Dynamic changes in host immune system and gut microbiota are associated with the production of SARS-CoV-2 antibodies

Recently, we read the article by Ng *et al* with great interest,[1] which identified several gut microbiota harbour the

potential to improve immune response and reduce adverse events following COVID-19 vaccines, and demonstrated that gut microbiota has the potential to complement the effectiveness of vaccines. Together with several recent studies, gut microbiota plays a key role in modulating immune responses of vaccination[2–4] and is related to the severity of COVID-19 patients,[5 6] however, the comprehensive assessment of host's response, particularly the role of gut microbiota in antibodies production is limited and should be seriously considered because the vaccination of SARS-CoV-2 is the most promising approach for curbing the COVID-19 pandemic.[4 7]

Therefore, we recruited 30 young volunteers (20–23 years old), including 15 male and 15 female volunteers, and collected 143 faecal and 120 blood samples at multiple time points to monitor their responses to Sinovac vaccine from multiple perspectives (figure 1A and online supplemental figure 1). Through routine blood test, flow cytometry and ELISA, the blood immunological indices, immune cell subsets and antibodies levels were measured, respectively, while by whole-genome shotgun sequencing, the structure of gut microbiota communities was profiled. Particularly, our results were compared with a published gut microbiota dataset derived from patients with SARS-CoV-2 infection (online supplemental material 1).

Interestingly, our results showed that a majority of healthy individuals can produce SARS-CoV-2 antibodies (90%, 96.67% and 80% of the subjects produce anti-(N+S) IgA, IgG and IgM antibodies, respectively, online supplemental table 1), at the end of 2 weeks after second dose of Sinovac vaccine. Moreover, the levels of these antibodies first increased over the first 2 weeks after the first dose and reached a peak 2 weeks after the second dose during the vaccination process (figure 1B).

In addition, the alterations in cytokines, lymphocytes and indicators of physiological and biochemical systems were measured to visualise the response of immune system of host (figure 1C-E, online supplemental tables 2–4). In addition, based on the taxonomical compositions of gut microbial communities across different vaccination time points, we found that the alpha diversities of the gut microbial communities did not significantly differ (figure 1F). However, the compositions of gut microbial communities during the vaccination process exhibited significant differences (analysis of similarities, Bray-Curtis dissimilarity, $p=0.015$, figure 1G), these gut microbial communities could be distinctly separated depending on the time points of vaccination (figure 1H), and the taxonomical compositions of gut microbial communities undergo changed (figure 1I).

Furthermore, the comparison of the gut microbiota of healthy individuals who vaccinated with Sinovac vaccine and COVID-19 patients with different clinical diagnoses, without accounting for factors such as age, suggest that the alterations of gut microbiota during vaccination were not as substantial as those caused by SARS-CoV-2 infection (figure 1J).

Finally, our results showed that the correlations among gut microbiota, cytokines, lymphocytes and SARS-CoV-2 antibodies (figure 1K and online supplemental figures 2, 3). In particular, we found that several gut microorganisms have a significant association with SARS-CoV-2 antibodies production. For example, *Prevotella copri* was negatively correlated with IgG, whereas *Clostridium leptum*, *Lactobacillus ruminis*, *Ruminococcus torques*, etc, presented a positive correlation with antibodies production (all $p<0.01$, figure 1K). Moreover, a variation partitioning analysis based on the metadata of body features and the compositions of gut microbial communities was performed, which showed that the production of antibodies is mainly affected by the gut microbiome (22%) and body features (18%, online supplemental table 5, online supplemental figure 4). These results suggest that gut microbiota plays an important role in the production of SARS-CoV-2 antibodies in young healthy individuals and the dynamic changes of immune system and gut microbiota and their associations with the production of SARS-CoV-2 antibodies in elderly population remain elusive and should be further investigated.

Overall, our study systematically investigated the dynamic changes of host, including lymphocytes, cytokines, gut microbiota and antibodies, and linked these factors to the production of antibodies. Our results provide an optional perspective for evaluating the safety and effectiveness of SARS-CoV-2 vaccines and settling the treatment of COVID-19 patients, and can alleviate the public's concerns and fears about the vaccination.

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Contributors MHa and SH designed the study. MH, WJ and SH recruited the healthy volunteers from School of Life Sciences, Anhui Medical University. MHa, YX, MHe, HL, XW, NZ and SaK collected the blood and fecal samples. MHa, YH, HG, YX, NZ and XC analysed the data of indicators obtained from routine blood tests, lymphocytes, cytokines and metagenomic sequencing data. HL and ML conducted the measurement of the indicators obtained from routine blood tests, lymphocytes and cytokines. MHa, YW, FW and SH organised the structure of the manuscript. MH, YH, HG and YX wrote the initial draft of the manuscript. All authors read, modified and approved the final manuscript.

