Low Prevalence of Interferon-α Autoantibodies in People Experiencing Long COVID Symptoms

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Short Title: Anti-interferon autoantibodies in Long COVID

ABSTRACT

Interferon (IFN)-specific autoantibodies have been implicated in severe COVID-19 and have been proposed as a potential driver of the persistent symptoms characterizing Long COVID, a type of post-acute sequelae of SARS-CoV-2 infection (PASC). We report than only two of 215 SARS-CoV-2 convalescent participants tested over 394 timepoints, 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 post-acute phase of the infection.

Keywords: COVID-19, SARS-CoV-2, post-acute sequalae of SARS-CoV-2 infection, post-COVID conditions, Long COVID, autoimmunity, interferon autoantibodies

INTRODUCTION

Post-acute sequelae of SARS-CoV-2 infection (PASC) or post-COVID 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 following 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 type 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 one study early in the pandemic, over 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 4/116 (3%) had detectable anti-IFN-α2 antibodies a minimum of 14-28 days following resolution of symptoms [10], in the early post-acute period. It has been argued that disruption of IFN pathways could be a contributor to severe illness in a significant proportion of cases of COVID-19 [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 which demonstrated an association between detection of IFN-α2-specific autoantibodies and the presence of pulmonary symptoms at timepoints during acute SARS-CoV-2 infection and 2-3 months following 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 post-acute cohorts, and the prevalence of IFN-specific autoantibody responses and their association with post-acute 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 SARS-CoV-2 convalescent participants over 394 unique time points in a diverse post-acute COVID-19 cohort, including 121 individuals with a variety of mild to severe Long COVID symptoms.

METHODS

Ethics. The study was approved by the UCSF institutional review board. All participants provided written informed consent.

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 following infection, and then quarterly thereafter. Plasma was obtained by centrifugation of EDTA whole blood samples. Samples were collected from April 21, 2020 through June 29, 2021. These samples represented all individuals in the cohort who enrolled prior to receiving a

SARS-CoV-2 vaccine. The median number of samples studied per participant was 2 (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 or more days from 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 cDNA sequence with a Flag-Myc tag (Origene#RC221091) was used as template in T7-promoter-based in itrotranscription/translation reactions (Promega, Madison, WI: #L1170) using [S35]-methionine (PerkinELmer, Waltham,MA; #NEG709A). Protein was column-purified using Nap-5 columns (GE Healthcare, Chicago, IL; #17–0853-01), incubated with 2.5-ul plasma or 1-ul anti-myc positive control antibody (CellSignal, Danvers, MA; #2272), and immunoprecipitated with Sephadex protein A/G beads (Sigma Aldrich, St. Louis, MO; #GE17-5280–02 and #GE17-0618–05, 4:1 ratio) in 96-well polyvinylidene difluoride filtration plates (Corning, Corning, NY; #EK-680860). The radioactive counts (cpm) of immunoprecipitated protein were quantified using a Microbeta Trilux liquid scintillation plate reader (Perkin Elmer). 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 greater than 4 standard deviations above the mean of pre-COVID-19 blood bank non-inflammatory controls as previously reported [10]. APS1 samples were used as positive controls.

RESULTS

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

IFN-α2-specific autoantibodies were detected in only two of 215 participants across all sample timepoints. Both were Latino males in their late 40s to early 50s with pre-existing diabetes and hypertension who experienced severe COVID-19 requiring hospitalization during the acute phase of infection (**Figure 1; Table 1**). The first participant had detectable anti-IFN abs at 87 and 115 days following acute infection. He had initially presented in Spring 2020 after suffering pulseless electrical activity (PEA) arrest requiring resuscitation and mechanical ventilation 10 days after onset of COVID-19 symptoms. Following hospitalization, he experienced intermittent viral shedding detected on clinical nucleic acid amplification testing for approximately 6 months and reported persistent anosmia and an intermittent neuropathic pain syndrome for at least 18 months. The second participant had detectable anti-IFN antibodies at 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 at least 12 months. In both cases, persistent symptoms were attributed to Long COVID, although we note that both individuals experienced complex hospital courses which could have resulted in post-hospitalization syndromes (e.g., post-intensive care unit syndrome), which can be difficult to disentangle from Long COVID.

Whereas these two individuals with IFN-α2-specific autoantibodies both went on to experience post-acute 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 COVID-19. Aside from the two 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 post-acute Long COVID symptoms, were uncommon in our post-acute COVID-19 cohort, including among individuals with Long COVID.

