ARTHRITIS & RHEUMATISM
Vol. 63, No. 8, August 2011, pp 2407–2415
DOI 10.1002/art.30399

Association of Endogenous Anti–Interferon-α Autoantibodies With Decreased Interferon-Pathway and Disease Activity in Patients With Systemic Lupus Erythematosus

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Objective. **Numerous observations implicate interferon-α (IFNα) in the pathophysiology of systemic lupus erythematosus (SLE); however, the potential impact of endogenous anti-IFNα autoantibodies (AIAAs) on IFN-pathway and disease activity is unclear. The aim of this study was to characterize IFN-pathway activity and the serologic and clinical profiles of AIAA-positive patients with SLE.**

Methods. **Sera obtained from patients with SLE (n = 49), patients with rheumatoid arthritis (n = 25), and healthy control subjects (n = 25) were examined for the presence of AIAAs, using a biosensor immunoassay. Serum type I IFN bioactivity and the ability of AIAA-positive sera to neutralize IFNα activity were determined using U937 cells. Levels of IFN-regulated gene expression in peripheral blood were determined by microarray, and serum levels of BAFF, IFN-inducible chemokines, and other autoantibodies were measured using immunoassays.**

Results. **AIAAs were detected in 27% of the serum samples from patients with SLE, using a biosensor immunoassay. Unsupervised hierarchical clustering analysis identified 2 subgroups of patients, IFN^{low} and IFN^{high}, that differed in the levels of serum type I IFN bioactivity, IFN-regulated gene expression, BAFF, anti–ribosomal P, and anti-chromatin autoantibodies, and in AIAA status. The majority of AIAA-positive patients had significantly lower levels of serum type I IFN bioactivity, reduced downstream IFN-pathway activity, and lower disease activity compared with the IFN^{high} patients. AIAA-positive sera were able to effectively neutralize type I IFN activity in vitro.**

Conclusion. **Patients with SLE commonly harbor AIAAs. AIAA-positive patients have lower levels of serum type I IFN bioactivity and evidence for reduced downstream IFN-pathway and disease activity. AIAAs may influence the clinical course in SLE by blunting the effects produced by IFNα.**

Interferon-α (IFNα) is a member of the family of type I IFNs that, in humans, consists of 13 IFNα genes and single genes encoding IFNβ, IFNϵ, IFNκ, and IFNω. Type I IFNs bind to and initiate a signaling cascade through a common type I IFN receptor, whereas IFNγ is the only type II IFN and signals through a distinct receptor (1). Type I IFNs play a key role in the innate immune response, particularly antiviral responses. IFNα has multiple immunomodulatory activities, including maturation of dendritic cells and activation and enhancement of B cell survival and differentiation (2).

Numerous studies have implicated IFNα in the pathophysiology of systemic lupus erythematosus (SLE), an autoimmune disease characterized by multiple circulating autoantibodies and chronic inflammation affecting multiple organs. Elevated levels of IFNα protein and/or activity have been detected in SLE patient sera (3,4) and are associated with higher anti–double-stranded DNA (anti-dsDNA) and anti–RNA binding

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Submitted for publication December 1, 2010; accepted in revised form April 5, 2011.

protein antibody titers and disease activity scores (5–9). Some patients with malignancies who were treated with IFNα developed a lupus-like syndrome, with production of autoantibodies and the appearance of clinical features characteristic of SLE such as malar rash and proteinuria (10,11). In addition, patients with SLE have increased expression of IFN-regulated genes (collectively referred to as the IFN gene signature) in the peripheral blood as compared with healthy control subjects (12,13). In adult patients with SLE, high expression of the IFN gene signature is correlated with increased disease severity, including renal and central nervous system involvement (8,12,13).

Given the proposed central role of IFNα in SLE (2,14), therapeutics targeting the type I IFN pathway for the treatment of SLE are being developed (15,16). Rontalizumab is a humanized IgG1 monoclonal antibody (mAb) specific for human IFNα. The safety, tolerability, and pharmacokinetics of rontalizumab in patients with SLE are currently under investigation (15). Based on an initial observation that sera from rontalizumab-naive patients with SLE exhibited high and variable interpatient signals in an enzyme-linked immunosorbent assay (ELISA) developed to detect rontalizumab, we explored whether this activity was attributable to preexisting endogenous anti-IFNα autoantibodies (AIAAs) in SLE patient sera. We also aimed to determine whether this activity was associated with differences in measures of downstream IFN-pathway activity and SLE disease activity.

PATIENTS AND METHODS

Patients and control subjects. Sera from one cohort of patients with SLE (n = 32) and healthy control subjects (n = 30) and from a pool of healthy donors were purchased from Bioreclamation; disease and disease activity information for these patients was not available. Sera obtained from these subjects were used for characterization of AIAAs in initial ELISAs. The second cohort of patients (n = 49) was recruited from the outpatient clinics and inpatient services of the University of Michigan Hospitals and Clinics (Ann Arbor), and all of the patients fulfilled at least 4 of the American College of Rheumatology (ACR) revised criteria for the diagnosis of SLE (17). Sera from these patients were characterized for AIAA status, using both surface plasmon resonance (SPR) assays and ELISAs. All patients had signed an informed consent form approved by the institutional review board. In addition, sera from 25 patients with rheumatoid arthritis (RA) and 25 healthy control donors were characterized using SPR immunoassays; sera from an additional set of 10 healthy donors were used to set cutoff criteria. A majority (20 of 25) of the RA sera were from patients who fulfilled the ACR 1987 revised criteria for the classification of RA (18); the remaining 5 samples were purchased from Bioreclamation, and disease activity information for these patients was not available. The aforementioned healthy donor sera (n = 35) were provided by the Genentech blood donor program (South San Francisco, CA). Healthy donor blood samples used as controls in the IFN-regulated gene expression analysis (n = 20) and the peripheral blood mononuclear cells (PBMCs) and healthy control sera used for the neutralization assays were also provided by the Genentech blood donor program. All patients with RA and all healthy control subjects had signed an informed consent form.

