



ORIGINAL RESEARCH

Idiopathic inflammatory myopathies
lack neutralising autoantibodies to
type- I, II and III interferonsAnish Behere ¹, Hedvig Mildner,¹ Irene Peralta Garcia,^{2,3,4} César Pérez Bucio,¹
Ingrid Lundberg ^{2,3,4}, Begum Horuluoglu,^{3,4} Nils Landegren^{1,4}

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BH and NL contributed equally.

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¹Science for Life Laboratory, Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden

²Department of Gastro, Dermatology and Rheumatology, Karolinska University Hospital, Stockholm, Sweden

³Division of Rheumatology, Department of Medicine, Solna, Karolinska Institutet, Stockholm, Sweden

⁴Center for Molecular Medicine, Karolinska Institutet, Stockholm, Sweden

Correspondence to

Dr Anish Behere;
anish.behere@imbim.uu.se

ABSTRACT

Objective To determine whether autoantibodies against interferons are present and play a role in disease modulation in idiopathic inflammatory myopathies (IIMs).

Methods We screened for autoantibodies against a large number of interferons (IFNs) and other cytokines in a cross-sectional observational cohort of Swedish patients with anti-synthetase syndrome (n=51) and dermatomyositis (n=48), matched together with blood donors (n=100) from general population, using both planar and suspension-based multiplex assays. A single patient with autoimmune polyendocrine syndrome, type-1 (APS-1), known to harbour autoantibodies that neutralise type-I interferons, was included as a reference biological positive. The functional ability of autoantibodies to neutralise type-I interferons was tested in vitro, using an IFN- α / β responsive cell reporter assay.

Result The initial screening of plasma samples indicated a repertoire of autoantibodies in IIM patients against a number of common myositis-specific and myositis-associated antigens. On screening for autoantibodies against type-I, II or III interferons, we did not find any evidence of anti-IFN autoantibodies being present in any of the IIM patient subgroups or the blood donors from general population. Additionally, none of the tested plasma samples, except the APS-1, exhibited neutralisation of physiological concentration IFN- α 2, further confirming a complete lack of functional autoantibodies against IFN- α subtypes in this cohort.

Conclusions We did not detect neutralising autoantibodies against IFN- α and autoantibodies against other types of IFNs in a Swedish cohort of IIM patients. These findings contrast with the presence of autoantibodies against type-I IFNs in other systemic autoimmune diseases, such as systemic lupus erythematosus, characterised by type-I IFN overactivation.

INTRODUCTION

Type-I interferons (IFNs) play a key role in innate immunity and in antiviral response in particular. However, type-I IFNs have also been found to play a detrimental role in promoting autoimmunity by enhancing antigen presentation, stimulating the production of autoantibodies and increasing the

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Neutralising autoantibodies against type-I interferons are known to modulate interferon-driven inflammation in systemic autoimmune diseases such as systemic lupus erythematosus, but their role in idiopathic inflammatory myopathies (IIMs) has not been determined.

WHAT THIS STUDY ADDS

⇒ This study comprehensively screened for autoantibodies against type- I, II and III interferons in Swedish patients with anti-synthetase syndrome and dermatomyositis, finding no evidence of either binding or neutralising anti-IFN autoantibodies in these IIM cohorts. This contrasts with other autoimmune diseases and suggests a distinct immunological profile in IIMs.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The absence of anti-IFN autoantibodies in IIMs may influence future biomarker and therapeutic strategies, supporting the notion that neutralising anti-IFN autoantibodies are not universally involved across all systemic autoimmune diseases. This insight helps refine disease classification and suggests the need for IIM-specific pathogenic models and treatment approaches.

survival of autoreactive lymphocytes. Chronic activation of type-I IFN pathways can trigger or perpetuate autoimmunity in conditions such as systemic lupus erythematosus (SLE) and Sjögren's disease (SjD) by creating a sustained inflammatory environment that disrupts self-tolerance mechanisms.¹

Idiopathic inflammatory myopathies (IIMs) are another systemic autoimmune condition encompassing several subtypes, including dermatomyositis (DM), polymyositis (PM), anti-synthetase syndrome (ASyS), inclusion body myositis (IBM) and immune-mediated necrotising myopathies (IMNM).² The IBM and IMNM patients present predominant

muscle involvement, whereas patients with DM and ASyS present additional extra-muscular features, that is, skin and lung involvement and associations with elevated levels of type-I IFN signatures in muscle, blood and skin.^{3,4}

Autoantibodies against type-I IFNs can develop as a result of IFN treatment or spontaneously and have been documented across an increasing range of diseases.⁵ Recent findings point towards a detrimental role of autoantibodies against type-I IFNs in COVID-19 and other viral diseases by impairing the host response during infection.⁶ Conversely, these autoantibodies may play a beneficial role in systemic autoimmune diseases by dampening type-I IFN overactivations, as supported by findings in SLE.^{7,8} Given earlier observations of autoantibodies against type-I IFNs in SLE and SjD, here we aimed to determine whether these autoantibodies are present and play a role in disease modulation in IIMs.

We screened for potential presence of autoantibodies against type-I, II, III IFNs in a Swedish cohort of patients with ASyS (n=51) and DM (n=48), and compared them to blood donors (BDs) (n=100) from a general population. Additionally, we included a patient with autoimmune polyendocrine syndrome, type I (APS-1), known to harbour autoantibodies that neutralise type-I IFNs, as a positive control. Plasma samples were screened for autoantibodies against multiple antigens, including a number of commonly observed myositis-specific autoantibody (MSA), myositis-associated autoantibody (MAA) targets, IFNs and other cytokines. We took advantage of two different multiplex screening approaches, using a planar protein microarray and a bead-based suspension assay for a rigorous investigation. The functional ability of autoantibodies to neutralise type-I IFNs, using a representative IFN- α 2, was tested employing a reporter assay with IFN- α / β responsive HEK293 cell model.

PATIENTS AND METHODS

Patients and study design

This was a cross-sectional observational study employing a design to identify anti-IFN autoantibody profiles in patients with ASyS and DM. The stored plasma samples were selected from a cohort followed in a specialised myositis clinic at Karolinska University Hospital, Stockholm, Sweden. IIM diagnosis was established by an experienced rheumatologist. The recruitment and sample collection of patients was carried out in the period between years 1996 and 2014 and between years 2001 and 2021 for ASyS and DM subtypes, respectively. In the case of multiple samples, the one closest to IIM diagnosis was selected. Diagnosis of ASyS was made according to Connors' criteria: presence of an autoantibody against tRNA synthetase plus myositis, and/or Raynaud's phenomenon and/or arthritis and/or interstitial lung disease and/or mechanic's hands. DM patients were classified as probable or definite according to the 2017 European Alliance of Associations for Rheumatology/American College of Rheumatology classification

