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BRIEF REPORT

Low Prevalence of Interferon α Autoantibodies in People Experiencing Symptoms of Post–Coronavirus Disease 2019 (COVID-19) Conditions, or Long COVID

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Interferon (IFN)–specific autoantibodies have been implicated in severe coronavirus disease 2019 (COVID-19) and have been proposed as a potential driver of the persistent symptoms characterizing "long COVID," a type of postacute sequelae of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. We report that only 2 of 215 participants with convalescent SARS-CoV-2 infection tested over 394 time points, including 121 people experiencing long COVID symptoms, had detectable IFN-α2 antibodies. Both had been hospitalized during the acute phase of the infection. These data suggest that persistent anti-IFN antibodies, although a potential driver of severe COVID-19, are unlikely to contribute to long COVID symptoms in the postacute phase of the infection.

Keywords. COVID-19; autoimmunity; long COVID; post-COVID conditions; postacute sequalae of SARS-CoV-2 infection.

Postacute sequelae of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (PASC) or post–coronavirus disease 2019 (COVID-19) conditions include incident medical diagnoses, such as diabetes, cardiovascular events, stroke, and mental health issues, as well as chronic or persistent physical symptoms not attributable to another cause. Often referred to as "long COVID," these symptoms represent one type of PASC and can lead to marked morbidity and functional limitations in the months after acute infection [1]. The mechanisms underlying long COVID are unknown, and elucidating its cause has become a major research priority [2].

Interferons (IFNs) play vital roles in innate antiviral immune responses, and SARS-CoV-2 infection has been shown to hamper type I and III IFN responses [3]. Furthermore, inborn errors in type 1 IFN pathways and autoantibodies that neutralize type I IFNs have been identified in some individuals with COVID-19 who require a high level of care during acute infection, but not in those with milder initial disease [4–9]. For example, in a study early in the pandemic, >10% of patients with severe COVID-19 exhibited neutralizing autoantibodies to type 1 IFNs early in the disease course [4]. A more recent study of blood donors previously hospitalized with COVID-19 showed that 4 of 116 (3%) had detectable anti–IFN-α2 antibodies a minimum of 14–28 days after resolution of symptoms [10], in the early postacute period. It has been argued that disruption of IFN pathways could be a contributor to severe illness in a significant proportion of COVID-19 cases [9].

The presence of IFN-specific autoantibodies has also been proposed as a potential driver of PASC, including long COVID [11]. This hypothesis gained much attention following a provocative study that demonstrated an association between detection of IFN-α2–specific autoantibodies and the presence of pulmonary symptoms at time points during acute SARS-CoV-2 infection and 2–3 months after the initial presentation [12]. The authors argued that these antibodies might be uniquely associated with respiratory long COVID symptoms. This observation has not yet been confirmed in other postacute cohorts, and the prevalence of IFN-specific autoantibody responses and their association with postacute symptoms over longer periods of time consistent with current case definitions of long COVID is not known.

Further in-depth study of specific autoimmune mechanisms is critical, given the broad therapeutic potential of targeting various autoreactive immune responses. For this reason, we measured the presence of anti–IFN-α2 autoantibodies in plasma from 215 individuals with convalescent SARS-CoV-2 infection over 394 unique time points in a diverse postacute COVID-19 cohort, including 121 individuals with a variety of mild to severe long COVID symptoms.

METHODS

Ethics

The study was approved by the University of California, San Francisco, institutional review board. All participants provided written informed consent.

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Study Cohort and Sample Acquisition

Samples and in-depth demographic, symptom and clinical data were obtained from the Long-term Impact of Infection with Novel Coronavirus (LIINC) study (NCT04362150), which includes diverse participants with nucleic acid–confirmed SARS-CoV-2 infection, many of whom experience post-COVID conditions, such as long COVID. The cohort procedures, including recruitment, approaches to measurement, and follow-up, have been described in detail elsewhere [13]. Briefly, individuals with a history of nucleic acid–confirmed SARS-CoV-2 infection were enrolled and completed in-person visits at our research center in San Francisco; most were sampled approximately monthly from enrollment until 4 months after infection, and then quarterly thereafter. Plasma was obtained by centrifugation of ethylenediaminetetraacetic acid whole-blood samples. Samples were collected from 21 April 2020 through 29 June 2021. These samples represented all individuals in the cohort who enrolled before receiving a SARS-CoV-2 vaccine. The median number of samples studied per participant was 2 (interquartile range [IQR], 1.5–2).