ELISAs. Recombinant human IFNα4 (generated in *Escherichia coli* at Genentech) was added to 96-well plates at a concentration of 1 μg/ml in phosphate buffered saline (PBS). The wells were blocked with PBS, 0.5% bovine serum albumin, 0.05% Tween 20, and 0.05% ProClin 300, pH 7.4, for 1 hour at room temperature. After 3 washes with PBS–Tween 20 (0.05%; wash buffer), 1:100-diluted samples were added to the blocked plates and incubated at room temperature for 1 hour. Following 3 washes with wash buffer, horseradish peroxidase–conjugated sheep anti-human IgG antibodies (The Binding Site) were added at a concentration of 0.04 μg/ml and incubated for 1 hour at room temperature. After washing, 3,3′,5,5′-tetramethylbenzidine (TMB; Kirkegaard & Perry) was added, and the reaction was stopped by the addition of 100 μl of 1*M* phosphoric acid after a 14-minute incubation. The optical density at 450 nm of each well was measured on a SpectraMax Plus384 Microplate Reader (Molecular Devices). Rontalizumab (an anti-human IFNα mAb) added to a pool of healthy donor sera was used as a positive control. Sera from healthy donors were used as a negative control. Samples were deemed positive for AIAAs if the signal in the ELISA was greater than the mean +2 SD signal generated by sera from a panel of 30 individual healthy donors.

Surface plasmon resonance. The carboxyl groups on Sensor Chip CM5 surfaces were first activated with a mixture of 0.2*M* 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and 0.05*M* *N*-hydroxysuccinimide for 7 minutes at a flow rate of 10 μl/minute. Human growth hormone (somatropin) was immobilized on flow cell 1 to serve as a reference cell; IFNα4 was immobilized on either flow cell 2 or flow cell 3. The immobilization levels of somatropin and IFNα4 were ~2,500 response units (RU). Unreacted active sites were blocked with 1*M* ethanolamine injected at a flow rate of 5 μl/minute for 7 minutes.

SLE sera (n = 49), healthy donor sera (n = 25), and RA patient sera (n = 25) were diluted 1:20 into buffer containing 0.01*M* HEPES (pH 7.4), 0.15*M* NaCl, 3 m*M* EDTA, and 0.05% surfactant P20 and analyzed at a flow rate of 5 μl/minute for 5 minutes, using a BIAcore T100 instrument and associated software (GE Healthcare). A sample was deemed reactive if the response ($\text{binding}_{\text{IFN}\alpha4} - \text{binding}_{\text{somatropin}}$) was greater than the mean signal +1.65 SD generated by 10 additional individual healthy control sera processed and analyzed as described above (upper 95% confidence limit) (19). Samples deemed reactive were analyzed with a second method using immobilized IFNα4. A sample was deemed positive for AIAAs if the addition of anti-human IgG antibodies (Binding Site) caused an increase of at least an additional 100 RU. This cutoff was set above the mean signal caused by the addition of anti-human IgG antibodies to sera from healthy control sub-

jects (n = 10) and patients with SLE (n = 4) that were negative for significant binding to IFNα.

Measurement of autoantibodies. Serum samples were analyzed using the Quanta Plex SLE Profile 8 fluorescent immunoassay (Inova Diagnostics). Quasiquantitative measurement of IgG antibodies directed against ribosomal P, chromatin, histone, SSA, SSB, RNP, and Sm was performed. Serum samples were run on a Luminex 100 IS flow cytometry system according to the manufacturer's protocol. Anti-dsDNA antibodies were measured using a Quanta Lite dsDNA ELISA (Inova Diagnostics).

Measurement of serum BAFF and chemokine levels. The antibodies used in the BAFF ELISA were developed by Genentech. A mouse mAb to human BAFF was used as a capture reagent, and a biotin-conjugated mouse mAb to BAFF and streptavidin–HRP conjugate (GE Healthcare) were used for detection. Signal was detected by the addition of TMB substrate and was read spectrophotometrically at 450 nm. A custom 4-plex prototype 96-well ELISA using the Multi-Spot platform (Meso Scale Discovery) was used to measure serum levels of IFNγ-inducible 10-kd protein (IP-10), IFN-inducible T cell α chemoattractant, B lymphocyte chemokine (BLC), and monocyte chemoattractant protein 1 (MCP-1). Standard curves were generated using recombinant protein for each chemokine.

Type I IFN bioassay and neutralization assays. Type I IFN bioactivity in sera from patients with SLE was measured using human U937 cells stably expressing an Mx1 promoter–luciferase reporter construct (U937-Mx1-Luc). A standard curve of recombinant type I IFN was used as a positive control, and healthy donor sera and RPMI 1640 medium were used as negative controls. After incubation with patient sera (50%) in the presence of RPMI 1640 with 10% fetal bovine serum (FBS), cell lysate luciferase levels were measured using the Luciferase Assay System Reagent Kit and a GloMax 96 Microplate Luminometer (Promega).

