Temporal Dynamics of Anti-Type 1 Interferon Autoantibodies in COVID-19 Patients

Elana R. Shaw¹, Lindsey B. Rosen¹, Aristine Cheng¹,², Kerry Dobbs³, Ottavia M. Delmonte¹, Elise M. N. Ferré¹, Monica M. Schmitt¹, Luisa Imberti³, Virginia Quaresima³, Michail S. Lionakis¹, Luigi D. Notarangelo¹, Steven M. Holland¹, Helen C. Su¹,*

¹Laboratory of Clinical Immunology and Microbiology, NIAID, Bethesda, United States
²Infectious Diseases Division, Internal Medicine, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan
³CREA Laboratory, Diagnostic Department, ASST Spedali Civili di Brescia, Brescia, Italy

* Corresponding author: Helen C. Su, Building 10CRC, Room S-3940, 10CRC Center Dr., MSC 1456, Bethesda, MD 20892-1456, 301-451-8783 (phone), hsu@niaid.nih.gov
Abstract:

Binding levels and neutralization activity of anti-type 1 interferon (T1IFN) autoantibodies peaked during acute COVID-19 and markedly decreased thereafter. Most patients maintained some ability to neutralize T1IFN into convalescence despite lower levels of binding IgG. Identifying these autoantibodies in healthy individuals before they develop critical viral disease may be challenging.

Keywords: COVID-19, IFN-α, IFN-ω, autoantibodies, convalescence
Background:

Anti-cytokine autoantibodies (autoAbs) can phenocopy Mendelian defects in immune-signaling pathways to cause susceptibility to severe disease [1]. We previously reported that neutralizing autoAbs against type 1 interferons (T1IFNs), primarily IFN-α2 and/or IFN-ω, were present in 101 out of 987 critically ill COVID-19 patients from whom plasma or serum were obtained during hospitalization for acute disease, but in none of 663 asymptomatic/mildly symptomatic patients [2]. During this initial screening, the anti-T1IFN autoAbs showed high fluorescence intensity (FI) in a multiplex binding assay; patient plasma diluted to 10% were able to neutralize at least 10 ng/mL of IFN-α2 and/or IFN-ω.

Prior to the COVID-19 pandemic, neutralizing anti-T1IFN autoAbs of similar potency had been identified in patients with autoimmune polyendocrine syndrome type-1 (APS-1), hypomorphic RAG mutations, thymoma, and other diseases [3-5]. Within our cohort of acute COVID-19 patients, no causal genetic defect or underlying co-morbidity has yet been found to account for the anti-T1IFN autoAbs. In two critical COVID-19 patients who had samples available from before infection, neutralizing anti-T1IFN autoAbs were already present [2]. However, it is currently not known whether the neutralizing anti-T1IFN autoAbs in most patients pre-date critical COVID-19 infection as they do in APS-1, RAG-deficient, and thymoma patients, nor whether they persist after acute COVID-19 has resolved.

Methods:

Serum or plasma were collected longitudinally from 13 patients from Italy with acute COVID-19 who were critically ill and from 10 COVID-19 negative patients with either APS-1, RAG deficiency, or thymoma who had detectable autoAbs against IFN-α2 and/or IFN-ω. Twelve of the 13 acute COVID-19 patients had been included as part of a larger cohort in which single autoAb measurements were
reported during early hospitalization [2]. Acute COVID-19 patients were followed for an average of 147 (71-236) days after hospital admission for COVID-19 and other patients were followed for an average of 269 (65-451) days post their respective diagnoses. Clinical and/or demographic data were collected (Supplemental Tables 1-2). All subjects were recruited following protocols approved by local Institutional Review Boards (IRBs) of Comitato Etico Provinciale (NP 4000 – Studio CORONAlab) and the National Institute for Allergy and Infectious Diseases (NIAID). All protocols followed local ethics recommendations and informed consent was obtained when required.

Patient sera or plasmas diluted 1:100 in phosphate buffered saline (PBS) were screened for binding levels of anti-T1IFN autoAbs using a multiplex particle-based assay in which magnetic beads of differential fluorescence were covalently coupled to recombinant human IFN-α2, IFN-β, or IFN-ω at lysine residues. The FI of the bound PE-conjugated secondary anti-human IgG Fc was previously shown to be proportional to IgG binding levels of anti-T1IFN autoAbs [6]. FI measurements of anti-T1IFN autoAbs were corrected for background FI using a well containing PBS alone. A corrected FI value over the threshold of 3 standard deviations above the mean of the 1,230 healthy controls screened for our previous report (1,268 FI for anti-IFN-α2 autoAbs, or 1,391 FI for anti-IFN-ω autoAbs) was defined as high [2]. Alternatively, to determine Ig isotypes, secondary biotinylated anti-human IgG, IgM, IgA, or IgE Fc were used and levels were revealed using PE-conjugated streptavidin.