criteria.^{2,9} MSA and MAA were analysed as per clinical routine by line blot (Euroimmune, Lübeck, Germany) and Multiplex (Luminex; for anti-nuclear antibodies (ANA) specificities including Jo1, U1RNP, Ro52, Ro60 and La) assay. Every positive test by Addressable Laser Bead Immuno Assay for ANA, line blot (Euroimmun, Lübeck, Germany), immunoprecipitation or ELISA for myositis specific autoantibodies (Jo-1, PL-7, PL-12, EJ, OJ, Mi-2, SRP, MDA5, TIF1 γ , SAE1) and MAA (SSA, SSB, U1 RNP, Ku, Pm-Scl) was recorded for each patient. Patients with unknown status for MSA and MAA autoantibodies were excluded. After recruitment and routinely at clinical visits, patients provided antecubital plasma samples from which sera were obtained (10 min centrifugation at 2000 \times g) and stored (-80°C) until measurements were performed. All patients were recruited at the myositis unit at Karolinska University Hospital after obtaining written informed consent forms for study participation. The healthy control group comprised plasma samples obtained from anonymous BDs from a general population with 50:50 males to females' distribution with median age 44 years old (IQR: 30–57 years of age) collected at Uppsala Academic Hospital's Clinical Immunology and Transfusion Medicine Laboratory at Uppsala, Sweden during the month of February and March 2023.

Experimental design

An autoantibody profiling in the IIM cohort was conducted using a dual screening approach: (1) In-house printed planar custom microarrays containing ~500 duplicate features including autoantigens and full-length human cytokines; (2) A suspension-based Luminex assay, by coupling a subset ~95 antigens to multiple beads (please see online supplemental material 1, for antigen details). Briefly, the autoantibody profiling using protein microarray and multiplex bead assays was performed using a similar procedure as described previously.¹⁰

Autoantibody profiling with custom microarrays

An autoantibody profiling in the IIM cohort was conducted using custom microarrays containing ~500 duplicate features including autoantigens and full-length human cytokines. On the first day, microarrays were incubated for 90 min at room temperature in 5 mL of blocking buffer (containing 2% bovine serum albumin and 0.05% Tween-20 in phosphate-buffered saline, PBS) to prevent non-specific binding. Patient and BD plasma samples were then diluted at a ratio of 1:2000 in the same blocking buffer and incubated with the arrays overnight (min 16 hours) at room temperature. On the following day, custom microarrays underwent five consecutive 5 min washes of wash buffer (PBS with 0.05% Tween-20). Subsequently, they were transferred into a new plate for a 90 min incubation with a secondary antibody included Alexa Fluor 647-conjugated goat anti-human IgG (1:2000 dilution, Thermo Fisher Scientific, #A-21445) in 5 mL of blocking buffer. After this incubation, the arrays were washed again using the same steps. As a final step, the

microarrays were rinsed for 30s with deionised water and dried using a tabletop microarray centrifuge for 30s. Scanning was performed within 1 hour of drying using the Genepix 4000B fluorescence scanner (Axon Instruments), with image acquisition and data analysis conducted via GenePix Pro V.5.1.0.19 or GenePix Pro V.7 software using a custom microarray Genepix Array List (GAL) file. Autoantibody data from 99 patients diagnosed with IIM and 24 BD controls included in the same run was processed and analysed concurrently. Post-processing procedures are detailed in the data analysis section below. Autoantibody positivity was defined based on two thresholds: (1) signal intensity exceeding the mean of the BD group by at least five standard deviations (mean+5SD) and (2) a minimum mean fluorescence intensity (MFI) value over 750 arbitrary units (AUs).

Development of the multiplex bead assay

Colour-coded magnetic beads (MagPlex, Luminex, Austin, Texas, USA) were used to immobilise a total of ninety-three antigens (see online supplemental material table 1, for antigen details). Additionally, two bead types were coated with anti-human IgG (309-005-082, Jackson ImmunoResearch, West Grove, Pennsylvania, USA) and the Epstein-Barr virus protein EBNA1 (ab138345, Abcam, Cambridge, UK) as loading controls, while one bead was intentionally left uncoated as a technical control for assessing background noise from individual samples. All the antigens were diluted to ~80 µg/mL in 100 mM 2-(N-morpholino) ethanesulfonic acid (MES) buffer at pH 4.5 (Sigma-Aldrich) and assigned a unique colour code for identification. The carboxylated surfaces of 1 million beads per antigen type were activated in 100 µL of phosphate buffer with 0.5 mg of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (ProteoChem, Hurricane, Utah, USA) and 0.5 mg of N-hydroxysulfosuccinimide (Thermo Fisher Scientific). Activated beads were then incubated for 2 hours with the antigen solution and subsequently incubated overnight in a blocking buffer (Blocking Reagent for ELISA, Roche, with 0.1% ProClin, Sigma-Aldrich). Finally, all bead types were pooled to create the multiplex antigen bead assay.

Autoantibody profiling with the bead-based suspension assay

The plasma samples were thawed at 4°C, diluted 1:50 in an assay buffer (3% bovine serum albumin, Saveen-Werner, Limhamn, Sweden, and 5% non-fat milk, Sigma-Aldrich, in PBS containing 0.05% Tween-20, Thermo Fisher Scientific) using an automated liquid handling system (EVO150, TECAN, Männedorf, Switzerland). Each diluted sample was added to a 384-well microtiter plate (Greiner BioOne, Kremsmunster, Austria) containing the antigen beads and incubated for 1 hour at room temperature. After incubation, beads were washed in PBS-T and immunocomplexes were fixed with 0.2% paraformaldehyde for 10 min. Beads were then incubated with R-phycoerythrin-conjugated anti-human IgG (H10104, Invitrogen, Carlsbad, California, USA)

at a concentration of 0.4 µg/mL in PBS-T for 30 min. Signals were detected on a FlexMap3D instrument using Luminex xPONENT software (Luminex Corp.). The assay runs were conducted to include all samples, with a positive APS-1 serum biological control replicates (4x) and four seronegative technical samples per run. The biological negative samples were the hundred BD control plasma samples representing background levels across antigens and used to set seropositivity cut-off values.

Plasma IFN-α measurement with ELISA

To measure the concentration of IFN-α in plasma samples, we used a commercially available kit, ELISA Pro: Human IFN-α (#3425-1HP, Mabtech, Nacka Strand, Sweden) which is able to detect the following human IFN-α subtypes 1/13, 2, 4, 5, 6, 7, 8, 10, 14, 16 and 17 simultaneously. In brief, we diluted the plasma samples 1:1 in ready-to-use ELISA dilution buffer from the kit and performed the experiment by carefully following the manufacturer's guidelines. The elevated IFN-α response was determined by calculating the 98th percentile value from IFN-α levels measured in BD group, while excluding all the values below the assay's lower limit of detection (LLOD, 2 pg/mL).