The ability of AIAA-positive SLE sera to neutralize recombinant IFNα activity was assessed using U937-Mx1-Luc cells cultured in RPMI 1640 medium with 10% FBS. Cells were incubated for 24 hours in the presence of recombinant IFNα2 (Sigma) and 33% patient sera, healthy donor sera, or medium alone, then lysed and assayed as described above for luciferase induction. To assess the ability of AIAA-positive patient sera to neutralize endogenous IFNα activity, peripheral blood was obtained from a healthy donor, and PBMCs were isolated on a Ficoll-Hypaque gradient (Sigma). PBMCs were cultured in RPMI 1640 medium supplemented with 2 m*M* L-glutamine, 10 m*M* HEPES, 1 m*M* sodium pyruvate, 100 units/ml penicillin, 0.1 mg/ml streptomycin, and 10% FBS in the presence or absence of 5 μg/ml CL097 (InvivoGen). After 24 hours of incubation, cells were pelleted, and supernatant was collected. U937-Mx1-Luc cells were grown in the presence of PBMC supernatant diluted 1:4 with fresh RPMI 1640 and incubated in the presence of 33% patient sera, healthy donor sera, or medium alone for 24 hours, then lysed and assayed for luciferase induction as described above. Recombinant IFNα2 in RPMI 1640 was also used as a reference standard.

IFN-regulated gene expression levels. PBMCs were isolated from the patients with SLE (University of Michigan cohort). RNA was extracted using an RNeasy Mini Kit (Qiagen) following the manufacturer's protocol, with on-column DNase treatment. RNA was then profiled on HG-U133 microarrays, and data were processed using MAS5 software (Affymetrix). Microarray data for all samples were hierarchically clustered by the Pearson correlation similarity metric and by average linkage node summarization. Three genes (*EPSTI1*, *HERC5*, and *TYK1*) were chosen to represent the IFN gene signature. The expression level of each of these genes in each sample was standardized by dividing it by the mean expression level in age-matched healthy control subjects (n = 20). These 3 standardized expression measurements were then averaged for each sample, yielding a single numerical score, the IFN signature metric (ISM) value.

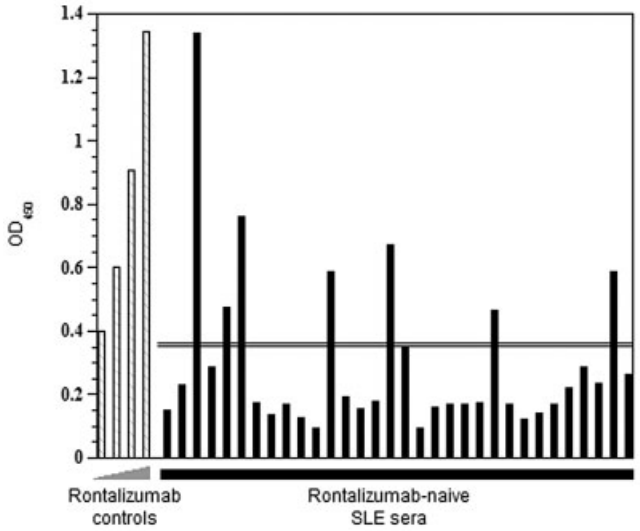

Figure 1. Identification of endogenous anti–interferon α (IFNα) autoantibodies in sera from patients with systemic lupus erythematosus (SLE). Sera obtained from rontalizumab-naive patients with SLE (n = 32) and from healthy donors (n = 30) were tested using an enzyme-linked immunosorbent assay originally designed to detect rontalizumab, a monoclonal antibody specific for human IFNα. Signals generated by rontalizumab (0.31–2.5 μg/ml) spiked into a pool of healthy donor sera are represented by the hatched bars (controls). The double horizontal lines show the mean (+2 SD) signal generated by sera from 30 healthy donors. OD_{450} = optical density at 450 nm.

Hierarchical cluster analysis. Data were scaled and displayed in a heat map by $\log_2$ transformation and median centering such that the transformed data had a median value of 0 and a variance of 1. A hierarchical clustering algorithm (20) using Euclidean distance similarity metric and average linkage method was applied, and data were displayed using Java TreeView software (21).

Statistical analysis. Wilcoxon's, Pearson's chi-square, and Mann-Whitney tests were performed using JMP software (SAS). Benjamini and Hochberg correction for multiple testing was used when appropriate. Samples from the following patients were excluded from individual statistical analyses due to a lack of specific data points: patients 64, 109, and 149 (SLE Disease Activity Index [SLEDAI] score [22]), and patients 83, 110, 118, and 149 (C3 and C4 levels).

