Long Term Disruption of Cytokine Signalling Networks are Evident Following SARS-CoV-2 Infection

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To the Editor,

The current pandemic caused by the SARS-CoV-2 virus has so far infected more than 130 million people worldwide, resulting in approximately 3 million deaths. While the current clinical and public health priorities are designed to limit severe acute and fatal episodes of the disease, and to quickly roll out vaccines to the general population, it has become apparent that there may also be significant detrimental long-term effects following SARS-CoV-2 infection that impact daily functioning and quality of life[1]. The mechanisms underpinning the post-acute sequelae of SARS-CoV-2 infection's long-lasting symptoms can include direct effects of the infection (e.g. endothelial damage, lung fibrosis) or indirect effects associated with changes in the microbiome or abnormalities in inflammatory and immune signalling pathways stimulated by the infection[2,3].

In order to examine the potential long-term immune changes that occur following elimination of the primary infection, we examined the levels of 52 cytokines and growth factors (using MSD multiplex kits) in the serum of patients that attended follow-up post-COVID infection clinics at Cork University Hospital, Cork, Ireland

(The Clinical Research Ethics Committee of the Cork Teaching Hospitals approved this study and all patients provided informed consent). All patients had been hospitalised for PCR-proven SARS-CoV-2 infection (median in-patient stay of 5.5 days, range 1 day to 24 days) during the first wave of the pandemic in Ireland (March-May 2020). 38 serum samples were obtained from 24 patients (median age 53.5 years, 11 female) at 3-9 months following hospital discharge. Clinical severity ranged from mild to critical during hospitalisation and the most common symptoms at follow-up clinics were fatigue and/or dyspnoea (supplementary Table S1). Sera obtained prior to the pandemic from 29 healthy volunteers (median age 43.2 years, 14 female) were analysed in parallel.

Of the 52 analytes measured, 19 were significantly elevated in post-COVID patient sera compared to healthy controls (supplementary Table S2). These 19 mediators are illustrated as dot plots in Figure 1 and Figure 2. One group of mediators, c-reactive protein (CRP), serum amyloid A (SAA), Interleukin-1 receptor antagonist (IL-1RA), IL-6, IL-8, IL-15, IL-16, monocyte chemotactic protein (MCP)-1 and MCP-4, can be broadly categorised as being associated with ongoing inflammatory responses (Figure 1a)[4]. These mediators remained as elevated in samples taken 6-9 months following hospital discharge as those levels observed 3-6 months following discharge ($p<0.05$ versus controls, ANOVA). A second group of mediators, vascular endothelial growth factor (VEGF-A), soluble tyrosine-protein kinase receptor Tie-2 (Tie-2), soluble intercellular adhesion molecule (ICAM-1) and basic fibroblast growth factor (bFGF), can be generally associated with endothelial dysfunction, remodelling and angiogenesis (Figure 1b)[5]. The remaining elevated mediators are associated with patterns of lymphocyte polarisation. Elevated IL-4, macrophage-derived chemokine (MDC) and thymic stromal lymphopoietin (TSLP) sera levels indicate activation of T_H2 responses (Figure 2a), while IL-17A, macrophage inflammatory protein (MIP)-3α and IL-12/23p40 indicate ongoing T_H17 activity (Figure 2b). Other indicators of T_H2-associated activities are just outside statistical significance (IL-5, $p=0.06$; supplementary Table S2). While T_H1 responses are well described to be upregulated during acute infection[6], the levels of these mediators (e.g. IFN-γ, IP-10) decrease following elimination of the virus and are at control levels in our cohort of post-COVID patients (supplementary Table S2).

Our data suggests that there are long term immunological consequences following SARS-CoV-2 infection, at least in those that had acute symptoms severe enough to require hospitalisation. While the relatively low number of patients included in our study at this stage does not allow us to perform subgroup analysis, it is possible that these immune mediators may associate with clinically meaningful disease variables and ultimately may be of therapeutic value, if findings are replicated in future studies. Of particular interest is the elevation in T_H2-associated mediators. Could this response be a component of the mucosal repair mechanisms that occur following viral damage, or does this indicate new T_H2-associated pathological immune activity that might underpin an increased risk of developing allergy or asthma? Clearly the potential immune mechanisms underpinning the emerging post-COVID clinical entities will become increasingly more important to understand as the health care systems adapt to caring for large numbers of COVID-19 survivors during the coming months and years.

Figure Legends

Figure 1. Proinflammatory and Endothelial Mediators in Post-COVID Patients.

Proinflammatory mediators (a) and angiogenesis-associated factors (b) are elevated in sera from patients 3-6 months and 6-9 months post-COVID hospital discharge compared to levels in sera from healthy controls. Using ANOVA, the differences are statistically significant for CRP ($p=0.018$), SAA ($p=0.007$), IL-6 ($p=0.001$), IL-8 ($p=0.001$), MCP-1 ($p=0.040$), MCP-4 ($p=0.017$), IL-1RA ($p=0.001$), IL-15 ($p=0.031$), IL-16 ($p=0.034$), VEGF-A ($p=0.011$), Tie-2 ($p=0.046$), sICAM-1 ($p=0.040$) and bFGF ($p=0.001$). Results are illustrated on a log scale and the mean +standard error are indicated for each group.

Figure 2. T_H2 and T_H17 Cytokines in Post-COVID Patients.

Sera from patients 3-6 months or 6-9 months post-COVID hospital discharge are significantly different compared to healthy controls for T_H2 cytokines (a) and T_H17 cytokines (b). Using ANOVA, the differences are statistically significant for IL-4 ($p=0.035$), MDC ($p=0.003$), TSLP ($p=0.003$), IL-17A ($p=0.006$), MIP-3α

(p=0.004) and IL-12/23p40 (p=0.001). Results are illustrated on a log scale and the mean + standard error are indicated for each group.

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