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SPECIALTY SECTION

This article was submitted to
T Cell Biology,
a section of the journal
Frontiers in Immunology

RECEIVED 20 March 2022

ACCEPTED 30 August 2022

PUBLISHED 12 October 2022

CITATION

Wang L, Li J, Jiang S, Li Y, Guo R,
Chen Y, Chen Y, Yu H, Qiao Q,
Zhan M, Yin Z, Xiang Z, Xu C and Xu Y
(2022) COVID-19 vaccination
influences subtypes of $\gamma\delta$ -T cells
during pregnancy.
Front. Immunol. 13:900556.
doi: 10.3389/fimmu.2022.900556

COVID-19 vaccination influences subtypes of $\gamma\delta$ -T cells during pregnancy

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Up to now, there has been insufficient clinical data to support the safety and effects of vaccination on pregnancy post COVID-19 vaccination. The $\gamma\delta$ -T cells are considered an important component in the immune system to fight against viral infection and exhibit critical roles throughout the pregnancy period. However, the immunological roles of $\gamma\delta$ -T cells in pregnant women with the COVID-19 vaccination remain unclear. Therefore, the objective of this study is to investigate the alteration of frequency and expression pattern of activation receptors and inhibitory receptors in $\gamma\delta$ -T cell and its subsets in peripheral blood samples collected from non-pregnant vaccinated women, vaccinated pregnant women, and unvaccinated pregnant women. Our findings indicated that the frequency of CD3⁺ $\gamma\delta$ -T⁺ cells is lower in vaccinated pregnant women than in unvaccinated pregnant women. But no significant difference was found in the frequency of CD3⁺ $\gamma\delta$ -T⁺ cells between non-pregnant vaccinated women and vaccinated pregnant women. In addition, there were no significant differences in the frequencies of CD3⁺ $\gamma\delta$ -T⁺V δ 1⁺T cells, CD3⁺ $\gamma\delta$ -T⁺V δ 2⁺T cells, CD3⁺ $\gamma\delta$ -T⁺V δ 1⁺V δ 2⁺T cells, and V δ 1⁺T cell/V δ 2⁺T cell ratio between the pregnant women with or without COVID-19 vaccination. Similar results were found after comparing non-pregnant and pregnant women who received the COVID-19 vaccine. However, there was a significant difference in the fraction of V δ 1⁺V δ 2⁺T cells in CD3⁺ $\gamma\delta$ -T⁺ cells between non-pregnant vaccinated women and vaccinated pregnant women. The frequency of NKG2D⁺ cells in V δ 2⁺T cells was not significantly different in the vaccinated pregnant women when compared to that in unvaccinated pregnant women or non-pregnant vaccinated women. But the percentage of NKG2D⁺ cells in V δ 1⁺T cells was the lowest in pregnant women after COVID-19 vaccination. Furthermore, down-regulation of NKP46 and NKP30 were found in V δ 2⁺T and

V δ 1⁺T cells in the vaccinated pregnant women, respectively. After the vaccination, up-regulation of PD-1 expression in V δ 1⁺T cells and V δ 2⁺T cells indicated $\gamma\delta$ -T cells could respond to COVID-19 vaccination and display an exhausted phenotype following activation. In conclusion, COVID-19 vaccination influences subtypes of $\gamma\delta$ -T cells during pregnancy, but the side effects might be limited. The phenotypical changes of V δ 1⁺T cells and V δ 2⁺T cells will be a promising predictor for evaluating the clinical outcome of the COVID-19 vaccine.

KEYWORDS

COVID-19, vaccination, pregnancy, $\gamma\delta$ -T cells, V δ 1⁺ T cells, V δ 2⁺ T cells

Introduction

Due to a lack of clinical and scientific knowledge, the challenge of the Coronavirus Disease 2019 (COVID-19) pandemic has exposed the limitations of our understanding about severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as well as the immune response during viral infection and vaccination (1). Pregnant women especially are at increased risk of severe illness from COVID-19. There have been a few studies that demonstrated the efficacy and safety of COVID-19 vaccines in pregnant women, who have been excluded from the clinical trials because of the ethical issues (2). However, we still struggle with a complete understanding about whether vaccination could modulate the immune response of pregnant women (3). During normal human pregnancy, $\gamma\delta$ -T cells have been reported to play a role in this process (4).