IFN-α2 neutralisation reporter assay

To assess IFN-α2 signalling, we employed HEK-Blue IFN-α/β cells (#hkb-ifnabv2, InvivoGen), a specialised HEK 293 cell line that exclusively responds to IFN-α/β through IFNAR_{1/2} binding, subsequently inducing the secreted embryonic alkaline phosphatase (SEAP) under ISG54 promoter control, into the culture medium. The assay protocol was performed according to manufacturers' guidelines. Cells were stimulated with 1 ng/mL recombinant IFN-α2 (#HY-P7022, MedChemExpress). To evaluate the IFN-α2 neutralising capability, serum or plasma samples from patients and BDs were diluted 1:10 and compared against an anti-IFN-α2 antibody (#hifn-mab1-02, InvivoGen) at a concentration starting from 0.5 µg/mL. Plasma samples were diluted in DMEM and pre-incubated at 37°C for 2 hours in the presence of IFN-α2 in a total volume of 40 µL. Following this, HEK-Blue IFN-α/β cells (50 000 cells/well) were added to the culture plates at a final volume of 160 µL and incubated overnight at 37°C. After a minimum of 18 hours, 20 µL of supernatant was transferred from each well to 180 µL of freshly prepared QUANTI-Blue solution (#rep-qbs, InvivoGen). The substrate reaction was developed for at least 1 hour at 37°C, and SEAP activity was quantified by measuring absorbance at 630 nm using a Tecan plate reader. Baseline SEAP activity was calculated from seronegative replicates with IFN-α2 stimulation, while total inhibition of a SEAP activity was calculated from IFN-α2 -unstimulated, seronegative replicates.

Data analysis

Data analysis and visualisation were performed using R (V.3.6.1) and RStudio (V.1.2.1335), along with packages

Table 1 Clinical information and patient demographics

Plasma samples	Total patients	Anti-synthetase syndrome	Dermatomyositis
n	99	51	48
Female, n (%)	65 (66)	36 (71)	29 (60)
Males, n (%)	34 (34)	15 (29)	19 (40)
Age (in years) at diagnosis, median (IQR)	50 (40–62)	50 (41–64)	51 (38–61)
Cancer within 3 years of IIM diagnosis, n (%)	24 (24)	7 (14)	17 (35)
Treatment prior to sample extraction, n (%)	37 (37)	22 (43)	19 (40)
Sample extraction at diagnosis (<3 months from diagnostic date), n (%)	63 (63)	28 (55)	35 (73)

IIM, idiopathic inflammatory myopathies.

such as BiocManager limma, tidyverse, pheatmap, viridis, patchwork, gridExtra, WriteXLS, ropls, ggplot2, ggforce, ggthemes, ggVennDiagram and reshape2 as well as custom functions for data import and quality checks. The data were acquired as MFI, expressed as AUs for both microarray and Luminex experiment. Seropositivity cut-off values were determined per antigen as the mean plus five standard deviations (rounded to the nearest integer) of the BDs for both microarray and Luminex runs. The data obtained from the cell experiment were plotted using Prism V.6.0 software (GraphPad Software, La Jolla, California, USA). The neutralisation assay using the HEK-Blue cell model and the use of half-maximal inhibitory concentration (IC_{50}) value threshold as a cut-off criterion for neutralising activity is based on a previously described report after validation in our lab.^{11 12} In this cross-sectional study, there were no missing data for any of the variables included in the analysis.

RESULTS

Clinical characteristics of the IIM patients

We collected a total of 99 plasma samples from patients with IIM (see table 1), comprising 51 with ASyS and 48 with DM. The overall cohort was 66% female, with a slightly higher proportion of females in the ASyS group (71%) compared with the DM group (60%). The median age at diagnosis was similar between groups: 50 years (IQR 41–64) in ASyS and 51 years (IQR 38–61) in DM. Cancer within 3 years of diagnosis was more prevalent in the DM group (35%) than in ASyS (14%). Prior to sample extraction, 37% of all patients had received treatment, with comparable rates in both subgroups (ASyS: 43%; DM: 40%). Notably, 63% of samples were collected within 3 months of diagnosis, with a higher proportion in the DM group (73%) than ASyS (55%).

Plasma IFN α and MSA/MAA autoantibody status in IIM patients

We sought to characterise a repertoire of autoantibodies against a variety of myositis-specific and myositis-associated (MSA and MAA) targets prior to assessing other potential IFN autoantibody targets with custom microarrays. Our analysis revealed an autoantibody

repertoire towards the following antigens in the patients: AARS1 (PL12), EXOSC10 (PM/ScI.100), EXOSC9 (PM/ScI.75), IFIH1 (MDA5), Jo-1, La.SSB., POLR3A, RNP.Sm (RNP-Smith antigen complex), RNP.Sm.FREE (RNP, free of Smith antigen), RO.SSA, RO60, SAE1, SNRNP70, SNRPA, SNRPB, SNRPC, SNRPD3, SRP68, SRP72, SSB, TRIM21 (RO52), TRIM33 (TIF1 γ). Out of these 22 autoantibodies, 13 were overlapping between ASyS and DM, while 5 and 4 were not shared between IIM subtypes (see figure 1A).

In parallel, plasma IFN- α levels in patients with IIM and BDs were quantified using commercially bought ELISA kits. Although the majority of samples were below the LLOD (2 pg/mL) of this ELISA assay, two patients with ASyS and three patients with DM exhibited elevated (>46.7 pg/mL) plasma IFN- α levels, whereas one BD had elevated plasma IFN- α levels (see online supplemental material figure 1). On plotting all the detected MSA and MAA-positive patients against corresponding detectable plasma IFN- α levels, no strong association between plasma IFN- α levels and specific autoantibody positivity was observed (see figure 1B).

Additionally, a supervised multivariate OPLS-DA model (see online supplemental material figure 2, for model descriptors) based on the MSA and MAA immunoreactivity MFI values and plasma IFN- α concentration values achieved partial separation between ASyS and DM subgroups along the first predictive component, indicating distinct underlying autoantibody profiles. Despite modest predictive performance ($Q^2=0.163$), the clustering patterns suggest meaningful biological differentiation among the patient cohorts (see figure 1C). Additionally, this descriptive analysis indicated Jo-1, TRIM21 and RO60 autoantibody targets as top three class-descriptor variables for patients with ASyS; whereas, IFIH1 (MDA5), TRIM33 (TIF1 γ) and SNRPB autoantibody targets were top three descriptors for patients with DM (see figure 1D). However, plasma IFN- α levels had very low predictive value for distinguishing between ASyS and DM patients (figure 1D).