The neutralizing activity of anti–T1IFN autoAbs was determined by assessing STAT1 phosphorylation on Y710 (pSTAT1) in healthy control (HC) peripheral blood mononuclear cells (PBMC) after 15 minutes of stimulation with 10 ng/mL of IFN-α2 or IFN-ω in the presence of 10% HC or patient serum or plasma. The level of pSTAT1 was assessed in CD14+ monocytes and
normalized to that of 10% HC plasma. 0-20% pSTAT1 was classified as neutralizing, 20-65% pSTAT1 as partially neutralizing, and >65% pSTAT1 as not neutralizing [2]. A protein G column was used to deplete IgG from the plasma and the IgG-depleted flow-through fraction was collected and pSTAT1 was assessed and compared to that of total plasma [2]. To determine the median inhibitory concentration (IC₅₀), patient plasma/serum collected at a given timepoint was serially diluted 2- to 10-fold into human serum AB to assess pSTAT1 in the presence of patient plasma at final concentrations ranging from 10% to 0.000625%. The IC₅₀ was interpolated from the sigmoidal four parameter curve where X = Log (Concentration). Data were analyzed using FlowJo version 10 and graphed in GraphPad Prism 8.

**Results:**

Consistent with previous observations of cross-reactivity against structurally and phylogenetically related T1IFN [2], in this study eight COVID-19 patients had autoAbs against both IFN-α2 and IFN-ω, two against only IFN-α2, and three against only IFN-ω; none had autoAbs against IFN-β (Supplemental Tables 3-4). In the critically ill COVID-19 patients with anti-T1IFN autoAbs, binding IgG FI peaked during acute infection and markedly decreased thereafter (Figure 1A). The highest binding IgG FI was generally observed on the first available sample, which was obtained between 0 and 24 days after hospital admission. Binding IgG FI predominated over IgM, while IgA or IgE FI were minimal (Supplemental Figures 1-14). In general, the anti-T1IFN binding IgG FIs in the acute COVID-19 cohort halved every ~2 weeks. This contrasted with the stable high FI anti-T1IFN IgG observed in APS-1 and thymoma patients regardless of B cell depleting therapy with rituximab, and in RAG-deficient patients despite hematopoietic stem cell transplantation (Figure 1A, Supplemental Tables 3-4).

The neutralizing activity of anti-T1IFN autoAbs in acute COVID-19 patients, normalized to HC, also tended to decrease over time, tracking with the drop in detectable IgG binding (Supplemental Figures 2-14). Three out of the 10 patients who showed high binding FI of anti-IFN-α2 autoAbs
during acute illness dropped to sub-threshold in convalescence. Plasma diluted to 10% from two of these patients lost the ability to neutralize 10 ng/mL of IFN-α2 by ~40 and ~54 days into our follow-up. The third patient maintained the ability to partially neutralize 10 ng/mL of IFN-α2 up to 218 days after hospital admission, despite having sub-threshold binding FI. Three out of the 11 patients who showed high binding FI of anti-IFN-ω autoAbs during acute illness dropped to sub-threshold and two out of those three maintained the ability to neutralize 10 ng/mL of IFN-ω at 10% plasma (Figure 1B, Supplemental Tables 3-4). In all three patients who lost detectable IgG FI but maintained neutralizing activity, in vitro preincubations to deplete IgG removed the neutralizing activity completely (Supplemental Figure 15).

To further characterize changes in neutralizing strength of anti-T1IFN autoAbs in COVID-19 patients during disease and/or convalescence, IC_{50}s were determined against 10 ng/mL of the respective cytokine. For most patients, the neutralizing strength of the autoAbs decreased sharply after acute infection (Figure 1C and Supplemental Tables 3-4).

Discussion:

By following patients from acute disease through convalescence, we now show that the anti-T1IFN autoAb responses in most of the tested COVID-19 patients were highly dynamic, in sharp contrast to the stable high FI anti-T1IFN IgG in APS-1, thymoma, and RAG-deficient patients. Interestingly, the COVID-19 patients with only anti-IFN-ω autoAbs showed a different pattern, with binding FI and/or neutralizing activity that remained constant or slightly increased over time (Supplemental Table 4), suggesting different inductive conditions.

Most COVID-19 patients maintained some ability to neutralize T1IFN into convalescence. In several patients, this neutralizing activity persisted even after binding FI dropped to below detectable. We suspect that their neutralizing autoAbs recognized an epitope on IFN that was blocked by coupling the IFN to beads at lysine residues for our multiplex particle-based assay. Alternative explanations,
such as binding IgA FI or autoAbs against the common T1IFN receptor, IFNAR1/2, seem unlikely given that neutralizing activity was not only depleted by in vitro preincubation to deplete IgG specifically (Supplemental Figure 15), but also did not extend against all type I IFN tested including IFN-β (data not shown).

