthy controls (HC) in panel (A) were collected from 20 adolescents with only two-dose inactivated vaccination. The geometric mean with a 95% confidence interval (CI) or geometric mean alone is shown in the above panels. Values of GMT were shown at the bottom of the panel (A). Values of GMT with reduction times compared to D614G, and the prevalence of detectable NAbs titers above 30 were shown in the panel (B). The black dashed line indicates the threshold for detectable NAbs titers (ID_{50} = 30). A Kruskal–Wallis test adjusted with the FDR method for multiple comparisons was performed in panel (A). Wilcoxon matched-pairs signed rank test for comparisons between D614G and each of the Omicron subvariants was performed in panel (B). $p < 0.05$ was considered statistically significant. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. GMT, geometric mean titers.

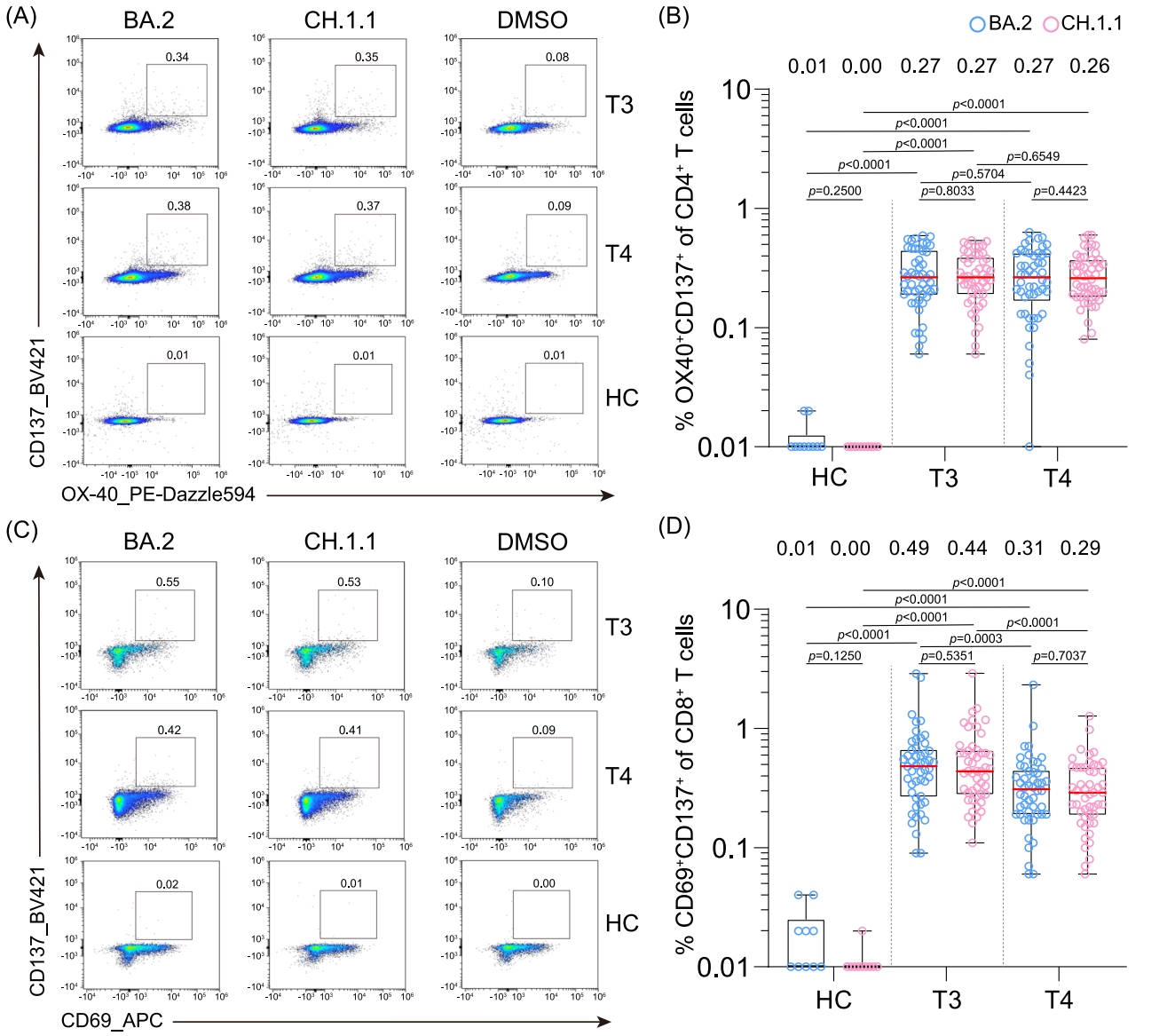

FIGURE 3 Spike-specific AIM+ CD4+ or CD8+ T cell responses against Omicron BA.2 and CH.1.1 in adolescents. The percentage of AIM$^+$ (OX40$^+$CD137$^+$) of CD4$^+$ T cells (A and B) and AIM$^+$ (CD69$^+$CD137$^+$) of CD8$^+$ T cells (C and D) after stimulation of PBMCs with Omicron BA.2 or CH.1.1 spike-specific Mega peptide pools (MPs). PBMCs were collected from the adolescents at 6- and 12-month post-Omicron BA.2.3 breakthrough infection (BTI) with or without subsequent Omicron BA.5 reinfection (RI). PBMCs of HCs were collected from 10 adolescents with only two-dose inactivated vaccination. Graphs show individual responses of AIM$^+$ CD4$^+$ or CD8$^+$ T cell responses against Omicron BA.2 