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CONCISE REPORT-ARTICLE - COVID-19 AND RELATED TOPICS

Cross-reactive cellular, but not humoral, immunity is detected between OC43 and SARS-CoV-2 NPs in people not infected with SARS-CoV-2: Possible role of cT_{FH} cells

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Abstract

Multiple questions about SARS-CoV-2 humoral and cellular immunity remain unanswered. One key question is whether preexisting memory T or B cells, specific for related coronaviruses in SARS-CoV-2-unexposed individuals, can recognize and suppress COVID-19, but this issue remains unclear. Here, we demonstrate that antibody responses to SARS-CoV-2 antigens are restricted to serum samples from COVID-19 convalescent individuals. In contrast, cross-reactive T cell proliferation and IFN-γ production responses were detected in PBMCs of around 30% of donor samples collected prepandemic, although we found that these prepandemic T cell responses only elicited weak cT_{FH} activation upon stimulation with either HCoV-OC43 or SARS-CoV-2 NP protein. Overall, these observations confirm that T cell cross-reactive with SARS-CoV-2 antigens are present in unexposed people, but suggest that the T cell response to HCoV-OC43 could be deficient in some important aspects, like T_{FH} expansion, that might compromise the generation of cross-reactive T_{FH} cells and antibodies. Understanding these differences in cellular responses may be of critical importance to advance in our knowledge of immunity against SARS-CoV-2.

KEYWORDS
COVID-19, cross-reactive immunity, HCoV-OC43, SARS-CoV-2

Abbreviations: COVID-19, Coronavirus disease 2019; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; HCoVs, human coronaviruses; NP, Nucleocapsid protein; S, Spike; IFN-γ, Interferon-gamma; IL17-A, Interleukin 17A; IL-10, Interleukin 10; PBMCs, Peripheral blood mononuclear cells; ACE2, Angiotensin-Converting Enzyme 2; cTFH, Circulating T follicular helper cells; RBD, Receptor binding domain.

1 | INTRODUCTION

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), comprises a set of clinical features varying from asymptomatic to severe symptoms and even death.[1] T/B cell cross-reactive responses between SARS-CoV-2 and the endemic human coronaviruses (HCoVs) HKU1, 229E, NL63, and OC43 have been suggested to influence SARS-CoV-2 immunity and in vitro studies have shown notable T-cell reactivity to SARS-CoV-2 antigens in 25–30% of healthy, noninfected individuals.[2–5] In contrast, cross-reactive humoral immunity against SARS-CoV-2 antigens, principally directed to the S2 domain of the spike glycoprotein, is present in few healthy donor samples.[6,7] The seasonal HCoVs belong to the *Alpha*- (229E, NL63) and *Beta*- (HKU1, OC43) coronavirus genus and are around 30% homologous to SARS-CoV-2 spike or nucleocapsid proteins. Infection with these HCoVs typically causes mild respiratory symptoms and generates short-lived antibody responses that cannot block reinfection only 12 months after infection.[8,9] It has been suggested, but is not known for certain, that the presence of cross-reactive cellular or humoral responses might modulate the development of COVID-19.[10,11] The vast majority of published studies assessing specific SARS-CoV-2 T-cell immune recognition use sets of overlapping 10 amino-acid peptides that span part, or the whole, proteome of SARS-CoV-2. In this study, we directly assessed the SARS-CoV-2 T and B cell responses using full-length recombinant protein antigens in sets of sera and PBMCs collected prepandemic as well as from convalescent COVID-19 patients. Thus, the present work extends knowledge about humoral and cellular immunity to SARS-CoV-2 and investigates immunologic cross-reactivity between coronaviruses from a different point of view.

2 | RESULTS AND DISCUSSION

2.1 | Absence of cross-reactive antibody responses in SARS-CoV-2 unexposed donors

We first determined the serum antibody responses to SARS-CoV-2 and HCoV-OC43 nucleocapsid protein antigens in a cohort of 24 serum samples collected from healthy blood donors between 2010 and 2014. Serum samples from 7 convalescent COVID-19 blood samples were included in these experiments as positive controls. We used OC43 nucleoprotein (NP) as a representative betacoronavirus antigen that was phylogenetically closer to SARS-CoV-2 and demonstrated the highest degree of amino acid sequence overlap among the common endemic coronaviruses (Figure S1). In general, we could detect a wide variety of immunoglobulin responses to OC43 NP antigen in healthy and convalescent samples, ranging from undetectable HCoV-OC43 antibodies to those with medium or high titer antibody responses, perhaps dependent on the B cell capacity and the period elapsed after infection. However, SARS-CoV-2 NP-specific antibodies were only detected in sera from convalescent COVID-19 patients (Figure 1). These serologic observations are consistent with other reports showing little antibody cross-reactivity between SARS-CoV-2 and endemic coronaviruses in healthy prepandemic donors[6,12] and potentially suggest that antibody responses to these viral proteins are independent.

