


Cytokine autoantibodies in SARS-CoV-2 prepandemic and intrapandemic samples from an SLE cohort

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ABSTRACT

Cytokine autoantibodies, particularly those directed to type I interferon (T1IFN), have been reported to portend an increased risk of severe COVID-19. Since SLE is one of the conditions historically associated with T1IFN autoantibodies, we sought to determine the prevalence of cytokine autoantibodies in our local cohort of 173 patients with SLE prepandemic and intrapandemic, of which nine had confirmed exposure to SARS-CoV-2. Autoantibodies to 16 different cytokines, including T1IFN, were measured by an addressable laser bead immunoassay. None of the 9 patients with confirmed exposure to SARS-CoV-2 had autoantibodies to T1IFN and none had severe COVID-19 symptoms, necessitating hospitalisation. Hence, we could not confirm that T1IFN autoantibodies increase the risk for severe COVID-19. In addition, the cytokine autoantibody pattern did not differ between those with and without evidence of SARS-CoV-2 exposure.

INTRODUCTION

Autoantibodies directed to cytokines, including type I interferon (T1IFN), have been reported in a wide variety of infectious and autoinflammatory diseases including in up to 27% of patients with SLE.^{1–3} A remarkable finding reported during the SARS-CoV-2 pandemic was that pre-existing autoantibodies directed to T1IFN were associated with a higher risk of developing severe COVID-19.^{4–6} Given the importance of IFN in host viral defences,⁷ these observations raised the question of whether anti-T1IFN autoantibodies are associated with an increased risk of severe COVID-19 in SLE, a disease in which T1IFN plays a key role.⁸

METHODS

We examined the prevalence of cytokine autoantibodies in prepandemic and intrapandemic sera from a SLE cohort using a commercially available 15-PLEX anticytokine array (Luminex Corporation, Austin, Texas, USA), which included detection of

Key messages

What is already known about this subject?

- Recent evidence indicated that the presence of neutralising anti-type 1 interferon (T1INF) antibodies is associated with severe COVID-19 outcome. Previous studies reported antibodies to a wide range of cytokines in SLE, including T1INF.
- We sought to determine the prevalence of cytokine antibodies including anti-T1INF in patients with SLE, exposed versus unexposed to COVID-19.

What does this study add?

- In a cohort of patients with SLE examining prepandemic and intrapandemic antibodies to cytokines, none of the COVID-19 exposed patients (although all had a mild infectious course) had antibodies to T1INF compared with 3.7% of those without COVID-19 exposure (not statistically significant).
- Autoantibodies to other interferons and cytokines were not associated with exposure to SARS-CoV-2.

How might this impact on clinical practice?

- Measurement of antibodies to T1INF in SLE may be an important biomarker identifying SLE at risk of severe COVID-19. A larger study including patients with SLE with a more severe COVID-19 course and the impact of neutralising autoantibodies and vaccines are needed.

IgG autoantibodies directed to IFN-gamma (γ), IFN-beta (β), B cell activating factor (BAFF)/B lymphocyte stimulator (Blys), tumour necrosis factor alpha (α), granulocyte colony stimulating factor, interleukin (IL)-1 α , IL-6, IL-8, IL-10, IL-12 (p40), IL-15, IL-17A, IL-17F, IL-18 and IL-22. In addition, autoantibodies to IFN- α (IFN- α 2b) were detected by an addressable laser bead immunoassay (ALBIA) using the full-length purified human protein (Abcam, Cambridge, UK) following protocols as previously published.⁹ A cut-off of 500 median fluorescence intensity was established at two standard deviations above the mean of age-matched healthy controls,

Table 1 Frequency of cytokine autoantibodies among prepandemic and intrapandemic samples stratified by SARS-CoV-2 exposure (positive RT-PCR test or positive SARS-CoV-2 serology)

