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Research Article

STAT5-c-Myc-axis regulates B cell metabolism in vaccinated individuals and COVID-19 recovered patients

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ABSTRACT

SARS-CoV-2 infection and vaccination both trigger immune responses. The former leads to naturally acquired immunity, while the latter induces active immunity through artificial means. However, the distinct immune effects of vaccination and infection, as well as their underlying mechanisms, require further clarification. In this study, we compared the peripheral B cell differentiation, serological differences and the expression level of BCR signaling molecules between the vaccinated and recovered group. The vaccinated group exhibited reduced RBD-specific B cell differentiation and lower CD86 signal intensity on memory B cells, but enhanced BCR signaling in B cells. Regarding metabolic signaling, the vaccinated group had elevated expression levels of pS6, c-Myc, pmTOR, and pSTAT5, suggesting that the STAT5-c-Myc axis plays a role in regulating B cell metabolism. Additionally, proteome microarray analysis revealed that the serum of the vaccinated group contained higher levels of IgG antibodies against the SARS-CoV-2 N-Nter protein and IgA antibodies specific to the SARS-CoV-2 S1 protein. In summary, these findings indicate that the vaccinated group develops a more robust coronavirus-specific immune response, with enhanced BCR signaling and metabolic activity compared to the recovered group. These insights might contribute to the optimization of SARS-CoV-2 vaccine design.

INTRODUCTION

COVID-19, resulting from infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, manifests with a spectrum of symptoms and varying degrees of severity (Wang et al., 2023). It often causes long-term complications (Liu et al., 2023), such as acute respiratory distress syndrome-related pulmonary fibrosis (Zheng et al., 2023). Therefore, the development of safe and effective vaccines is crucial in the fight against the COVID-19 pandemic (Krammer, 2020; Tregoning et al., 2021). Cheong et al. found that COVID-19 destroys the body's immune system, leading to long-term changes in the innate immune system (Cheong et al., 2023). Currently, there is a lack of standardized criteria or definitions for comparing populations who have recovered from SARS-CoV-2 infection and vaccinated populations.

The infection of SARS-CoV-2 affects human metabolism. Li et al. discovered that SARS-CoV-2 spike protein triggers inflammation and apoptosis by modulating autophagy through ROS-driven inhibition of the PI3K/AKT/mTOR signaling pathway (Li et al., 2021). However,

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Appelberg et al. demonstrated that SARS-CoV-2 infection leads to the activation of the AKT/mTOR pathway while inhibiting HIF-1α (Appelberg et al., 2020). This suggests that the AKT/mTOR signaling pathway is not singularly altered in COVID-19. Bi et al. conducted proteomic and metabolomic analyses of urine and serum samples from patients with COVID-19 and healthy controls, and found that urinary proteins could enable the classification of the severity of patients with COVID-19 (Bi et al., 2022). They found that CXCL14 was detected only in urine and was significantly down-regulated in severe cases, consistent with the reduction in lymphocyte counts. As lymphocytes contribute to severe sequelae of SARS-CoV-2 (Sen Chaudhuri and Sun, 2024), such as pulmonary fibrosis (Zheng et al., 2023), urinary CXCL14 might be a biomarker for predicting the severity of COVID-19.

Both SARS-CoV-2 infection and vaccination profoundly influence the human immune system (Castro Dopico et al., 2022). Vaccination is particularly critical for patients with impaired renal function and other comorbidities, who require enhanced protective measures (Huang et al., 2023). Liu et al. reviewed that vaccination can reduce the incidence of long COVID-19 (Liu et al., 2023). Besides, Zhang et al. conducted a comprehensive evaluation of neutralizing antibody levels, memory B cells, $CD8^+$ T cells, and $CD4^+$ T cells in populations vaccinated with mRNA-1273, BNT162b2, NVX-CoV2373, and Ad26.COV2.S over 0–6