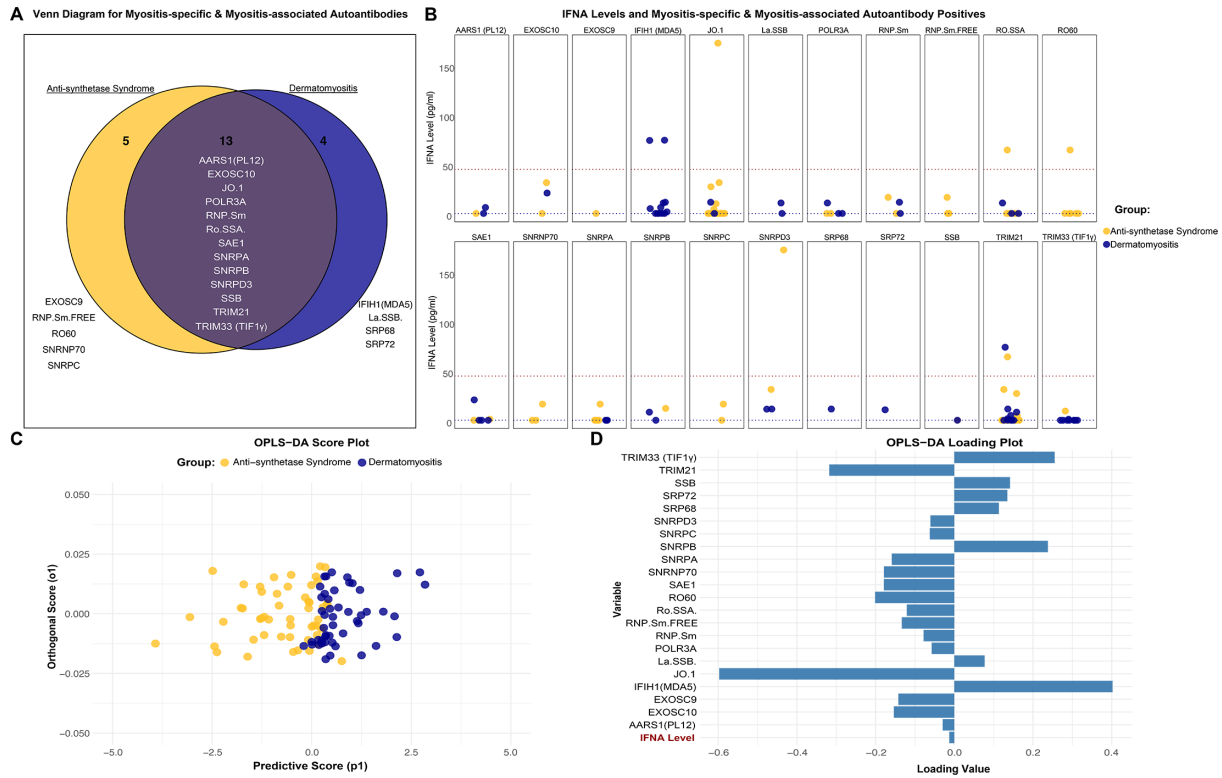


Figure 1 (A) Venn diagram showing a repertoire of autoantibodies against top 22 myositis-specific and myositis-associated antigens (MSS and MAA); out of these 22 autoantibodies, 13 are overlapping in both anti-synthetase syndrome (ASyS, n=51) and dermatomyositis (DM, n=48), while 5 and 4 are non-overlapping and unique to either ASyS or DM, respectively. (B) A scatterplot panel displaying IFN- α levels (pg/mL) in plasma samples from ASyS and DM patients on y-axes, while all the patients positive for different autoantibodies from ASyS (yellow) and DM (dark-blue) are plotted on x-axes. The dotted red line represents an arbitrary cut-off threshold for elevated IFN- α levels that was determined by calculating the 98th percentile value from blood donor (n=100) group, while the dotted blue line represents a lower limit of reliable IFN- α detection assay. (C) A representative OPLS-DA (Orthogonal Projections to Latent Structures Discriminant Analysis) score plot showing a separation of ASyS (yellow, n=51) and DM (dark-blue, n=48) patients based on top 22 MSA and MAA autoantibody status and IFN- α levels. (D) The corresponding OPLS-DA loading plot displaying all the discriminatory class variables (autoantibody status in black; IFN- α level in red).

Effect of immunosuppressive treatment in IIM patients

To investigate the impact of conventional immunosuppressive treatment (with agents such as prednisone, methotrexate, azathioprine and mycophenolate mofetil) on immunological markers, we performed a sensitivity analysis comparing treated and untreated individuals across the described 22 antigen-specific autoantibodies and type-I interferon (IFN- α) levels in plasma. We applied multiple testing correction using both the Benjamini-Hochberg false discovery rate (FDR) and the Bonferroni procedure to account for the increased risk of false positives.

Among the parameters analysed, IFN- α levels (see figure 2A) showed a statistically significant decrease in the treated subgroup (mean=2.04) compared with the untreated subgroup (mean=10.82; p=0.033; FDR-adjusted p=0.041; Bonferroni-adjusted p=0.66). This suggests a consistent and marked suppressive effect of treatment on type-I interferon signalling, even after controlling for FDR. While Bonferroni correction was more conservative and rendered this result non-significant, the FDR-adjusted result supports biological relevance.

Jo-1 autoantibody signal (see figure 2B) showed a suggestive but non-significant trend (raw p=0.066; FDR-adjusted p=0.082; Bonferroni-adjusted p=1.0), with lower levels observed in treated individuals, particularly within the ASyS subgroup. Several other antigens, including SNRPB, SNRPA and SAE1, demonstrated moderate treatment-associated differences, though these did not reach significance under either correction method (adjusted p>0.1).

We next assessed whether the effect of treatment varied by disease subtype (ASyS vs DM) using linear models with interaction terms. A stronger treatment effect on IFN- α levels was observed in DM, as confirmed by model diagnostics (see online supplemental material figure 3, for model diagnostics) and visualised via stratified boxplots and violin plots (figure 2). Untreated DM samples displayed the highest IFN- α levels and variability, while treated samples showed marked suppression. In contrast, Jo-1 autoantibodies remained elevated predominantly in ASyS with modest reductions in treated individuals.

Collectively, these results emphasise the modulatory role of standard of care immunosuppressive treatment,

