LETTER TO EDITOR

Transient Increase of Pre-existing Anti-IFN-α2 Antibodies Induced by SARS-CoV-2 Infection

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To the Editor,

Bastard et al. reported that autoantibodies neutralizing type I interferons (IFN)s are found in at least 18% of deceased COVID-19 patients [1]. They also showed that the prevalence of neutralizing anti-type I IFN antibodies in the general population increases with age, with antibodies to IFN-α2 and/or IFN-ω present in 0.17% of people < 70 years and in at least 4% of people over 80 years [1], suggesting that the autoantibodies predate COVID-19 [1]. In four [1] and two [2] COVID-19 patients for whom pre–COVID-19 samples were available, autoantibodies against IFN-α2 and/or IFN-ω were present before SARS-CoV-2 infection.

It is, however, unknown whether SARS-CoV-2 infection enhances antibody formation to type I IFNs in patients with pre-existing antibodies or might even trigger antibody formation in patients without pre-existing antibodies. Comparatively, little is known about the fluctuation of antibody levels over time. In order to address these questions, we studied anti-IFN-α2 antibodies in controls and in patients with severe COVID-19 [disease severity class IV (according to reference [3]): respiratory failure requiring mechanical ventilation, shock, organ failure that requires ICU care] for whom pre- and post-COVID-19 left-over serum samples

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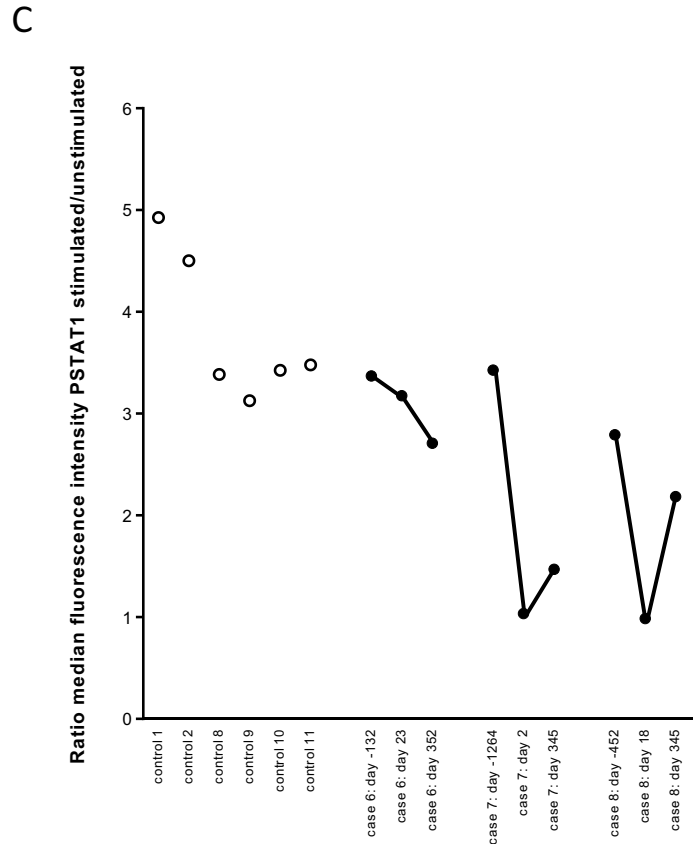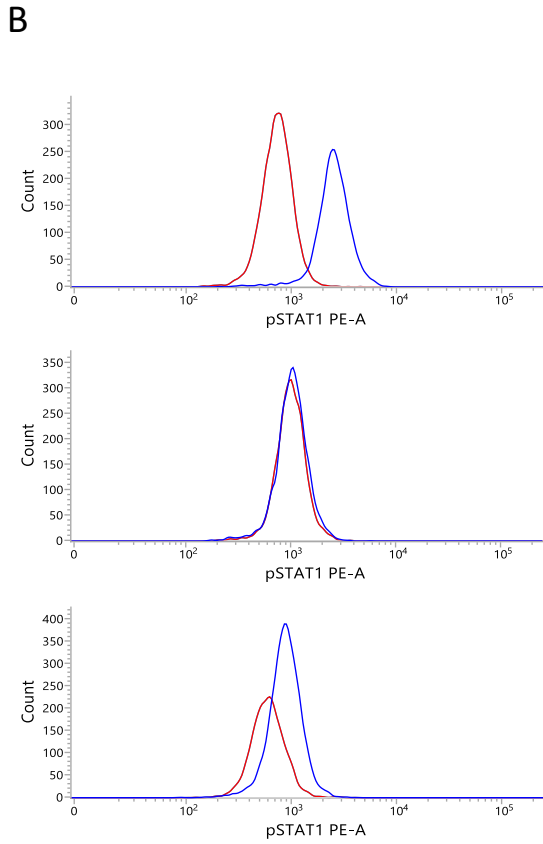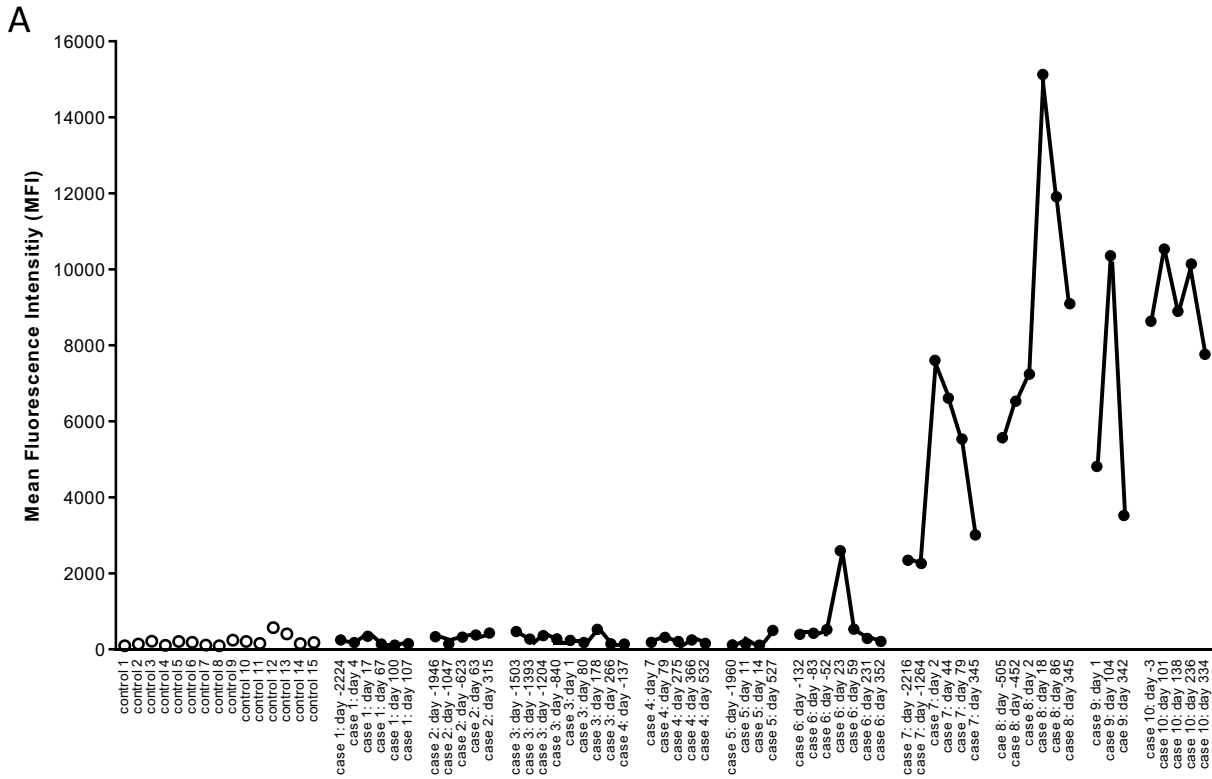
(from routine laboratory analyses) were available. The study was approved by the Ethics Committee of the University Hospitals Leuven, Belgium (S64799, S63708, S63881).

