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LETTER TO THE EDITOR OPEN ACCESS

Evaluating IL1RA-Autoantibodies Across SARS-CoV-2-Related Diseases

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To the editor,

Infections with SARS-CoV-2 can lead to a range of clinical manifestations, including mild to critical COVID-19 pneumonia and multi-system inflammatory syndrome in children (MIS-C). Additionally, rare adverse events following immunisation (AEFI) with COVID-19 vaccines have been reported, with myocarditis as a significant concern. Studies have revealed an important role for autoantibodies in these conditions, most prominently the role of pre-existing type-I interferon autoantibodies in severe COVID-19 pneumonia [1]. These studies have prompted a newfound interest in studying cytokine autoantibodies to investigate their pathophysiological roles in COVID-19-related conditions and to understand adverse events following immunisation (AEFI) with COVID-19 vaccines. Recently, three studies from the same group reported very high frequencies of neutralising IL1RA autoantibodies in different conditions involving SARS-CoV-2 infection, including MIS-C (in 13/22 (62%) patients) [2], severe-to-critical COVID-19 pneumonia (in 32/64 (50%) patients) [3] and vaccine-associated myocarditis (in 9/12 (75%) males < 21 years old) [4]. IL-1 receptor antagonist (IL1RA) is a naturally occurring anti-inflammatory protein that competitively inhibits the binding of pro-inflammatory cytokines IL-1α and IL-1β to the IL-1 receptor (IL1R1), thereby blocking their downstream signalling. It plays a critical role in regulating immune responses and preventing excessive inflammation, particularly in autoinflammatory conditions. IL1RA is produced by various cell types, including monocytes, macrophages, and epithelial cells, and its recombinant form (anakinra) is used therapeutically in hyperinflammatory conditions, including MIS-C and COVID-19. Thus, it was conceivable that IL1RA autoantibodies could play a pathogenic role in the studied diseases. While we and many others have been able to confirm the earlier findings on neutralising type-I interferon autoantibodies and their association with severe COVID-19 (prevalent in ~10% of severe cases) [5–8], there is a lack of independent reports exploring the association of IL1RA autoantibodies with COVID-19.

We aimed to independently assess the presence of IL1RA autoantibodies in an international cohort, enrolling patients with MIS-C ($n = 28$), severe COVID-19 ($n = 48$) and cardiac AEFI ($n = 47$), the