Long COVID Assessment

We assessed for the presence of long COVID cross-sectionally at each visit. For this study, we defined long COVID as the presence of COVID-attributed symptoms at a visit occurring ≥60 days after initial COVID-19 symptom onset.

IFN-Specific Autoantibody Measurement

Anti–IFN-α2 antibodies were measured using a previously published immunoprecipitation method [8, 10]. Briefly, a sequence-verified plasmid containing full-length IFNA2 complementary DNA sequence with a Flag-Myc tag (Origene no. RC221091) was used as template in T7-promoter-based in vitro transcription/translation reactions (Promega; no. L1170) using [S35]-methionine (PerkinElmer; no. NEG709A). Protein was column purified using NAP-5 columns (GE Healthcare; no. 17–0853-01), incubated with 2.5 µL of plasma or 1 µL of anti–myc-positive control antibody (CellSignal; no. 2272), and immunoprecipitated with Sephadex protein A/G beads (Sigma Aldrich; nos. GE17-5280–02 and GE17-0618–05; 4:1 ratio) in 96-well polyvinylidene difluoride filtration plates (Corning; no. EK-680860). The radioactive counts (cpm) of immunoprecipitated protein were quantified using a Microbeta Trilux liquid scintillation plate reader (PerkinElmer). Antibody index for each sample was calculated as follows: (sample cpm value − mean blank cpm value)/(positive control antibody cpm value − mean blank cpm value). Positive signal was defined as >4 standard deviations above the mean of pre–COVID-19 blood bank noninflammatory controls, as reported elsewhere [10]. Autoimmune polyglandular syndrome type 1 samples were used as positive controls.

RESULTS

As shown in Table 1, 215 unique participants were assessed and biospecimens collected at 394 time points from 0.5 to 14.7 months after symptom onset (median, 94 days; IQR, 52–124 days). Ninety participants (42%) were female, and 48 (22%) had been hospitalized during the acute phase of COVID-19; of those hospitalized, 16 (40%) had been in the intensive care unit, and 6 (15%) required mechanical ventilation. Of the 394 participant time points, 272 time points representing 185 unique individuals occurred ≥60 days after COVID-19 symptom onset, allowing for assessment of the presence of long COVID at the visit. A total of 121 unique participants at 182 distinct time points met long COVID criteria, defined as ≥1 COVID-19–attributed symptom ≥60 days after initial symptom onset. The cohort experiencing long COVID was highly symptomatic. Among time points at which participants endorsed long COVID symptoms, the median number of symptoms at any time point was 5 (IQR, 2–8). Sixty-four unique participants at 91 time points endorsed ≥5 symptoms; 22 unique individuals at 29 time points endorsed ≥10 symptoms.

IFN-α2–specific autoantibodies were detected in only 2 of 215 participants across all sample time points. Both were Latino men in their late 40s to early 50s, who had preexisting diabetes and hypertension and experienced severe COVID-19 requiring hospitalization during the acute phase of infection (Figure 1 and Table 1).

The first participant had detectable anti-IFN antibodies at 87 and 115 days after acute infection. He had initially presented in spring 2020 after suffering pulseless electrical activity arrest requiring resuscitation and mechanical ventilation 10 days after onset of COVID-19 symptoms. Following hospitalization, he experienced intermittent viral shedding, detected with clinical nucleic acid amplification testing, for approximately 6 months and reported persistent anosmia and an intermittent neuropathic pain syndrome for ≥18 months.

The second participant had detectable anti-IFN antibodies 41 and 90 days after acute infection and was hospitalized in early 2021, requiring supplemental oxygen. He subsequently developed long COVID, with fatigue, shortness of breath, concentration difficulties, headache, vision changes, and peripheral neuropathy that have persisted for ≥12 months. In both cases, persistent symptoms were attributed to long COVID, although we note that both individuals experienced complex hospital courses that could have resulted in posthospitalization syndromes (eg, post–intensive care unit syndrome), which can be difficult to disentangle from long COVID.