2.2 | SARS-CoV-2 antigen-specific T cell expansion is detected in healthy donor samples

To assess T-cell cross-reactivity between coronaviruses, viral antigen-induced T-cell proliferation was determined in PBMCs collected from the prepandemic healthy donors (B1–B14) available for study. PBMCs from COVID-19 convalescent individuals (C1–C6) were used as positive controls. OC43 and SARS-CoV-2 NPs were used to stimulate T-cells. Additionally, we also evaluated the potential of the SARS-CoV-2 spike (S) protein, directly involved in viral entry,[13] and the SARS-CoV-2 nonstructural protein $3CL^{pro}$, that potently stimulates humoral immunity.[14] $CD4^+$, $CD8^+$, and $CD3^+$ specific T-cell proliferation was assessed by cytometry analysis of CTV dilution (Figure 2(A)). For all antigens and T-cell populations tested, higher levels of proliferation were detected in convalescent patients compared with unexposed donors. Nevertheless, T-cell proliferation after exposure to SARS-CoV-2 antigens was observed for some of the prepandemic donors tested, and strikingly, expanded T cells from these donors mostly belonged to the memory compartment when PBMCs were stimulated either with OC43 or SARS-CoV-2 NPs (Figure 2(B)). In total, we found detectable T cell responses to SARS-CoV-2 NP, S or $3CL^{pro}$ in 5 prepandemic donors (Figure 2(C)). Among them, B3, B7, and B8 demonstrated considerable T cell proliferation against all of these proteins, whereas B1, B4, B6, and B12 made weak responses to at least 1 SARS-CoV-2 protein.

Thus, antigen-specific T-cell proliferation assays support the observation that preexisting SARS-CoV-2 T-cell immunity is present in some unexposed people. Preexisting T-cells responding to SARS-CoV-2 spike and NPs were mainly found in the $CD8^+$ compartment, contrary to what is expected using soluble exogenous proteins as antigens. This observation is interesting as, if assuming cross-reactivity between HCoVs, there is no clear enrichment for presentation through HLA-I alleles within these antigens (Figure S2). In line with this, convalescent individuals also showed similar but stronger trends in T-cell subset proliferation against these antigens, clearly showing the expansion capacity of SARS-CoV-2-specific $CD8^+$ T-cells when stimulated in vitro.[15]

2.3 | Cross-reactive T cells efficiently produce IFN-γ

Cytokine production after antigen-specific or nonspecific restimulation was also measured to gain insight into which $CD4^+$ T cell subsets were involved in the immunity to SARS-CoV-2. To this end, PBMCs from the proliferation assays were rested in IL-2 and then