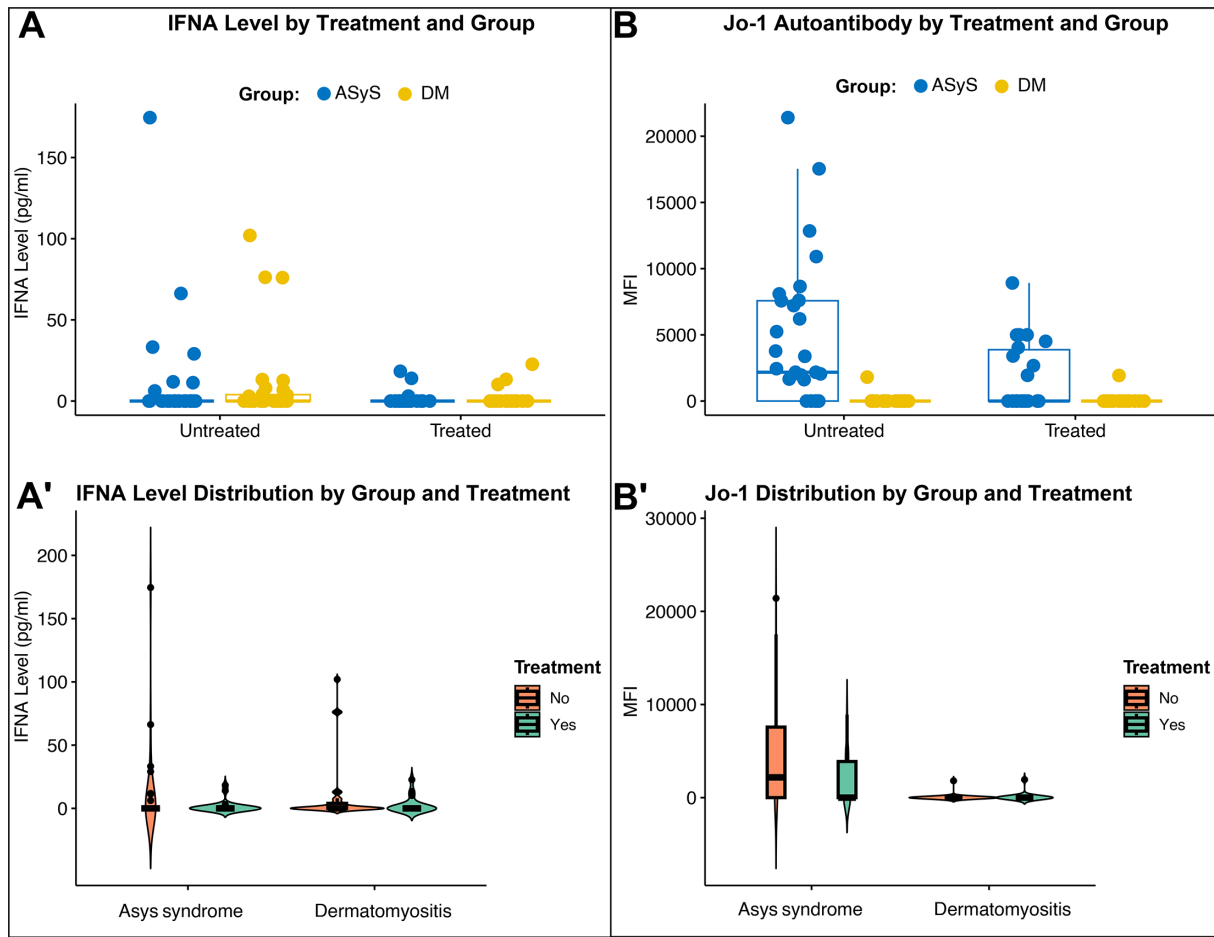


Figure 2 Immunosuppressive treatment-associated changes in IFN- α and Jo-1 autoantibody response levels across disease subtypes. Boxplots (top panel, A, B) and violin plots (bottom panel, A', B') depict the distribution of IFN- α (left) and Jo-1 (right) autoantibody response levels in treated versus untreated individuals, stratified by disease subtype (ASyS and DM). In the boxplots, jittered points (in colour) represent individual sample values, with distinct colour coding by disease group. Violin plots further illustrate the density distribution of the data, using a red-green colour scheme to distinguish untreated (red) and treated (green) groups. Notably, IFN- α levels were substantially lower in treated DM cases, while Jo-1 autoantibody response was more pronounced in ASyS and showed a trend towards reduction following treatment. ASyS, anti-synthetase syndrome; DM, dermatomyositis; MFI, mean fluorescence intensity.

particularly in downregulating IFN- α -associated pathways, and revealed disease subtype-specific responses. The integration of interaction models and multiple hypothesis corrections reinforces the robustness of our findings, while also highlighting the differential biological context of autoantibody and plasma IFN- α levels in treatment response.

A lack of autoantibodies against interferons in IIM patients

Next, we used a bead-based suspension assay approach to confirm potential presence or lack of autoantibodies against interferons and different cytokines and to validate a few of the common autoantibodies in patients with ASyS or DM against, for example, Jo-1, TRIM21/Ro52 (see online supplemental material table 1). Importantly, none of the BDs (n=100) showed a collective elevated immunoreactivity against the mentioned autoantigens, but exhibited high immunoreactivities against viral protein controls, for example, EBNA1 and HSV1gDN1

collectively, similar to patients (figure 3A,B), exhibiting robustness of this multiplex assay.

On analysing the cohort for autoantibodies against type- I, II or III interferons and their corresponding immunoreactivity MFI values, we did not find any evidence of elevated anti-IFN autoantibody response in any of the patient subgroups. The presence of elevated autoantibody response against IFN- α and ω subspecies including $\alpha_{1,2,4,5,7,8,10,16,17}$ and ω_1 in patients with ASyS or DM and BDs was limited to $\leq 1.5\%$, similar to previous reports indicating their prevalence in the general population.¹³ Furthermore, none of the tested ASyS, DM samples exhibited neutralisation capability to inhibit moderate IFN- $\alpha 2$ concentration (1 ng/mL), confirming the lack of functional autoantibodies against IFN- $\alpha 2$ (figure 3C). Additionally, we did not find elevated autoantibody response against type II and III IFNs, that is, IFN- γ or IFN- λ (IL-29, IL-28A/B) in ASyS or DM patients and BDs. Similarly, autoantibody responses to other

