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Short communication

T helper profile in pregnant women recovered from COVID-19

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ABSTRACT

T helper (Th) cell subsets play distinct and important roles during pregnancy. This work was focused on investigating the Th and cytokine profile in pregnant women recovered from COVID-19. To this aim, the frequency of Th1, Th2, Th17 subsets and the level of associated cytokines were analysed in pregnant women recovered from COVID-19 and in matched non-pregnant women. Principal component analysis highlighted a significant impact of pregnancy on Th profile with an increase of ex-Th17 subset and a parallel decrease of Th1 population. These modulations may participate in both preserving the pregnancy and reducing the risk of severe infection.

1. Introduction

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), spreads rapidly worldwide and, on March 2020, the World Health Organization has declared the novel coronavirus outbreak a global pandemic.

Generally, pregnant women are susceptible to viral infections (Kourtis et al., 2014), but their particular immunological profile could play a protective role in reducing the severity of SARS-CoV-2 infection. During normal pregnancy the immune system undergoes a series of changes to protect mother and foetus from pathogens, however avoiding the induction of a detrimental immune response against the allogeneic foetus (Abu-Raya et al., 2020). T helper subsets (Th1, Th2, Th9, Th17 and Th22) exert specific roles during pregnancy, aimed to avoid obstetrical complications and to maintain an effective immunosurveillance against infections. Th1 inflammatory immune cells are dominant during peri-implantation period but early shifted toward a Th2 anti-inflammatory immune profile, playing a key role in maintaining the maternal-foetal tolerance. Moreover, Th17 cells mediate protective immunity against extracellular microbes at the maternal-foetal interface (Wang et al., 2020), while ex-Th17 subset is the result of Th17 cells transition through a differentiation step characterized by the production of both IL-17 and IFN-γ (Basdeo et al., 2017). Unfortunately, no data about ex-Th17 during pregnancy are available.

Several studies reported that SARS-CoV-2 infected pregnant women are not at higher risk of adverse outcome compared with non-pregnant women (Vizheh et al., 2021) and have rare adverse perinatal outcomes (Donati et al., 2022). Pregnant women with COVID-19 infection showed a lower expression of pro-inflammatory and anti-inflammatory cytokines (Chen et al., 2021a) and displayed an effective endogenous control of inflammation (De Biasi et al., 2021).

The patients recovered from SARS-CoV-2 infection are characterized by a prevalence of symptoms that may persist after the resolution of infection associated with immune disorders. Data about the immunological recovery after SARS-CoV-2 infection in pregnant women are still missing. Few evidences suggest that pregnant women recovered from COVID-19 displayed a reduced but functional lymphoid immune response, and pathways related to T or B cell activation, migration and virus defence were upregulated (Chen et al., 2021b). Moreover, SARS-CoV-2 infection induced a physiological anti-viral T cell response in pregnant women that included the expansion of specific Treg cells (Hsieh et al., 2022).

The complex network of CD4+ T helper cells (Th) that participates in finely balancing the immune responses at the maternal-foetal interface (Wang et al., 2020) is poorly explored in pregnant women recovered from COVID-19 and can represent a signature of a protective response generated during the infection. For this reason, the aim of our study was