Human $\gamma\delta$ T cells can be divided into three subgroups, V δ 1, V δ 2 and V δ 3, according to the structural differences of γ and δ chains. Although $\gamma\delta$ -T cells represent only a small fraction of T lymphocytes (1-10%) (5), they have a variety of immune functions, such as resistance to virus infection (6), inflammatory regulation, tissue homeostasis (6), helping B cells to produce antibodies (7), and even maintenance of successful pregnancy (6). Some studies reported that, during normal human pregnancy, $\gamma\delta$ -T cells secrete some anti-inflammatory cytokines to reduce nature killer activity (8, 9). Indeed, $\gamma\delta$ -T cells regulate the release of inflammatory factors such as IFN- γ , TNF- α , granzyme A/B, and perforin by regulating the expression of surface-activated receptors such as NKG2D, NKP30, NKP46, and the inhibitory receptor PD-1 to regulate their cytotoxic functions (10-13). In addition, low cytotoxic activity of $\gamma\delta$ -T cells is necessary during normal pregnancy (14). Most importantly, the imbalance between V δ 1⁺ T cells and V δ 2⁺ T cells was observed in adverse pregnancy (15).

Therefore, in this study, we aimed to interrogate the alternation of subtypes of $\gamma\delta$ -T cells in pregnant women after COVID-19 vaccination, which could provide a better understanding of the role of $\gamma\delta$ -T cells in pregnant women after COVID-19 vaccination, and in turn can further help us to monitor and evaluate the safety and efficacy of COVID-19 vaccines during the pregnancy period.

Materials and methods

Study population

The research objects were selected from pregnant women vaccinated against COVID-19 who went to Tianhe Campus of the Third Affiliated Hospital of Sun Yat-sen University in Guangzhou, China from August 2021 to February 2022. These women did not confirm their pregnancies until after they had received the COVID-19 vaccine. The inclusion criteria included: (1) age of 18-35 years; (2) singleton pregnancy; (3) the onset of pregnancy as calculated by crown-rump length (CRL) on NT ultrasound, with at least one dose of vaccine after pregnancy; and (4) signing an informed consent form, providing vaccination information and confirming their participation in the study. The exclusion criteria included: (1) the gestational age of the documented prenatal examination is not 11-13+6 weeks; (2) other vaccines have been received within 1 year, such as HPV vaccine and hepatitis B vaccine; or (3) complications with basic diseases, requiring long-term medication. The peripheral blood of the patients enrolled in this study was collected to perform immunological assays. The study included 27 vaccinated pregnant women, 11 unvaccinated pregnant women, and 20 non-pregnant vaccinated women. The clinical and demographic characteristics and vaccination scheme of pregnant women were described in Tables 1, 2, respectively. This study was approved

TABLE 1 Basic characteristics of vaccinated and unvaccinated pregnant women.

Groups	Non-pregnant vaccinated group	Un-vaccinated group	Vaccinated group
Included	20	11	27
Age (median and range)	24 (23-35)	29 (26-33)	29 (28-32)
Previous history of SARS-CoV-2 infection	No	No	No
Hypertension	No	No	No
Diabetes	No	No	No
Gestational age of laboratory tests (weeks, median and range)	No	12.4 (12.3-12.7)	12.9 (12.6-13.3)
NT (mm, mean and standard deviation)	No	1.4 (0.4)	1.4 (0.4)
Gestational age of laboratory tests (weeks, median and range)	No	12.4 (12.3-12.7)	12.9 (12.6-13.3)

Gestational age is equal to the number of gestational days divided by 7.

by the Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University in Guangzhou.

Immune cell phenotype analyzing by flow cytometry

Collected peripheral blood samples were analyzed using flow cytometry. Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-Paque centrifugation, then stained by the following antibodies: anti-human CD3-APC-H7 (BD biosciences, clone: SK7), anti-human TCR $\gamma\delta$ -BV421 (BD biosciences, clone: 11F2), anti-human PD-1-BB515 (BD biosciences, clone: EH12.1), anti-human NKP46-BV510 (BD biosciences, clone: 9E2/NKP46), anti-human NKP30-Alexa Fluor[®] 647 (BD biosciences, clone: P30-15), anti-human NKG2D-PE-Cy[™]7 (BD biosciences, clone: 1D11), anti-human TCR V δ 2-PE (BD biosciences, clone: B6), and anti-human TCR V δ 1-PerCP-Vio700 (Miltenyi Biotec, clone: REA173). Data was analyzed using FlowJo 10.1 software (Tree Star Inc., Ashland, OR, USA).

Statistical analysis

Statistical analyses were performed using GraphPad Prism (GraphPad Software, Inc.). All results are expressed as the mean \pm SEM (standard error of the mean). To analyze the difference in $\gamma\delta$ T cells and its subsets between vaccinated women and unvaccinated women, Mann-Whitney U tests were performed.