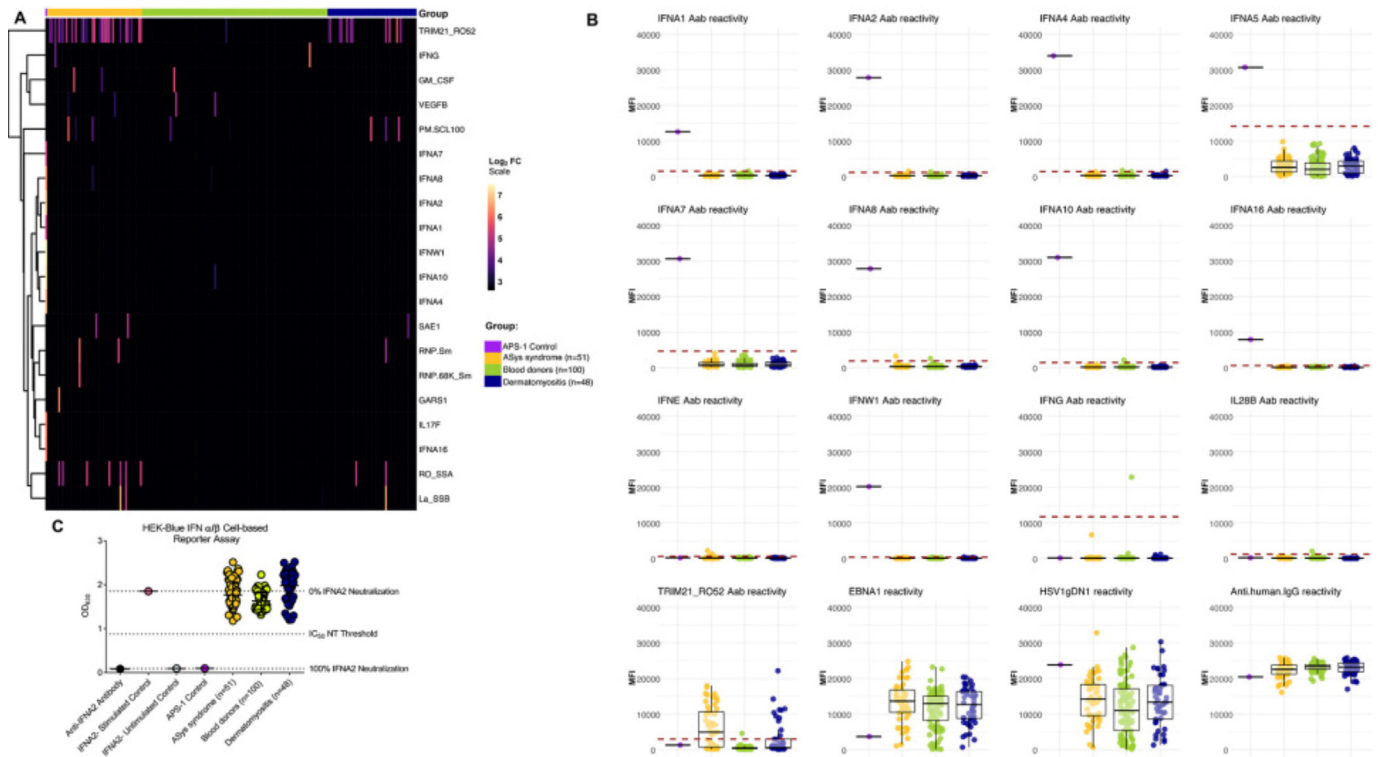


Figure 3 (A) A heatmap showing the top 20 unique antigen-specific reactivities with \log_2 fold-change above the blood donors (light-green, n=100) in this cohort of anti-synthetase syndrome (yellow, n=51) and dermatomyositis (dark-blue, n=48) patients, a single auto-immune polyendocrine syndrome, type-I (APS-1) patient (purple, n=1) is used as a reference biological positive for autoantibodies against type-I IFNs. (B) A scatterplot panel displaying mean fluorescence intensity (MFI) units of immunoreactivity against 16 key antigens including type I, II and III interferons, autoantigens, viral protein controls and total IgG levels evaluated by a bead-based multiplex assay. The dotted dark red line represents an arbitrary cut-off threshold for an elevated autoantibody response, which was determined by calculating five times SD above the MFI value from blood donor group. (C) Neutralisation activity of samples against moderate (1 ng/mL) IFN- α 2 concentration was assessed using a HEK Blue IFN- α/β cell reporter assay; the resultant secreted embryonic alkaline phosphatase (SEAP) release in cell media is proportional to IFN- α 2 mediated cell stimulation and was measured at 630 nm. Samples with response below the half-maximal inhibitory concentration threshold for SEAP activity (IC_{50} value) were considered a threshold for positivity for IFN- α 2 neutralisation.

tested cytokines were largely absent in ASyS and DM patients or BDs (see online supplemental material table 1). The bead-based assay confirmed the earlier results from custom microarray experiment (see online supplemental file 3), exhibiting absence of increased reactivity to interferons whereas, similar amount of autoantibody positives to myositis antigen, for example, TRIM21/Ro52 (in Microarray: 25 in ASyS, 10 in DM; vs in Luminex: 28 in ASyS, 11 in DM).

DISCUSSION

In this study, we comprehensively investigated the presence of neutralising anti-IFN autoantibodies in patients with IIM, specifically those diagnosed with ASyS and DM using three different state-of-the-art autoantibody screening approaches. Our findings reveal a complete absence of neutralising autoantibodies against IFN- α subtypes in this Swedish cohort. This result is in stark contrast to SLE and certain other systemic autoimmune diseases, where autoantibodies targeting type-I IFNs are prevalent and can modulate disease activity.^{7 8 14} Some studies have shown that approximately 10%–15% of

individuals with SLE harbour neutralising autoantibodies targeting IFN- α subtypes, which may interfere with the type-I IFN signalling cascade—an axis known to drive inflammation and contribute to the pathogenesis of SLE.^{7 8} Interestingly, the presence of these autoantibodies in SLE has been variably associated with less severe clinical manifestations or lower IFN gene signatures in some cohorts, although their overall functional relevance remains an area of ongoing research.

The absence of neutralising autoantibodies against type-I IFNs in patients with IIM is notable, as both ASyS and DM subgroups are conditions associated with an upregulated type-I IFN signature in the circulation and in tissues. This suggests that, unlike SLE, where endogenous IFN-neutralising mechanisms have been observed, patients with IIM may lack such immune-modulating factors. Although it is worth noting that SLE is more commonly diagnosed later than IIM, particularly due to its heterogeneous and often subtle early clinical features.^{15 16} Additionally, a few of the prospective studies report type-I IFN signatures are often elevated years before SLE diagnosis, even in asymptomatic individuals

who later develop the disease.¹⁷ The implications of this difference remain to be fully elucidated, but it raises intriguing questions about the underlying mechanisms that drive type-I IFN activation in different autoimmune diseases.

Although our study did not directly measure IFN- β signatures or circulating IFN- β levels, prior work has suggested that IFN- β may be a dominant effector cytokine in patients with DM, particularly in tissue compartments.^{18 19} The lack of autoantibodies against IFN- β observed across autoimmune and infectious cohorts may reflect its limited systemic bioavailability and its cell-type-specific production, primarily by stromal and epithelial cells, including myoblasts and endothelial cells, in an autocrine or paracrine fashion.^{20 21} This contrasts with the more immunogenic IFN- α subtypes, which are frequent targets of neutralising autoantibodies.^{22 23}

The intracellular and rapid nature of IFN signalling may insulate this pathway from antibody-mediated interference. The IFNAR engagement and STAT1/STAT2 phosphorylation occur swiftly at the cell membrane, offering limited extracellular antigenic exposure compared with more sustained cytokines like IL-6 or TNF.^{20 24–26} The rarity of anti-IFN autoantibodies in IIM patients could also reflect immunological thresholds that are not met in this disease, unlike in APS-1 or severe COVID-19, where genetic predispositions such as AIRE mutations or TLR pathway variants enable breach of tolerance and emergence of neutralising autoantibodies.^{23 27 28}