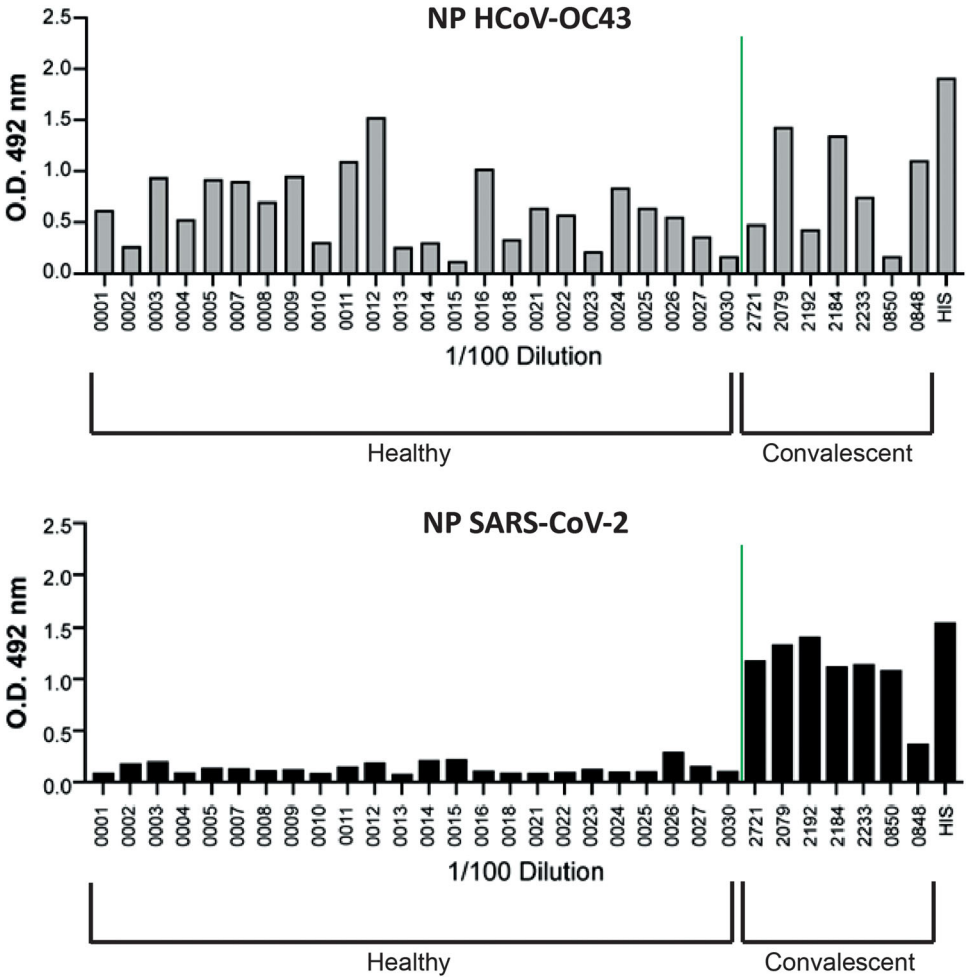

FIGURE 1 Humoral response against HCoV-OC43 and SARS-CoV-2 NP antigens. ELISAs were performed with 0.5 μg/ml of recombinant protein diluted in 0.1 M borate-buffered saline and detected with rabbit anti-human IgG. OD 492 values for OC43 and SARS-CoV-2 NP ELISAs are represented in healthy and convalescent donors

restimulated by exposure to freshly thawed autologous PBMCs pre-pulsed with coronavirus antigens. IFN-γ, IL17-A, and IL-10 T cell production were analyzed in convalescent patients (C1-C6), as well as in some prepandemic donors (B1–B8), that included SARS-CoV-2 antigen responders and nonresponders (in terms of proliferation). After reexposure to the same antigen used in the initial stimulation, we could detect IFN-γ producing T-cells in convalescent samples, but also in some healthy donors, especially in those that expanded against SARS-CoV-2 antigens (Figure 3(A)). Very few IL17-A and IL10 producing cells were observed in these experiments. After restimulation with a different antigen, for example, when cultures stimulated with SARS-CoV-2 $3CL^{pro}$ were restimulated with SARS-CoV-2 NP, very low responses were obtained, showing that T-cell stimulation was antigen specific. When OC43 NP expanded cultures were tested for cytokine production after restimulation with SARS-CoV-2 NP antigen and vice versa, IFN-γ production was consistently triggered in both directions for COVID-19 convalescent donors and those prepandemic donors who had responded in the first round of stimulation. Finally, PBMCs from donors who had T-cells that proliferated on exposure to the SARS-CoV-2 spike protein contained T-cells able to produce IFN-γ after restimulation by recombinant SARS-CoV-2 receptor binding domain (RBD), that mediates binding to the human ACE2 receptor (Figure 3(B)).[13]