Results

The frequencies of $\gamma\delta$ T cell and its subsets in pregnant women after COVID-19 vaccination

The aim of this study was to investigate whether COVID-19 vaccination influences the distribution of total $\gamma\delta$ T cells, V δ 1⁺T cells, V δ 2⁺T cells, and V δ 1⁻V δ 2⁻T cells in the peripheral blood

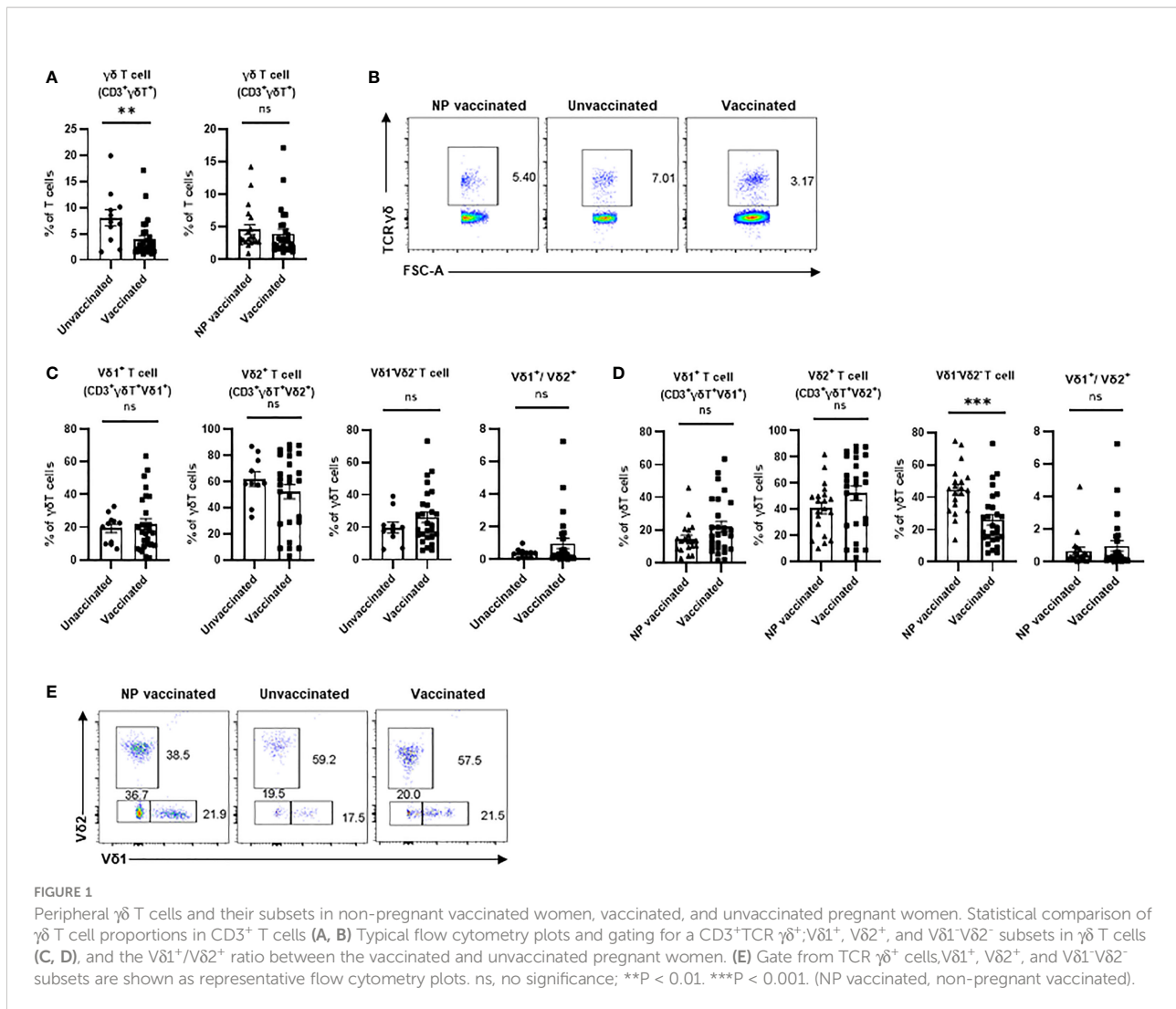
samples of 58 individuals enrolled in this study. Total $\gamma\delta$ T cell was identified among CD3⁺ lymphocytes using flow cytometry (Figures 1A, B). The results indicated that frequency of total $\gamma\delta$ T cells was markedly higher in unvaccinated pregnant women compared to vaccinated pregnant women, but there was no significant difference between non-pregnant vaccinated women and vaccinated pregnant women. Furthermore, to investigate the impact of COVID-19 vaccination on frequencies of $\gamma\delta$ T cells subsets, we next compared V δ 2⁺T cells, V δ 1⁺T cells, and V δ 1⁻V δ 2⁻T cells' frequencies as well as V δ 1/V δ 2 ratio in non-pregnant vaccinated women, vaccinated pregnant women, and unvaccinated pregnant women. The results indicated that there were no significant differences in frequencies of V δ 1⁺T, V δ 2⁺T cells, and V δ 1⁻V δ 2⁻T cells in CD3⁺ $\gamma\delta$ ⁺ T cells between vaccinated pregnant women and unvaccinated pregnant women (Figures 1C, E). However, compared with non-pregnant vaccinated women, the proportion of V δ 1⁻V δ 2⁻T cells in CD3⁺ $\gamma\delta$ ⁺ T was significantly reduced in vaccinated pregnant women (Figure 1D). Additionally, V δ 1/V δ 2 ratio was similar in these women with or without vaccination (Figure 1D).

