Virologica Sinica 39 (2024) 699–701

Contents lists available at ScienceDirect

Virologica Sinica

journal homepage: www.keaipublishing.com/en/journals/virologica-sinica
www.virosin.org

Letter

Correlation between circulating T follicular helper cell levels after infection and a decreased risk of COVID-19 re-infection

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Dear Editor,

As mass vaccination has been accomplished worldwide and a growing number of individuals have already been infected by SARS-CoV-2, understanding the incidence of subsequent re-infection is urgently required for comprehending the transmission dynamics of COVID-19 (Bobrovitz et al., 2023). Additionally, current SARS-CoV-2 variants cause widespread escape from the neutralizing antibody (nAb) elicited by current vaccines. Meanwhile, durable neutralization and memory B cell responses can be predicted by rapid $CD4^+$ T cell responses, particularly strong circulating T follicular helper (cTfh) cell responses, in individuals infected with SARS-CoV-2 (Gong et al., 2020; Boppana et al., 2021; Narowski et al., 2022). Although nAb titers are known as predictors of protection against SARS-CoV-2 variants (Khoury et al., 2021), identifying more potential factors that could predict the risk of re-infection following primary infection and vaccination has become quite important in shaping prevention strategies (Chemaitelly et al., 2023).

We collected sera specimens and PBMCs from 25 volunteers residing on a university campus in Guangzhou, a city in southern China, with close quarters and constant personal contact (Fig. 1A). All participants received the three-dose CoronaVac inactivated vaccine by July 2022, before being infected during the massive SARS-CoV-2 Omicron BA.5 outbreak in December 2022, with only mild symptoms. According to national genomic surveillance data from the Centers for Disease Control and Prevention (CDC), BA.5, BF.7, BQ.1, BQ.1.1, EG.5.1, XBB, XBB.1.16, XBB.1.5 and XBB.1.9 have been circulating in China (Fig. 1B). Following the second XBB variant wave in May–June 2023, the confirmed re-infection occurred in 12 (48%) of 25 individuals (re-infection group), while the remaining 13 (52%) individuals were uninfected (single-infection group) at the beginning of July 2023 (Fig. 1A and Supplementary Table S1). Confirmation of re-infection was based on both antigen tests and nAb titers (Methods in Supplementary Materials) (Ma et al., 2020; Zhang et al., 2023).

We conducted a retrospective study using blood samples collected at one and three months post symptom onset (PSO) after the first wave of Omicron BA.5 to assess possible factors associated with the risk of re-infection between the single-infection group (mean age, 34 years; 15% men) and the re-infection group (mean age, 28 years; 8% men) (Fig. 1A and Supplementary Table S1). We measured anti-spike antibodies and nAbs against SARS-CoV-2, which decreased over time, while only in re-infection group, nAbs against D614, BA.5, XBB and EG.5.1 increased from three months to five months (Fig. 1C and D and Supplementary Figs. S2A–D). Interestingly, at one month PSO, nAbs against BA.5 rather than other variants in the single-infection group were significantly higher than those in the re-infection group (Fig. 1E and Supplementary Fig. S3).

Furthermore, we conducted a thorough analysis of different T cell populations in the single-infection and re-infection groups using enzyme-linked immune absorbent spot (ELISpot) and intracellular cytokine staining (ICCS) assays. No significant difference observed in the two groups in terms of the counts and percentages of spike-specific $CD8^+$ and $CD4^+$ T cells and various phenotypes of spike-specific T cells (Fig. 1F and G, Supplementary Fig. S4). Unexpectedly, upon further investigation of the $CD4^+$ T cell subtypes, we found that the participants in the single-infection group exhibited a higher proportion of cTfh cells ($CXCR5^+PD\text{-}1^+CD4^+$ cells) at one month PSO (Fig. 1H), but not at three months PSO. Furthermore, this increase in nAb titers significantly correlated with the proportion of cTfh cells, highlighting the potential

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https://doi.org/10.1016/j.virs.2024.06.002
Received 25 January 2024; Accepted 30 May 2024
Available online 7 June 2024