These analyses showed that SARS-CoV-2 responding T-cells produce much more IFN-γ than IL-17A or IL-10 and also demonstrated clear evidence for bidirectional T-cell cross-reactivity between HCoV-OC43 and SARS-CoV-2 NPs in pandemic and prepandemic samples. Thus, our work is consistent with those articles that found T-cell cross-reactivity against SARS-CoV-2 peptides with homology to human endemic coronaviruses.[2,3] These data also argue against the idea that coronavirus $CD4^+$ T-cell cross-responses are practically absent and of low avidity,[16] although our observations do suggest a major role for $CD8^+$ subset that, strikingly, was overexpanded in COVID-19 patients in response to OC43 NP (Figure 2(A)). Consistent with previous work, examples of healthy donor T-cell IFN-γ production against RBD were also detected.[3] Conversely, the prepandemic samples with the greatest expansion and IFN-γ production against OC43 NP (donors B3, B7, and B8) also demonstrated reactivity against all SARS-CoV-2 antigens tested (Figure 3(C)), probably indicating a strong and diverse epitope immunity versus HCoV-OC43 that consequently triggers a wider SARS-CoV-2 antigen spectrum.

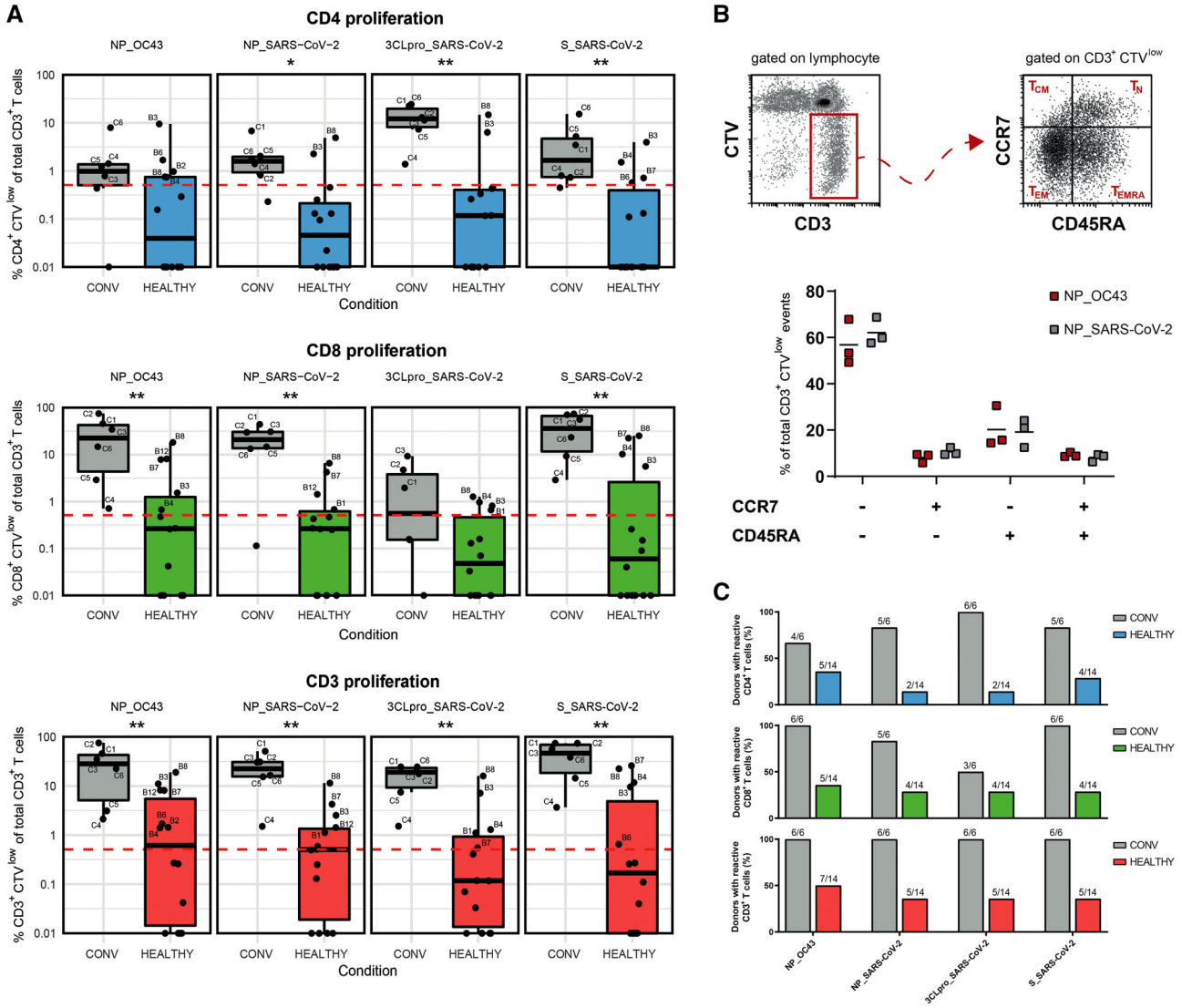

FIGURE 2 T cell proliferation assays. PBMCs were labeled with the cytoplasmic dye Cell Trace Violet (CTV—5 μM) and pulsed with a specific recombinant protein antigen (10 μg/ml) to evaluate the specific proliferation after culture for 6 days. (A) $CD4^+$, $CD8^+$, and $CD3^+$ T cell proliferation were assessed by cytometry analysis for CTV dilution within the total $CD3^+$ population. Samples with proliferation values above 0.5% are labeled. Two-sided Mann–Whitney test ($^*p < 0.05$, $^{**}p < 0.01$). (B) Expression of CCR7 and CD45RA markers within the $CD3^+CTV^{low}$ events detected in healthy samples responding to both OC43 and SARS-CoV-2 NP. (C) Percentage of convalescent or healthy donors with detectable $CD4^+$, $CD8^+$ or $CD3^+$ proliferation against the different antigens