Phenotypical changes of V δ 2⁺T cells in vaccinated pregnant women

It is well known that the activating and inhibitory receptors define the degree of immune cell maturation and responsiveness to stimuli, so we next investigated the frequencies of NKG2D⁺, NKp30⁺, NKp46⁺, and PD-1⁺ cells in V δ 2⁺T cells. We found no significant difference in the proportions of NKG2D⁺ V δ 2⁺T cells among these three groups (Figure 2A). $\gamma\delta$ T cell subsets usually express activating natural killer (NK) receptors, such as NKp30 and NKp46, which are involved in regulating immunological functions of $\gamma\delta$ T cell and its subsets. There was no significant difference in percentage of NKp30⁺V δ 2⁺T cells between vaccinated pregnant women and unvaccinated pregnant women (Figure 2B). In addition, a similar result was found in comparison of vaccinated pregnant women and non-pregnant vaccinated women (Figure 2B). By contrast, there was no

TABLE 2 Scheme of vaccination of vaccinated pregnant women.

Vaccinated pregnant women	Pregnancy time	Date of laboratory tests	Scheme of vaccination	
			Date of 1st dose	Date of 2nd dose
Vg-1	2021/05/30	2021/08/27	2021/07/27	–
Vg-2	2021/06/03	2021/08/27	2021/06/12	–
Vg-3	2021/05/31	2021/08/27	2021/05/22	2021/06/29
Vg-4	2021/05/25	2021/08/30	2021/05/23	2021/06/22
Vg-5	2021/06/08	2021/08/30	2021/06/13	2021/04/30
Vg-6	2021/05/30	2021/08/30	2021/06/10	2021/07/13
Vg-7	2021/05/29	2021/08/30	2021/06/23	–
Vg-8	2021/05/29	2021/08/30	2021/06/02	2021/05/04
Vg-9	2021/06/04	2021/09/01	2021/07/01	–
Vg-10	2021/05/31	2021/09/01	2021/04/14	2021/06/07
Vg-11	2021/06/04	2021/09/02	2021/05/29	2021/06/26
Vg-12	2021/06/06	2021/09/06	2021/05/10	2021/06/09
Vg-13	2021/06/13	2021/09/10	2021/05/06	2021/06/16
Vg-14	2021/06/06	2021/09/10	2021/05/09	2021/06/12
Vg-15	2021/06/16	2021/09/15	2021/07/16	–
Vg-16	2021/06/25	2021/09/18	2021/05/29	2021/06/25
Vg-17	2021/06/26	2021/09/22	2021/04/22	2021/07/08
Vg-18	2021/06/28	2021/09/26	2021/06/23	2021/07/19
Vg-19	2021/06/26	2021/09/27	2021/06/28	2021/07/20
Vg-20	2021/06/26	2021/09/27	2021/05/30	2021/06/26
Vg-21	2021/06/24	2021/09/27	2021/05/29	2021/06/29
Vg-22	2021/07/15	2021/10/12	2021/07/11	2021/08/07
Vg-23	2021/07/24	2021/10/12	2021/07/25	2021/08/24
Vg-24	2021/07/06	2021/10/11	2021/07/19	–
Vg-25	2021/07/23	2021/10/14	2021/06/29	2021/07/21
Vg-26	2021/07/20	2021/10/15	2021/06/20	2021/07/28
Vg-27	2021/07/17	2021/10/19	2021/07/20	–
Np-1	–	–	2021/04/22	2021/05/27
Np-2	–	–	2021/04/22	2021/05/25
Np-3	–	–	2021/04/01	2021/05/25
Np-4	–	–	2021/04/08	2021/05/07
Np-5	–	–	2021/04/24	2021/05/11
Np-6	–	–	2021/04/02	2021/05/31
Np-7	–	–	2021/04/02	2021/04/30
Np-8	–	–	2021/04/02	2021/04/30
Np-9	–	–	2021/04/02	2021/04/30
Np-10	–	–	2021/04/02	2021/04/30
Np-11	–	–	2021/04/02	2021/04/30
Np-12	–	–	2021/04/02	2021/04/30
Np-13	–	–	2021/04/02	2021/04/30
Np-14	–	–	2021/04/02	2021/04/30
Np-15	–	–	2021/04/02	2021/04/30
Np-16	–	–	2021/03/11	2021/04/12
Np-17	–	–	2021/03/11	2021/04/12
Np-18	–	–	2021/03/11	2021/04/12
Np-19	–	–	2021/03/11	2021/04/12
Np-20	–	–	2021/03/11	2021/04/12



