Brief report

Autoantibodies to type I interferons in patients with systemic mastocytosis

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Background: Autoantibodies to type I interferons have been identified in association with a variety of inflammatory and autoimmune diseases. Type I interferons have demonstrated inhibitory effects on mast cell proliferation and degranulation. Systemic mastocytosis (SM) is a disease characterized by increased mast cell burden and mediator release. Whether autoantibodies to type I interferon are present in the sera of patients with SM, and if so, whether they correlate with characteristics of disease, is unknown.

Objective: The purpose of this study was to determine whether autoantibodies to type I interferons are observed in the sera of patients with SM, and if so, whether they correlate with biomarkers of disease severity.

Methods: We analyzed sera from 89 patients with SM for concentrations of autoantibodies to type I interferon by using a multiplex particle-based assay and signal neutralization capacity by using a STAT1 activity assay and then compared these measurements with those in a database of information on 1284 healthy controls.

Results: Our cohort was predominantly female (57.3%), with a median age of 56 years. Of the cohort members, 13 produced autoantibodies to IFN-β, 3 to IFN-ω, and 0 to IFN-α. None of the 13 sera demonstrated signal neutralization. Neither autoantibody concentration nor signaling inhibition measurements correlated with tryptase concentrations or *D816V* allele burden.

Conclusion: Although a small subpopulation of patients with SM have autoantibodies to type I interferons, there was no correlation between autoantibody production and signaling inhibition. These data are consistent with the conclusion that autoantibodies to type I interferon do not play a significant role in the pathogenesis or severity of SM. (J Allergy Clin Immunol Global 2024;3:100273.)

Key words: *Autoantibodies, type I interferons, cytokines, mastocytosis, mast cell, inflammatory, autoimmune, COVID-19*

INTRODUCTION

Antibodies to type I interferons have been identified in association with various diseases, including SLE,[1] thymoma and/or myasthenia gravis,[2] autoimmune polyendocrinopathy syndrome type 1,[3] psoriasis,[4] and chronic liver disease,[5] in which they have been implicated in disease pathology and prognosis. Recently, the presence of autoantibodies to type I interferons were identified as playing a significant role in disease outcome in a subset of patients with coronavirus disease 2019 (COVID-19) infection. This group showed that at least 10% of patients experiencing life-threatening COVID-19 pneumonia produced neutralizing levels of autoantibodies against type I interferon, thereby inhibiting the antiviral response that type I interferon induces in a variety of cell types.[6]

Systemic mastocytosis (SM) is a disease characterized by the aberrant expansion and accumulation of clonal mast cells in 1 or more organ systems, which is associated with mediator release and results in a constellation of symptoms, including flushing, pruritis, anaphylaxis, musculoskeletal pain, nausea, vomiting, diarrhea, and abdominal pain.[7] Notably, type I interferon has been demonstrated to inhibit mast cell proliferation, degranulation, and bone marrow infiltration, which helped provide a rationale for use of interferons in the treatment of patients with mastocytosis.[8,9] Furthermore, the critical role of type I interferons in steady-state mast cell homeostasis was demonstrated in an animal model deficient in the receptor complex for type I interferon and displayed exacerbated systemic anaphylaxis after sensitization, increased histamine in the circulation; and increased secretory granule synthesis and release.[10] On the basis of these findings indicating that type I interferons influence mast cell homeostasis, we hypothesized that autoantibodies to type I interferons may play a role in disease activity.

RESULTS AND DISCUSSION

Following informed consent on National Institutes of Health protocol 19-I-0277, sera were drawn from 89 adult patients with SM. Detection of anticytokine autoantibodies was conducted through a multiplex particle-based assay in which magnetic beads with differential fluorescence were covalently coupled to recombinant human proteins. Sera with a