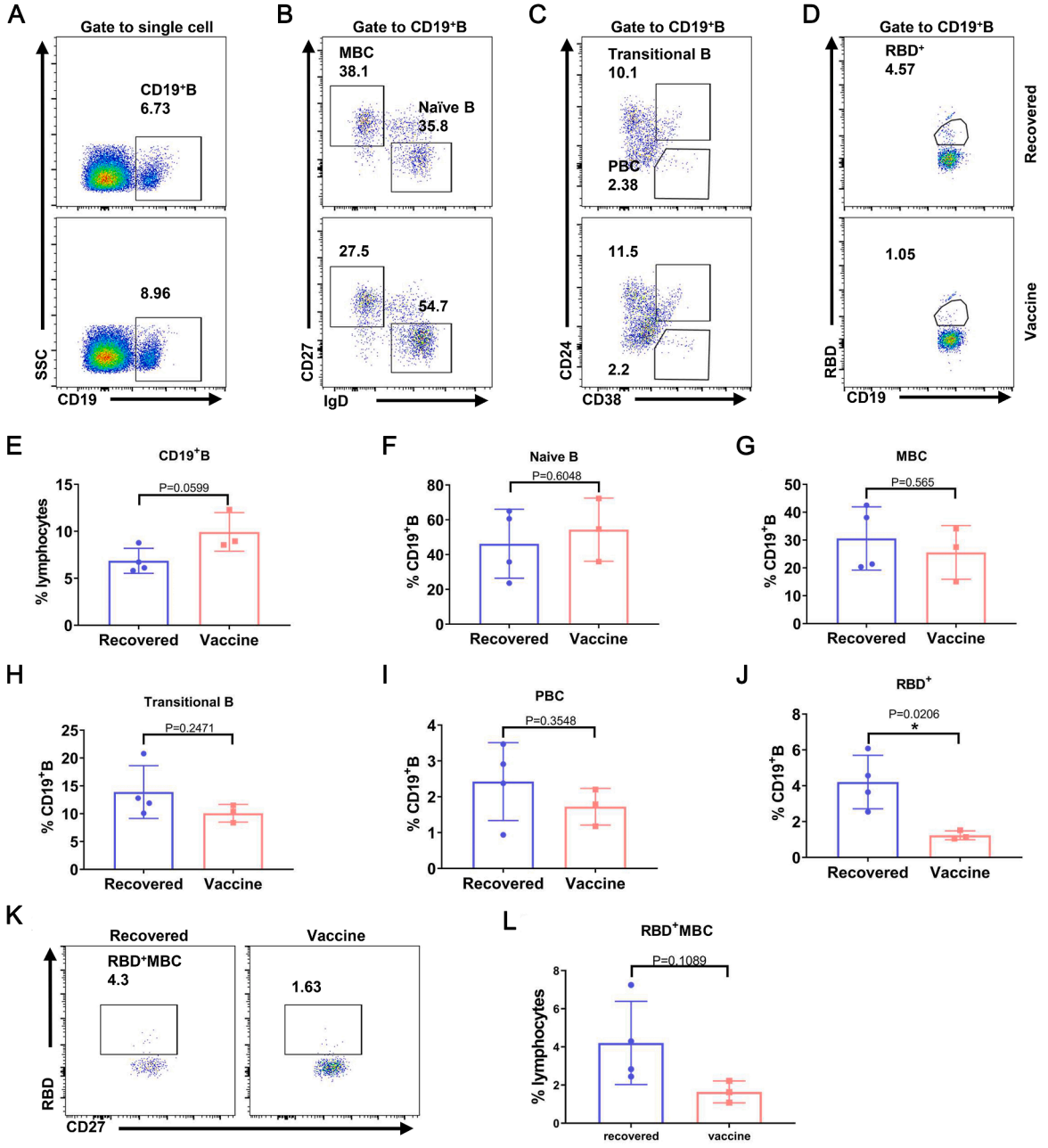

Fig. 1. Peripheral B cell homeostasis and dynamics. **A** Representative flow cytometry plots of $CD19^+$B of PBMC from the recovered individuals and vaccinated individuals. **B–D** Representative flow cytometry plots of memory B cells (MBC), naïve B cells, transitional B cells, plasmablast, and RBD^+ B cells among $CD19^+$B cells from the recovered population and vaccinated population. **E** The percentage of $CD19^+$B among PBMC from the recovered population and vaccinated population. Recovered = 4, vaccinated = 3. **F–J** The percentage of naïve B cells, MBC, transitional B cells, plasmablast (PBC), and RBD^+ B cells among $CD19^+$B cells from the recovered population and vaccinated population. Recovered = 4, vaccinated = 3. **K** Representative flow cytometry plots of RBD $^+$ MBC among $CD27^+$MBC from the recovered population and vaccinated population. **L** The percentage of RBD^+MBC among $CD27^+$MBC from the recovered population and vaccinated population. Recovered = 4, vaccinated = 3. Dots represent individual samples. Statistical analyses were performed using student's *t*-test. *P < 0.05.

months. They found that the intensity of memory B cell responses induced by the four vaccines, in descending order, was: mRNA-1273 > BNT162b2 > Ad26.COV2.S > natural infection > NVX-CoV2373. They elucidated the correlation between cellular immunity and humoral immunity induced by these four vaccines (Zhang et al., 2022).

He et al. identified that natural infection enhanced B cell receptor (BCR) clonal expansion compared with the vaccinated SARS-CoV-2-inactivated virus vaccine (He et al., 2022). BCR can directly bind to antigenic molecules, activate downstream signaling pathways, and further induce B cell proliferation and differentiation to produce antibodies. It has also been shown that complete and booster inoculations help to clear the Omicron BA.2 variant of the virus (Huang et al., 2023). However, the differential immune responses elicited by vaccination and SARS-CoV-2 infection remain unclear. Therefore, our study aims to investigate these differences in immune responses to vaccination and infection with SARS-CoV-2, with the goal of informing strategies to optimize vaccination efforts.

Here, we compared the peripheral B cell differentiation, serological differences and the expression level of BCR signaling molecules between the vaccinated and recovered group. We found that the vaccinated group has stronger BCR signal and coronavirus specific immune response. The results of PKMito and CellRox suggest that the vaccinated group had higher metabolic level. We also examined the expression level of pmTOR, pS6, c-Myc and pSTAT5, and these metabolic molecules in the vaccinated group were increased, which suggests that the STAT5-c-Myc axis regulates B cell metabolism.