significant difference in the percentage of $NKp46^+V\delta2^+$ T cells between vaccinated pregnant women and unvaccinated pregnant women. But compared with non-pregnant vaccinated women, the percentage of $NKp46^+V\delta2^+$ T cells was significantly decreased in vaccinated pregnant women (Figure 2C). Furthermore, we supposed that $\gamma\delta$ T cell would exhibit the exhausted phenotype after vaccination. Actually, the frequency of $PD-1^+V\delta2^+$ T cells was significantly elevated in vaccinated pregnant women compared to unvaccinated pregnant women. However, there was no difference between non-pregnant vaccinated women and vaccinated pregnant women (Figure 2D).

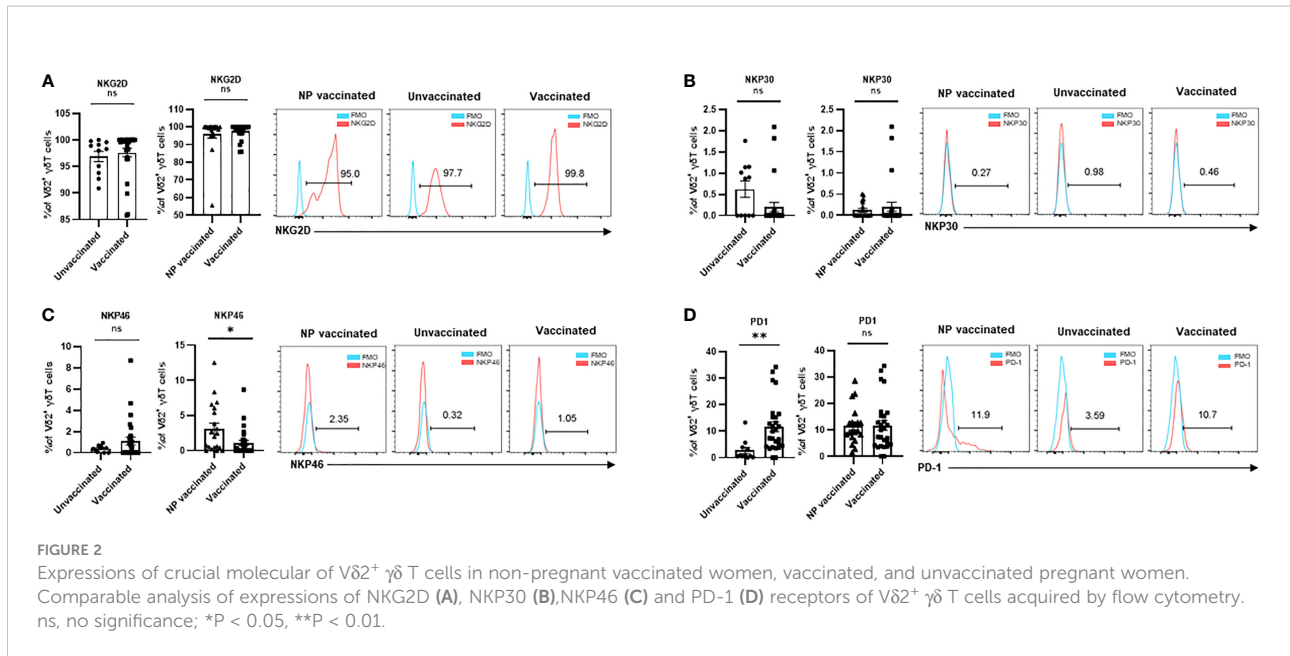
Phenotypical changes of $V\delta1^+$ T cells in vaccinated pregnant women

To investigate the alteration of expression pattern of activating and inhibitory receptors in $V\delta1^+$ T cells after COVID-

19 vaccination in the pregnant women, the frequencies of $NKG2D^+$, $NKp30^+$, $NKp46^+$, and $PD-1^+$ cells in $V\delta2^+$ T cells were determined. Unsimilar to $V\delta2^+$ T cells, the frequencies of $NKG2D^+$ and $NKp30^+$ cells in $V\delta1^+$ cells were much lower in vaccinated pregnant women than that in unvaccinated pregnant women (Figures 3A, B). In addition, a significant difference in the frequency of $NKG2D^+V\delta1^+$ cells was found between non-pregnant vaccinated women and vaccinated pregnant women. Similar to $V\delta2^+$ T cells, the frequency of $NKp46^+$ cells were no different in all three groups (Figure 3C). Finally, $PD-1^+V\delta1^+$ T cells were significantly increased in the pregnant women after vaccination (Figure 3D).