or CH.1.1 MPs plotted as background-subtracted DMSO negative controls. Boxplots indicate median and interquartile range (IQR). Wilcoxon matched-pairs signed rank test and Mann–Whitney test were performed in panels (B and D), and $p < 0.05$ was considered statistically significant. AIM, activation-induced cell marker; HC, healthy controls; PBMCs, peripheral blood mononuclear cells.

3.4 | Omicron BA.5 exposure promotes the differentiation of virus spike-specific memory T-cells

To explore the differentiation patterns of Omicron BA.2 and CH.1.1 spike-specific memory T cells, we selected two surface markers, CD45RA and CCR7, to subdivide spike-specific memory T cells into central memory T cells (TCM, CD45RA$^-$CCR7$^+$), naïve T cells (Tnaive, CD45RA$^+$CCR7$^+$), effector memory T cells (TEM, CD45RA$^-$CCR7$^-$), and terminally differentiated effector memory T cells (TEMRA, CD45RA$^+$CCR7$^-$) by detecting the expression levels on virus-specific AIM$^+$ T cells (Figure 5A). The results showed that TEM and TCM constituted the primary subsets of Omicron BA.2 and CH.1.1 spike-specific CD4$^+$ T cells, with a slightly or significantly higher proportion of TEM and a lower proportion of Tnaive at T4 compared to that at T3 (Figure 5B). Regarding virus spike-specific CD8$^+$ T cells, the main subsets identified as TEM and TEMRA for both Omicron BA.2 and CH.1.1 MPs. At T4, there was a slight or significant increase in the percentage of TEM subset, while the percentage of TCM subset showed a slight or significant reduction compared to that at T3 for spike-specific memory CD8$^+$ T cells (Figure 5C).