RESULTS

Elevated IFNα binding activity in sera from patients with SLE. To assess the pharmacokinetics of rontalizumab, an anti-human IFNα mAb, in patients with SLE, we developed an ELISA specific for anti-IFNα IgG antibodies (see Patients and Methods). A majority of sera obtained from rontalizumab-naive patients with SLE exhibited low background signal in the ELISA. However, a subset of these sera (7 of 32) demonstrated elevated and highly variable signals in the ELISA relative to healthy donor sera (Figure 1). The signals in the ELISA produced by the sera from these 7 patients with SLE were higher than those generated by 0.31 μg/ml of rontalizumab spiked into a pool of healthy donor sera (Figure 1). Preincubation of ELISA-positive SLE sera (n = 3) with excess recombinant IFNα caused a large decrease in signal (60–85%) in the ELISA as compared with a small change in signal (−4–3%) in sera preincubated with human growth hormone as a control (data not shown). In addition, preincubation of these ELISA-positive SLE sera with protein A/G resulted in a large reduction in signal in the ELISA (data not shown). Taken together, these observations demonstrated that endogenous anti-IFNα IgG autoantibodies were being detected in sera obtained from rontalizumab-naive SLE patients, using an ELISA method.

High prevalence of AIAAs in SLE patient sera. To assess the potential influence of AIAAs on clinical signs and symptoms of disease, we determined the AIAA status for an independent cohort of female patients with SLE (n = 49) for whom associated clinical and laboratory data were available. We first tested whether AIAA status could be identified with greater sensitivity using an SPR-based immunoassay with IFNα as a capture reagent (see Patients and Methods). The SPR platform has been shown, in some cases, to detect biologically significant antibodies when classic ELISA methods could not (23,24). Using an anti-IFNα SPR immunoassay, 27% of the SLE sera tested (13 of 49) were positive for AIAAs of the IgG isotype (Figures 2A and B). The normal control sera (n = 25) and a majority (24 of 25; 96%) of the RA sera tested demonstrated negligible reactivity to IFNα (Figure 2C). Only one of the 25 RA serum samples tested (4%) showed reactivity to IFNα (data not shown). Human growth hormone was used to evaluate nonspecific binding by sera from patients with SLE (n = 49) (Figure 2D) and sera from patients with RA (n = 25) and healthy control subjects (n = 25; results not shown).

Serologic and clinical profile of SLE patients with AIAAs. We next determined the levels of type I IFN bioactivity and additional downstream measures of

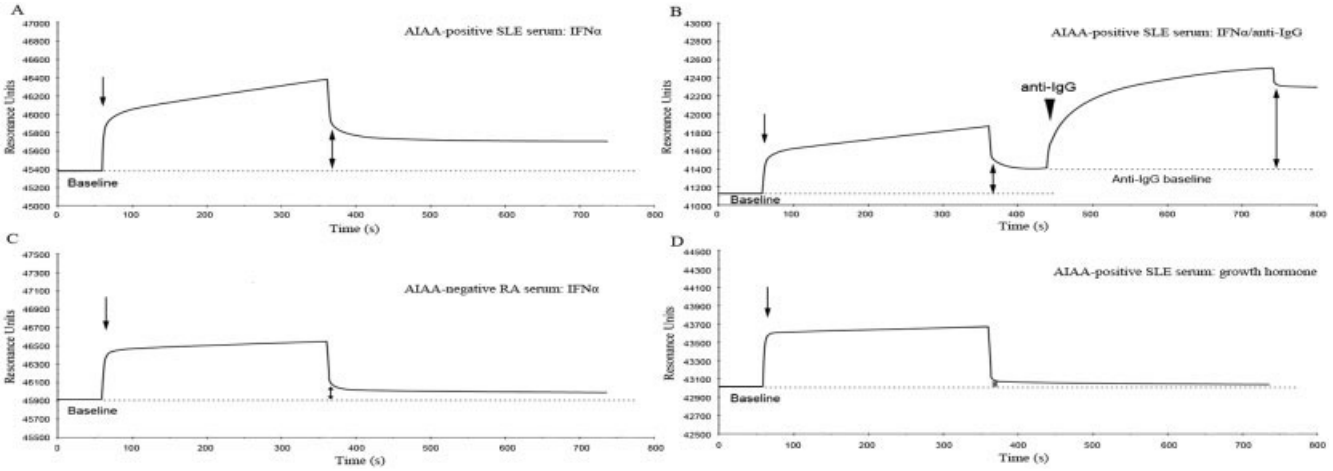

Figure 2. Detection of endogenous anti–interferon-α (IFNα) autoantibodies (AIAAs) in sera from patients with systemic lupus erythematosus (SLE), using surface plasmon resonance. Binding to human IFNα or human growth hormone by sera from patients with SLE and patients with rheumatoid arthritis (RA) was measured using a surface plasmon resonance–based immunoassay. Representative profiles of binding of AIAA-positive SLE sera to IFNα (**A**), AIAA-positive SLE sera to IFNα, with secondary detection using anti-human IgG antibodies (**B**), AIAA-negative RA sera to IFNα (**C**), and AIAA-positive SLE sera to human growth hormone (**D**) are shown. The dotted lines represent the signal generated by either IFNα (**A, B,** and **C**) or human growth hormone (**D**) alone (baseline); the anti-IgG baseline is the signal generated by IFNα and the anti-IgG antibody reagent. **Arrows** denote the times when serum was injected. **Double-headed arrows** represent the net amount of material bound. The **arrowhead** indicates the time at which anti-IgG antibodies were injected.