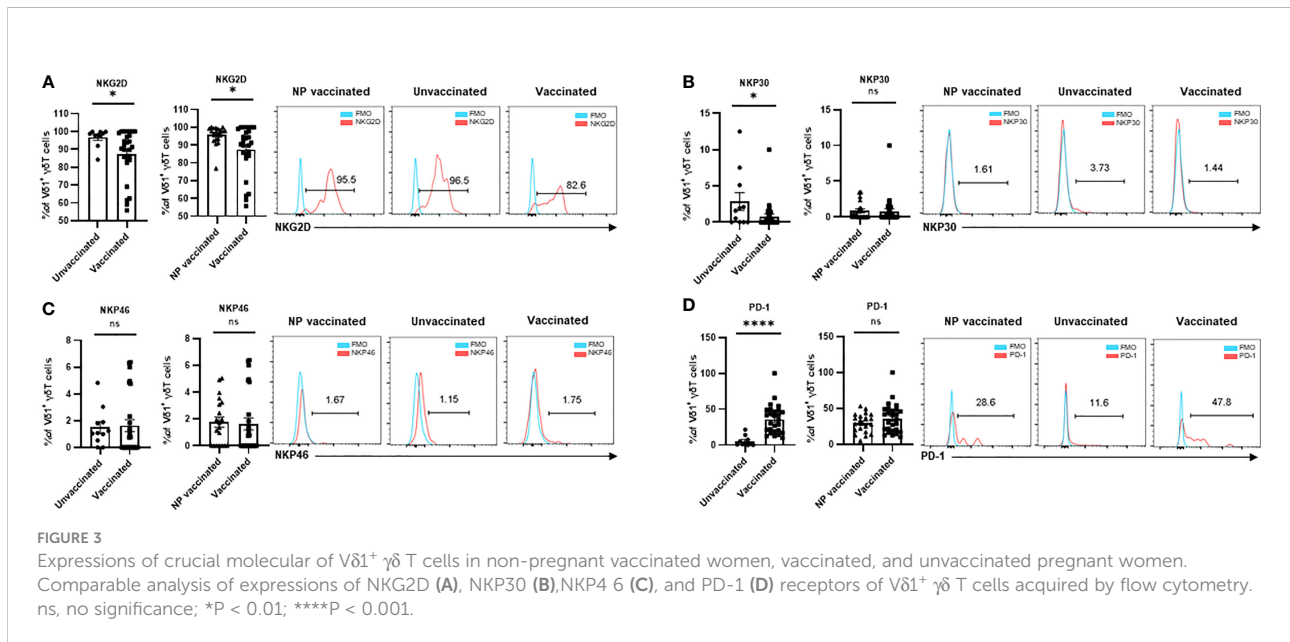
Discussion

Our study demonstrated that frequencies of total $\gamma\delta$ T cell ($CD3^+\gamma\delta^+$ T cells) was significantly decreased in the pregnant



women with COVID-19 vaccination compared to that in the pregnant women without COVID-19 vaccination. Furthermore, analysis of the frequencies of $V\delta 1^+$ T cells and $V\delta 2^+$ T cells in total $\gamma\delta$ T cells indicated that there were no significant differences between vaccinated pregnant women and unvaccinated pregnant women. Importantly, no significant difference was found on the $V\delta 1/V\delta 2$ ratio between these two groups of women who were with or without COVID-19 vaccination. For evaluation of activated and exhausted phenotypes in of $V\delta 1^+$ T cells and $V\delta 2^+$ T cells after COVID-19 vaccination in the pregnant women, the frequencies of $NKG2D^+$,

$NKP30^+$, $NKP46^+$, and $PD-1^+$ cells in these two subsets were analyzed. Our findings suggested that $V\delta 1^+$ T cells and $V\delta 2^+$ T cells developed an exhausted phenotype post COVID-19 vaccination. These results demonstrate that COVID-19 vaccination exhibits a certain degree of influence on the frequency of total $\gamma\delta$ T cell and alteration of phenotype of $V\delta 1^+$ T cells and $V\delta 2^+$ T cells. However, the $V\delta 1/V\delta 2$ ratio is similar between vaccinated pregnant women and unvaccinated pregnant women, which indicates that vaccination did not break the important balance between these two main subsets of peripheral $\gamma\delta$ T cells. Taken together, the results suggest



COVID-19 vaccination influences subtypes of $\gamma\delta$ -T cells without effects on pregnancy.