RESULTS

The recovered population has a stronger virus-specific B cell differentiation

To identify the immune profile differences between the recovered and vaccinated populations, we collected peripheral blood from the infected and three-dose inactivated vaccine-immunized individuals separately for flow cytometry testing. We labeled B cells in lymphocytes using the CD19 antibody and discovered that the percentage of $CD19^+$ B cells in lymphocytes was not statistically different between the two populations (Fig. 1A and E). We further analyzed the B cell subpopulation composition. After gating to $CD19^+$ B cells, we used CD27 and IgD expression to differentiate $CD27^+IgD^-$ memory B cells (MBCs) and IgD^+CD27^- naïve B cells, CD38 and CD24 expression to differentiate $CD24^+CD38^+$ transitional B cells and $CD24^-CD38^+$ precursor B cells, and RBD expression to differentiate RBD-specific B cells. No significant difference in the proportion of naive B, MBC, transitional B and PBC cells were found in the peripheral blood B cells of the recovered and vaccinated populations (Fig. 1B, C and 1F–I). Interestingly, the proportion of RBD-specific B cells in the recovered population was higher than that in the vaccinated population (Fig. 1D and J), and the proportion of RBD-specific MBC cells was also slightly higher than that in the vaccine-immunized population (Fig. 1K and L). These results indicate that there is no difference between the effects of infection or immunization with SARS-CoV-2 on nonspecific B cell subsets in peripheral blood, but virus-specific B cell differentiation is stronger in recovered populations than in vaccine-immunized populations.

No significant difference in RBD-specific B cell immune response between recovered and vaccine-immunized populations

To explore the differences in B cell function between the vaccine immunization group and the recovered group, we used flow cytometry to analyze the expression of three antigen-presentation molecular markers, CD80, CD86 (a B cell activation marker), and HLA-DR, on MBC, $CD19^+RBD^+$ B cells, and RBD^+CD27^+ B cells, respectively. We found no significant differences in the expression of CD80 on the surface of MBC, RBD-specific B cells, and RBD-specific MBC subsets between the recovered and vaccinated population (Fig. 2A and 2D–F). The expression of CD86 on the surface of MBC subsets in the recovered population was higher than that in the vaccinated population. However, there was no notable discrepancy in the expression of CD86 on the surface of RBD-specific MBC subsets and RBD-specific B cell between the recovered and vaccinated populations (Fig. 2B, 2G–I). At the same time, we examined the expression of HLA-DR and found no significant difference in the mean fluorescence intensity (MFI) of HLA-DR on the surface of MBC, RBD-specific B cells, and RBD-specific MBC subsets in the recovered and vaccinated populations. (Fig. 2C 2J–L). These results suggested that the activation of memory B cells was increased in the recovered population compared to the vaccinated population, but there was no significant difference in RBD-specific immune responses between the recovered and vaccine-immunized populations.

The recovered population has weak BCR signal and STAT5-cMyc mediated cell metabolism

In addition to understanding the differences in B cell subpopulation composition and function between the two populations, we explored the underlying molecular mechanism. BCR signaling is important for B cell activation. Peripheral blood B cells from the two populations were stimulated with soluble antigens [biotin-conjugated F(ab')2 anti-human Ig (M + G) plus streptavidin)] for 5 min, and the expression differences of key molecules in the BCR signal were compared by Western blotting. We found that the expression of pSHIP-1 and pBLNK in B cells of the vaccinated population was significantly higher than that in the recovered population (Fig. 3A), and the expression of metabolic signals pmTOR and pS6 was also significantly higher than that of the recovered population (Fig. 3B). Furthermore, we also detected the expression of the key metabolic regulatory factor c-Myc, and found that its expression in B cells of the vaccinated population was higher than that of the recovered population (Fig. 3C). STAT5 is an upstream transcriptional regulator of c-Myc, and its expression in B cells of the vaccinated population is also higher than that of the recovered population (Fig. 3A and C). Given the metabolic changes observed in B cells from two populations, we examined the production of ROS and mitochondrial levels using flow cytometry. We found that the production of ROS and the number of mitochondria in B cells of the vaccinated population were higher than those in the recovered population (Fig. 3D–G). These results indicate that the BCR signaling and cellular metabolism of B cells in the recovered population are weaker.

Increased levels of IgM, IgA, and IgG antibodies specific to SARS-Cov-2 after vaccination

Enhanced B cell activation may cause increased humoral response. To understand the differences in SARS-CoV-2-specific antibody responses between vaccinated and recovered patients, we analyzed serum samples from the vaccinated and recovered groups using a SARS-CoV-2 protein microarray containing 3 structural proteins, 5 auxiliary proteins, and 12 nonstructural proteins (Jiang et al., 2020). No significant difference in the titers of S1, N, N-Nter, C-Cter, and E protein-specific IgM antibodies was found in the serum of these two groups (Fig. 4A). And the titers of NSP14 specific antibodies in the serum of the vaccinated population were higher than those of the recovered population, while there was no significant difference in the titers of other NSP subtype specific IgM antibodies (Fig. 4B). The titers of N-Nter specific IgG antibodies in the serum of the vaccinated population were significantly higher than those in the recovered population, rather than other structural/nonstructural protein subtypes (Fig. 4C and D). Furthermore, the titers of S1 protein specific IgA antibodies in the serum of the vaccinated population were significantly higher than those in the recovered population, while there were no significant differences in other structural/nonstructural protein subtypes (Fig. 4E and F). These results suggest