Anti-IFN-α2 antibodies were measured by a Luminex bead-based assay as previously described [4] with minor modifications (See Supplemental Data). In order to evaluate whether the autoantibodies neutralized IFN-α2, CD14-stained blood (200 μL) from a healthy control was incubated with 10% healthy control or patient serum and unstimulated or stimulated with 10 ng/mL IFN-α2 (Miltenyi Biotec) for 15 min at 37 °C. Cells were fixed, permeabilized and stained for intracellular phosphorylated STAT1 [using PE-labeled mouse anti-PSTAT1 (pY701), BD]. Cells were acquired on a BD Lyric instrument with gating on CD14-positive monocytes.

Anti-IFN-α2 antibodies were measured in samples from 15 healthy non-infected individuals (median age 31, age range 25–61, males/females ratio 6/9) and in 52 patients with severe COVID-19 (median age 67, age range 38–91, males/females ratio 38/14). In this latter population, 8/52 had anti-IFN-α2 antibodies. From the 52 patients, pre- and post-SARS-CoV-2 infection samples from eight patients, irrespective of having anti-IFN-α2 antibodies, were selected based on sample availability. In addition, post-infection samples from two patients with severe COVID-19 and IFN-α2 antibodies were included for whom no pre-infection samples were available. The median age of ten included patients was 68 years (age range 50–83 years old) and the male/female ratio 6/4.