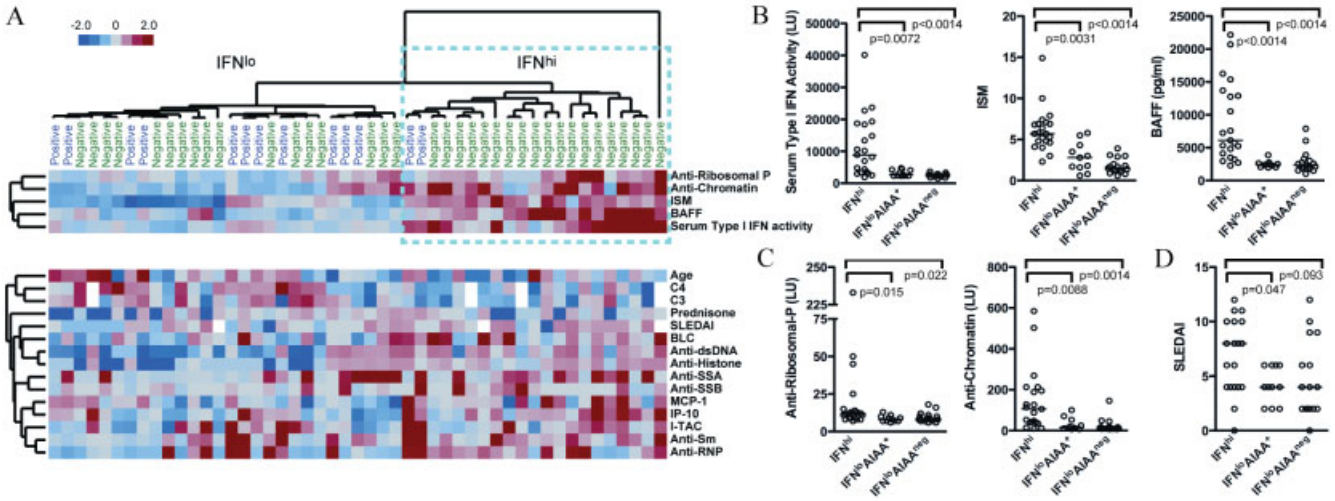

Figure 3. Reduced levels of IFN-regulated metrics, autoantibodies, and disease activity in AIAA-positive patients with SLE. **A,** Top, Unsupervised hierarchical clustering of analytes with decreased mean levels in AIAA-positive patients (purple) versus AIAA-negative patients (green). Two distinct clusters were revealed: IFN^{high} and IFN^{low}. Bottom, Supervised clustering analysis of the remaining analytes and clinical parameters, showing their relative levels in patients in the IFN^{high} and IFN^{low} groups. Data are displayed in a heat map, with grey indicating average values, blue indicating lower-than-average values, and red indicating higher-than-average values. White boxes indicate missing data. **B–D,** Serum type I IFN bioactivity levels, IFN signature metric (ISM) values, and serum BAFF levels (**B**), anti–ribosomal P and anti-chromatin antibody levels (**C**), and SLE Disease Activity Index (SLEDAI) scores (**D**) in the IFN^{high} group (n = 21), the IFN^{low}/AIAA-positive group (n = 11), and the IFN^{low}/AIAA-negative group (n = 17). For the SLEDAI score, data were not available for 2 patients in the IFN^{high} group and for 1 patient in the IFN^{low}/AIAA-negative group. Horizontal lines indicate the medians. Wilcoxon's test was used to compare groups. The *P* values shown were adjusted for multiple testing using Benjamini and Hochberg correction. LU = luciferase units; BLC = B lymphocyte chemokine; anti-dsDNA = anti–double-stranded DNA; MCP-1 = monocyte chemoattractant protein 1; IP-10 = IFNγ-inducible 10-kd protein; I-TAC = IFN-inducible T cell α chemoattractant (see Figure 2 for other definitions).

IFN-mediated signaling in this cohort of patients. IFNα protein levels were not directly measured in SLE patient sera due to a lack of available reagents that could accurately measure all IFNα subtypes (25). Instead, sera were tested for type I IFN bioactivity using a sensitive cell-based reporter assay (see Patients and Methods). Peripheral blood was examined for levels of expression of IFN-regulated genes (ISM value) using microarrays. Patient sera were also examined for levels of type I IFN–inducible chemokines. In addition, because other investigators have demonstrated an association of type I IFN activity and the levels of autoantibodies to nucleic acids and nucleic acid–associated antigens (7–9), sera were tested for levels of autoantibodies to histone, ribosomal P, SSA, SSB, Sm, RNP, dsDNA, and chromatin.

An unsupervised hierarchical clustering analysis of AIAA status and levels of serum type I IFN bioactivity, the ISM, BAFF, and anti–ribosomal P and anti-chromatin autoantibodies (mean levels of each analyte were lower in AIAA–positive versus AIAA-negative patients; data not shown) clustered the patients into 2 distinct subgroups, IFN^{low} and IFN^{high} (Figure 3A). The IFN^{low} group contained the majority of AIAA-positive patients (11 of 13; 85%) and also approximately half of the AIAA-negative patients (17 of 36; 47%). In contrast, the IFN^{high} group was predominantly composed of AIAA-negative patients (19 of 21; 90%). Some of the other available clinical and laboratory features for each patient are shown in the lower panel of Figure 3A.

We then compared clinical parameters across the 3 identified subgroups: IFN^{high}, IFN^{low}/AIAA positive, and IFN^{low}/AIAA negative (Table 1). The IFN^{low}/AIAA-positive group had a lower percentage of patients with serologic manifestations and a higher median age (at time of blood collection) compared with the IFN^{high} group (Table 1), but these differences were not significant ($P > 0.05$). In addition, musculoskeletal, mucocutaneous, and renal manifestations, as well as medication profiles, appeared to be similar between the IFN^{high}, IFN^{low}/AIAA-positive, and IFN^{low}/AIAA-negative subgroups (Table 1 and data not shown). Last, no correlation was observed between individual clinical features or medications and AIAA-positive status, as determined by ELISA versus SPR assays (data not shown).