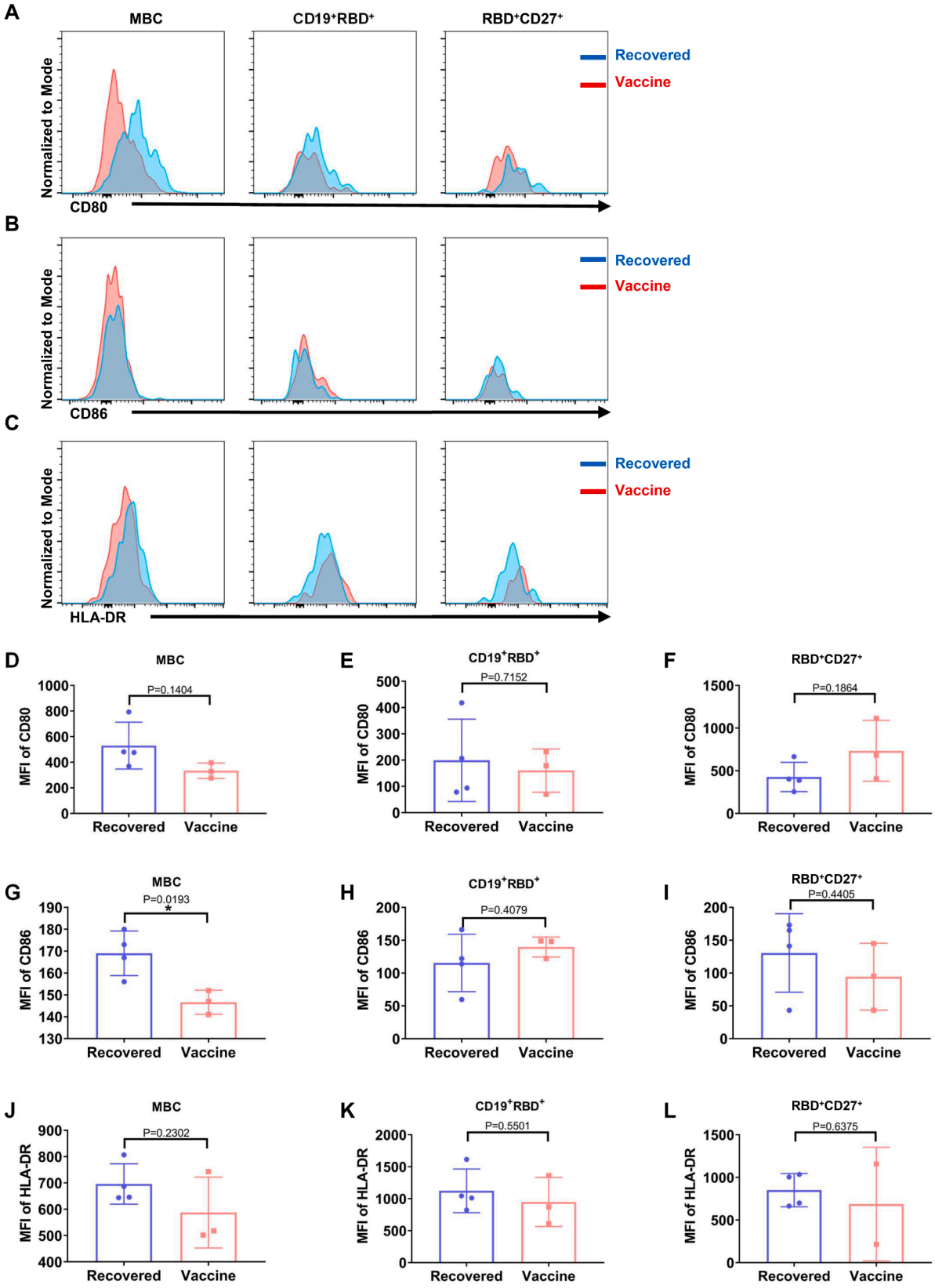

Fig. 2. Peripheral B cell immune response. **A–C** Flow cytometry analysis of CD80, CD86, and HLA-DR from the memory B cell (MBC), $CD19^{+}RBD^{+}$, and $RBD^{+}CD27^{+}$ subsets from the recovered population and vaccinated population. The representative histogram of CD80, CD86, and HLA-DR from the MBC, $CD19^{+}RBD^{+}$, and $RBD^{+}CD27^{+}$ subsets are shown. **D–L** Quantification of the mean fluorescence intensity (MFI) of CD80, CD86, and HLA-DR on the MBC, $CD19^{+}RBD^{+}$, and $RBD^{+}CD27^{+}$ (RBD^{+}MBC) subsets from the recovered population and vaccinated population (In panel **D–K**: recovered = 4, vaccinated = 3; In panel **L**: recovered = 4, vaccinated = 2). Dots represent individual samples. Statistical analyses were performed using student's *t*-test. $^{*}P < 0.05$.