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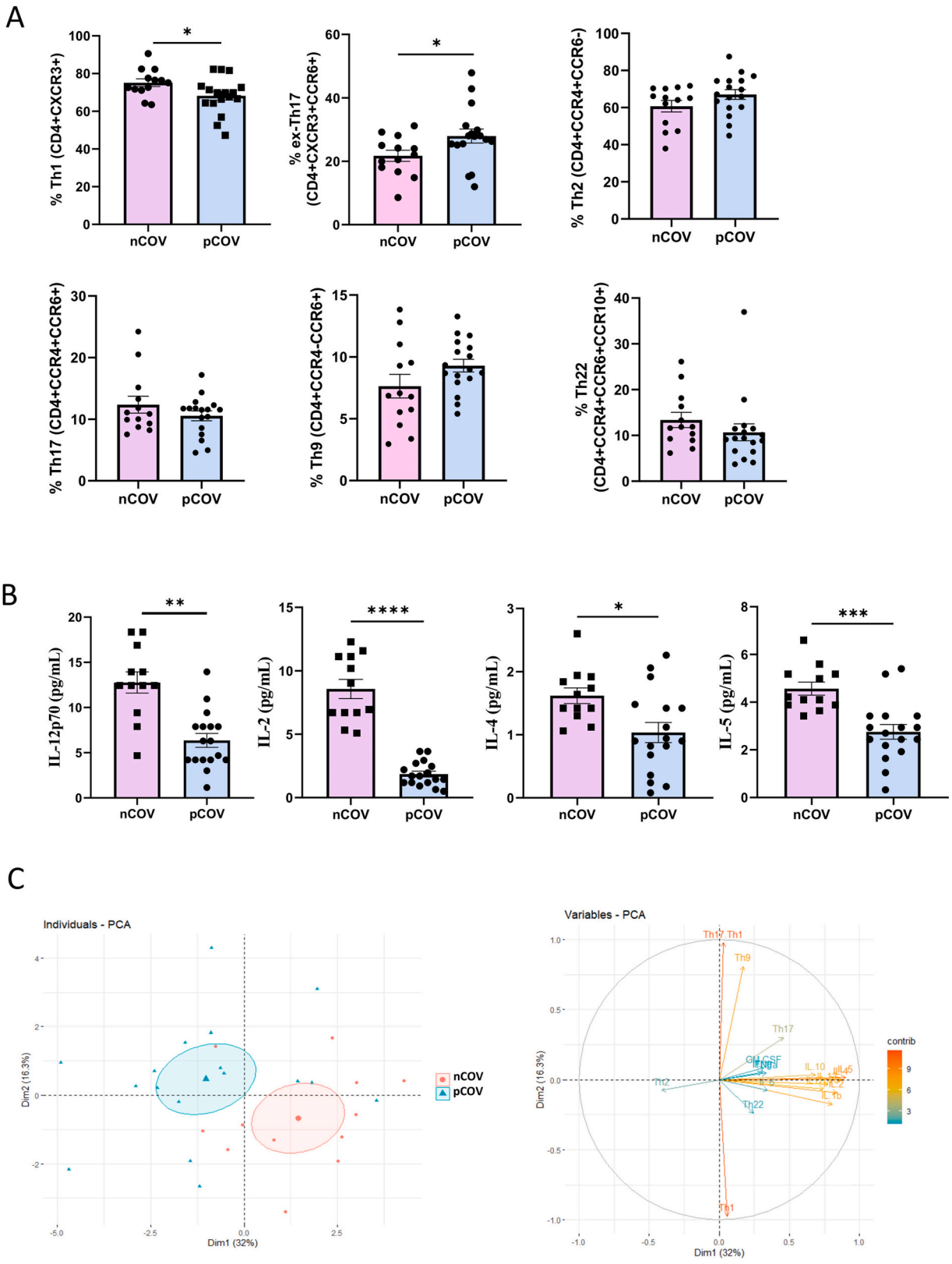

Fig. 1. A–B: Frequency of Th-expressing cells (B) and Th1/Th2 cytokine levels (IL-2, IL-12, IL4 and IL-5) (B) in pregnant (pCOV) and non-pregnant women recovered from COVID-19 (nCOV). Statistical differences were assessed by Mann-Whitney test. C: Principal Component Analysis of the immunological parameters of patients recovered from coronavirus disease 2019 (COVID-19) patients. Score plot of individuals (Left): pregnant (pCOV) and non-pregnant women recovered from COVID-19 (nCOV) are marked in blue and red colours respectively; confidence ellipses are drawn to better appreciate differences between groups. Graph of variables (right): directions and magnitude of each vector indicate the contribution of the corresponding mediator levels to principal component 1 and principal component 2.

to evaluate the Th cell profile, cell frequency and cytokines production in pregnant women recovered from COVID-19.

2. Material and methods

2.1. Subjects

In this study, 17 pregnant women (pCOV) and 12 matched non-pregnant women (nCOV) recovered from COVID-19, were enrolled at the National Institute for Infectious Diseases "L. Spallanzani" in Rome. All subjects showed mild clinical symptoms (82 % paucisyntomatics, 12 % developed COVID-19 pneumonia, 6 % asymptomatic) and no significant differences were reported between pCOV and nCOV (age 35.7 years ±5.1 vs 37.9 years ±3.9; Lymphocytes 1447.1 cells/mmc ±343.7 vs 1666.5 cells/mmc ±418.1).

2.2. Plasma and peripheral blood mononuclear cells isolation

Plasma samples were obtained from the peripheral blood by speed centrifugation from 10 min at 1800 rpm and immediately stored at −80 °C. Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood by density gradient centrifugation (Lympholyte-H; Cedarlane).

2.3. Flow cytometry

The characterization of T helper cell subsets was evaluated on PBMCs by flow cytometry. Briefly, PBMCs were stained for 20 min at 4 °C in the dark with a cocktail of surface antibodies: anti-CXCR3 FITC, anti-CCR10 PE, anti-CCR4 PE-VIO 770, anti-CCR6 APC (MILTENYI), anti-CD3 V500 (BD BIOSCIENCES) and anti-CD4 PACIFIC BLUE (BECKMAN COULTER). After washing (PBS 1X) and fixing (1% paraformaldehyde), cells were acquired using FACSLyric (Becton-Dickinson BD) and analysed BD FACSuite (Becton-Dickinson BD).

2.4. Luminex

The plasma levels of 11 cytokines, chemokines, and growth factors, was evaluated using the Luminex based multiplex bead technology (Human Th1/Th2 11-plex Fixed Panel: GM-CSF, IFN-gamma, IL-1 beta/IL-1F2, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12 p70, IL-13 and TNF-alpha, biotechne; Human IL-17 immunoassay, biotechne). The assay was performed according to manufacturer's instruction. Plates were measured using the Bio-Plex MagPix System and analyzed with the Bio-Plex Manager version 6.0 (BioRad Laboratories, CA, USA).