	Prepandemic			Intrapandemic		
	Exposed patients with SLE* n=9 (%)	Unexposed patients with SLE† n=164 (%)	P value‡	Exposed patients with SLE* n=9 (%)	Unexposed patients with SLE† n=164 (%)	P value‡
IFN γ	0 (0)	17 (10.4)	0.658	1 (11.1)	8 (4.9)	0.961
IFN- α 2b	0 (0)	6 (3.7)	1.000	0 (0)	6 (3.7)	1.000
IFN β	0 (0)	0 (0)	NA	0 (0)	0 (0)	NA
BAFF	0 (0)	8 (4.9)	1.000	1 (11.1)	10 (6.1)	1.000
TNF- α	0 (0)	3 (1.8)	1.000	0 (0)	1 (0.6)	1.000
G-CSF	0 (0)	4 (2.4)	1.000	1 (11.1)	1 (0.6)	0.205
IL-1 α	0 (0)	0 (0)	NA	0 (0)	0 (0)	NA
IL-6	2 (22.2)	13 (7.9)	0.381	1 (11.1)	8 (4.9)	0.961
IL-8	0 (0)	1 (0.6)	1.000	0 (0)	1 (0.6)	1.000
IL-10	0 (0)	10 (6.1)	0.976	1 (11.1)	9 (5.5)	1.000
IL-12	0 (0)	4 (2.4)	1.000	0 (0)	3 (1.8)	1.000
IL-15	0 (0)	0 (0)	NA	0 (0)	1 (0.6)	1.000
IL-17A	1 (11.1)	6 (3.7)	0.813	1 (11.1)	2 (1.2)	0.367
IL-17F	0 (0)	5 (3.0)	1.000	0 (0)	2 (1.2)	1.000
IL-18	0 (0)	0 (0)	NA	0 (0)	0 (0)	NA
IL-22	1 (11.1)	7 (4.3)	0.891	1 (11.1)	7 (4.3)	0.891
Any	3 (33.3)	46 (28.0)	1.000	2 (22.2)	38 (23.1)	1.000

*Exposure to SARS-CoV-2 defined as positive RT-PCR test and/or positive serology (n=9).

†Not exposed to SARS-CoV-2 defined as negative RT-PCR test, negative serology or not assessed (n=164).

‡The p values for the respective χ^2 comparisons. A value of $p < 0.003$ is considered statistically significant based on the Bonferroni correction. BAFF, B cell activating factor; G-CSF, granulocyte colony stimulating factor; IFN, interferon; IL, interleukin; NA, not applicable; RT-PCR, reverse transcriptase PCR assay for SARS-CoV-2 viral RNA; TNF, tumour necrosis factor.

following the requirements for validation of laboratory tests in the accredited laboratory (MitogenDx) that performed these tests. SLE-related autoantibodies (anti-double stranded DNA (dsDNA), anti-Smith (Sm), anti-U1-ribonucleoprotein (RNP), anti-ribosomal P, anti-SSA/Ro60, anti-Ro52/ (tripartite motif containing-21) TRIM21 and antichromatin) were detected in the prepandemic and intrapandemic sera by commercially available multianalyte immunoassays as previously published.^{9 10}

Prepandemic serum samples from the SLE cohort were biobanked prior to 01 January 2020 and intrapandemic samples were collected from 15 March 2020 to 31 January 2021; all patients were unvaccinated at the time of serum collection.¹¹ All samples were tested for SARS-CoV-2 antibodies using an ELISA measuring IgA and IgG antispike 1 (S1) protein (Euroimmun AG, Lübeck, Germany) and an assay detecting IgG antibodies to nucleocapsid (N), S1 receptor binding domain (RBD) and S1 (XMAP: Luminex Corporation, Austin, Texas, USA).¹¹ RT-PCR tests were performed if clinically indicated and results collected retrospectively through to 31 January 2021.

Cytokine autoantibodies were compared between patients with SLE with SARS-CoV-2-positive serology and/or RT-PCR confirmed SARS-CoV-2 infection (SARS-CoV-2 serology methods previously published)¹⁰ vs the remaining cohort using a χ^2 test. The Bonferroni method

was used to correct for multiple comparisons (17), with a corrected α of 0.0029 being considered statistically significant. Potential associations between each cytokine autoantibody and each SLE-related autoantibody in the prepandemic and intrapandemic periods were assessed using a χ^2 test, with a Bonferroni corrected α of 0.00045 being considered significant to account for the 112 comparisons.