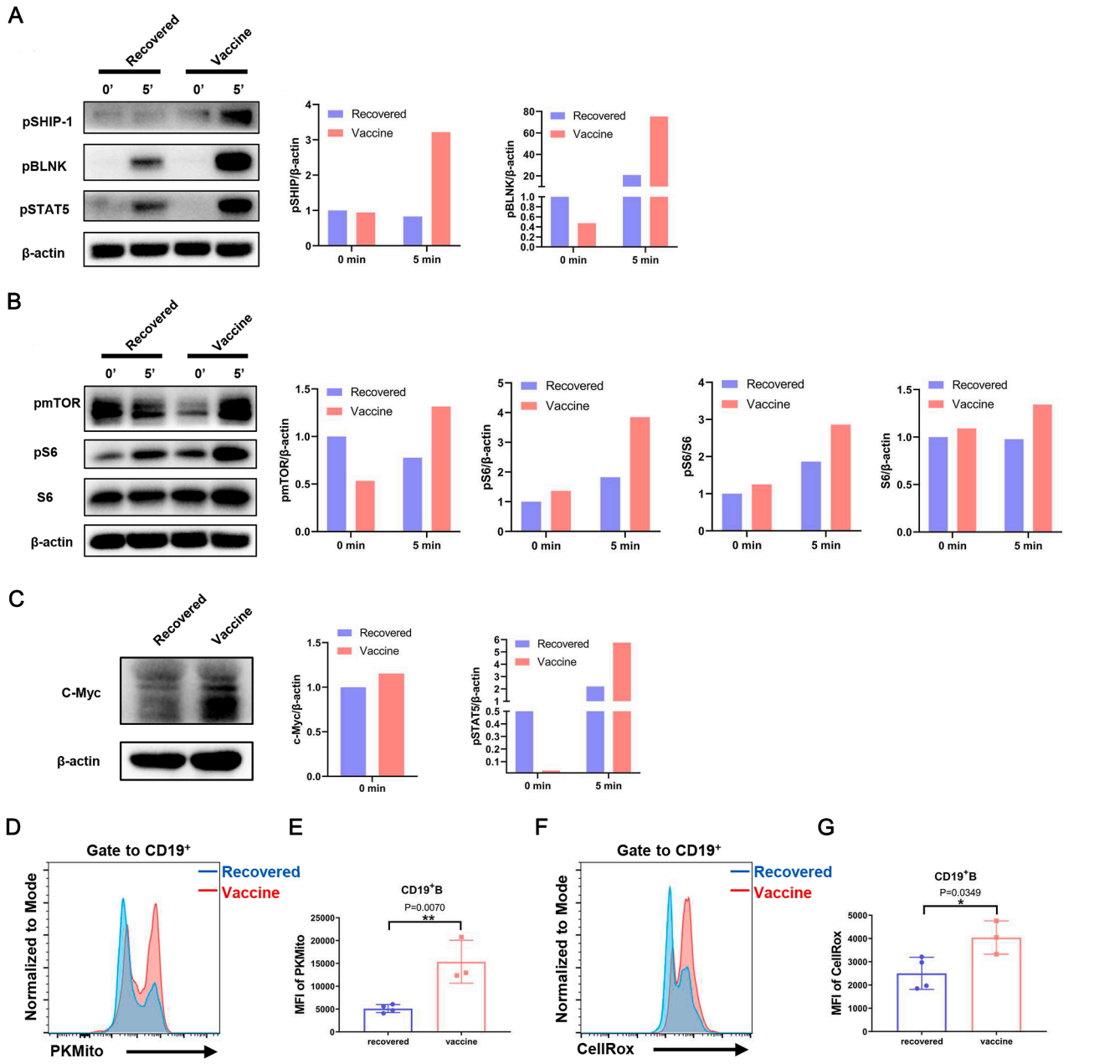

Fig. 3. BCR signal and STAT5-cMyc mediated cell metabolism. **A, B** PBMC were stimulated with or without biotin-conjugated F(ab')2 anti-human Ig (M + G) (10 μg/mL) plus streptavidin (20 μg/mL) at 37 °C for 5 min. Immunoblot for pSHIP-1, pBLNK, pSTAT5, pmTOR, pS6, and S6 protein expression in PBMC from the recovered population and vaccinated population. **C** Immunoblot for c-Myc protein expression in PBMC from the recovered population and vaccinated population. The relative quantification of protein expression levels in panels **A**–**C** was performed with β-actin or total protein as internal controls. **D–G** Flow cytometry analysis of PKMito and CellRox in $CD19^+$B cells of recovered population and vaccinated population. The mean fluorescence intensity (MFI) of PKMito and CellRox in $CD19^+$B cells was shown in histogram. Recovered = 4, vaccinated = 3. Dots represent individual samples. Statistical analyses were performed using student's *t*-test. $^*P < 0.05$; $^{**}P < 0.01$.

that the vaccinated population has an increased humoral immune response than recovered populations.

DISCUSSION

In our study, we have elucidated immune distinctions between the vaccinated and infected cohorts, with a specific focus on B cells. Our findings demonstrate that the vaccinated group exhibited weaker RBD-specific B cell differentiation and memory B cell activation compared to the infected group, but they had elevated metabolic signaling and increased production of specific antibodies. Diverging from prior investigations, we delved not only into memory B cells but also explored various B cell subpopulations, offering a novel perspective on B cell homeostasis disparities between the vaccinated and infected cohorts.