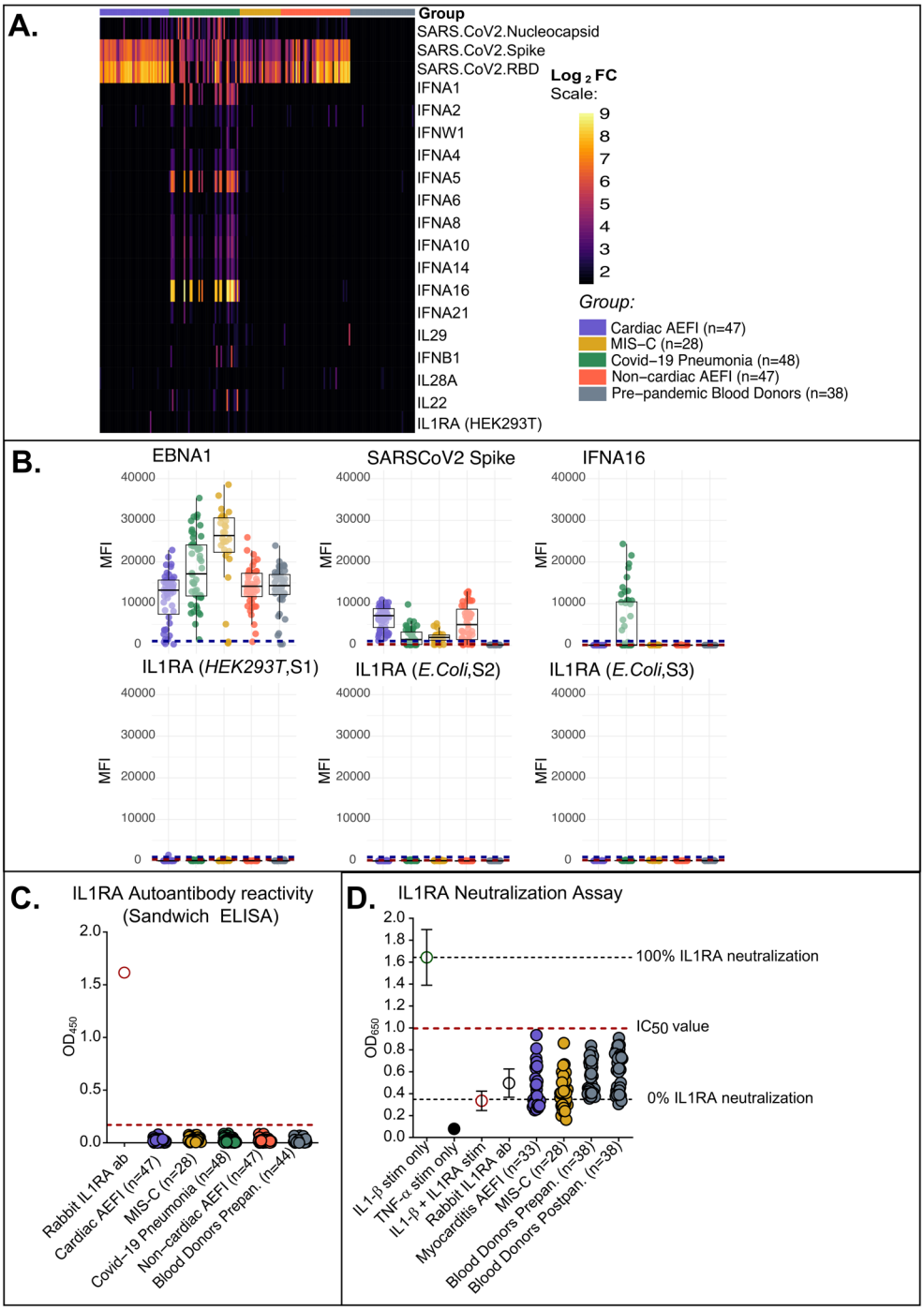

FIGURE 1 | Patients with multisystem inflammatory syndrome in children (MIS-C, $n = 28$), COVID-19 vaccine-associated myocarditis and perimyocarditis (Cardiac AEFI, $n = 47$), severe COVID-19 pneumonia ($n = 48$), COVID-19 vaccine-associated thrombosis (Non-cardiac AEFI, $n = 47$) plasma samples and blood donor samples were tested for autoantibodies against IL1RA. (A) A heatmap showing the unique antigen-specific reactivities with log2 fold-change above the pre-pandemic blood donor group (grey, $n = 38$), from a multiplex bead assay experiment. Elevated and shared immunoreactivity against multiple type-I interferons was observed in the severe COVID-19 pneumonia patient group. However, none of the disease groups showed elevated immunoreactivity against IL1RA (HEK293T cell-expression system) (B) In the same experiment, IL1RA protein from three sources, as well as technical controls in the form of a representative type-I interferon (IFNA16), SARS-CoV-2 Spike protein and Epstein–Barr virus Nuclear Antigen (EBNA1) were included. The red-dotted threshold line indicates mean + 5 SD over MFI value of pre-pandemic blood donor group, while blue-dotted line indicates lower limit of detection of the bead assay. (C) IL1RA autoantibodies were measured using a sandwich ELISA with human IL1RA protein. The red-dotted threshold line indicates mean + 7 SD over OD value at 450 nm of pre-pandemic blood donors' group. (D) Samples were tested for IL1RA neutralisation using a cell-based IL1 reporter assay. HEK-Blue IL-1β cells were stimulated with recombinant IL-1β in the presence of IL1RA, with or without rabbit anti-IL1RA antibody or samples. The resultant release of SEAP in proportion with IL1R-mediated cell stimulation was measured at 650 nm using manufacturer reagents. The red-dotted threshold line indicates IC_{50} threshold calculated based on IL-1β sole stimulation versus IL-1β + IL1RA co-stimulation. IC_{50}, half maximal inhibitory concentration; MFI, Mean Fluorescence Intensity; OD, optical density; SD, standard deviation; SEAP, Secreted Embryonic Alkaline Phosphatase.

latter comprising cases of myocarditis ($n=33$) and perimyocarditis ($n=14$). We also included patients with non-cardiac AEFIs ($n=47$), such as thrombotic and blood coagulation-related AEFIs, and blood donors from before ($n=44$) and during the pandemic ($n=38$) as controls (see Supporting Information for patient details). A multiplex bead-based immunoassay was used to screen for autoantibodies against recombinant IL1RA proteins from three separate sources, alongside interferons and a few other cytokines, EBV nuclear antigen (EBNA1), and SARS-CoV-2 antigens as technical positive controls (see Supporting Information for details). An elevated antigen response was defined as a mean fluorescent intensity (MFI) value exceeding five standard deviations above the mean MFI value of the blood donor group. Expectedly, elevated responses were detected against type-I interferons (in severe COVID-19 pneumonia) and viral antigens (across all groups) (Figure 1A,B). However, increased IL1RA reactivity was not detected in any of the samples (Figure 1A,B), with the exception of a single borderline positive patient in cardiac AEFI group. To further assess the potential presence of IL1RA autoantibodies, we performed a sandwich ELISA. The results showed no differences [one-way ANOVA: F (DFn, DFd): F (4, 203) = 2.089; *P*-value = 0.0835, non-significant] in absorbance values between different groups, indicating an overall absence of IL1RA autoantibodies (Figure 1C). Finally, we conducted a cell-based IL1β signalling assay to assess the functional capacity of samples to neutralise physiological levels of IL1RA. None of the analysed samples were able to neutralise ILRA activity and to restore baseline IL1β signalling in the cells. Plasma samples from patients with myocarditis ($n=33$, including five males < 21 years of age) and MIS-C ($n=28$) did not differ in their IL1RA-blocking response from those of pre- or post-pandemic blood donors (Figure 1D).

There is a rapidly growing understanding of how anti-cytokine autoantibodies can impair immune function and contribute to disease. Identifying and validating these autoantibodies is essential not only for advancing mechanistic insight but also for diagnostics and treatment. Our study, employing various established assays and multiple IL1RA protein sources in a large cohort, found no evidence of IL1RA autoantibodies being present in patients with severe COVID-19 pneumonia, MIS-C or vaccine-associated myocarditis. Differences in patient characteristics relative to the previous reports (e.g., inclusion criteria, sex and age) might exist. However, given the size of our cohort and the high frequency of IL1RA autoantibody positivity previously reported, such differences are unlikely to explain the negative findings in our investigation. While over 50 publications have now confirmed the presence of type-I IFN autoantibodies in severe COVID-19 pneumonia [1], the reports of IL1RA autoantibodies in COVID-19 and AEFI have not been independently verified, to the best of our knowledge. On the contrary, a Dutch group recently reported failure to reproduce results on IL1RA autoantibodies in a MIS-C cohort, even when similar assessment criteria as that of original findings was applied [9]. Our results, together with the current lack of corroborating published evidence, raise questions about the relevance of IL1RA as an autoantibody target in COVID-19 and AEFI.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that supports the findings of this study are available in the Supporting Information of this article.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.