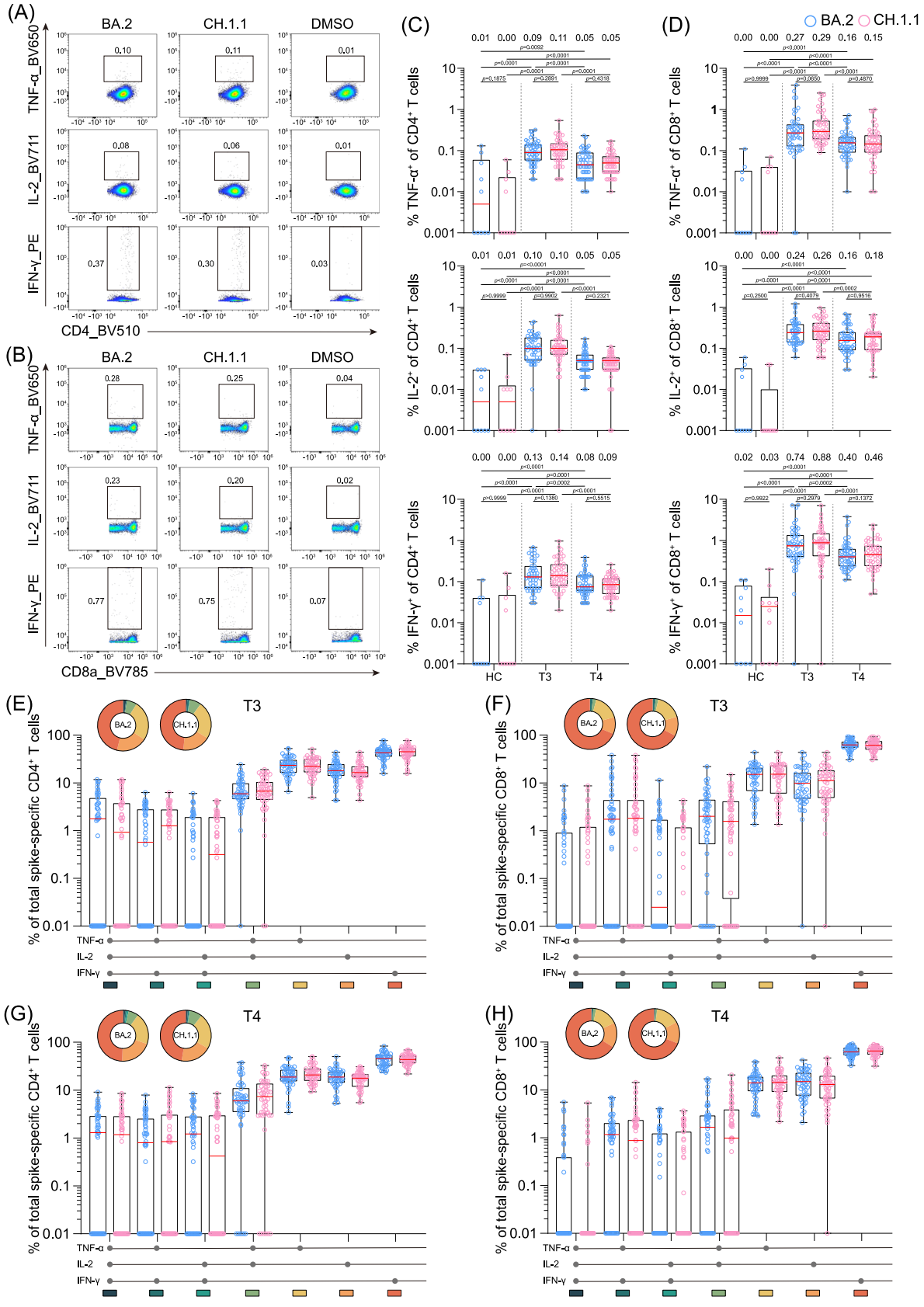

FIGURE 4 (See caption on next page).

Considering the RI status, we found that the phenotypic differentiation of spike-specific memory $CD4^+$ or $CD8^+$ T cells was comparable between the RI group and NRI group as a whole (Supporting Information S1: Figure S7A–D). Further comparison between these two groups revealed that the NRI group exhibited a higher percentage of TEM subset at T3 compared to the RI group, while no significant difference in TEM subset was observed at T4, indicating a correlation between lower TEM subset and susceptibility to Omicron BA.5 RI (Supporting Information S1: Figure S7E–H).