This uniqueness of our study population underscores the significance of our findings in comparing the differences in human immune responses between infected and vaccinated individuals. However, it is important to note that there may be differences in long-term immune responses in both recovered individuals and vaccinated individuals, compared to the individuals we studied, including differences in infection severity, underlying disease, age, and reinfection. In particular, with the global surge of Omicron (B.1.1.529) variants, almost everyone has been infected and vaccinated, and their immune responses may have changed again. Studies have shown that a hybrid of immunity generated

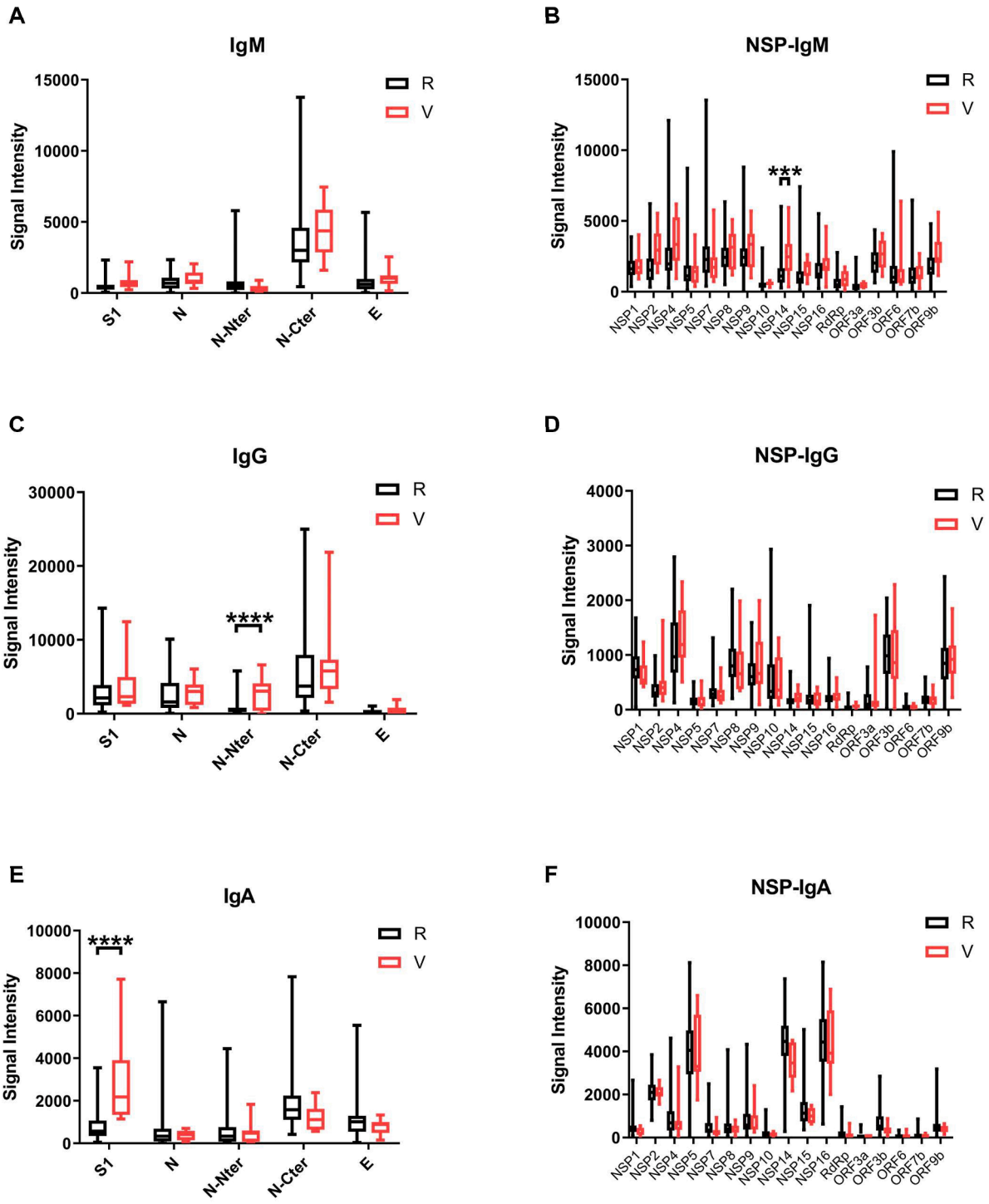

Fig. 4. Antibody specific immune response. Serum proteome microarray was used to probe IgM, IgG or IgA antibodies against SARS-CoV-2 in all samples collected from 59 recovered individuals (R) and 11 vaccinated individuals (V). **A, C and E** IgM, IgG or IgA specific antibodies response to S1, N, N-Nter, C-Cter and E proteins of SARS-CoV-2. **B, D, and F** IgM, IgG or IgA specific antibodies response to NSP protein of SARS-CoV-2. Recovered = 59, vaccinated = 11. The results were shown as mean ± SD in different groups. Statistical analyses were performed using multiple *t* tests. ***$P < 0.001$; ****$P < 0.0001$.

by previous infection and booster vaccination is the most protective against symptomatic Omicron infection compared to previous infection and vaccination alone (Altarawneh et al., 2022; Bobrovitz et al., 2023).