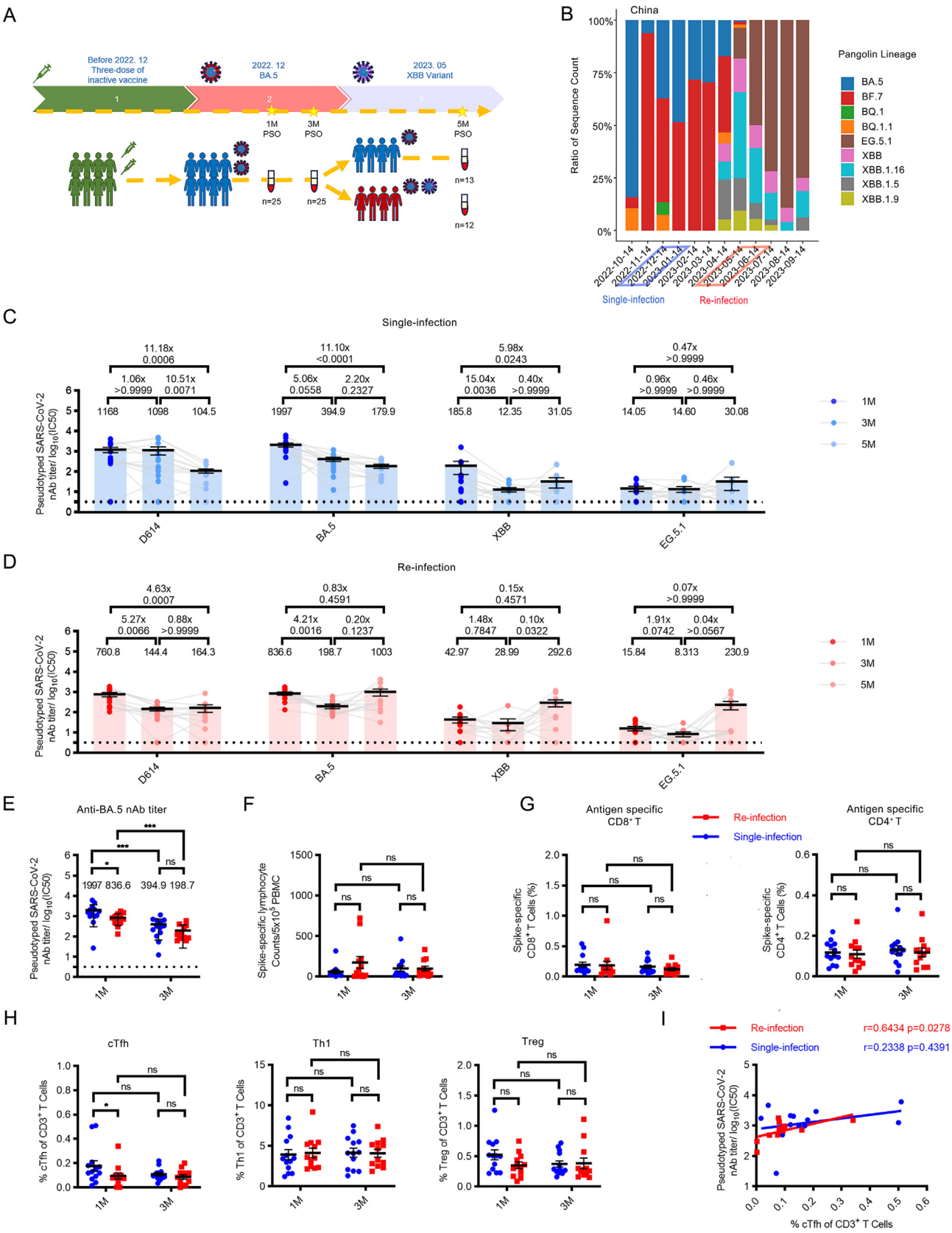

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Fig. 1. Variations in antibody levels and T-cell responses among convalescents with diverse outcomes of re-infection. **A** Schematic of the recruited cohort for the single-infection group (n = 13) and the re-infection group (n = 12), along with experimental procedures. Sera samples of the single-infection group (n = 13) receiving three-dose inactivated CoronaVac vaccine following BA.5 breakthrough infection and the re-infection group (n = 12) receiving three-dose inactivated CoronaVac vaccine following both BA.5 and the XBB lineage breakthrough infections were collected at three distinct time points (at one month, three months and five months post symptom onset, PSO). **B** Frequencies of BA.5, BF.7, BQ.1, BQ.1.1, EG.5.1, XBB, XBB.1.16, XBB.1.5 and XBB.1.9 deposited in GISAID. **C, D** nAb titers against SARS-CoV-2 D614, BA.5, XBB and EG.5.1 of sera from individuals in the single-infection group (n = 13) (**C**) and the re-infection (n = 12) (**D**) at three distinct time points are shown. **E** The comparison of neutralizing titers against BA.5 in sera from the single-infection group (n = 13) and the re-infection group (n = 12) at one month and three months PSO were showed. **F–H** PBMC samples were collected from both the single-infection group (n = 13) and the re-infection group (n = 12) at one month and three months PSO. PBMCs were stimulated with Omicron BA.5 spike (subunit 1 + subunit 2) peptides pool. The counts of BA.5 spike-specific lymphocytes expressing IFN-γ were determined by enzyme-linked immune absorbent spot (ELISpot) assays (**F**). The percentages of BA.5 spike-specific IFN-γ^+ $CD8^+$ T cells and BA.5 spike-specific IFN-γ^+ $CD4^+$ T cells in total $CD8^+$ and $CD4^+$ T cells, respectively, were determined by intracellular cytokine staining (ICCS) assay (**G**). **H** The percentages of circulating follicular helper T cells (cTfh, $CXCR5^+PD\text{-}1^+CD4^+$ cells) (left), circulating T helper 1 cells (Th1, T-bet^+CD4^+ cells) (Middle) and circulating regulatory T cells (Treg, $Foxp3^+CD4^+$ cells) (right) in total $CD4^+$ T cells were assessed by ICCS. **I** Correlation analysis of the frequencies of cTfh cells and nAb titers against Omicron BA.5 between the single-infection group (n = 13) and the re-infection group (n = 12) at one month PSO. Experiments were conducted independently in triplicates. Data represented as mean ± SEM. Adjusted *P* values were calculated by Wilcoxon signed-rank tests, Mann–Whitney U tests, Friedman tests and Spearman's rank correlation. *, $P < 0.05$; ***, $P < 0.001$; ns, not significant.

