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LETTER

Mast cell activation is associated with post-acute COVID-19 syndrome

To the Editor,

As of July 2021, over 30-million Americans have recovered from documented COVID-19 infection, and prevalence studies suggest up to twice as many may be undocumented.[1] For the survivors of COVID-19, chronic morbidity is common, with reports of fatigue, brain fog, body aches, and loss of smell lasting for months following acute symptoms.[2] These persistent symptoms have been referred to as post-acute sequelae of COVID-19 (PASC), post-acute COVID-19 syndrome, long-COVID, and "long-hauler syndrome." The etiopathogenesis of PASC remains unclear, but inflammation may play a role[3,4] along with metabolic disturbances[5] and autoantibodies.[6]

TABLE 1 Patient Characteristics

Patient characteristics	PASC[a] $n = 13$	PAAC[b] $n = 13$	Healthy controls $n = 20$	P-value[c]
Age, median (range)	49 (24–73)	48 (22–59)	36 (18–51)	0.5670
Female sex, *n* (%)	12 (92%)	11 (85%)	6 (30%)	1.0000
Race				
Black, *n* (%)	1 (8%)	0 (0%)	0 (0%)	1.0000
Caucasian/non-Hispanic, *n* (%)	12 (92%)	11 (85%)	20 (100%)	1.0000
Hispanic, *n* (%)	0 (0%)	2 (15%)	0 (0%)	1.0000
SARS-CoV-2 ELISA IgG, median (range)	4.3 (0.3–11.7)	3.8 (1.2–6.7)	N/A[d]	0.2434
Days between positive PCR test and serum sample collection, median (range)	62 (39–305)	34 (22–322)	N/A[d]	0.1851
Self-reported long-term symptom, *n* (%)	13 (100%)	0 (0%)	N/A[d]	N/A
Fatigue	11 (85%)	0 (0%)		
Body aches	6 (46%)	0 (0%)		
Change or loss in taste/smell	6 (46%)	0 (0%)		
Anxiety	4 (31%)	0 (0%)		
Shortness of breath	4 (31%)	0 (0%)		
Brain fog	3 (23%)	0 (0%)		
Headaches	3 (23%)	0 (0%)		
Sore throat	2 (15%)	0 (0%)		
Tachycardia	2 (15%)	0 (0%)		
Anemic	1 (8%)	0 (0%)		
Dyspnea	1 (8%)	0 (0%)		
Joint pain	1 (8%)	0 (0%)		
Insomnia	1 (8%)	0 (0%)		

Abbreviations: PAAC, post-acute asymptomatic of COVID-19; PASC, post-acute sequelae of COVID-19.

[a]PASC patients were defined as SARS-CoV-2 negative with persistent symptoms for at least ~1 month after confirmed positive infection.

[b]PAAC patients were defined as SARS-CoV-2 negative with no symptoms after confirmed positive infection.

[c]Unpaired two-tailed t test between PASC vs PAAC performed on numerical values; Fisher's exact test performed on proportions.

[d]Data are not collected as part of study.