Our study found that RBD-specific B cells in the recovered population were more differentiated, but RBD-specific antibody titers were lower, which may be related to the time of sample collection in the recovered population. The titer and neutralization ability of anti-RBD antibodies in serum decreases six months after infection, but memory B cells can still maintain RBD-specific responses *in vitro* (Abayasingam et al., 2021). Moreover, through an in-depth analysis of B cell metabolic pathways and multi-faceted antibody specificity, we conducted a pioneering comparison of the underlying biological mechanisms shaping B cell variances in the vaccinated and infected groups. Activation of the mTOR pathway induces p70 S6 kinase activation and subsequent S6 ribosomal protein phosphorylation that triggers a cascade of metabolic up-regulation. It has been previously reported that STAT5 is involved in

mTOR pathway activation and induces the expression of MYC by IL-2–STAT5 axis in T cells, while the mTOR signaling pathway and MYC are two well-known key metabolic regulators (Villarino et al., 2022). We measured PKMito and CellRox levels in both the vaccinated and recovered groups, finding that the vaccinated group exhibited a higher metabolic rate. After stimulation with F(ab')2, we analyzed the expression levels of pmTOR, pS6, c-Myc, and pSTAT5, all of which were higher in the vaccinated group compared to the recovered group. Our findings suggest that the STAT5-c-Myc-mTOR axis plays a key role in regulating the metabolic differences in B cells between these two populations. Our exploration of B cell metabolic alterations post-vaccination and infection suggests a distinct advantage of vaccination in bolstering B cell immunity, thereby laying a robust groundwork for the formulation of vaccination strategies and furnishing a theoretical framework for future vaccine development endeavors. Our findings also have implications for vaccine optimization. For instance, we discovered that the vaccinated population exhibited stronger metabolic pathways, such as STAT5 and c-Myc. Future vaccines could potentially target molecules within these pathways to enhance immune response and protection against infection.

B cell linker (BLNK) is one of B cell adaptors (Niiro and Clark, 2002) and it can fine-tune BCR signals by efficiently connecting SYK and BTK with PLCγ2 (Fu et al., 1998; Hashimoto et al., 1999). SH2-domain-containing inositol polyphosphate 5′ phosphatase (SHIP) can be recruited by B cell co-receptors because they have immunoreceptor tyrosine-based inhibitory motifs (ITIMs) in their cytoplasmic tails, thereby inhibiting BCR signaling (Cyster and Goodnow, 1995; Chacko et al., 1996). We gave stimulation to B cells from the convalescent and vaccinated group, and detected the expression of BLNK, a key protein in the BCR signaling pathway, and SHIP-1, a key protein in the negative feedback loop. The result showed that the expression of pBLNK and pSHIP-1 was significantly higher in the vaccinated group than that in the convalescent group. Our results may establish that the vaccinated group can produce stronger BCR signals and the immune system is more capable of mounting an immune response to antigens. pSHIP-1 signaling enhancement may be due to the enhancement of positive BCR signaling resulting in a secondary enhancement of negative signaling, which regulates the maintenance of B cell homeostasis.

CONCLUSIONS

In this study, we observed similar peripheral B cell differentiation in both vaccinated and recovered individuals. However, vaccinated individuals exhibited decreased RBD-specific B cell differentiation, as well as reduced expression of CD86 on the surface of MBC, but with stronger BCR signaling. Metabolically, this group also showed increased expression of pmTOR, pS6, c-Myc, and pSTAT5, suggesting regulation of B cell metabolism via the STAT5-c-Myc axis. Additionally, using serum proteome microarray, we found higher levels of IgM, IgG and IgA antibodies specific to SARS-CoV-2 in the vaccinated group. Our study established a link axis among B cell activation-cell metabolism-antibody production and revealed the difference of this link axis between infected and vaccinated populations. It is worth investigating whether the intervention on B cell activation or cell metabolism have an impact on antibody production, which could provide strategies for vaccine design of COVID-19.

MATERIALS AND METHODS

Participants

The recovered population and vaccine-immunized population were collected from Jing Zhou Hospital. The recruited recovered participants included individuals with mild COVID-19 symptoms who were discharged after obtaining two consecutive negative SARS-CoV-2 nucleic acid RT-PCR test results, following the diagnostic criteria outlined in the fifth edition of the National Health Commission of China's diagnosis and treatment guidelines. And peripheral blood samples are taken at 24–26 weeks after initial diagnosis when CT scan is free of white lung symptoms and is not vaccinated. The vaccine-immunized population was vaccinated with 3 doses of inactivated SARS-CoV-2 vaccine (Vero cell) from Wuhan Institute of Biological Products Co., Ltd, and the peripheral blood samples were collected 8–14 days after the third dose. The Ethics Committee of Huazhong University of Science and Technology approved this study (ethics number S093), and written informed consent was secured from all subjects prior to specimen collection.