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Abbreviations used
FI: Fluorescence intensity
MCA: Mast cell activation
SM: Systemic mastocytosis

fluorescence intensity (FI) greater than the mean plus 3 SDs of previously assessed healthy control sera[6,11] (FI > 1865, FI > 446, and FI > 2277, for IFN-α-2b, IFN-β, and IFN-ω, respectively [n = 1284] for anti–IFN-α, anti–IFN-β, and anti–IFN-ω, respectively) were considered "positive" in that they met the required threshold for functional testing for the respective type I interferon subtype. The neutralizing activities of anti-interferon autoantibodies were determined by assessing STAT1 phosphorylation in PBMCs from a healthy control after stimulation with appropriate cytokines in the presence of 10% healthy control or patient sera. The neutralizing capacity of autoantibody-positive patient sera was measured as a percentage of the stimulation index (stimulated over unstimulated condition) normalized against that of the healthy control sera. Less than 20% phosphorylated STAT1 activity was considered blocking, 20% to 65% phosphorylated STAT1 activity was considered partially blocking, and greater than 65% phosphorylated STAT1 activity was considered to not have blocking effects on signaling.

In this cohort of 89 patients with SM, patient age ranged from 20 to 79 years, with a median of 56 years (Table I). Of the 89 patients, 51 were female and 38 were male. The distribution of self-reported race/ethnicity was as follows: 1 patient was Asian, 1 was Black, 84 were White, and 3 did not provide race/ethnicity information. The median level of serum tryptase, which is a marker of mast cell burden,[12] was 80.7 ng/mL, with an interquartile range of 42.2 to 149.0 ng/mL. According to the World Health Organization criteria for SM subgroups,[13] 69 patients had indolent SM,[6] 16 had smoldering SM, and 4 patients has aggressive SM,[6] with 84 patients having concurrent maculopapular cutaneous mastocytosis. The majority of adult patients with SM harbored an activating mutation (*D816V*) in *KIT*. The total proportion of patients in the entire cohort in whom any form of *KIT D816V* analysis was performed was 84 of 89 (in either the peripheral blood or bone marrow), of whom 76 were positive (90.5%). Using allele-specific quantitative PCR to measure *D816V* allele burden in peripheral blood, we determined that 49 of 60 patients (81.7%) who were evaluated were positive (a median of 1.331 and an IQR of 0.257-3.385) (Table I).[14]

No patients met the threshold for functional testing for autoantibodies against IFN-α. In all, 13 patient samples reached the threshold for autoantibodies against IFN-β and 3 patient samples reached the threshold for autoantibodies against IFN-ω, with 2 patients meeting the threshold for both. Those patients whose sera met the threshold for autoantibodies to any type I interferon subtype were subsequently analyzed for neutralizing effects *in vitro* by using 10% sera (Fig 1 and Table II).

No patient sera blocked or partially blocked STAT1 signaling activity when stimulated with 10 ng/mL of IFN-β, with the percentage of signaling ranging from 71% to 125% of the median healthy control signaling value (ie, 103%). Similarly, no patients had blocking or partially blocking activity against IFN-ω (range 103%-136%, median 111%) (Fig 1 and Table II). Type I

TABLE I. Patient demographics

Characteristic (n = 89)	Value
Age (y), median (IQR)	56 (44-65)
Sex, % (no.)	
Female	57.3% (51)
Male	42.7% (38)
Race and ethnicity, % (no.)	
Asian	1.1% (1)
Black	1.1% (1)
Unknown	3.4% (3)
White	94.4% (84)
Tryptase level (ng/mL), median (IQR)	80.70 (42.2-149.0)
Diagnoses, % (no.)	
Indolent SM	77.5% (69)
Smoldering SM	18.0% (16)
Aggressive SM	4.5% (4)
D816V analyses, % (no./no.)	
Performed in either bone marrow or peripheral blood via any method	94.4% (84/89)
Performed in bone marrow	88.1% (74/84)
Performed in peripheral blood	98.8% (83/84)
Positive in either bone marrow or peripheral blood via any method	90.5% (76/84)
Positive in bone marrow	90.5% (67/74)
Positive in peripheral blood	72.3% (60/83)
Performed in peripheral blood via ASqPCR	67.4% (60/89)
Positive in peripheral blood via ASqPCR*	81.7% (49/60)
Median (IQR)	1.33 (0.26-3.39)
MCA-related symptoms, % (no.)	
Anaphylaxis	40.5% (36)
Gastrointestinal symptoms	92.1% (82)
Flushing	76.4% (68)

ASqPCR, Allele-specific quantitative PCR; *IQR*, interquartile range.
*Cutoff used was 0.03%.

interferon autoantibody concentrations, as measured by the multiplex particle-based assay, did not correlate with serum tryptase levels (for IFN-α level, $P = .33$ and $R^2 = 0.01$; for IFN-β level, $P = .10$ and $R^2 = 0.03$; and for IFN-ω level: $P = .20$ and $R^2 = 0.02$) (Table II). Thus, according to this assay, which is the most relevant analysis, there was no evidence of a functional consequence.