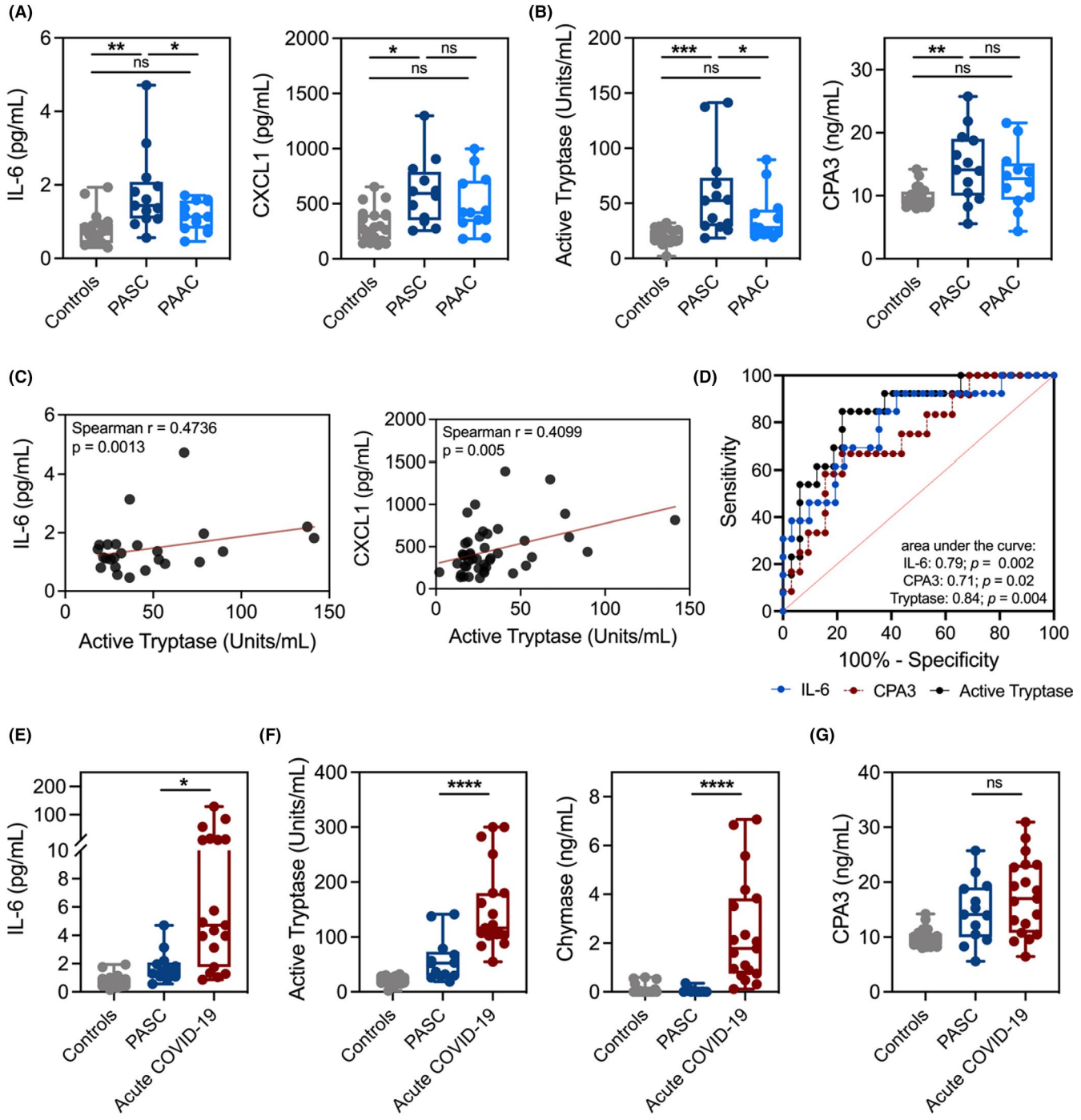

FIGURE 1 PASC patient sera display a distinct profile of elevated inflammatory cytokines and mast cell-derived proteases. (A–B) Cytokines, chemokines, or mast cell-derived proteases in sera from symptomatic PASC (dark blue; $n = 13$), PAAC (light blue; $n = 13$), or control (gray; $n = 19$–20) groups. (C) Spearman correlations for levels of active tryptase, IL-6 and CXCL1 from panels A, B. (D) ROC curves for active tryptase, CPA3, and IL-6 using PASC versus PAAC and controls. (E–G) Levels of cytokines and mast cell-derived proteases in sera from PASC patients (light blue; $n = 13$), acute COVID-19 patients (red; $n = 19$), or controls (gray; $n = 19$–20). $^{*}P < 0.05$;$^{**}P < 0.01$;$^{***}P < 0.001$;$^{****}P < 0.0001$; ns=not significant; PAAC, post-acute asymptomatic COVID-19; PASC, post-acute sequelae of COVID-19