In this study, we found that the frequency of total $\gamma\delta$ -T cells was similar between the pregnant women and non-pregnant women after COVID-19 vaccination (Figure 1A). In addition, there were no significant differences in frequencies of $V\delta 1^+$ T cells and $V\delta 2^+$ T cells between these two groups of individuals. A similar result was also found in the $V\delta 1^+/V\delta 2^+$ ratio (Figure 1C). However, the frequency of $V\delta 1^-V\delta 2^-$ T cells was much higher in the non-pregnant vaccinated women compared with that in vaccinated pregnant women (Figure 1D). Furthermore, our data indicated that the proportions of $NKP46^+V\delta 2^+$ T cells and $NKG2D^+V\delta 1^+$ T cells were lower in the vaccinated pregnant women than that in non-pregnant vaccinated women (Figures 2C, 3A). By contrast, no significant differences were found in the percentages of $NKG2D^+$, $NKP30^+$, and $PD-1^+$ cells in $V\delta 2^+$ T cells and $NKP30^+$ and $NKP46^+$ cells in $V\delta 1^+$ T cells between the non-pregnant vaccinated women and vaccinated pregnant women (Figures 2A, B, D; Figures 3B-D). These data further demonstrated that pregnancy is not a potential influencing factor for our findings, and this will help us to achieve more confident conclusions.

In humans, $V\delta 1^+$ T cells and $V\delta 2^+$ T cells are the two major subsets of $\gamma\delta$ T cells which are identified by the $V\delta$ chains. $V\delta 1^+$ T cells constitute the majority of T cells in the thymus and mucosal tissues, and $V\delta 2^+$ T cells are predominant in the peripheral blood (16). As the main $\gamma\delta$ T cell subsets, $V\delta 1^+$ T cells and $V\delta 2^+$ T cells exhibit different immunological functions. $V\delta 1^+$ T cells display regulatory and effector features, and $V\delta 2^+$ T cells exert a cytotoxic activity targeting pathogenic characteristics. According to previous studies, the frequency of peripheral $\gamma\delta$ T cells is higher in women with a successful pregnancy compared to women with pregnancy failure (9). In addition, one study suggested that $V\delta 1^+$ T cells could produce IL-10 to down-regulate the cytotoxic NK cells during pregnancy. In healthy pregnant women, the predominant subpopulation of peripheral $\gamma\delta$ T cells is $V\delta 1^+$ T cells, whereas $V\delta 2^+$ T cells is the most frequent subset in women with recurrent miscarriage (16). Therefore, this evidence demonstrated that an imbalance of $V\delta 1/V\delta 2$ ratio leads to adverse pregnancy outcome (15, 16). It has been reported that $\gamma\delta$ T cells (mostly expressing $V\delta 2$) are able to destruct influenza A virus-infected cells as efficient as $CD8^+$ T cells or NK cells in a polycytotoxic manner and by releasing IFN- γ against infected cells *in vitro* (17). In several contexts, including infection with Mtb, malaria, influenza, and HIV and vaccination with BCG and live attenuated influenza, there are clear patterns of $\gamma\delta$ T-cell expansion, particularly of the $V\delta 2^+$ subset, in response to both infection and vaccination (18). However, there is no literature report on the effect of other vaccines after pregnancy on $\gamma\delta$ T cells' function. In the future, we could detect the difference in $\gamma\delta$ T cells' function between other vaccines and the COVID-19 vaccine during pregnancy, so as to

explore whether this result is specific to the COVID-19 vaccine. In our study, COVID-19 vaccination did not change $V\delta 1/V\delta 2$ ratio in the pregnant women, which indicates the impact of vaccination might not cause an adverse outcome.

NK cell activating receptors, such as $NKG2D$, $NKP30$, and $NKP46$, are widely involved in regulating NK functions during pregnancy (19). Additionally, one recent study suggested that the frequency of $NKG2D^+V\delta 2^+$ T cells was negatively correlated with a successful clinical pregnancy (8). Our data demonstrate that no significant difference was found on the percentages of $NKG2D^+$ cells in $V\delta 1^+$ T cells and $V\delta 2^+$ T cells, which suggest that COVID-19 vaccination did not induce the highly activated peripheral $\gamma\delta$ T cells in the pregnant women. Because of the activated $\gamma\delta$ T cells, a high level of inflammation is considered as the major cause of abortion (15). Additionally, frequencies of $NKP30^+$ and $NKP46^+$ cells in $V\delta 1^+$ T and $V\delta 2^+$ T were largely similar between vaccinated pregnant women and unvaccinated pregnant women. In summary, these data suggested that the immunological functions of $\gamma\delta$ T cells were not altered after COVID-19 vaccination in the pregnant women.