When tested for signal neutralization of autoantibodies against IFN-β, STAT1 signaling activity had a slightly positive correlation with tryptase value ($P = .05$ and $R^2 = 0.32$). However, given the small R^2 value, this is unlikely to be of clinical significance. In testing for neutralization of IFN-ω through measurement of STAT1 signaling activity, STAT1 signaling activity as a percentage of the stimulation index normalized against healthy control sera had no correlation with tryptase level ($P = .98$ and $R^2 < 0.01$). (Table II).

Mast cell *KIT D816V* mutation detection values did not correlate with levels of autoantibodies against any subtypes of type I interferon (for autoantibodies against IFNα vs D816V, $P = .78$ and $R^2 < 0.01$; for autoantibodies against IFN-β vs D816V, $P = .88$ and $R^2 < 0.01$; and for autoantibodies against IFN-ω vs D816V, $P = 1.00$ and $R^2 < 0.01$). Additionally, STAT1 activity was not correlated with *KIT D816V* detection values ($P = .28$ and $R^2 = 0.36$) (Table II). When autoantibodies against type I interferon levels in those who were positive for presence of the *KIT D816V* mutation in peripheral blood were compared with the levels in those who were negative for presence of the same

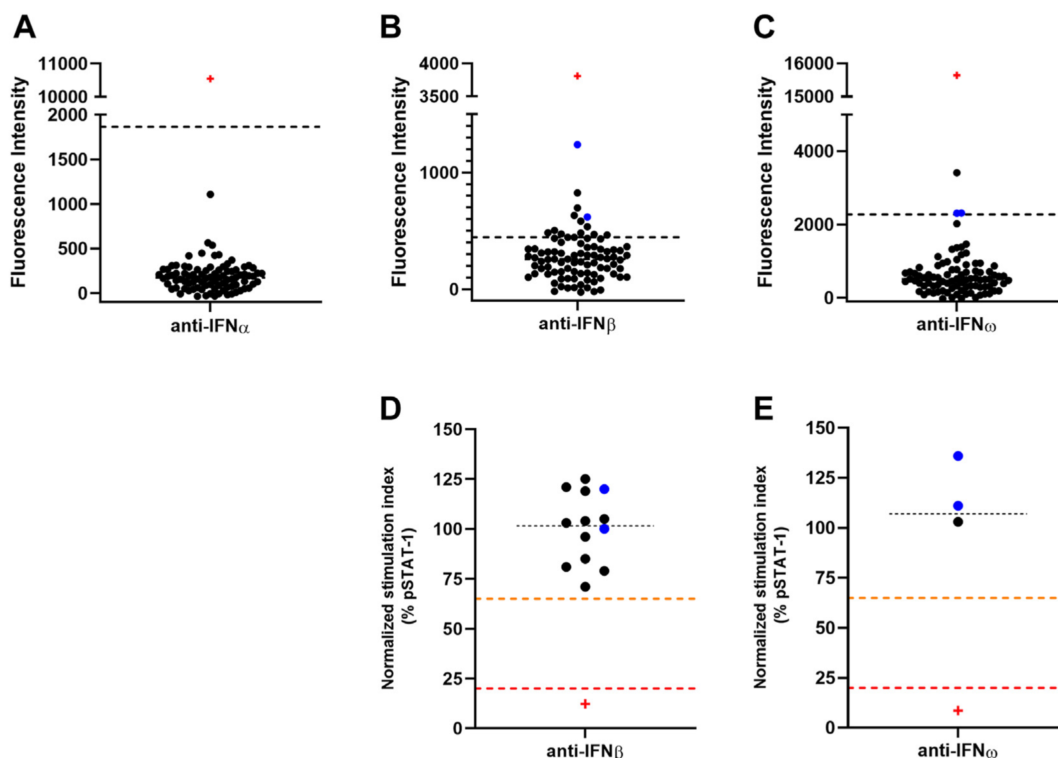

FIG 1. Binding activity and neutralization of autoantibodies to type I interferon in patients with SM. **A-C,** Scatter plot for the distribution of concentrations of autoantibodies to IFN-α (**A**), IFN-β (**B**), and IFN-ω (**C**). **D** and **E,** Scatterplot of the normalized stimulation index following stimulation by the respective type I interferon subtype for patients who met threshold for autoantibody production, with less than 20% considered blocking, 20% to 65% considered partially blocking, and greater than 65% considered not blocking. AIRE.23C plasma sample was used as the positive control for percentage of phosphorylated STAT1 anti–IFN-β (9.73%) and anti–IFN-ω (8.54%), as denoted by the red crosses in (**D** and **E**). **B-D** and **E,** Blue dots indicate the same patients.

TABLE II. Concentrations and neutralization of autoantibodies to type I interferons in sera of patients with SM

Results of assays of autoantibodies to IFN	IFN-α	IFN-β	IFN-ω
Patients with levels above cutoff for binding to type I interferons, no. (%)	0	13 (14.6%)	3 (3.4%)
Patients positive for neutralizing autoantibodies, no.	0	0	0
Normalized stimulation index (% phosphorylated STAT1), median (IQR)		103.0 (83.0-119.5)	111.0 (103.0-136.0)
Correlations, *P* value (R^2)			