Serologic parameters were then examined across the 3 identified subgroups (Figures 3B and C and

Table 1. Clinical characteristics of the IFN/AIAA subgroups*

		IFN^{low}	
	IFN^{high} (n = 21)	AIAA positive (n = 11)	AIAA negative (n = 17)
Age, median (range) years	34 (23–57)	43 (20–59)	35 (23–56)
Ethnicity			
White	18 (86)	7 (64)	9 (53)
African American	3 (14)	4 (36)	7 (41)
Other	0	0	1 (6)
Clinical manifestations			
Serologic	19 (91)	6 (55)	12 (71)
Musculoskeletal	7 (33)	5 (46)	7 (41)
Mucocutaneous	6 (29)	3 (27)	3 (18)
Renal	2 (10)	0 (0)	3 (18)
Medications			
Prednisone	17 (81)	8 (73)	14 (82)
Dosage, median mg/day	10	15	17.5
Antimalarial	13 (62)	9 (82)	7 (41)
Immunosuppressive	15 (71)	8 (73)	7 (41)

* Except where indicated otherwise, values are the number (%). All patients were female. IFN = interferon; AIAA = anti–IFNα autoantibody.

Table 2). After Benjamini and Hochberg correction for multiple testing, levels of serum type I IFN bioactivity, the ISM value, serum BAFF levels, and levels of anti-chromatin, anti-histone, and anti–ribosomal P autoantibodies were all significantly higher ($P \leq 0.042$) (Figures 3B and C and Table 2) in the IFN^{high} subgroup

Table 2. Serologic and clinical profiles of patients with SLE in the IFN^{high}, IFN^{low}/AIAA-positive, and IFN^{low}/AIAA-negative subgroups*

		IFN^{low}		IFN^{high} vs. IFN^{low}/ AIAA positive		IFN^{high} vs. IFN^{low}/ AIAA negative	
Parameter	IFN^{high}	AIAA positive	AIAA negative	P†	Adjusted P‡	P†	Adjusted P‡
BAFF, pg/ml	6,107 (2,291–22,170)	2,447 (1,974–3,872)	2,338 (1,043–7,892)	<0.0001	<0.0014	<0.0001	<0.0014
IFN signature metric	5.74 (2.3–14.9)	2.83 (0.6–5.8)	1.5 (0.6–3.9)	0.0006	0.0031	<0.0001	<0.0014
Serum type I IFN bioactivity§	8,839 (1,895–40,144)	2,704 (2,291–4,785)	2,620 (1,491–3,709)	0.002	0.0072	<0.0001	<0.0014
Autoantibody profile, LU							
Anti-histone	4 (1–10)	1 (0–7)	1 (0–8)	0.0236	0.042	0.001	0.0045
Anti-chromatin	106 (10–585)	15 (8–101)	13 (8–146)	0.0027	0.0088	0.0002	0.0014
Anti–ribosomal P	12 (7–233)	8 (6–13)	8 (6–18)	0.0049	0.0147	0.0086	0.022
Anti-dsDNA	395 (40–826)	125 (19–577)	82 (20–698)	0.0543	0.075	0.0014	0.0056
Anti-SSB	5 (4–195)	5 (4–16)	5 (4–13)	0.1187	0.153	0.504	0.550
Anti-SSA	33 (6–310)	9 (5–306)	7 (6–328)	0.461	0.519	0.813	0.813
Anti-Sm	10 (4–159)	5 (4–181)	5 (4–22)	0.510	0.540	0.02	0.040
Anti-RNP	31 (4–200)	6 (3–244)	6 (3–280)	0.330	0.383	0.020	0.040
C4, mg/dl	8 (3–24)	19 (8–138)	23.5 (4–49)	0.0177	0.040	0.011	0.026
C3, mg/dl	74 (39–141)	107 (35–133)	104 (49–157)	0.0275	0.045	0.022	0.042
SLEDAI	8 (0–12)	4 (2–6)	4 (0–12)	0.0297	0.047	0.07	0.093
BLC, pg/ml	1,281 (549–6,208)	812 (373–3,127)	686 (346–2,242)	0.0391	0.059	0.026	0.044
IP-10, pg/ml	258 (96–5,207)	149 (41–1,317)	148 (66–747)	0.052	0.075	0.0005	0.003
MCP-1, pg/ml	455 (93–2,689)	374 (203–742)	294 (68–702)	0.2581	0.310	0.007	0.019
I-TAC, pg/ml	106 (31–930)	101 (28–875)	86 (20–542)	0.579	0.595	0.168	0.209

* Values are the median (range). All data are from 49 female patients with systemic lupus erythematosus (SLE), with the exception of the SLE Disease Activity Index (SLEDAI; n = 46) and C3 and C4 (n = 45). All statistical analyses were based on available data. IFN = interferon; AIAA = anti-IFNα autoantibody; LU = luminex units; anti-dsDNA = anti–double-stranded DNA; BLC = B lymphocyte chemokine; IP-10 = IFNγ-inducible 10-kd protein; MCP-1 = monocyte chemoattractant protein 1; I-TAC = IFN-inducible T cell α chemoattractant.
† By Wilcoxon's test.
‡ After Benjamini and Hochberg correction for multiple testing.
§ Luciferase units.