3.5 | Correlations between potential influence factors and immune responses

Finally, we conducted an analysis to determine the correlation between sex and initial clinical symptoms with antibody and T cell responses. Our findings revealed that males exhibited significantly higher NAbs titers against Omicron BA.5, BA.2.75.2, and XBB.1.5 subvariants compared to females at T2 (Supporting Information S1: Figure S8A). As expected, asymptomatic patients showed significantly higher NAbs titers against some Omicron subvariants at each visited time point (Supporting Information S1: Figure S8B). For virus-specific T cell responses, only a few differences were identified between these factors (Supporting Information S1: Figure S8C,D). Overall, the influence of sex and initial clinical symptoms on the responses of antibody and T cell was limited.

4 | DISCUSSION

In this study, we investigated the persistence of antibody and T cell responses in a prospective cohort of adolescents approximately 1 year post-Omicron BA.2.3 BTI with or without subsequent Omicron BA.5 RI. Our results revealed that Omicron BA.2.3 BTI elicited higher levels of virus-specific IgG and NAbs titers compared to those observed in vaccinated HCs. Over the course of follow-up, both IgG and NAbs titers gradually declined but remained detectable against previous Omicron BA.2 and BA.5 subvariants. However, notable neutralization resistance was observed against emerging Omicron BQ.1.1, BA.2.75.2, CH.1.1, XBB.1.5, XBB.1.16, EG.5.1, and JN.1 subvariants and most of the adolescents possessed diminished NAbs titers against these Omicron subvariants 6-month post-BTI. Importantly, there is a significant correlation between lower levels of IgG and NAbs titers and subsequent Omicron BA.5 RI, which can effectively reduce the antigenic distances between the D614G and each of the detected Omicron subvariants. What's more, robust virus-specific T cell responses were observed in most of the individuals against both Omicron BA.2 and CH.1.1 MPs. Interestingly, exposure to Omicron BA.5 promotes phenotypic differentiation of virus-specific memory T cells, leading to an increase of TEM subset for both $CD4^+$ and $CD8^+$ T cell responses.

Previous studies have demonstrated that Omicron BA.1 or BA.2 BTI leads to a significant increase in NAbs titers against the D614G and Omicron subvariants compared to primary vaccination or infection at early convalescent (1–3 months),[2,4,6,25,26] and the NAbs titers gradually decrease at 6–8 months post-BTI.[27,28] In this study, we confirm these findings that Omicron BA.2 BTI induces IgG and NAbs titers against Omicron subvariants more effectively than vaccination alone in HCs, and these titers remained detectable for previous Omicron subvariants (BA.2, BA.5, and BF.7) at 12-month post-BTI. However, with the emergence of various Omicron subvariants, neutralization resistance continues to be strengthened. Following Omicron BA.2 BTI, the NAbs titers remain high against D614G, BA.1, and BA.2,[2–6,25–28] while slightly decrease against BA.4/5 and BA.2.12.1,[4–6,26–28] and show apparently immune escape against BA.2.75 and BQ.1.1.2,[25,28] Consistent with previous studies mentioned above, we also found that higher NAbs titers are induced by Omicron BA.2.3 BTI against the D614G and previous Omicron BA.2, BA.5, and BF.7 subvariants, while significant lower NAbs titers are generated against newly emerging Omicron subvariants, including BQ.1.1, BA.2.75.2, CH.1.1, XBB.1.5, XBB.1.16, EG.5.1, and JN.1. What's more, we identified that the lower levels of IgG and NAbs titers are closely related to subsequent Omicron BA.5 RI. Thus, an updated COVID-19 vaccine targeting a more recent circulating variant is needed to combat the newly emerging SARS-CoV-2 variants.