FI vs tryptase	.33 (0.01)	.10 (0.03)	.20 (0.02)
STAT1 activity vs tryptase		.05 (0.32)	.98 (<0.01)
FI vs KIT *D816V* allele burden	.78 (<0.01)	.88 (<0.01)	1.00 (<0.01)
STAT1 activity vs KIT *D816V* allele burden		.28 (0.36)	
D816V positive vs negative, *P* value (*U* value)*	.50 (236.5)	.35 (218)	.48 (233)

FI, Fluorescent intensity; *IQR*, interquartile range.
*Mann-Whitney *U* test comparison.

mutation, no significant differences were found (when comparing IFN-α levels, $P = .50$; when comparing IFN-β levels, $P = .35$; and when comparing IFN-ω levels, $P = .48$) (Table II).

No significant differences were found when analyzing concentrations of autoantibodies against IFN-α, IFN-β, and IFN-ω, as measured by the multiplex particle-based assay with comparator SM subgroups (aggressive SM, smoldering SM, and indolent SM), nor were concentrations of autoantibodies correlated with age or use of medication, including cytoreductive agents (n = 3) (data not shown). Regarding mast cell activation (MCA)-related symptoms, 40.5% of our cohort had a history of anaphylaxis, 92.1% reported chronic gastrointestinal symptoms (abdominal pain, diarrhea, nausea, and/or vomiting), and 76.4% reported consistent flushing episodes (Table I.). Within the group of patients who were positive for autoantibodies to IFN-β, there was no difference in the proportion of patients who had a history of MCA-related symptoms and those who did not. Thus, MCA-related symptoms were not associated with elevations in concentrations of autoantibodies to IFN-β. The signal blocking measurements were also not significantly different when SM

was classified by SM subgroup (data not shown). Thus, no patterns relating autoantibodies against type I interferon to any metrics associated with disease severity in mastocytosis were found.[15]

To our knowledge, this is the first study to report measurement of serum levels of autoantibodies to type I interferon in patients with SM and attempt to correlate these findings with disease manifestations or severity of illness. Our results demonstrate that although significant levels of autoantibodies against type I interferon subtypes were identified in 14 of 89 patients with SM (14.6%) and although there is a 1.1% positivity rate for IFN-β in the general population, no apparent inhibition of type I interferon signaling via autoantibodies to type I interferon was found.[6] These findings support the conclusion that autoantibodies to type I interferon do not have a significant role in the pathogenesis or manifestations of SM.

