Persistent SARS-CoV-2 infection in asymptomatic young adults

Dear Editor,

While most of the patients infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) cleared the virus within a few weeks of infection, some people have a persistent infection or persistent shedding viral RNA in the long term. The persistent detection of viral RNA in clinical specimens is unlikely to reflect either relapse or reinfection in most cases because the replication-competent virus is generally not recoverable after ten days following symptom onset in mild to moderate cases of COVID-19 and after 20 days in severe or immunocompromised cases. However, the evidence from several studies revealed the shedding of infectious virus up to 70–105 days after the first positive detection in immunocompromised patients, and viruses sampled at different time points showed different dominant genetic mutations. Moreover, persistent SARS-CoV-2 infection within immunocompromised individuals could accumulate more mutations during convalescent plasma therapy with evidence of reduced susceptibility to neutralizing antibodies. While rare SNVs were observed for B.1 viruses, a high mutational load in the B.4 viruses enriched nonsynonymous SNVs (Supplementary Fig. 2a, b). We observed a significantly lower cycle threshold (Ct) value of the B.1 viruses than the B.4 viruses (Supplementary Fig. 2c, p = 0.00204), consistent with the previous study. While rare SNVs were observed for B.1 viruses, a high mutational load in the B.4 viruses enriched nonsynonymous SNVs in spike gene compared to the open reading frame (ORF) 1a and ORF1b genes (Supplementary Table 2).

To further evaluate the difference of antibody response between PCs and NPCs after the first positive RT-PCR detection, we measured spike (S) and receptor-binding domain (RBD) binding IgG antibodies and pseudovirus neutralizing antibodies (NAb) (Supplementary methods). We found that both PCs and NPCs displayed low endpoint titer (<1000) of S- and RBD-IgG antibodies and no obvious changes of geometric mean endpoint titer throughout infection over time (Fig. 1b). For comparison, serum samples with similar time points (≤7, ≤14, 15–21, and 28–42 days and 2–3 months post-symptom onset (PSO)) from 20 symptomatic cases (referred to SCs) were included (Supplementary Table 1). We found that both PCs and NPCs had significantly lower geometric mean endpoint titer of anti-S and anti-RBD IgG antibodies than SCs (Fig. 1b). A pattern of NAb that closely resembled anti-S and anti-RBD IgG responses was observed, but no significant difference was observed with SCs three months PSO (Fig. 1b). We observed a significant correlation between NAb and binding IgG antibodies (Supplementary Fig. 3). As expected, we observed a minimal reactivity of anti-S or anti-RBD IgG antibodies in the serum of healthy controls (HCs), but no serum samples from HCs tested positive for NAb (Supplementary Fig. 4). These findings indicate that asymptomatic cases induced a relatively low antibody response related to SCs.

We next measured the SARS-CoV-2-specific memory B cells secreting IgG and T cells secreting IFN-γ using an enzyme-linked immunospot (ELISpot) assay (Supplementary methods). The numbers of RBD-specific IgG memory B cells were significantly higher among cases than HCs (Fig. 1c). However, no significant differences were observed among case groups (Fig. 1c). RBD-specific IgG memory B cells were detected in 4 (66.7%) of 6 PCs two months after the first RT-PCR positive detection, 22 (91.7%) of 24 NPCs, and 15 (75%) of 20 SCs three months after infection (Fig. 1c), while we observed more specific T cells secreting IFN-γ in NPCs and SCs than HCs, 6 PCs showed a similar mean number of T cells secreting IFN-γ with HCs (Fig. 1d). Regarding the responder of subjects to SARS-CoV-2, we observed T cell response detectable in 83.3% (5/6), 66.7% (16/24), and 75% (15/20) of the six PCs, 24

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NPCs, and 20 SCs, respectively (Fig. 1d). While there are significant correlations between anti-S and -RBD IgG antibodies and the numbers of RBD-specific IgG$^+$ memory B cells, an absence correlation between the numbers of T cells secreting IFN-γ and antibody levels (Supplementary Fig. 5a, b). These results indicate that most PCs and NPCs developed and maintained specific B and T cells two months after the first RT-PCR positive detection.

We further assessed immune status in PCs and NPCs two months after first positive RT-PCR detection by immunophenotyping B cells, monocytes, NK cells, and T cells using flow cytometry (Supplementary methods). We observed a decreased frequency of B cells in both NPCs and SCs than HCs, but NPCs showed a higher frequency than SCs, of which SCs had more plasma cells than HCs (Fig. 1e). The CD14$^+$CD16$^+$ proinflammatory intermediate monocytes level was significantly decreased in NPCs compared to SCs and HCs (Fig. 1e). A significantly higher frequency of CD56$^+$CD16$^+$ NK cells in SCs than HCs was observed (Fig. 1e). While the composition of overall T cells, CD4$^+$, and CD8$^+$ T cells were similar between all groups (Fig. 1e), there was a marked increase of Tregs in PCs and NPCs compared to SCs and HCs (Fig. 1e). On the contrary, both PCs and NPCs have significantly reduced HLA-DR$^+$CD38$^+$ CD4 and CD8$^+$ T cells compared with SCs and HCs and lower frequency of terminal effect and/
or central memory CD4 T cells (Fig. 1e). In addition, we observed a significant negative correlation between the percentage of CD4 Tregs and HLA-DR∗CD38∗CD4+ T cell (r = −0.41, p = 0.002) and CD8+ T cells (r = −0.41, p = 0.003) (Fig. 1f). These findings indicated a dysregulation of Treg and activated CD4+ and activated CD8+ T cells even recovered from the disease.

In summary, despite a small cohort sample size of PCs, failure isolation of the virus, and cross-section assessment of immune status in asymptomatic cases two months after first positive RT-PCR detection, our data indicate that certain PCs shed infectious viruses in the long term. Moreover, our data suggest that PCs had relatively low antibody response but considerable memory B and T cells and upregulation of CD4 Tregs two months after infection. Further studies are needed to assess the kinetics of memory B and T cells and upregulation of CD4 Tregs two months after infection.

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AUTHOR CONTRIBUTIONS
M.J.M., W.C.C., and H.B.S. conceived the study. S.F.Q. and H.B.L. collected samples and performed molecular testing. M.J.M. and L.Y. performed immunological testing; M.N., M.J.M., W.C.C., and H.B.S. performed molecular testing, M.J.M. and L.Y. performed immunological testing; M.N., and R.Z.Y. analyzed the sequence data; M.J.M., Q.S.F., and M.N. drafted the manuscript. All authors reviewed and approved the final manuscript.

ADDITIONAL INFORMATION
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