2.4 | OC43-reactive circulating T follicular helper cells (cT_{FH}) appear low in healthy people

The previous experiments indicated that SARS-CoV-2 reactive T cells could be found in PBMCs collected prepandemic, including specific T cell cross-reactivity between SARS-CoV-2 and HCoV-OC43 NPs antigens. Nevertheless, antibodies able to bind SARS-CoV-2 NP antigen were not detected in serum samples from unexposed donors. As T follicular helper cells are an important link between cellular and humoral immunity,[17] the responsiveness of antigen-specific cT_{FH} ($CD3^+CD4^+PD\text{-}1^{high}CXCR5^+$) cells was analyzed by assessing the induction of expression of the CD69 marker in PBMCs from either 10 COVID-19 convalescent patients incubated with SARS-CoV-2 NP or 21 prepandemic individuals incubated with OC43 NP. Overall, samples from convalescent COVID-19 donors demonstrated clear $CD4^+$ and cT_{FH} responses to SARS-CoV-2 NP, whereas healthy samples triggered only mild $CD4^+$ activation and a limited cT_{FH} response to OC43 NP (Figures 4(A) and 4(B)). The proportion of cT_{FH} cells in PBMCs upon stimulation was highly increased in these COVID-19 convalescent donors, and marked differences in cT_{FH} responses were observed between COVID-19 samples suffering from mild or severe disease (Figure S3), consistent with data reporting that SARS-CoV-2-specific antibody titers are higher in patients that have experienced severe disease.[18,19] Moreover, for the majority of COVID-19