DISCLOSURE STATEMENT

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Key messages

- **Autoantibodies to type I interferon have been implicated in autoimmune, inflammatory, and (more recently) COVID -19 pathology and progression.**
- **Whether autoantibodies to type I interferon and blocking of type I interferon signaling participate in the pathology and disease manifestation of mastocytosis, which is characterized by aberrant numbers and activity of mast cells (whose proliferation and degranulation are inhibited by type I interferon), has been investigated.**
- **The investigation found no significant correlations between production of autoantibodies to type I interferon and objective measures of disease burden or severity in mastocytosis, suggesting a lack of a significant role for anti-interferon autoantibodies in disease pathology and manifestations.**

REFERENCES

1. Slavikova M, Schmeisser H, Kontsekova E, Mateicka F, Borecky L, Kontsek P. Incidence of autoantibodies against type I and type II interferons in a cohort of systemic lupus erythematosus patients in Slovakia. J Interferon Cytokine Res 2003;23:143-7.
2. Meager A, Wadhwa M, Dilger P, Bird C, Thorpe R, Newsom-Davis J, Willcox N. Anti-cytokine autoantibodies in autoimmunity: preponderance of neutralizing autoantibodies against interferon-alpha, interferon-omega and interleukin-12 in patients with thymoma and/or myasthenia gravis. Clin Exp Immunol 2003;132:128-36.
3. Meager A, Visvalingam K, Peterson P, Möll K, Murumägi A, Krohn K, et al. Anti-interferon autoantibodies in autoimmune polyendocrinopathy syndrome type 1. PLoS Med 2006;3:e289.
4. Bergman R, Ramon M, Wildbaum G, Avitan-Hersh E, Mayer E, Shemer A, Karin N. Psoriasis patients generate increased serum levels of autoantibodies to tumor necrosis factor-alpha and interferon-alpha. J Dermatol Sci 2009;56:163-7.
5. Ikeda Y, Miyake K, Toda G, Yamada H, Yamanaka M, Oka H. Detection of anti-interferon-alpha 2a antibodies in chronic liver disease. J Gastroenterol Hepatol 1989;4:411-8.
6. Bastard P, Rosen LB, Zhang Q, Michailidis E, Hoffmann H-H, Zhang Y, et al. Autoantibodies against type I IFNs in patients with life-threatening COVID-19. Science 2020;370:eabd4585.
7. Valent P, Akin C, Metcalfe DD. Mastocytosis: 2016 updated WHO classification and novel emerging treatment concepts. Blood 2017;129:1420-7.
8. Casassus P, Caillat-Vigneron N, Martin A, Simon J, Gallais V, Beaudry P, et al. Treatment of adult systemic mastocytosis with interferon-α: results of a multicentre phase II trial on 20 patients. Br J Haematol 2002;119:1090-7.
9. Kluin-Nelemans HC, Jansen JH, Breukelman H, Wolthers BG, Kluin PM, Kroon HM, Willemze R. Response to interferon alfa-2b in a patient with systemic mastocytosis. N Engl J Med 1992;326:619-23.
10. Kobayashi T, Shimabukuro-Demoto S, Tsutsui H, Toyama-Sorimachi N. Type I interferon limits mast cell–mediated anaphylaxis by controlling secretory granule homeostasis. PLoS Biol 2019;17:e3000530.
11. Gupta S, Tatouli IP, Rosen LB, Hasni S, Alevizos I, Manna ZG, 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.
12. Sperr WR, Jordan JH, Fiegl M, Escribano L, Bellas C, Dirnhofer S, et al. Serum tryptase levels in patients with mastocytosis: correlation with mast cell burden and implication for defining the category of disease. Int Arch Allergy Immunol 2002;128:136-41.
13. Valent P, Akin C, Hartmann K, Alvarez-Twose I, Brockow K, Hermine O, et al. Updated diagnostic criteria and classification of mast cell disorders: a consensus proposal. Hemasphere 2021;5:e646.
14. Hoermann G, Sotlar K, Jawhar M, Kristensen T, Bachelot G, Nedoszytko B, et al. Standards of genetic testing in the diagnosis and prognostication of systemic mastocytosis in 2022: recommendations of the EU-US Cooperative Group. J Allergy Clin Immunol Pract 2022;10:1953-63.
15. Graf I, Herndlhofer S, Kundi M, Greiner G, Sperr M, Hadzijusufovic E, et al. Incidence of symptomatic Covid-19 infections in patients with mastocytosis and chronic myeloid leukemia: a comparison with the general Austrian population. Eur Jf Haematol 2023;110:67-76.