Despite extensive mutations and reduced neutralizing ability against emerging Omicron subvariants, the virus-specific T cells responses induced by vaccination or infection are robust and able

FIGURE 4 Spike-specific ICS+ CD4+ or CD8+ T cell responses against Omicron BA.2 or CH.1.1 in adolescents. The percentage of TNF-α^+, IL-2^+, and IFN-γ^+ for $CD4^+$ T cells (A and C) and the percentage of TNF-α^+, IL-2^+, and IFN-γ^+ for $CD8^+$ T cells (B and D) after stimulation of PBMCs with Omicron BA.2 or CH.1.1 spike-specific Mega peptide pools (MPs). Comparison of the polyfunctional profiles between Omicron BA.2 and CH.1.1 spike-specific $CD4^+$ T cells (E and G) or $CD8^+$ T cells (F and H) at 6-month and 12-month. PBMCs were collected from the adolescents at 6- and 12-month post-Omicron BA.2.3 breakthrough infection (BTI) with or without subsequent Omicron BA.5 reinfection (RI). PBMCs of HCs were collected from 10 adolescents with only two-dose inactivated vaccination. Graphs show individual responses of ICS^+ $CD4^+$ or $CD8^+$ T cell responses against Omicron BA.2 or CH.1.1 MPs plotted as background-subtracted DMSO negative controls. Boxplots indicate median and interquartile range (IQR). Each response pattern (i.e., any possible combination of IFN-γ, IL-2, or TNF-α expression) is color-coded, and data is summarized in the pie charts in panels (E–H). No significant differences were observed between pies using a permutation test for ICS^+CD4^+ or ICS^+CD8^+ T cell responses. Wilcoxon matched-pairs signed rank test and Mann–Whitney test were performed in panels (C–H), and $p < 0.05$ was considered statistically significant. HC, healthy controls; ICS, intracellular staining; PBMCs, peripheral blood mononuclear cells.