association between cTfh cell levels and the strength of the humoral immune response (Fig. 1I).

cTfh cells play a pivotal role in the generation of antibodies and diverse memory B cells (Yu et al., 2022). Previous reports have indicated that the increased percentage of cTfh cells at early memory time points was an indicator of less severe COVID-19 disease progression (Zhang et al., 2021; Yu et al., 2023). In this study, we demonstrate that the elevated level of cTfh responses after primary infection is positively associated with the reduced risk of re-infection. Our findings indicate that the immune response of cTfh cells is correlated with the production of neutralizing antibodies, ultimately contributing to long-term protection against SARS-CoV-2. Our study has several limitations. First, stringent cohort selection criteria and the limited time frame for sample collection (all samples were collected within a specific period following the initial wave of infection, with all participants having achieved full immunity) imposed objective constraints that hindered the expansion of our cohort to a larger sample size. Secondly, ethical considerations and the preferences of our volunteers further constrained the number of cells we were able to obtain, presenting challenges in conducting more extensive validation and further exploration.

In conclusion, our study elucidates the dynamics and cross-reactivity of hybrid immunity against SARS-CoV-2 variants. We find a positive correlation between cTfhs and a reduced risk of SARS-CoV-2 re-infection. These findings will contribute to delaying the progression of multi-round of SARS-CoV-2 infections and shaping improved vaccine strategies.

Footnotes

This work was supported by the National Key R&D Program of Department of Science and Technology of China (2022YFC0870700), the Important Key Program of Natural Science Foundation of China (NSFC) (92169201), the Exchange Program of NSFC (82150710553), the Emergency Key Program of Guangzhou National Laboratory (EKPG21-24), and the Key R&D Program of Department of Science and Technology of Guangdong (2022B1111020004) to H.Z. Natural Science Foundation of China (92169108 and 32000613), Guangdong Natural Science Funds for Distinguished Young Scholar (2023B1515020005) to Y.Z. The authors declare that they have no conflict of interest. The Ethics Review Boards of Sun Yat-sen University approved this study. All participants provided written informed consent with approval of the Ethics Committees.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.virs.2024.06.002.

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