Our findings of anti-IFN antibodies limited to two 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 sizeable 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 that require critical care for severe illness

(>10%) [4]. In most cases, the autoantibodies are thought to pre-date the illness. These antibodies are uncommon in healthy controls (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 hospitalization during the acute phase of COVID-19 [1]. The assessment of anti-IFN antibodies in the post-acute 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 (*e.g.,* cough and sputum production) in a cohort comprised mostly of previously hospitalized individuals 2-3 months following symptom onset [12]. The majority of our post-acute participants (~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 more prevalent in females [1], while IFN-specific autoantibodies are more commonly identified in males.

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, the presence of these antibodies is unlikely to be a primary driver of Long COVID symptoms, in particular those who were not hospitalized during the acute phase of COVID-19.

These data do not, however, 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 etiologies 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.

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FIGURE CAPTIONS

Figure 1. Interferon (IFN)-α2 autoantibody responses in 215 unique participants over 394 longitudinal timepoints with convalescent COVID-19. 121 participants experienced post-acute sequelae of SARS-CoV2 infection. Square and triangle data points represent 2 unique individuals that had repeatedly detectable IFN α2 autoantibodies 41 to 115 days following onset of initial symptoms. Positive controls represents sample from individuals with Autoimmune Polyglandular Syndrome Type ; APS1) and negative control samples are from uninfected individuals. Bars represent mean and standard deviations.

	All Participants	Participants with sampling >60 days following onset of symptoms		IFN-α2 Autoantibody + Participants
		No Long COVID Symptoms	Long COVID Symptoms	
N	215	64	121	2
Age [median (IQR)]	46 (36-55)	48 (39-58)	46 (38-55)	51 (46-56)
Sex at birth [N (%)]				
Female	90 (42)	24 (38)	57 (47)	0 (0)
Male	125 (58)	40 (63)	64 (53)	2 (100)
Race/ethnicity [N (%)]				
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 [N (%)]	48 (22)	14 (22)	29 (24)	2 (100)
Body mass index [N (%)]				
< 25	67 (31)	22 (34)	37 (31)	0 (0)
25 to 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 [N (%)]				
Autoimmune disease	13 (6)	1 (2)	9 (7)	0 (0)
Cancer treated within past 2 years	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 blood pressure	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 [N (%)]	64 (30)	16 (25)	38 (31)	1 (50)
Long COVID with >5 symptoms [N (%)]	-	0 (0)	64 (35.0)	1 (50)
Long COVID with >10 symptoms [N (%)]	-	0 (0)	22 (11.9)	1 (50)

Table 1. Participant demographics, medical history, and COVID-19 disease course. Note: Of 215 participants contributing samples, 185 had at least one specimen beyond 60 days from initial infection. This included the 2 IFN antibody positive individuals. 30 individuals had samples available only between 28 and 59 days following infection. Abbreviations: IFN = interferon; IQR = interquartile range.

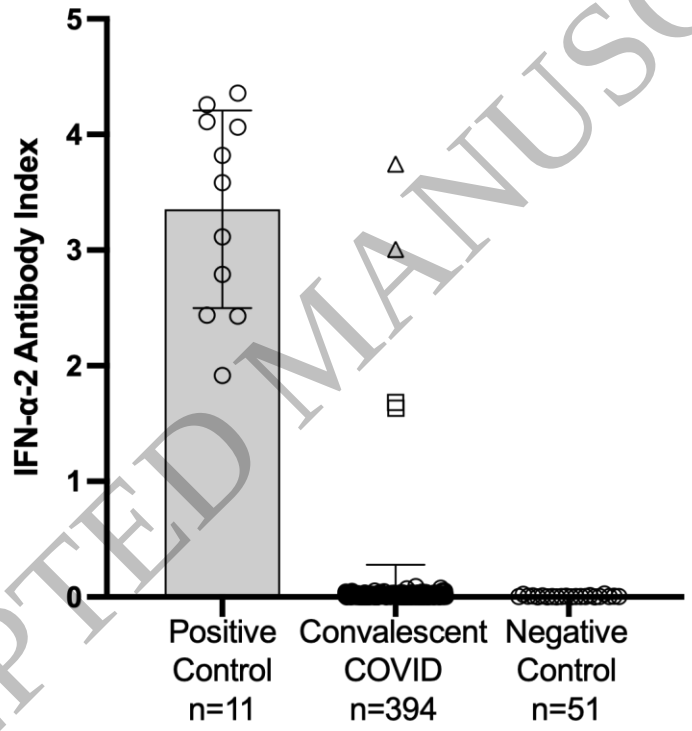

Figure 1
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