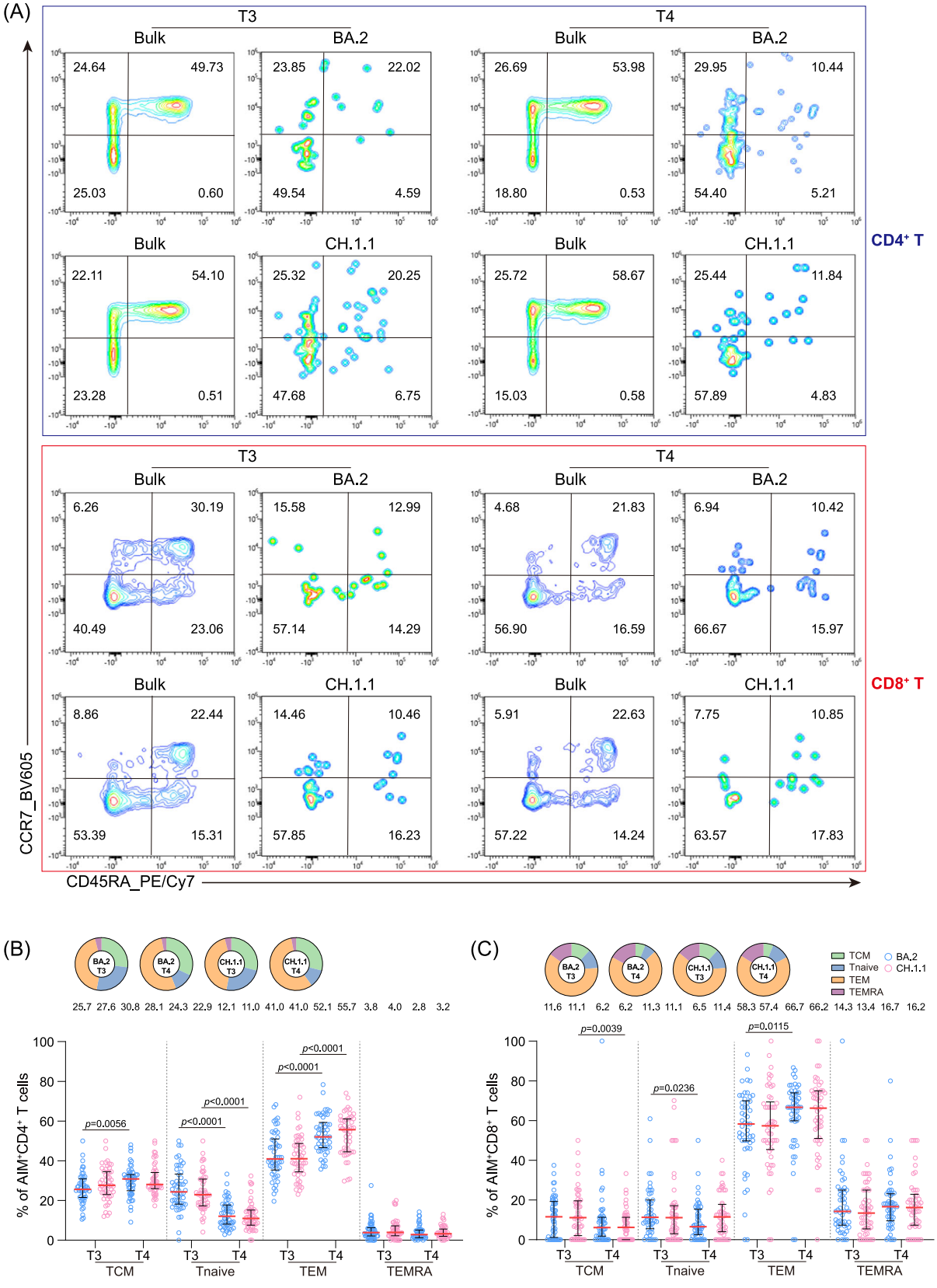

FIGURE 5 (See caption on next page).

to cross-recognize the different SARS-CoV-2 variants, including the Omicron variant.[14,15,20,29,30] The Omicron BA.2- and CH.1.1-spike genes were selected to synthesize the corresponding MPs in this study, taking into account the previous variant of infection and the circulating variant at that time. These two variants differ by 13 amino acids, which affect 10.0% (25/252) of peptide sequences. Also, we calculated that the percentage of different peptide sequences between WT and each of the Omicron subvariants (BA.2 or CH.1.1) is 21.8% (55/252) and 26.2% (66/252), respectively. Similar with these reported studies, we clarified that well-recognized T cell responses exist between the previous circulating Omicron BA.2 and the emerging Omicron CH.1.1, regardless of visited time points and RI status. Interestingly, the AIM^+CD4^+ T cell responses remained stable, while AIM^+CD8^+ T cell responses and ICS^+ $CD4^+$ or $CD8^+$ T cell responses were significantly reduced at 12-month compared to those at 6-month post-Omicron BA.2.3 BTI. What's more, the T cell responses are not strengthened in the RI group (2-month post-RI) than those in the NRI group (6-month post-BTI). One possible reason is that the T cell responses may be not significantly enhanced by additional antigen exposures.[21,31] Notably, the adolescents with Omicron BA.5 RI in this study were asymptomatic, which was distinctly different from the status post-Omicron BA.2.3 BTI. Therefore, combined with the findings of a recent study,[32] we speculate that another possible reason is that the virus-specific $CD4^+$ or $CD8^+$ T cells are rapidly activated, reaching the peak within 1 week post-antigen exposure and then dropping to a low level about 1 month post-RI.