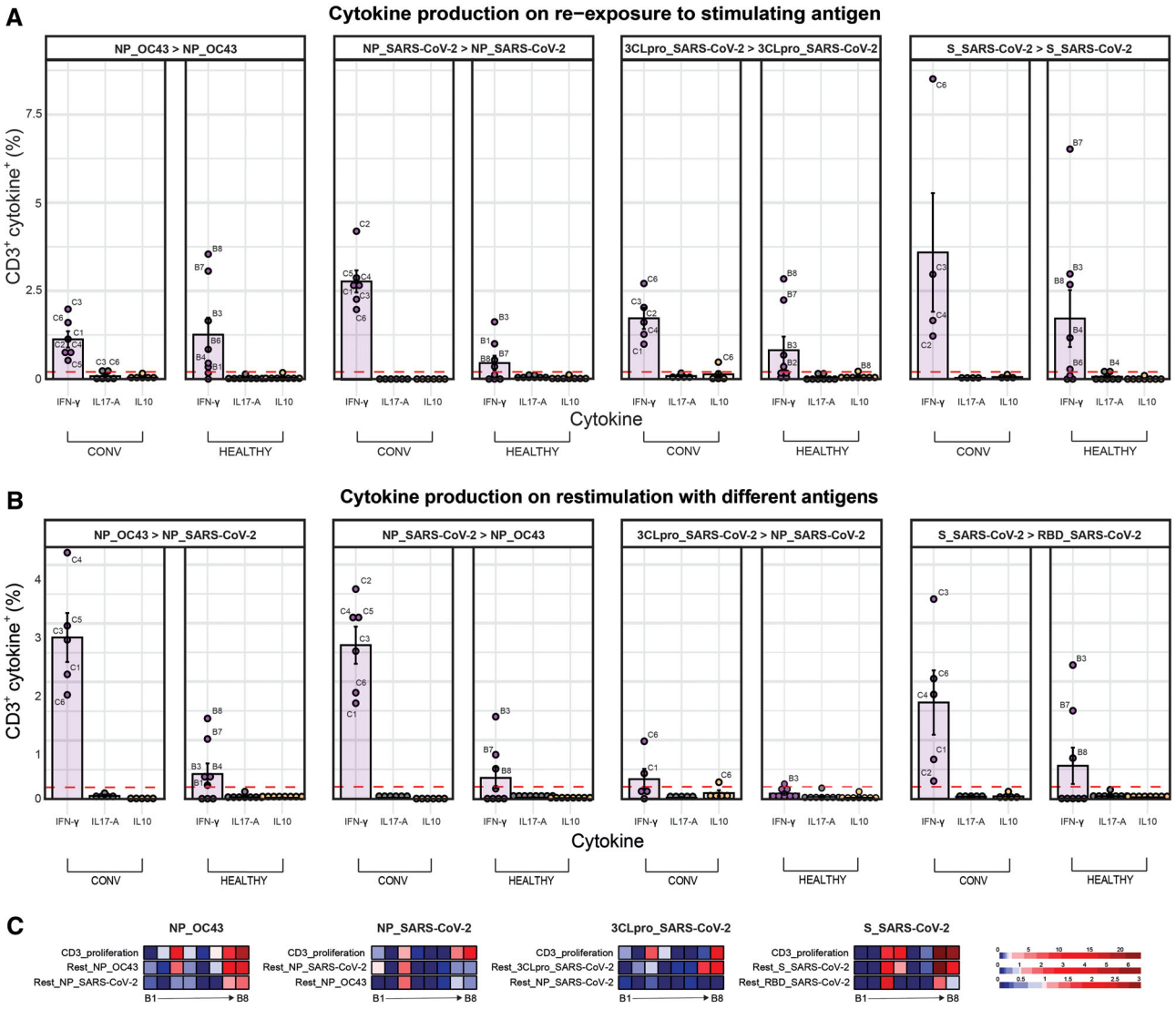

FIGURE 3 T cell cytokine production assays. Proliferating PBMCs were rested in culture with IL-2 (5 U/ml) and then restimulated by exposure to freshly thawed autologous PBMCs pre-pulsed with different coronavirus antigens (10 μg/ml). (A) IFN-γ, IL17-A, and IL-10 T cell production were analyzed after antigen reexposure and (B) after restimulation with different antigens. Samples with positive events above 0.2% were labeled. Bar plots showing mean values ± SEM. (C) Heatmaps showing tendencies of $CD3^+$ proliferation and IFN-γ production after restimulation with the same or different antigens for B1–B8 healthy samples

convalescent patients, levels of SARS-CoV-2 NP-specific antibodies and cT_{FH} cells correlated (Figure 4(C)), supporting the key role of these cells in humoral immunity.

It is conceivable that the presence of cT_{FH} migrating from secondary lymphoid tissue after recent infection might contribute to the higher frequencies of SARS-CoV-2 cT_{FH} cells detected in the COVID-19 convalescent patients; however, humoral immunity to seasonal coronaviruses like OC43 is short lived and reinfections are common, suggesting that cT_{FH} would likely also be frequent in our panel of healthy donors, many of whom have considerable titers of anti-OC43 NP antibodies (Figure 1). To try and assay antigen-specific cT_{FH} reactivity when the subject is not undergoing an active immune response, we compared HCoV cT_{FH} reactivity and cross-reactivity to OC43 and SARS-CoV-2 NPs with cT_{FH} responses made by healthy donors against the M1 antigen of another seasonal respiratory virus, influenza. In these experiments, the NP-specific cT_{FH} responses observed in COVID-19 convalescent donors were markedly higher than the responses of prepandemic donors to either OC43 or SARS-CoV-2 NPs (Figure 4(D)), supporting the idea that $CXCR5^+CD4^+$ from COVID-19 patients are enriched in migrating cT_{FH}. Nevertheless, in healthy samples, the frequency of SARS-CoV-2 and OC43 NP-responsive cT_{FH} among $CD4^+$ T cells responding to those antigens was significantly lower than the frequency of cT_{FH} detected after stimulation with the Influenza A antigen (Figure 4(D)), consistent with the suggestion that cT_{FH} responses to the coronavirus NP antigens are comparatively weak.