Peripheral blood samples

We collected the peripheral blood samples from the recovered population and vaccine-immunized population. Peripheral blood mononuclear cells (PBMCs) were separated using a Ficoll density gradient (GE Healthcare, 17-1440-02). In summary, peripheral blood was diluted at a 1:1 ratio in RPMI 1640 medium, plated on the upper layer of Ficoll, and centrifuged at an 800 $\times g$ density gradient for 20 min. Then, aspirate the lymphocytes in the middle layer and wash with 5 vol of RPMI 1640 medium. The cells were then counted and stored in gas-phase liquid nitrogen.

Flow cytometry and antibodies

For surface staining, isolated PBMCs were stained with antibodies include: FITC anti-CD19 (Biolegend, 302206), APC anti-CD27 (Biolegend, 302810), BV510 anti-IgD (Biolegend, 348205), PE anti-CD24 (Biolegend, 311106), Pacific Blue anti-CD38 (Biolegend, 356628), PE-Cy7 anti-CD80 (BD Bioscience, 561135), PE anti-CD86 (Abcam, ab77226), Percp-Cy5.5 anti-HLA-DR (BD Bioscience, 552764). For RBD-specific B cell staining, the PBMCs were surface-labeled with Biotin anti-RBD (Absin, 04759) and then stained with the secondary antibody DyLight 405 streptavidin (Jackson ImmunoResearch, 016-470-084).

Immunoblot analysis

Isolated PBMCs were stimulated with or without biotin-conjugated F(ab')2 anti-human Ig (M + G) (Jackson ImmunoResearch, 109-066-127) at 10 μg/mL plus streptavidin (Jackson ImmunoResearch, 016-000-114) at 20 μg/mL at 37 °C for 5 min. Then, we lysed the cells using RIPA buffer (P0013B, Beyotime). Subsequently, the cell lysates were added to the loading buffer (Biosharp, BL-502A) and boiled for 10 min. And then, we used SDS-PAGE to separate the protein, which was probed for the antibodies include: anti-pmTOR (Cell Signaling Technology (CST), 5536S), pS6 (CST, 4856S), S6 (CST, 2217S), pSHIP-1 (CST, 3941S), pBLNK (CST, 62144S), pSTAT5 (CST, 4322S), and β-actin (Proteintech, 60008-1-IG-10).

Mito and ROS detection

For ROS analysis, isolated PBMCs were staining with anti-CD19 and CellROX Green (Invitrogen, C10444) in PBS at 37 °C for 20 min. Next, we washed the cells using RPMI 1640 (Gibco, 11875500B1) and analyzed by flow cytometry. For Mito analysis, isolated PBMCs were staining with anti-CD19 and PKMito Red (Genvivotech, PKMDR-1) in RPMI 1640 with 10 % fetal bovine serum at 37 °C for 20 min. Then the cells were washed with RPMI 1640 and analyzed by flow cytometry.

Microarray-based serum analysis

We used the SARS-CoV-2 proteome microarray to analyze serum, following a previously described method (Jiang, H.-W. et al., 2020). In short, the frozen arrays were first brought to room temperature. Then, they were blocked with 3 % BSA in PBS containing 0.1 % Tween 20 for 3 h. Serum samples were diluted 1:200 in PBS with 0.1 % Tween 20 (PBST) and mixed with 0.1 mg/mL eGFP and 0.5 mg/mL total *E. coli*

lysate. Each subarray was then incubated overnight at 4 °C with 200 μL of either the diluted serum or buffer alone. After washing with PBST, the arrays were incubated for 1 h at 37 °C with Alexa Fluor 647-conjugated donkey anti-human IgM (Jackson ImmunoResearch, 709-605-073), Cy3-conjugated goat anti-human IgG (Jackson ImmunoResearch, 109-165-008), and goat anti-human IgA-AF488 (SouthernBiotech, 2050–30) in PBST. Next, the microarrays were rinsed with PBST, air-dried at 37 °C, and scanned using the LuxScan 10K-A (CapitalBio Corporation). The scanning parameters were set to 95 % laser power/PMT 550 for IgM and IgG, and 95 % laser power/PMT 480 for IgA. Fluorescence intensity data was then extracted using GenePix Pro 6.0 software (Molecular Devices).

Statistical analysis

Statistics were presented using mean ± SD and evaluated with Prism® 8.0 (GraphPad™). The unpaired two-tailed student *t*-test compared the differences between the two cohorts. Multiple *t* tests compared the differences more than two cohorts. Significance was defined as $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $****P < 0.0001$.

DATA AVAILABILITY

All the data generated during the current study are included in the manuscript.

ETHICS STATEMENT

This study received approval from the Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology, China (Approval number: S093). Written informed consent was secured from all subjects prior to specimen collection.

AUTHOR CONTRIBUTIONS

Lu Yang: conceptualization, data curation, writing-original draft. Linhua Wang: investigation, data curation. Qian Liu: data curation. Xu Zhang: Collect samples. Yuexin Luo: writing-original draft. Junbiao Xue: methodology. Xinpu yang: methodology. Maria G Byazrova: conceptualization. Alexander V Filatov: conceptualization. Sheng-Ce Tao: Supervision. Wei Xiao: project administration. Chaohong Liu: conceptualization, writing-review & editing, supervision.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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