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Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval The study protocols were all approved by the Biomedical Ethics Committee of Anhui Medical University (No 2021H021). Participants gave

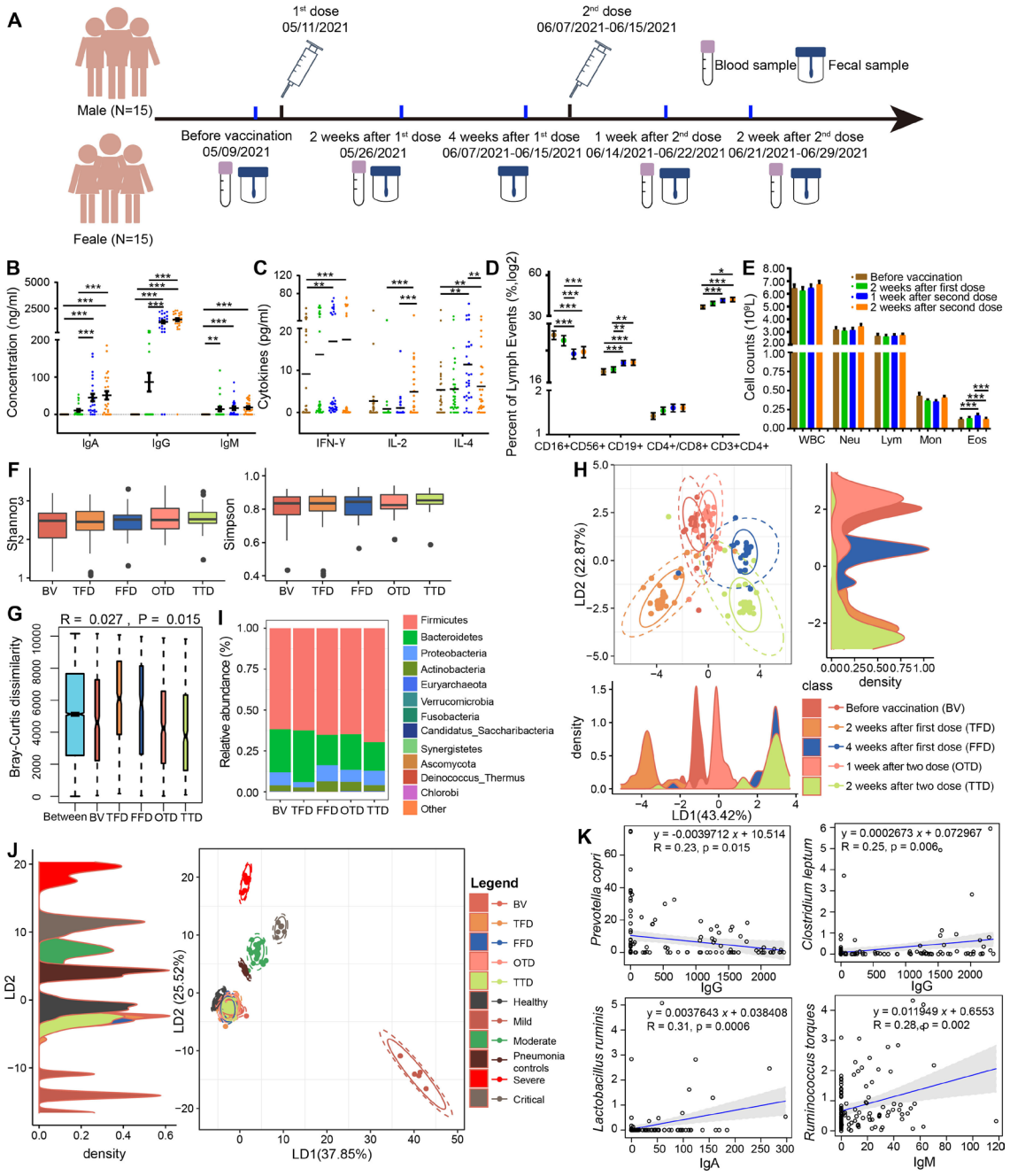

Figure 1 The response of healthy individuals during the vaccination of two dose of SARS-CoV-2 vaccine and the interplay between host immune systems and gut microbiota that contributes to the production of SARS-CoV-2 antibodies. (A) Study design for collecting the faecal and blood samples from 30 healthy individuals to explore the dynamics changes of host immune systems, gut microbiota and the production of SARS-CoV-2 antibodies. Dynamic changes in SARS-CoV-2 antibodies, cytokines, lymphocytes and indicators obtained from routine blood tests. (B) Concentrations of IgA, IgG and IgM detected at different time points during the vaccination process. The differences between different time-points were assessed by two-way ANOVA, and two-sided exact p values are reported. (C) Concentrations of IFN-γ, IL-2 and IL-4 measured at different time points during the vaccination process. (D) The levels of NK cells, B cells and CD4+T cells and the CD4+/CD8+ratio are illustrated in chronological order. (E) Dynamic changes in the counts of white cell count, neutrophils (Neu), lymphocyte (Lym), monocytes (Mon) and eosinophils (EOSs) during the vaccination process. (F) The alpha diversities, including the Shannon and Simpson indices, of the human gut microbial communities did not significantly differ among different time points during the vaccination process. (G) A significant difference in the human gut microbial compositions was found among different time points during the vaccination process according to their Bray-Curtis dissimilarity at the species level. (H) Based on the taxonomic compositions of all 143 samples at the species level, LDA can successfully separate the human gut microbial communities at different time points during the vaccination process. The density curves in the bottom and right panels show the distribution of the human gut microbial communities along the LD1 and LD2 axes, respectively. (I) Compositional differences in the gut microbiota among different time points during the vaccination process visualised with the average relative abundances at the phylum level. (J) Comparison of the taxonomic structure of the human gut microbiota among unvaccinated healthy individuals, healthy individuals at different time points during the vaccination process, and COVID-19 patients with different clinical diagnoses. (K) Correlations between the production of antibodies against SARS-CoV-2 and gut microbiota.*, p<0.05; **, p<0.01; ***, p<0.001; ANOVA, analysis of variance; LDA, linear discriminant analysis.

informed consent to participate in the study before taking part.

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