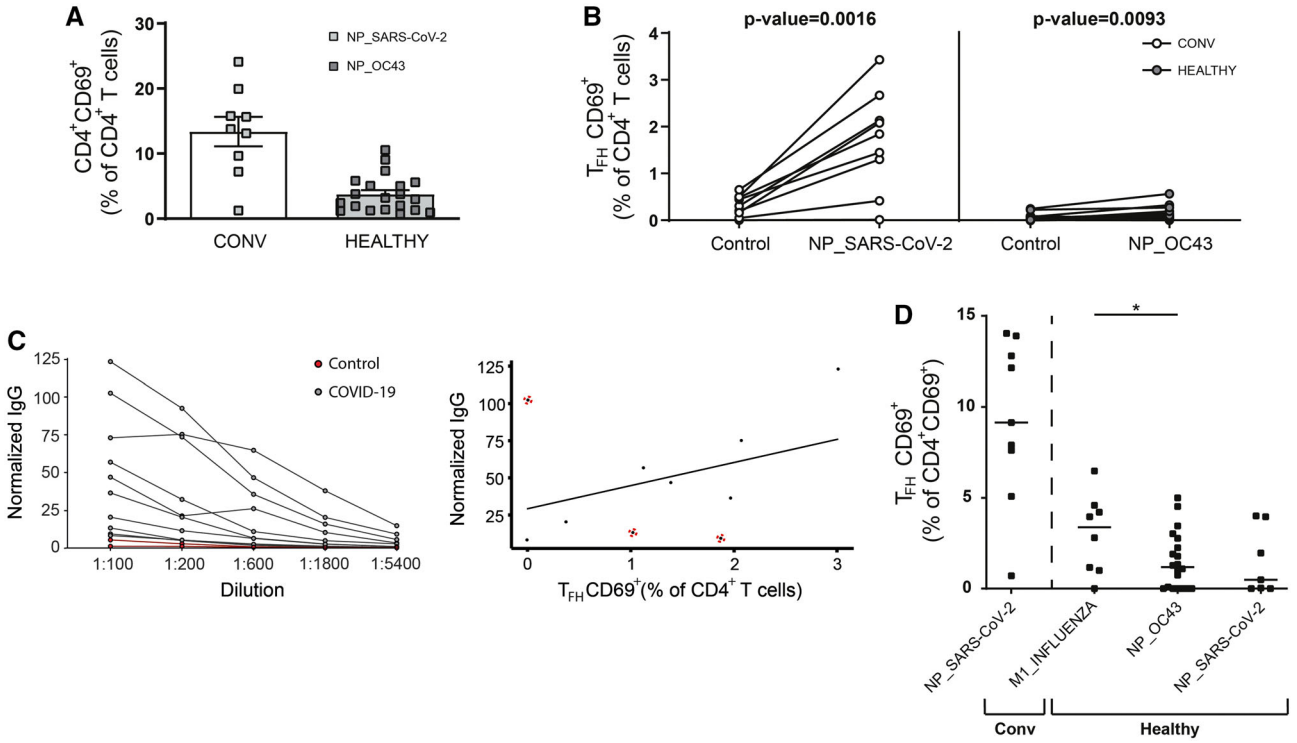

FIGURE 4 cT_{FH} cell activation in response to SARS-CoV-2 and OC43 NP antigens. PBMCs were cultured at 5×10^5 cells/well with the selected antigen at 10 μg/ml during 48 h. Early antigen response was detected through flow cytometry. (A) Expression of CD69 in $CD4^+$ T-cells from stimulated convalescent or healthy samples. (B) Paired plots comparing the proportion of $CD69^+$ expressing cT_{FH} cells within the total $CD4^+$ subpopulation in control and stimulated conditions. Paired sample *t*-test (*p* values are shown). (C) An ELISA directed against SARS-CoV-2 NP was performed in COVID-19 samples for different dilutions of sera, detecting titers of IgG that were normalized to the values obtained for a great responder (C3 sample). The maximum IgG level and the presence of $CD69^+$ T_{FH} cells in COVID-19 samples are represented in a scatter-plot. Data points outside the 95% confidence interval are highlighted in red circles. (D) cT_{FH} response within the $CD4^+CD69^+$ subset was analyzed in healthy samples that responded to SARS-CoV-2 NP or Influenza A H1N1 M1 proteins. Plot showing the magnitude of cT_{FH} activation in samples responding to OC43 NP, SARS-CoV-2 NP, and Influenza A H1N1 M1. Two-tailed *t*-test (*$p < 0.05$). Bar plots showing mean values ± SEM

Collectively, our results suggest the hypothesis that the absence of SARS-CoV-2 cross-reactive humoral immunity could reflect some qualitative aspect of the T-cell response involving the T_{FH}-cell subset. Comparison of the levels of IgG-specific for SARS-CoV-2 $3CL^{pro}$, NP or RBD with the T-cell responses of these same COVID-19 convalescent donors revealed shared patterns in humoral and cellular responses, especially when IgG levels were analyzed (Figure S4). These data suggest that SARS-CoV-2 antigen-specific T_{FH} cells, that aid B cell responses, were generated efficiently in these patients, indeed the magnitude of the T_{FH} response made by SARS-CoV-2 patients is comparable to that seen in vaccination trials for HIV or Ebola.[20,21] Moreover, strikingly, recent studies showed that SARS-CoV-2 mRNA vaccination induces cT_{FH} in blood and lymph nodes that are maintained at constant levels for at least 6 months.[22] In contrast, the levels of OC43 NP-specific $CD4^+$ cT_{FH} activation noted in PBMCs from OC43 NP responding prepandemic donors were lower than that induced by the M1 antigen from another seasonal virus like Influenza, and consequently, potential cT_{FH} cells cross-reactive with SARS-CoV-2 NP were rather insignificant. Interestingly, Meckiff et al.