Recently, our group and others identified evidence of mast cell (MC) activation in sera and lung tissue in patients with acute COVID-19 infection.[7] Whether this activation is persistent and contributes to the morbidity associated with PASC is unknown. To evaluate systemic inflammation and MC activation in PASC, we obtained serum from 13 adults with symptomatic PASC and a history of positive RT-PCR for SARS-CoV-2 between 39 and 305 days prior to collection (Table 1). The most reported symptoms among PASC patients were fatigue (84%), body aches (46%), loss of taste/smell (46%), anxiety (31%), shortness of breath (31%), brain fog (23%), and headaches

(23%). To control for inflammatory mediators attributable to acute SARS-CoV-2 infection, we obtained serum from age-matched post-acute asymptomatic COVID-19 (PAAC) patients with a history of positive RT-PCR for SARS-CoV-2 between 22 and 322 days prior to collection, as well as healthy controls (Table 1). We evaluated 11 mediators previously implicated in COVID-19 systemic inflammation.[8] Among the screened mediators, IL-6 and CXCL1 were significantly elevated in PASC sera compared to controls, whereas no significant difference was detected in PAAC sera relative to controls (Figure 1A). Notably, IL-6 levels were also significantly higher in sera from the PASC cohort compared to PAAC (Figure 1A). The following mediators showed no difference between PASC, PAAC, and control: IL-8, TNF, CCL2, CCL3, IL-17A, IL-33, and VEGF (Figure S1A–B). Next, we assessed levels of MC-derived proteases to evaluate MC activation. Active tryptase levels were significantly elevated in PASC sera compared to PAAC and heathy controls, highly suggestive of systemic MC activation (Figure 1B). Carboxypeptidase A3 (CPA3) levels were significantly elevated in PASC, but not PAAC sera compared to healthy controls (Figure 1B). In contrast, chymase levels were not significantly different across these populations (Figure S1C).

To determine whether the MC activation was associated with elevated IL-6 and CXCL1, we performed correlation analyses using sera levels of MC proteases from PASC, PAAC, and controls. Active tryptase levels showed a weak but significant correlation with both IL-6 and CXCL1 levels, whereas CPA3 levels demonstrated modest association (Figure 1C, Figure S1D). To assess the utility of serum IL-6 along with MC proteases as a diagnostic test for PASC, we assessed receiver operating characteristics for the outcome of PASC vs. PAAC + control. Notably, the active tryptase level was superior with an area under the curve of 0.84 (Figure 1D).

To further characterize the inflammatory profile identified in PASC patient sera, we compared cytokine, chemokine, and MC protease levels against sera from a previously published cohort of acute COVID-19 patients.[7] Sera from symptomatic PASC patients displayed significantly reduced inflammatory cytokines and chemokines compared to sera from acute COVID-19 patients (Figure 1E, Figure S2). Levels of active tryptase and chymase were also significantly lower in PASC patients compared to acute COVID-19 patients (Figure 1F). Notably, CPA3 levels in the serum were not significantly different between PASC and acute COVID-19 patients (Figure 1G), suggesting CPA3 levels may remain similarly elevated post-acute infection.

Taken together, our findings support a potential role for immune dysfunction associated with MC activation in a subgroup of patients with PASC. Findings from this study also suggest that MCs are differentially activated in acute SARS-CoV-2 infection compared to PASC. Additional studies are needed to determine if these differences are based on distinct populations of activated MCs or local environmental cues. Interestingly, IL-6 has been shown to increase MC proliferation and induce a more reactive phenotype[9] providing a possible link between elevated IL-6 levels and MC activation in PASC. While it remains unclear if MC activation is causative in PASC or simply a consequence, larger longitudinal studies to validate our findings and assess the natural history are critical. Additional limitations of our study include using unmatched healthy controls and lack of medical history from PASC and PAAC patients. Importantly, our findings highlight MCs as potential therapeutic targets for patients with PASC, which could be targeted with agents that (1) reduce MC-derived mediators,[10] (2) engage inhibitory receptors,[11] or (3) attenuate inflammation.[12]

KEYWORDS

mast cells, COVID, inflammation, innate immunity, long covid

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CONFLICT OF INTEREST

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