PATIENT AND PUBLIC INVOLVEMENT

Patients or the public were not involved in the design, conduct, reporting or dissemination plans of our research.

RESULTS

The SLE cohort consisted of 173 patients (94.8% female, mean age 48.5 years, mean disease duration 11.7 years, 42.8% non-White race/ethnicity, 83.2% prescribed hydroxychloroquine, 28.9% corticosteroids and 43.9% other immunomodulators).¹¹ Intrapandemic samples from nine patients had SARS-CoV-2-positive serology and/or RT-PCR confirmed SARS-CoV-2 infection (SARS-CoV-2 serology + and RT-PCR+ (n=3); SARS-CoV-2 serology + and RT-PCR not done (n=3); SARS-CoV-2 serology- and RT-PCR+ (n=3)), yielding 164 patients with no laboratory evidence of SARS-CoV-2 exposure. The frequency of

Table 2 Characteristics of SLE cohort patients with positive intrapandemic SARS-CoV-2 serology and/or positive RT-PCR

ID	Date of PCR test	Date serum collected	*Age, years	Sex	Race/ethnicity	Medications			Cytokine antibodies	
						At time of pre-pandemic serum sample	At time of Intrapandemic serum sample	Pre-pandemic sample	Intrapandemic sample	
SARS-CoV-2 antibody + and RT-PCR+										
SLE-A	Oct '20	Oct '20	40–45	M	Asian	Hydroxychloroquine	Hydroxychloroquine	NRR	NRR	NRR
SLE-B	Dec '20	Jan '21	40–45	F	Asian	Hydroxychloroquine, mycophenolate mofetil, corticosteroids	Hydroxychloroquine, mycophenolate mofetil, belimumab	IL-6, IL-17a	BAFF	
SLE-C	Apr '20	Oct '20	45–50	F	Asian	Hydroxychloroquine, mycophenolate mofetil, corticosteroids	Hydroxychloroquine, mycophenolate mofetil	NRR	NRR	NRR
SARS-CoV-2 antibody + and RT-PCR not done										
SLE-D	ND	Oct '20	60–65	F	White	None	None	IL-6	IFN γ , G-CSF, IL-10, IL-6, IL-22, IL-17a	
SLE-E	ND	Oct '20	45–50	F	White	Hydroxychloroquine, methotrexate	Hydroxychloroquine, methotrexate	NRR	NRR	NRR
SLE-F	ND	Oct '20	70–75	F	White	Hydroxychloroquine	Hydroxychloroquine	NRR	NRR	NRR
SARS-CoV-2 antibody – and RT-PCR+										
SLE-G	Dec '20	Nov '20	20–25	F	White	Hydroxychloroquine, azathioprine	Hydroxychloroquine, mycophenolate mofetil	NRR	NRR	NRR
SLE-H	Oct '20	Jun '20	50–55	F	Asian	Mycophenolate mofetil, corticosteroids	Methotrexate	NRR	NRR	NRR
SLE-I	Nov '20	Sep '20	45–50	F	White	Hydroxychloroquine	Hydroxychloroquine	IL-22	NRR	NRR

*Age shown as a 5-year interval to protect confidentiality of individual.
 BAFF, B cell activating factor; G-CSF, granulocyte colony stimulating factor; IFN, interferon; IL, interleukin; ND, not done; NRR, normal reference range; RT-PCR, reverse transcriptase PCR assay for SARS-CoV-2 viral RNA.