Similar with the documented studies,[29,33] TEM are the main phenotypic subsets of virus-specific $CD4^+$ or $CD8^+$ T cells 6-months post-BA.2.3 BTI. What's more, there was an observed increase in the TEM subset and a decrease in the Tnaive subset of virus-specific $CD4^+$ memory T cells were occurred between T3 and T4 in both the RI group and NRI group, suggesting that Omicron BA.5 exposure can promote the differentiation of virus-specific memory T cells, even though among non-seroconversion adolescents in NRI group.[34] In addition, the TEM subset is significantly higher in the NRI group compared to the RI group at T3 before RI, suggesting that this subset may play an important role in viral clearance and prevention of RI.[29,33]

This study has two limitations. First, our focus was primarily on virus spike-specific humoral and cellular immunity, without assessing the function of other key proteins. Second, we only obtained PBMCs during convalescent stages at 6- and 12-intervals, which limits our understanding of the dynamic changes in T cell responses during the acute stage post-Omicron BA.2.3 BTI or Omicron BA.5 RI. Therefore, more prospective studies with multiple followed-up time points and enough clinical samples are needed to confirm and expand the findings presented in this study.

In conclusion, we have demonstrated the dynamic changes in antibody and T cell responses about 1 year post-Omicron BA.2.3 BTI, with or without subsequent Omicron BA.5 RI, in adolescents. The emerging Omicron subvariants can extensively escape the humoral immunity elicited by the previous circulating Omicron subvariants BTI and RI, while the robust virus-specific T cell responses are observed in most of the adolescents against both the previous and current circulating Omicron subvariants. More importantly, additional antigen exposure following Omicron BA.2.3 BTI can promote the differentiation of memory T cells, which may play a crucial role in clearing the virus and preventing RI. Therefore, updated COVID-19 vaccines targeting a more recent circulating variant are needed to provide protection against the newly emerging SARS-CoV-2 variants among adolescents.

AUTHOR CONTRIBUTIONS

Li-Qun Fang, Guo-Lin Wang, Zeng-Qiang Kou, and Hui Wang conceived the study. Xiao-Lin Liu, Ti Liu, Jie Wu, Kai-Ge Du, and Zeng-Qiang Kou collected the samples and data. Guo-Lin Wang, Xin-Jing Zhao, Hong-Jing Gu, De-Yu Li, Sheng Zhang, Kai-Ge Du, and Shen Tian conducted the experiments. Xin-Jing Zhao, Guo-Lin Wang, Hong-Jing Gu, Sheng Zhang, Jin-Jin Chen, Qiang Xu, Chen-Long Lv, and Bao-Gui Jiang analyzed the data. Guo-Lin Wang, Li-Qun Fang, and Xin-Jing Zhao wrote the manuscript. All authors reviewed and approved the manuscript.

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FIGURE 5 Memory phenotype differentiation of Omicron BA.2- or CH.1.1-specific memory T cells in adolescents. (A) Flow cytometry plots in Omicron BA.2 and CH.1.1 MPs stimulated groups showing spike-specific memory $CD4^+$ and $CD8^+$ T cell phenotypes in ex vivo assay. Frequency of different spike-specific memory $CD4^+$ T cell (B) and $CD8^+$ T cell (C) phenotypes in Omicron BA.2 and CH.1.1 groups at 6- and 12-month. PBMCs were collected from the adolescents at 6- and 12-month post-Omicron BA.2.3 breakthrough infection (BTI) with or without subsequent Omicron BA.5 reinfection (RI). Bars indicate median and interquartile range (IQR). Each subset of memory phenotype differentiation is color-coded, and data is summarized in the pie charts in panels (B and C). No significant differences were observed between pies using a permutation test in each panel. Wilcoxon matched-pairs signed rank test and paired *t*-test were performed in panels (B and C), and $p < 0.05$ was considered statistically significant. PBMCs, peripheral blood mononuclear cells.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data described in the report can be made available from the corresponding author upon reasonable request and upon completion of required approvals.

ETHICS STATEMENT

The study was approved by the Institutional Review Boards of the Academy of Military Medical Science (IRB number: AF/SC-08/02.245) and the Shandong Center for Diseases Control and Prevention (IRB number: 2021-61). Written informed consent was obtained from all custodians of the participants.

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SUPPORTING INFORMATION

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

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