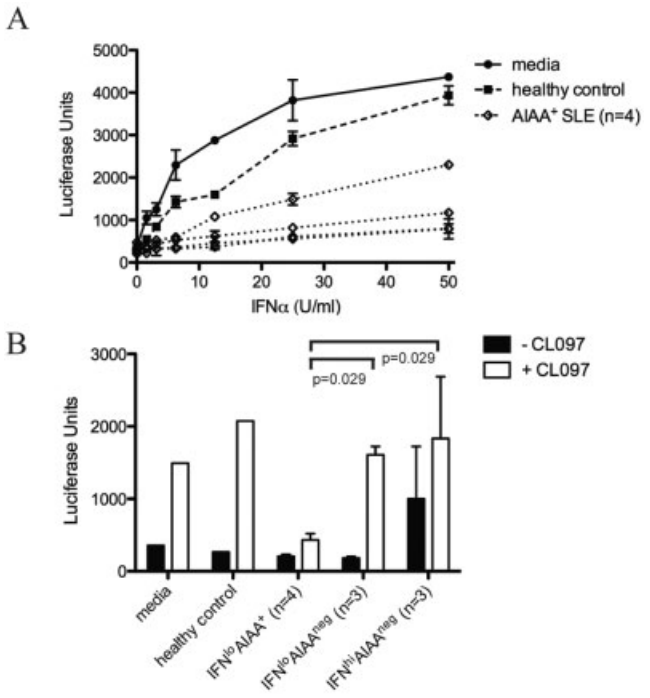

Figure 4. Sera from AIAA-positive patients with SLE neutralize type I IFN activity. **A,** U937 cells stably expressing an IFN-responsive luciferase reporter plasmid (U937-Mx1-Luc) were cultured in the presence of the indicated concentrations of recombinant IFNα and medium, healthy control sera, or sera from AIAA-positive patients with SLE. Luciferase activity was measured in cell lysates. Data are displayed as the mean ± SD for duplicate wells at each concentration. **B,** Peripheral blood mononuclear cells (PBMCs) from a healthy donor were incubated in the presence or absence of CL097. Following 24 hours of stimulation, supernatant was collected. U937-Mx1-Luc cells were cultured in the presence of PBMC supernatant and serum samples, as indicated. Luciferase activity was measured in cell lysates. Values for media and healthy control samples are the mean of duplicate wells. Values for the other groups are the mean ± SD. *P* values were determined by Mann-Whitney test. See Figure 2 for other definitions.

compared with both the IFN^{low}/AIAA-positive and IFN^{low}/AIAA-negative subgroups. Levels of anti-dsDNA antibodies, anti-RNP antibodies, and anti-Sm autoantibodies and serum IP-10, BLC, and MCP-1 levels were significantly higher ($P \leq 0.044$) (Table 2) in the IFN^{high} subgroup compared with the IFN^{low}/AIAA-negative subgroup but were not significantly different from those in the IFN^{low}/AIAA-positive subgroup. Scores for the measure of disease activity, SLEDAI, were lower, and C3 and C4 levels were significantly higher ($P < 0.047$) (Figure 3D and Table 2) in the IFN^{low}/AIAA-positive subgroup compared with the IFN^{high} subgroup. Similarly, C3 and C4 levels were significantly higher ($P < 0.042$) (Table 2) in the IFN^{low}/AIAA-negative subgroup compared with the IFN^{high} subgroup, but SLEDAI scores were not significantly different between these 2 subgroups (Figure 3D and Table 2).

Finally, we used a cell-based assay to assess whether endogenous AIAAs could directly influence IFNα activity. In an initial set of experiments, U937 cells stably expressing a type I IFN–responsive luciferase reporter (U937-Mx1-Luc) (see Patients and Methods) were incubated with recombinant IFNα2 in the presence or absence of sera from SLE patients with AIAAs as determined by ELISA. AIAA-positive SLE sera (n = 4) inhibited recombinant IFNα2 activity (59–83% inhibition of 50 units/ml of IFNα2 activity) as compared with normal healthy control sera (10% inhibition) (Figure 4A). To further characterize the ability of AIAAs to neutralize IFNα activity, we next investigated whether these AIAA-positive SLE sera could neutralize diverse endogenous IFNα subtypes produced by peripheral blood mononuclear cells (PBMCs) treated with the Toll-like receptor 7/8 agonist CL097 (see Patients and Methods). The level of endogenous type I IFN activity present in the supernatant from these PBMCs was comparable with the activity of 2.5 units/ml of recombinant IFNα2 (data not shown). U937-Mx1-Luc cells were incubated with PBMC supernatant in the presence of individual sera from AIAA-positive (n = 4), IFN^{high}/AIAA-negative (n = 3), and IFN^{low}/AIAA-negative (n = 3) patients with SLE, or with a pool of sera from healthy control subjects, or with medium alone (Figure 4B). AIAA-positive sera effectively inhibited endogenous type I IFN activity as compared with sera from IFN^{low}/AIAA-negative and IFN^{high}/AIAA-negative patients (both $P = 0.029$).