Accordingly, many previous studies suggested that exhaustion of $\gamma\delta$ T cells is accompanied by a decrease in the frequency of cells in different types of disease. For instance, in acute myeloid leukemia (AML), the data demonstrated that the proportion of total $\gamma\delta$ T cells was decreased in AML patients (20). Subsequently, in these $\gamma\delta$ T cells, the authors observed increased PD-1 expression and decreased $NKG2D$ expression, indicating highly activated or even exhausted states in the $\gamma\delta$ T cells at diagnosis of AML (20). Additionally, in the acute viral infection, a lower frequency of $V\delta 2^+$ T cells was also observed (21). Moreover, these $V\delta 2^+$ T cells highly expressed CD95, which in turn could lead to cell apoptosis that induces the loss of cells (21). Interestingly, this work demonstrated that the expression level of CTLA-4 exhaustion marker was elevated during acute viral infection. In line with these previous results, we also found that stimulation of COVID-19 vaccine induces the exhaustion status within the loss of $\gamma\delta$ T cells in pregnant women, suggesting the involvement of $\gamma\delta$ T cells in the complex network of protective response induced by COVID-19 vaccination. PD-1 expression is induced on activated T cells and is correlated to exhaustion status in anti-infection and anti-tumor responses (22, 23). In our study, we found that the frequency of $PD-1^+$ cells in $V\delta 1^+$ T and $V\delta 2^+$ T were much higher in vaccinated pregnant women compared to unvaccinated pregnant women. We suspect that $\gamma\delta$ T cells developed an exhausted phenotype following activation by COVID-19 vaccination. This switch from activation and exhaustion might be the reason why the frequency of $\gamma\delta$ T cells was decreased in the pregnant women with COVID-19 vaccination. Since the objective of this study is to investigate whether COVID-19 vaccination influences subtypes of $\gamma\delta$ -T cells during pregnancy, the changes of frequency and immunological phenotypes of $\gamma\delta$ -T cells were determined in this study. $\gamma\delta$ -T cells are well known as having

multiple functions in innate immune cells, suggesting they play important roles in anti-viral infection and immune response to vaccination. The results of our study could be considered as the clues to address the questions about the potential immunological functions of $\gamma\delta$ -T cell and its subsets involved in the immune activity associated with COVID-19 vaccination. This deserves further study in the future.

Despite the positives provided by this study, there are still some limitations. First, a further larger sample size study is warranted to validate these findings. And the effects of age, vaccination scheme, vaccine types, and other factors in impacting the immunological features of $\gamma\delta$ T cell should be further evaluated in pregnant women after COVID-19 vaccination. Second, in this study, we only determined the phenotypic changes of $\gamma\delta$ T cell subsets in the peripheral blood of individuals. The functional capacity of $\gamma\delta$ T cell and its subpopulations are rarely investigated in COVID-19 vaccination and should be determined in the future. Third, we found the phenotypic changes of $\gamma\delta$ T cell subsets by comparing the data collected in the pregnant women with or without COVID-19 vaccination. In the future, using samples from each vaccinated individual at different time points in the vaccination scheme will further help us to clarify the significance of functional alterations of $\gamma\delta$ T cell and its subsets in establishing protective immunity against COVID-19 infection after vaccination.

Taken together, our study suggests that $\gamma\delta$ T cell and its subsets could respond to COVID-19 vaccination and display an exhausted phenotype following activation. In addition, COVID-19 vaccination influences subtypes of $\gamma\delta$ -T cells during pregnancy, but the side effects are limited. Last but not least, the contribution of $\gamma\delta$ T cell and its subsets to the immunology of COVID-19 vaccination needs to be further investigated.

Data availability statements

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

This study was approved by the Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University in Guangzhou, PR China. The participants provided their written informed consent to participate in this study. The patients/participants provided their written informed consent to participate in this study.

Author contributions

CX and ZY designed the study; YX and ZX designed the study, performed the data analysis, and wrote the manuscript. LW and SJ enrolled the subjects and collected the peripheral blood, performed the experiments, and assisted in the preparation of the figures. JL, YL, and RG performed the data analysis and plotted the graphs. YYC, YC, HY, QQ, and MZ collected the peripheral blood and performed the experiments. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the National Natural Science Foundation of China (82070606) and the Natural Science Foundation of Guangdong Province, China (2021A1515011441). This work was also supported by the National Natural Science Foundation of China (32000616 and 31570898) and the Basic and Applied Basic Research Fund of Guangdong Province (2020A1515111203 and 2016A030313112).

Acknowledgments

We thank all participants and investigators involved in the study.

Conflict of interest

Author JL was employed by the company Guangzhou PuruiBiotechnology Co., Ltd, Guangdong, China.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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