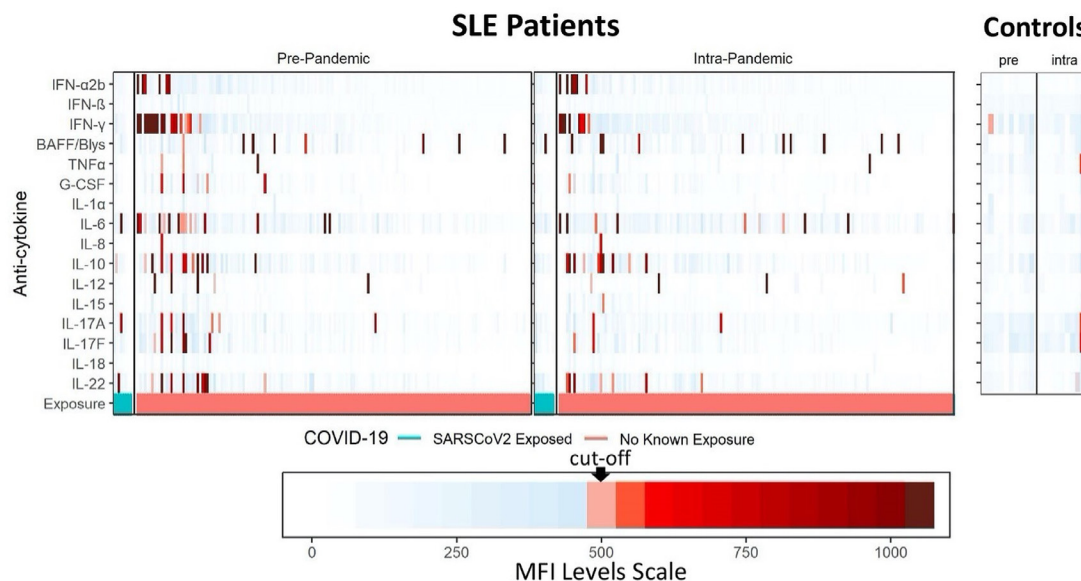


Figure 1 Heat map of cytokine autoantibody titres in pre-pandemic and intrapandemic patients with SLE who had evidence of SARS-CoV-2 exposure versus those who had no evidence of SARS-CoV-2 exposure. Apparently healthy controls are shown for comparison as a reference for established cut-offs. Each vertical column in the heat map represents an individual patient. The patients are sorted from left to right based on descending type I interferon (T1IFN) autoantibody titres. Therefore, the order of the patients in the pre-pandemic and intrapandemic panels are not the same. A supplemental figure where the pre-pandemic and intrapandemic results are vertically aligned is provided online supplemental figure 1. MFI, median fluorescence intensity.

cytokine autoantibodies in the pre-pandemic and intrapandemic samples stratified by SARS-CoV-2 exposure is shown in table 1, the patient characteristics and cytokine autoantibodies for the nine exposed patients are shown in table 2, and a graphical representation of the distribution of cytokine autoantibodies and their titers is shown in figure 1 (patients are sorted from left to right based on descending T1IFN autoantibody titres separately for pre-pandemic and intrapandemic samples).

In the pre-pandemic samples, 33.3% (3/9) of patients with serological and/or RT-PCR evidence of SARS-CoV-2 exposure versus 28.0% (46/164) without exposure had at least one cytokine antibody ($p=1.00$). In the intrapandemic samples, 22.2% (2/9) of exposed versus 23.1% (38/164) without exposure had at least one cytokine antibody ($p=1.00$). Overall, there was no difference in the frequency of cytokine autoantibodies in pre-pandemic compared with intrapandemic samples (28.3% (49/173) vs 23.1% (40/173), respectively; $p=0.33$). In the entire cohort of 173 patients, the most common cytokine autoantibodies in pre-pandemic samples were directed against IFN- γ (9.8%), followed by IL-6 (8.7%), IL-10 (5.8%) and BAFF (4.6%) (table 1 and figure 1) compared with 5.2% for IFN- γ and anti-IL-6, 5.8% for IL-10% and 6.4% for BAFF in intrapandemic samples. In the pre-pandemic samples, none of the exposed patients had autoantibodies to IFN- γ versus 10.4% (17/164) without exposure ($p=0.66$). In the intrapandemic samples, 11.1% (1/9) of the exposed patients had autoantibodies to IFN- γ versus 4.9% (8/164) without exposure ($p=0.96$). In the pre-pandemic and intrapandemic samples, none of the exposed patients had autoantibodies to T1IFN (IFN- α or IFN- β) versus 3.7% (6/164) without exposure had autoantibodies

to IFN- α and 0% without exposure had autoantibodies to IFN- β ($p=1.00$ and not applicable, respectively).