Whereas these 2 individuals with IFN-α2–specific autoantibodies both went on to experience postacute symptoms, the large majority (99%) of our cohort had no detectable anti-IFN antibodies at any time point. IFN-α2–specific autoantibodies were not identified in any individuals who were managed as outpatients for

Table 1. Participant Demographic and Medical History Characteristics and Coronavirus Disease 2019 Disease Course

Characteristic	Participants, No. (%)[a]			
		Participants with Sampling ≥60 d After Onset of Symptoms		
	All Participants (N = 215)	No Long COVID Symptoms (n = 64)	Long COVID Symptoms (n = 121)	IFN-α2 Autoantibody-Positive Participants (n = 2)
Age, median (IQR), y	46 (36–55)	48 (39–58)	46 (38–55)	51 (46–56)
Sex at birth				
Female	90 (42)	24 (38)	57 (47)	0 (0)
Male	125 (58)	40 (63)	64 (53)	2 (100)
Race/ethnicity				
Hispanic/Latino	67 (31)	13 (20)	43 (36)	2 (100)
White	106 (49)	33 (52)	62 (51)	0 (0)
Black/African American	11 (5)	4 (6)	6 (5)	0 (0)
Asian	21 (10)	9 (14)	7 (6)	0 (0)
Pacific Islander/Native Hawaiian	4 (2)	3 (5)	1 (1)	0 (0)
Declined to answer	6 (3)	2 (3)	2 (2)	0 (0)
Hospitalized during acute COVID-19	48 (22)	14 (22)	29 (24)	2 (100)
BMI[b]				
<25	67 (31)	22 (34)	37 (31)	0 (0)
25–30	68 (32)	24 (38)	36 (30)	1 (50)
>30	77 (36)	17 (27)	48 (40)	1 (50)
Missing	3 (1)	1 (2)	0 (1)	0 (0)
Medical history				
Autoimmune disease	13 (6)	1 (2)	9 (7)	0 (0)
Cancer treated within past 2 y	8 (4)	1 (2)	4 (3)	0 (0)
Diabetes	22 (10)	8 (13)	11 (9)	2 (100)
HIV/AIDS	47 (22)	9 (14)	30 (25)	0 (0)
Heart attack or heart failure	7 (3)	2 (3)	3 (2)	0 (0)
Hypertension or high BP	41 (19)	6 (9)	30 (25)	2 (100)
Lung disease	35 (16)	13 (20)	18 (15)	0 (0)
Kidney disease	4 (2)	1 (2)	2 (2)	0 (0)
Ever smoker	64 (30)	16 (25)	38 (31)	1 (50)
Long COVID symptoms				
≥5 Symptoms	…	0 (0)	64 (35.0)	1 (50)
≥10 symptoms	…	0 (0)	22 (11.9)	1 (50)

Abbreviations: BMI, body mass index; BP, blood pressure; COVID-19, coronavirus disease 2019; HIV, human immunodeficiency virus; IFN, interferon; IQR, interquartile range; long COVID, post–COVID-19 conditions.

[a]Data represent no. (%) of participants unless otherwise specified. Of 215 participants contributing samples, 185 had ≥1 specimen obtained >60 days after initial infection; these included the 2 IFN antibody–positive individuals, and 30 had samples available only 28–59 days after infection.

[b]BMI calculated as weight in kilograms divided by height in meters squared.

COVID-19. Aside from the 2 persons described above, IFN-α2–specific autoantibodies were not identified in any other individuals who went on to experience moderate-to-severe long COVID symptoms persisting for up to 2 years.

DISCUSSION

We found that anti–IFN-α2 antibodies, which have been identified as a contributor to severe acute COVID-19 and proposed as a contributor to postacute long COVID symptoms, were uncommon in our postacute COVID-19 cohort, including among individuals with long COVID.