Intrinsic regulatory mechanisms such as SOCS proteins, IRF repressors and nucleic acid-sensing feedback loops are key modulators of IFN signalling that may attenuate pathway activation intracellularly, reducing the need for extracellular neutralisation.^{24 29} This supports the view that the persistent IFN signature in IIM may reflect a tightly regulated endogenous state, rather than uncontrolled IFN overproduction.¹⁹ The absence of neutralising anti-IFN autoantibodies in IIM may thus stem from a fundamentally different immune architecture compared with diseases like SLE or APS-1, where tolerance is broken and IFNs become direct immunological targets. Further cell-specific and tissue-level interrogation, particularly in muscle and skin, will be essential for understanding the therapeutic potential of targeting this axis in myositis.³⁰

Our findings highlight the immunosuppressive treatment-associated modulation of IFN- α and Jo-1 autoantibody response across distinct IIM subtypes, with IFN- α suppression most prominent in DM and Jo-1 autoantibody response reduction more evident in ASyS. The pronounced decline in IFN- α levels among treated patients with DM suggests that type-I interferon signalling may be a particularly treatment-responsive axis in this group, potentially reflecting differences in baseline interferon activity or tissue tropism. In contrast, Jo-1 autoantibody response, which defines a hallmark serotype in ASyS, demonstrated only modest decreases, consistent with their role as a stable disease biomarker rather than a direct effector of dynamic immune activity. Notably,

the absence of detectable neutralising anti-IFN autoantibodies in both subtypes suggests that the observed treatment-related reduction in IFN- α is not confounded by inherent IFN-blocking mechanisms, thereby strengthening the interpretation that immunosuppression directly downregulates IFN- α pathway activity. However, potential biases remain, including uneven baseline IFN- α levels between subtypes and differences in treatment exposure or disease duration, which may influence treatment responsiveness. Stratifying analyses by disease subtype was critical to avoid masking these subtype-specific effects, and future studies should incorporate longitudinal profiling and mechanistic assays to disentangle primary vs secondary immunological shifts under therapy.

Our findings may have important therapeutic implications. Targeting the IFN pathway has emerged as a promising strategy in autoimmune diseases, with drugs such as anifrolumab demonstrating efficacy in SLE by blocking IFN- α receptor signalling. Given that IFN-driven pathology is also implicated in IIM, it remains uncertain whether the absence of natural, neutralising anti-IFN autoantibodies influences the responsiveness of IIM patients to such therapies. Although, in myositis patients especially in DM with skin-predominant manifestations, the disease activity is driven by IFN- β , over IFN- α signatures and a monoclonal antibody, dazukirbart, selectively blocking IFN- β has shown promising effects.³¹ Future studies should explore whether exogenous IFN- α/β specific blockade strategy yields similar therapeutic benefits in IIM as it does in SLE. Additionally, understanding why some autoimmune diseases develop neutralising anti-IFN autoantibodies while others do not could provide critical insights into disease pathogenesis and treatment selection.

A key limitation of our study is that it was conducted in a single cohort of Swedish patients, which may limit the generalisability of our findings to other populations with distinct genetic backgrounds or environmental exposures. Population-specific variation such as differences in HLA class II haplotypes, polymorphisms in IFNAR1 and IFNAR2, and differential expression of interferon-regulatory genes may significantly influence baseline IFN activity and immune responses, including the propensity to develop anti-IFN autoantibodies.^{32–34} These genetic factors have been associated with variation in disease phenotypes, interferon pathway activation and response to IFN-targeted therapies across both autoimmune and infectious disease settings.^{35 36} The absence of neutralising anti-IFN autoantibodies in our IIM cohort may therefore reflect underlying host-genetic differences that shape the immunologic landscape in Scandinavian populations. Expanding future studies to include diverse, multi-ethnic cohorts will be important to capture the full spectrum of IFN-related immune phenomena in IIM.

We also acknowledge the lack of longitudinal sampling in our cohort, which precludes evaluation of temporal changes in anti-IFN autoantibody status. While such

autoantibodies have been reported to be stable over time in certain contexts such as APS-1 and subsets of SLE,^{14 23 37} their dynamics in IIM remain unknown. It is plausible that anti-IFN autoantibodies, if present, may arise transiently in response to infection,³⁸ disease activity or immunomodulatory treatment. Longitudinal studies will be necessary to determine whether anti-IFN autoantibodies fluctuate over time in IIM, and if so, whether such changes correlate with clinical course or IFN pathway activity.

Additionally, while we comprehensively screened for neutralising antibodies against multiple IFN- α subtypes, our study does not rule out other potential mechanisms of IFN modulation, such as non-neutralising autoantibodies, epitope-specific differences or intracellular regulatory pathways that might dampen signalling independently of circulating autoantibody effects.

In conclusion, we report a complete absence of neutralising autoantibodies against IFN- α as well as other types of IFNs in a Swedish cohort with ASyS and DM patients. These findings contrast with the presence of autoantibodies against type-I IFNs in SLE and other systemic diseases characterised by type-I IFN overactivation. Understanding interferon status including its drivers and the presence of natural neutralising factors across disease groups is important for determining which patients are most likely to benefit from new anti-IFN treatments, such as anifrolumab and dazukirbart.

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Contributors AB, IL, BH and NL conceived and designed the study. AB, HM, IPG, CPB and BH conducted experiments, analysed data. AB wrote the manuscript. HM, IPG, CPB, IL, BH and NL provided feedback on the manuscript. AB is the guarantor. During the preparation of this work, the author(s) used ChatGPT-4 by OpenAI in order to edit contents of this manuscript and refine the language of this manuscript. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

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

Ethics approval This study involves human participants. The Swedish Ethical Review Authority approved the conduct of this study. All steps and processes conformed to the Declaration of Helsinki in its most recent amended form and the Good Clinical Practice guidelines. Patients were recruited at the myositis unit

at Karolinska University Hospital after obtaining written informed consent forms for study participation. This study involves human samples approved by the Stockholm Ethics Examination Authority (2005/792-31/4). The research use of plasma samples collected from anonymous healthy blood donors was approved by Swedish Ethical Review Authority (2016/1422-31/1; 2016/2553-31/2). Participants gave informed consent to participate in the study before taking part.

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Data availability statement Data are available on reasonable request. All data relevant to the study are included in the article or uploaded as supplementary information. The datasets regarding individual autoantibody status used in this study are not publicly available due to ethical considerations but can be requested from the corresponding author.