In two patients with pre-existing IFN-α2 autoantibodies (case 7 and case 8), higher antibody levels were observed during COVID-19 compared to pre-COVID-19. In both patients the antibody levels started to decline +/− 6–12 weeks after SARS-CoV-2 infection and further declined up to 11 months after infection (Fig. 1A). In both patients, the capacity of the serum to neutralize IFN-α2 was higher during infection than pre-infection, with complete abolishment of STAT1 phosphorylation in whole

◄**Fig. 1** Antibodies to IFN-α2 in controls and in COVID-19 patients. Panel **A**. Antibodies to IFN-α2 were measured in 15 healthy controls, in 6 patients with pre-COVID-19 antibody levels that were comparable to the antibody levels in the 15 controls (cases 1–6), in 2 patients with pre-COVID anti-IFN-α2 antibodies (cases 7–8) and in 2 patients with anti-IFN-α2 antibodies but for whom with no pre-COVID samples were available (cases 9–10). In COVID-19 patients, antibody levels were measured in pre-COVID-19 samples, during and post-COVID-19. The results are from a representative experiment out of 2 experiments. Controls are represented by open symbols, cases by filled symbols. In the X-axis, the number of days before infection or the number of days post-infection are indicated (first positive PCR for SARS-CoV-2 is day 0). Panel **B**. STAT-1 phosphorylation in the presence of 10% serum in unstimulated (red line) or stimulated (10 ng/mL IFN-α2) cells (blue line). The distribution of the fluorescence intensities is shown. The results for patient case 7 are shown with serum obtained before (day −1264), during (day 2) and after (day 345) SARS-CoV-2 infection. Panel **C**. Neutralizing capacity of 10% serum on whole blood STAT-1 phosphorylation. STAT-1 phosphorylation was evaluated in 6 controls and three patients (cases 6, 7, and 8). For each patient, three serum samples (pre-infection, during infection, and post-infection) were evaluated. The ratio of the median fluorescence intensity in IFN-α2-stimulated to unstimulated cells was calculated. A ratio of 1 indicates that IFN-α2-induced STAT1 phosphorylation was abolished, suggesting the presence of neutralizing antibodies. The days before or after the first positive PCR for SARS-CoV-2 are indicated (first positive PCR is day 0)

blood by serum obtained during acute COVID-19 infection (Fig. 1B, C). In the pre-infection samples, the neutralizing capacity was comparable to or marginally higher than the neutralizing capacity of the controls. It should be noted, however, that our conditions for detecting neutralizing autoantibodies (neutralization of 10 ng/mL IFN-α2) were more stringent than the conditions recently validated by Bastard et al. (neutralization of 100 pg/mL IFN-α2) [1]. It is thus possible that patients who had neutralizing activity pre-COVID-19 similar to controls may have antibodies capable of neutralizing lower but more physiologic levels of IFN-α2.

Six COVID-19 patients had pre-COVID-19 antibody levels that were comparable to the antibody levels in the 15 controls. SARS-CoV-2 infection (documented by positive PCR) did not induce anti-IFN-α2 antibodies in five (cases 1–5) of the six patients, whereas it did in one patient (case 6). In this patient, antibodies to IFN-α2 were increased 23 days after the first positive PCR for SARS-CoV-2 and declined to pre-COVID-19 levels 59 days after the first positive PCR. The serum obtained 23 days after the first positive PCR did not neutralize IFN-α2 (Fig. 1C).

Finally, we also evaluated two COVID-19 patients with anti-IFN-α2 antibodies for whom only post-COVID-19 serum samples were available. In one of these patients (case 9) a transient increase in anti-IFN-α2 antibody levels was observed upon SARS-CoV-2 infection, whereas in the other patient (case 10), antibody levels remained high over a long period (11 months). This patient presented with acute respiratory distress syndrome and required 83 days of intensive care.

The clinical features of the patients with increased anti-IFN-α2 antibodies are summarized in Supplemental Data Table 1. Interestingly, different auto-immune features were observed in two patients: one was known with auto-immune hepatitis under corticosteroid therapy and the other was diagnosed with myasthenia gravis during hospitalization. Of note, antibodies to IFN-α2 have previously been described in myasthenia gravis [5].

Our finding that anti-IFN-α2 antibodies transiently increase during COVID-19 infection is consistent with the observation of Shaw et al. [6] that binding levels and neutralization activity of autoantibodies to type I IFNs were highest during acute COVID-19 and decreased afterwards. Shaw et al. [6] also reported more stable fluorescence intensity of autoantibodies neutralizing type I IFNs in patients with autoimmune polyendocrine syndrome type-1, thymoma, and *RAG* deficiency and in patients with only anti-IFN-ω autoantibodies [6].

In conclusion, our findings indicate that SARS-CoV-2 infection can enhance pre-existing (neutralizing) anti-IFN-α2 antibodies and, albeit to a lesser extent, induce anti-IFN-α2 antibodies in a patient without pre-existing antibodies. In most cases, the increase was transient and antibody levels tended to return to pre-COVID-19 levels within 2–11 months. Our limited data endorse that the presence of anti-IFN-α2 antibodies can be associated with autoimmune disease.

Declarations

Consortium Leuven COVID-19 Study Group.

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