[23] also described a marked in vitro expansion of noncytotoxic T_{FH} cells in convalescence that was not found in T-cells from healthy donors that displayed cross-reactivity between SARS-CoV-2 and other coronaviruses. These differences in T_{FH} responses may contribute to explain, at least in part, why antibody cross-reactivity is generally absent in SARS-CoV-2 unexposed samples. An interesting idea that could be explored in future experiments is whether this paucity of circulating T_{FH} cells reflects polarization of the T-cell response after frequent, recurrent seasonal coronavirus infections that may also underlie the typical antibody waning observed.[8]

3 | LIMITATIONS OF STUDY

The present report is clearly limited in sample size and number of antigens studied. Moreover, for obvious reasons, T cell subpopulations are only assessed in peripheral blood through the detection of general or classical markers and not in secondary lymphoid tissues. However, the use of full-length antigens in prolonged assays may represent a more realistic approach even though clonotype specificities are not determined, and in this sense, the results are clear in highlighting that T_{FH} cells can be a key intermediary population that can influence the development of cross-reactive antibodies in SARS-CoV-2

unexposed people. It is possible that COVID-19 cT_{FH} cells may be more frequent in peripheral blood because of recent infection, while detecting cT_{FH} in healthy human donor peripheral blood tends to be more difficult unless they have undergone a recent response to HCoV. Further studies, using more antigens from other coronaviruses, would help to confirm that T_{FH} cells are underrepresented in the immune memory against common endemic coronaviruses, and importantly, would contribute to determine if this trend can also be present in reinfections with SARS-CoV-2.

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DISCLOSURES

J. M. R. F., J. M. C., H. T. R., and M. V. G. are inventors on the European patent "Assay for the detection of the Cys-like protease (Mpro) of SARSCoV-2" (EP20382495.8). The rest of the authors declare no potential conflict of interest.

AUTHORSHIP

A. F. G. J. performed experiments and analyzed data. Y. C. M., D. F. S., and J. M. R. F. prepared reagents and performed experiments. P. M. F. and F. S. M. selected and clinically characterized patients. F. S. M., M. V. G., and H. T. R. obtained financial support, conceived, and designed the study. A. F. G. J. and H. T. R. wrote the manuscript with revisions from all authors.

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SUPPORTING INFORMATION

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