Our findings of anti-IFN antibodies limited to 2 individuals previously hospitalized for COVID-19 are consistent with the published literature [9]. To date, most assessments of anti-IFN antibodies have been conducted during the acute phase of COVID-19. During the acute phase, anti–IFN-α2 antibodies have been observed in a sizable proportion of SARS-CoV-2–infected patients hospitalized with severe COVID-19, but such antibodies are not common in those with mild illness [4–9]. For example, it was previously reported that IFN-α2–specific antibodies are detected during acute SARS-CoV-2 infection in those who require critical care for severe illness (>10%) [4]. In most cases, the autoantibodies are thought to predate the illness. These antibodies are uncommon in healthy controls (prevalence, approximately 0.3%) [8].

While severity of illness is thought to be a predictor of long COVID, most individuals with this condition did not require

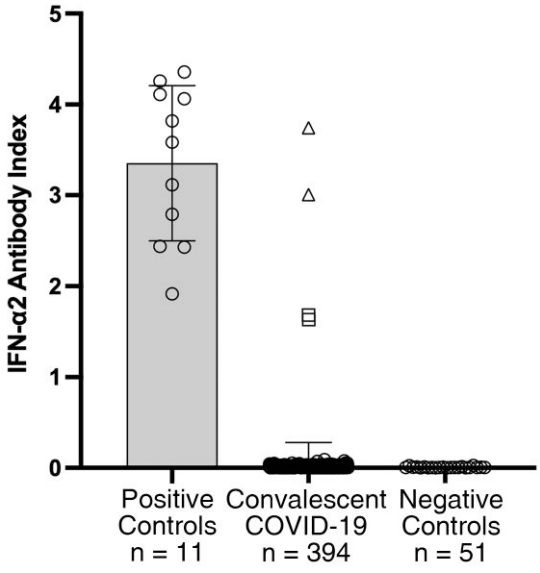

Figure 1. Interferon (IFN) α2 autoantibody responses in 215 unique participants with convalescent coronavirus disease 2019 over 394 longitudinal time points. Of these, 121 participants experienced postacute sequelae of severe acute respiratory syndrome coronavirus 2 infection. Square and triangle data points represent 2 unique individuals who had repeatedly detectable IFN-α2 autoantibodies 41–115 days after the onset of initial symptoms. Positive controls represent sample from individuals with autoimmune polyglandular syndrome type 1, and negative control samples are from uninfected individuals. Bars represent means and standard deviations.

hospitalization during the acute phase of COVID-19 [1]. The assessment of anti-IFN antibodies in the postacute phase has been more limited, but their presence has been proposed as a potential driver of long COVID symptoms [11]. In one PASC study, correlations were noted between the presence of these antibodies and pulmonary symptoms (eg, cough and sputum production) in a cohort comprising mostly previously hospitalized individuals 2–3 months after symptom onset [12]. The majority of our postacute participants (approximately 75%) had not been hospitalized, and none of these individuals exhibited anti-IFN autoantibodies, despite the presence of long COVID symptoms. Furthermore, many studies have reported that persistent symptoms are more prevalent in women [1], while IFN-specific autoantibodies are more commonly identified in men.

Type 1 IFN responses play a dual role in viral infection; while they exert antiviral activity during the acute phase of many infections, paradoxically they can contribute to the establishment of chronic infection through their immunoregulatory roles [14]. The growing indirect evidence in long COVID including the nature of symptoms, female preponderance, and upregulation of other cytokines [2], argues that excess signaling rather than inhibition might be more likely to contribute to this condition. Taken in context, our data suggest that while anti-IFN antibodies may contribute to severe SARS-CoV-2 infection, their presence is unlikely to be a primary driver of long COVID symptoms, particularly in those who were not hospitalized during the acute phase of COVID-19.

These data, however, do not negate the hypothesis that other autoreactive antibodies that develop during SARS-CoV-2 infection may target portions of the human proteome leading to tissue damage and the pathophysiological development of long COVID. For example, one study demonstrated the presence of autoantibodies targeting G protein–coupled receptors involved in cardiovascular and neurologic function in patients recovering from COVID-19 [15]. Overall, there are many challenges with studying causes of long COVID, given the heterogenous nature of the condition and inconsistent definitions used to describe the syndrome. This further emphasizes the need for larger mechanistic studies in precisely defined clinical cohorts.

Notes

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