Our results suggest that the increase in endogenous IFNs during early viral infection may trigger a memory response of high FI but transient autoAbs. Anti-IFN-α2 and anti-IFN-ω autoAbs in the same patient often followed different trajectories, suggesting a polyclonal response to different triggers (Supplemental Figures 2-14). Moreover, the persistent neutralizing activity despite undetectable binding IgG in several patients suggests that only a small fraction of the polyclonal autoAbs responsible for high binding FI during acute disease contribute to the neutralizing activity. Prospective studies enrolling COVID-19 patients from the time of their first positive PCR test are needed to better define these potential mechanisms.

Our previous report showed that individuals with anti-IFN-α2 and/or IFN-ω autoAbs constitute a group at significant risk for developing critical COVID-19 disease [2]. Identifying these individuals early in the course of disease for targeted intervention with compensatory therapeutics, such as inhaled IFN-β therapy, might alter their course of disease and improve outcome [7]. However, our findings indicate that the dynamic nature of these autoAbs may make diagnosis in healthy individuals before acute disease difficult, at least if the level of autoAb FI sought for diagnostics is that which we observe during acute disease. Neutralization activity may be a more appropriate diagnostic measure than binding FI since some convalescent patients with sub-threshold binding FI against T1IFN during convalescence continued to potently neutralize supraphysiological amounts of the respective IFN. Accordingly, a high-throughput method for detecting neutralizing activity against more physiological levels of IFN, which has been recently validated in COVID-19 patients, may be useful and appropriate [8].
NOTES

Funding:

This research was supported by the Intramural Research Program of the NIAID, NIH and by Regione Lombardia, Italy (project “Risposta immune in pazienti con COVID-19 e co-morbidità”).

Acknowledgements:

We thank Yu Zhang, Sarah Weber, Li Ding, Sandhya Xirasagar, Jason Barnett, and Helen Matthews for their assistance, and Polly Matzinger for helpful discussions.

Potential conflicts of interest.

H.C.S. is a shareholder in Amgen, Eli Lilly, and Pfizer. All other authors have no conflicts.
References:

1. Browne, S.K., *Anticytokine autoantibody-associated immunodeficiency*. Annu Rev Immunol, 2014. 32: p. 635-57.
2. Bastard, P., et al., *Autoantibodies against type I IFNs in patients with life-threatening COVID-19*. Science, 2020. 370(6515).
3. Walter, J.E., et al., *Broad-spectrum antibodies against self-antigens and cytokines in RAG deficiency*. The Journal of clinical investigation, 2015. 125(11): p. 4135-4148.
4. Meager, A., et al., *Anti-interferon autoantibodies in autoimmune polyendocrinopathy syndrome type 1*. PLoS Med, 2006. 3(7): p. e289.
5. Burbelo, P.D., et al., *Anti-cytokine autoantibodies are associated with opportunistic infection in patients with thymic neoplasia*. Blood, 2010. 116(23): p. 4848-4858.
6. Ding, L., et al., *Determination of Human Anticytokine Autoantibody Profiles Using a Particle-Based Approach*. Journal of Clinical Immunology, 2012. 32(2): p. 238-245.
7. Monk, P.D., et al., *Safety and efficacy of inhaled nebulised interferon beta-1a (SNG001) for treatment of SARS-CoV-2 infection: a randomised, double-blind, placebo-controlled, phase 2 trial*. The Lancet Respiratory Medicine, 2021. 9(2): p. 196-206.
8. Bastard, P., et al., *Autoantibodies neutralizing type I IFNs are present in ~4% of uninfected individuals over 70 years old and account for ~20% of COVID-19 deaths*. Sci Immunol, 2021. 6(62).
Figure Legend:

**Figure 1:** A. Anti-IFN-α2 and IFN-ω autoAb binding FI over time. Measured in days since hospital admission for acute COVID-19 patients (red) and days since diagnosis for APS-1 patients (dark blue), thymoma patients (purple), and RAG-deficient patients (black). B. Neutralizing activity of COVID-19 patients over time against 10 ng/mL of IFN-α2 and/or IFN-ω at a plasma concentration of 10%. Red lines on the graph indicate that binding FI against the respective cytokine dropped to sub-threshold. Black lines on the graph indicate that binding FI against the respective cytokine remained high throughout the course of follow-up. pSTAT1 index was normalized against that of 10% HC plasma in the same experiment. Neutralizing (orange region of the graph) was defined as 0-20% pSTAT1, partially neutralizing (yellow region of the graph) as 20-65% pSTAT1, and not neutralizing (blue region of the graph) as >65% pSTAT1. C. 1/IC₅₀ over time for COVID-19 patients who blocked signaling by 10 ng/mL of IFN-α2 or IFN-ω at 10% plasma at all timepoints.