When cytokine autoantibodies were analysed for associations with SLE-related autoantibodies, only in intrapandemic samples were there associations with p values less than 0.05: anti-IFN- α and anti-dsDNA (intrapandemic $p=0.015$ vs pre-pandemic $p=0.7035$); IL-12 and anti-dsDNA (intrapandemic $p=0.043$ vs pre-pandemic $p=0.816$); IL-12 and anti-ribosomal P (intrapandemic $p=0.009$ vs pre-pandemic $p=1.000$); and IL-12 and anti-Sm (intrapandemic $p=0.005$ vs pre-pandemic $p=1.000$). However, after correcting for multiple comparisons none of these associations met the reduced α ($\alpha=0.00045$) to be considered statistically significant.

DISCUSSION

The frequency of cytokine autoantibodies observed in our study is in keeping with previous publications that used similar assay techniques reporting that up to 27% of patients with SLE had cytokine autoantibodies.^{1,3} In the present study, none of the patients with SLE with confirmed exposure to SARS-CoV-2 had autoantibodies to T1IFN and, as we previously reported,¹¹ none of the 173 patients in our cohort, including the 9 patients with confirmed exposure, had severe COVID-19 symptoms necessitating hospitalisation. Hence, we could not confirm that T1IFN autoantibodies increase the risk for severe SARS-CoV-2. The overall observed frequency of anti-IFN γ autoantibodies in our study is consistent with a previous report of 11% in patients with SLE.¹ In an earlier communication, we reported that this SLE cohort had a lower rate of pre-pandemic seropositivity and a slightly

lower to similar rate of intrapandemic seropositivity compared with contemporaneous controls.¹¹ Granted, in the current report we did not study the neutralising effect of cytokine autoantibodies, but it would be difficult to explain T1IFN neutralising activity in the absence of detectable T1IFN cytokine autoantibodies. In addition, we found a non-significant association between IFN γ autoantibodies and dsDNA autoantibodies in intrapandemic samples, which is similar to a previous report that anti-IFN γ autoantibodies were associated with anti-dsDNA.¹ Further, due to the size of our cohort and its geographical context, our results may not be generalisable to a broader population of patients with SLE. Because of interest in vaccine responses in patients with SLE,^{12–15} current efforts are focusing on a possible relationship of cytokine autoantibodies to adverse or unusual reactions.

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Contributors All authors were involved in the concept and design, data analysis and interpretation, and editing for intellectual content. MJF and AEC are joint senior authors.

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Competing interests MJF is a consultant to and has received honoraria and/or travel support from Werfen (Barcelona, Spain, San Diego, CA). MJF is also Medical Director of Mitogen Diagnostics Corporation. MYC has received consulting fees from Janssen, AstraZeneca and MitogenDx. AEC has received consulting fees from AstraZeneca, Bristol Myers, Squibb and Glaxo Smith Kline. All other authors declare no conflict of interest.

Patient consent for publication Consent obtained directly from patient(s).

Ethics approval This study involves human participants and was approved by the University of Calgary Ethics Board (REB13-0425). Participants gave informed consent to participate in the study before taking part.

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Supplemental Material

Supplemental Figure 1 Heat map of cytokine autoantibody titers in pre- and intra-pandemic SLE patients who had evidence of SARS-CoV2 exposure versus those who had no evidence of SARS-CoV2 exposure. Apparently healthy controls are shown for comparison as a reference for established cut-offs. Each vertical column in the heat map represents an individual patient. This is a reconfiguration of **Figure 1** in the main manuscript where the results of individual pre- and intra-pandemic SLE are vertically aligned.