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ORCID iDs

Anish Behere <http://orcid.org/0000-0002-2424-3475>

Ingrid Lundberg <http://orcid.org/0000-0002-6068-9212>

REFERENCES

- Muskardin TLW, Niewold TB. Type I interferon in rheumatic diseases. *Nat Rev Rheumatol* 2018;14:214–28.
- Lundberg IE, Tjärnlund A, Bottai M, *et al*. 2017 European League Against Rheumatism/American College of Rheumatology classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups. *Ann Rheum Dis* 2017;76:1955–64.
- Argyriou A, Horuluoglu B, Galindo-Feria AS, *et al*. Single-cell profiling of muscle-infiltrating T cells in idiopathic inflammatory myopathies. *EMBO Mol Med* 2023;15:e17240.
- Bolko L, Jiang W, Tawara N, *et al*. The role of interferons type I, II and III in myositis: A review. *Brain Pathol* 2021;31:e12955.
- Bastard P, Gervais A, Le Voyer T, *et al*. Human autoantibodies neutralizing type I IFNs: From 1981 to 2023. *Immunol Rev* 2024;322:98–112.
- Casanova J-L, Peel J, Donadieu J, *et al*. The ouroboros of autoimmunity. *Nat Immunol* 2024;25:743–54.
- Gupta S, Tatouli IP, Rosen LB, *et al*. Distinct Functions of Autoantibodies Against Interferon in Systemic Lupus Erythematosus: A Comprehensive Analysis of Anticytokine Autoantibodies in Common Rheumatic Diseases. *Arthritis Rheumatol* 2016;68:1677–87.
- Bradford HF, Haljasmägi L, Menon M, *et al*. Inactive disease in patients with lupus is linked to autoantibodies to type I interferons that normalize blood IFN α and B cell subsets. *Cell Rep Med* 2023;4:100894.
- Connors GR, Christopher-Stine L, Oddis CV, *et al*. Interstitial lung disease associated with the idiopathic inflammatory myopathies: what progress has been made in the past 35 years? *Chest* 2010;138:1464–74.
- Le Voyer T, Parent AV, Liu X, *et al*. Autoantibodies against type I IFNs in humans with alternative NF- κ B pathway deficiency. *Nature New Biol* 2023;623:803–13.
- Vanker M, Särekannu K, Fekkar A, *et al*. Autoantibodies Neutralizing Type III Interferons Are Uncommon in Patients with Severe Coronavirus Disease 2019 Pneumonia. *J Interferon Cytokine Res* 2023;43:379–93.
- Behere A, Hallberg P, Cederholm A, *et al*. Evaluating IL1RA-Autoantibodies Across SARS-CoV-2-Related Diseases. *Scand J Immunol* 2025;102:e70039.
- Fernbach S, Mair NK, Abela IA, *et al*. Loss of tolerance precedes triggering and lifelong persistence of pathogenic type I interferon autoantibodies. *J Exp Med* 2024;221:e20240365.

- 14 Beydon M, Nicaise-Roland P, Mageau A, *et al.* Autoantibodies against IFN α in patients with systemic lupus erythematosus and susceptibility for infection: a retrospective case-control study. *Sci Rep* 2022;12:11244.
- 15 Kernder A, Richter JG, Fischer-Betz R, *et al.* Delayed diagnosis adversely affects outcome in systemic lupus erythematosus: Cross sectional analysis of the LuLa cohort. *Lupus (Los Angel)* 2021;30:431–8.
- 16 Namsrai T, Parkinson A, Chalmers A, *et al.* Diagnostic delay of myositis: an integrated systematic review. *Orphanet J Rare Dis* 2022;17:420.
- 17 Postal M, Vivaldo JF, Fernandez-Ruiz R, *et al.* Type I interferon in the pathogenesis of systemic lupus erythematosus. *Curr Opin Immunol* 2020;67:87–94.
- 18 Walsh RJ, Kong SW, Yao Y, *et al.* Type I interferon-inducible gene expression in blood is present and reflects disease activity in dermatomyositis and polymyositis. *Arthritis & Rheumatism* 2007;56:3784–92.
- 19 Greenberg SA. Type 1 interferons and myositis. *Arthritis Res Ther* 2010;12 Suppl 1:S4.
- 20 Plataniias LC. Mechanisms of type-I- and type-II-interferon-mediated signalling. *Nat Rev Immunol* 2005;5:375–86.
- 21 Mammen AL. Autoimmune myopathies: autoantibodies, phenotypes and pathogenesis. *Nat Rev Neurol* 2011;7:343–54.
- 22 Meisel C, Akbil B, Meyer T, *et al.* Mild COVID-19 despite autoantibodies against type I IFNs in autoimmune polyendocrine syndrome type 1. *J Clin Invest* 2021;131:e150867.
- 23 Bastard P, Rosen LB, Zhang Q, *et al.* Autoantibodies against type I IFNs in patients with life-threatening COVID-19. *Science* 2020;370:eabd4585.
- 24 Ivashkiv LB, Donlin LT. Regulation of type I interferon responses. *Nat Rev Immunol* 2014;14:36–49.
- 25 Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. *Cold Spring Harb Perspect Biol* 2014;6:a016295.
- 26 Bradley JR. TNF-mediated inflammatory disease. *J Pathol* 2008;214:149–60.
- 27 Meager A, Visvalingam K, Peterson P, *et al.* Anti-interferon autoantibodies in autoimmune polyendocrinopathy syndrome type 1. *PLoS Med* 2006;3:e289.
- 28 Bastard P, Orlova E, Sozaeva L, *et al.* Preexisting autoantibodies to type I IFNs underlie critical COVID-19 pneumonia in patients with APS-1. *J Exp Med* 2021;218:e20210554.
- 29 Yoshimura A, Naka T, Kubo M. SOCS proteins, cytokine signalling and immune regulation. *Nat Rev Immunol* 2007;7:454–65.
- 30 López De Padilla CM, Crowson CS, Hein MS, *et al.* Gene Expression Profiling in Blood and Affected Muscle Tissues Reveals Differential Activation Pathways in Patients with New-onset Juvenile and Adult Dermatomyositis. *J Rheumatol* 2017;44:117–24.
- 31 Benhadou F, Cogan E. Targeting IFN β in dermatomyositis. *The Lancet* 2025;405:99–101.
- 32 Fernando MMA, Freudenberg J, Lee A, *et al.* Transancestral mapping of the MHC region in systemic lupus erythematosus identifies new independent and interacting loci at MSH5, HLA-DPB1 and HLA-G. *Ann Rheum Dis* 2012;71:777–84.
- 33 Niewold TB. Interferon alpha as a primary pathogenic factor in human lupus. *J Interferon Cytokine Res* 2011;31:887–92.
- 34 Gallucci S, Meka S, Gamero AM. Abnormalities of the type I interferon signaling pathway in lupus autoimmunity. *Cytokine* 2021;146.
- 35 Pairo-Castineira E, Clohisey S, Klaric L, *et al.* Genetic mechanisms of critical illness in COVID-19. *Nature New Biol* 2021;591:92–8.
- 36 Zhang Q, Bastard P, Liu Z, *et al.* Inborn errors of type I IFN immunity in patients with life-threatening COVID-19. *Science* 2020;370:eabd4570.
- 37 Shaw ER, Rosen LB, Cheng A, *et al.* Temporal Dynamics of Anti-Type 1 Interferon Autoantibodies in Patients With Coronavirus Disease 2019. *Clin Infect Dis* 2022;75:e1192–4.
- 38 Babcock BR, Kusters A, Eddins DJ, *et al.* Transient anti-interferon autoantibodies in the airways are associated with recovery from COVID-19. *Sci Transl Med* 2024;16:eaddq1789.