DISCUSSION

In this study, we characterized IFN-pathway activity and both serologic and clinical features of AIAA-positive patients with SLE. Our results showed that ~25% of patients with SLE exhibit endogenous AIAAs; this percentage is significantly higher than previous estimates of 0–12% (26–29). We also demonstrated that these AIAAs can neutralize the bioactivity of IFNα. Moreover, we provided the first identification of a subgroup of patients with SLE who have AIAAs and reduced levels of serum type I IFN bioactivity, lower levels of IFN-regulated genes, and lower levels of autoantibodies to chromatin, histone, and ribosomal P protein. Last, we demonstrated that a majority of AIAA-

positive patients with reduced IFN-pathway activity have lower disease activity.

Previous studies have examined the relationship between AIAAs and disease activity (28,29). Von Wussow and colleagues reported that 3 patients with anti-IFNα–neutralizing antibodies had relatively inactive disease over a period of years, with no visceral involvement (28). However, Slavikova et al did not observe a correlation between the presence of these autoantibodies and lower disease activity (29). This discrepancy may be attributable to the methods used to detect AIAAs. For our analysis, an SPR-based immunoassay was used to detect AIAAs. This technology was chosen due to its ability to detect biologically and clinically significant, lower-affinity antibodies and was critical to our evaluation of the relationship of AIAAs with IFN-pathway activity and disease activity. Assessment of patient AIAA status using an ELISA (similar to that used by Slavikova et al) resulted in the characterization of only 10% of patients as AIAA positive; the remaining 17% of AIAA-positive patients with SLE were positive only by SPR assay (data not shown).

AIAA status and levels of IFN-pathway activity appear to define distinct subgroups of patients with SLE. The IFN^{low}/AIAA-negative subgroup may have disease that is driven by mechanisms other than IFNα. In contrast, the difference in profiles between the IFN^{high} and the IFN^{low} AIAA-positive subgroups suggests an association between decreased levels of serum type I IFN bioactivity and IFN-pathway activity and the presence of AIAAs in this group of patients with SLE. This hypothesis is supported by our observation that AIAAs can effectively neutralize the activity of both recombinant and endogenous forms of IFNα. AIAAs may mediate a reduction in BAFF expression (30,31); the decreased levels of BAFF may result in reduced levels of specific autoreactive B cells (32) that produce autoantibodies to histone, chromatin, and ribosomal P protein. AIAAs may therefore possess beneficial properties that mimic antibody-based therapeutics specifically engineered to neutralize IFNα. Consistent with this hypothesis, a recent phase I study of treatment with a fully human mAb specific for IFNα demonstrated a reduction of BAFF expression in peripheral blood as well as in skin lesions of patients with SLE (16).

Of interest, 2 of the AIAA-positive patients with SLE demonstrated relatively high levels of IFN-pathway activity and, accordingly, clustered with IFN^{high} patients. In these patients, IFNα may be present at levels in relative excess to AIAA levels, resulting in elevated IFN-pathway activity despite the presence of AIAAs. Alternatively, because our analysis focused on AIAAs with reactivity to IFNα4 (see Patients and Methods), the IFN-pathway activity in these patients may be driven by an IFNα subtype(s) that is not neutralized by the AIAAs present in the sera or potentially by IFNβ or IFNγ.

The current study has several limitations. First, the relatively small sample size did not allow us to definitively rule out the possibility that clinical covariates contributed to the differences seen between the subgroups with respect to IFN activity, although as shown in Tables 1 and 2, the groups appeared to be relatively well matched for most clinical features. Second, we have not yet performed longitudinal analysis of AIAA levels in patients who are positive for AIAAs. It has been shown that the ISM values and serum BAFF levels, 2 of the 5 components that define the IFN^{low} and IFN^{high} groups, are relatively stable over time (33,34). Additional investigation is needed to assess the longitudinal stability of the AIAA phenotype and the components that define IFN^{low} and IFN^{high} patient status as well as the relationship between AIAA status and disease activity over time. Third, we have not established that the SPR-based immunoassay identifies all patients who carry AIAAs, and thus there is some risk for misclassification with regard to AIAA status based on the assays described here. Finally, we have not yet determined the full immunoglobulin isotype spectrum of AIAAs; we will be testing in future experiments the possibility that IgM or IgA antibodies could be contributing.

In summary, we have shown that ~25% of patients with SLE have AIAAs, and that most of these patients have evidence of lower serum type I IFN bioactivity, reduced IFN-pathway activity, and lower disease activity levels. Characterization of endogenous AIAAs, together with characterization of IFN-pathway activity markers, may prove useful in predicting levels of disease activity in SLE and responses to therapeutic approaches that target type I IFN.

ACKNOWLEDGMENTS

We thank Drs. S. Chung and J. McBride for helpful comments on the manuscript, Dr. K. Hillan for helpful discussions, A. Paler-Martinez for anti–ribosomal P autoantibody analysis, and Drs. C. Lei and W. Forrest for assistance with the statistical analysis.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Morimoto had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Morimoto, Yang, Wolslegel, Wang, Quarmby, Wakshull, Townsend, Behrens.
Acquisition of data. Thibault Flesher, Yang, Wolslegel, Wang, Brady.
Analysis and interpretation of data. Morimoto, Thibault Flesher, Yang, Wolslegel, Wang, Brady, Abbas, Quarmby, Wakshull, Richardson, Townsend, Behrens.

ROLE OF THE STUDY SPONSORS

All of the authors, with the exception of Dr. Richardson, were employees of Genentech, Inc., and were involved in the study design, collection and analysis of data, manuscript writing, and the decision to publish the final version of the manuscript.

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