2.5. Statistical analysis

Principal Component Analysis (PCA) was performed to identify the relevant information and visualize variables with high contribution to the immunological T helper cells profile. Data were analyzed using RStudio software from http://www.rstudio.org with the libraries FactoMineR (for the analysis) and factoextra (for ggplot2-based visualization). Clusters of variables were identified using k-means clustering algorithm. To visualize a correlation matrix in R, we used the corrplot function and generated a heatmap object using correlation coefficients (computed using the Spearman correlation test) as input to the heatmap. The heatmap was produced with the R package heatmap3. Quantitative variables were compared with nonparametric Mann-Whitney test. A P values lower than 0.05 was considered statistically significant. Statistical analyses were performed using GraphPad Prism version 8.0 software.

3. Results and discussion

To verify the differences of Th cell profile in pregnant and non-pregnant women recovered from COVID-19 (pCOV and nCOV respectively), we analysed the frequency of Th1 (CD4+CXCR3+), ex-Th17 (CD4+CXCR3+CCR6+), Th2 (CD4+CCR4+CCR6-), Th17 (CD4+CCR4+CCR6+), Th9 (CD4+CCR4-CCR6+) and Th22 (CD4+CCR4+CCR6+CCR10+) subsets by flow cytometry. As showed in Fig. 1A, we observed an increase of ex-Th17 subset and a parallel decrease of Th1 cells in pCOV respect to nCOV (ex-Th17 median 28 % pCOV vs 22 % nCOV, $p = 0.03$; Th1 median 69 % pCOV vs 75 % nCOV, $p = 0.04$); in contrast, no significant differences were observed in Th2, Th9, Th17 and Th22 subsets.

The cytokines belonging to Th1 (IFN-gamma, IL-2, IL-12 p70 and TNF-alpha), Th2 (GM-CSF, IL-1 beta/IL-1F2, IL-4, IL-5, IL-6, IL-10 and IL-13) and Th17 (IL-17) families were quantified in plasma samples. We found a lower level of Th1 cytokines [(IL-12: median 6.3 pg/mL pCOV vs 12.4 pg/mL nCOV, $p = 0.002$; IL-2: median 1.7 pg/mL pCOV vs 8 pg/mL nCOV, $p = 0.0008$)] (Fig. 1B), suggesting a reduced inflammatory T cells response that may preserve successful pregnancy outcome. The frequency of Th2 cells was not significantly different between the groups (Fig. 1A), but the respective cytokine levels were lower in pregnant respect to non-pregnant women [(IL-4: median 0.9 pg/mL pCOV vs 1.5 pg/mL nCOV, $p = 0.01$; IL-5: median 2.7 pg/mL pCOV vs 4.2 pg/mL nCOV, $p = 0.0001$)] (Fig. 1B). IL-17 was not detected in both pregnant and non-pregnant women, and IFN-γ was comparable between the two groups (median 1.2 pg/mL pCOV vs 1.2 pg/mL nCOV, $p > 0.05$). The decrease of Th2 cytokine levels observed in pregnant women recovered from COVID-19 may have contributed in preventing the cytokine storm. Furthermore, the increase of ex-Th17 cells did not correspond to an increase of their plasmatic products such as IL-17 and IFN-γ, probably to avoid adverse pregnancy outcomes. No significant differences were observed for other cytokines, chemokines and growth factors analysed.

In order to define the major trend inherent to the Th cell profile in pregnant and non-pregnant women recovered from COVID-19, principal component analysis (PCA) was performed. Data showed a segregation of pCOV and nCOV groups with Th1 and ex-Th17 cell subsets, representing the variables with the higher contribution to the variance (Fig. 1C). Interestingly, a possible antimicrobial role of CXCR3 and CCR6 expressing Th17 subset has been proposed in the lung of *M. tuberculosis* infected macaques (Shanmugasundaram et al., 2020). This suggests that the increase of circulating ex-Th17 subset may mirror their recruitment in the infected lung, mediating Th1 and Th17 protective tissue immunity, and can play an important role in controlling SARS-CoV-2 infection. Therefore, the absence of plasmatic IL-17 can be at least partially explained by the recruitment of ex-Th17 in the lung, resulting in a cytokine compartmentalization in the infected tissue. The functional characterization of circulating ex-Th17 cells expanded in pregnant women recovered from COVID-19 needs further evaluations. On the other hand, the lower levels of Th1 response may preserve pregnancy and counteract the parallel increase of protective ex-Th17 subset, avoiding the risk of severe COVID-19. The lack of a control group of pregnant non-COVID-19 women did not allow us to have conclusive data about the impact of pregnancy on Th response during COVID-19. Nevertheless, our data clearly demonstrated that during the recovered phase after COVID-19, the pregnancy shapes a different Th and cytokine profile that could contribute to the definition of a mild clinical phenotype.

In conclusion, our data indicate that the particular adaptation and cytokine regulation of Th cell profile in pregnant women may prevent pregnancy complications and risk of severe outcome during SARS-CoV-2 infection.

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