Type III Interferons in Systemic Lupus Erythematosus

Association Between Interferon λ3, Disease Activity, and Anti-Ro/SSA Antibodies

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Objective: The aim of this study was to assess associations between serum type III (λ) interferons (IFN-λ) and disease activity in systemic lupus erythematosus (SLE).

Methods: Serum levels of IFN-λ1, IFN-λ2, and IFN-λ3 were measured in 93 SLE patients and 67 healthy individuals. The associations with overall disease activity, organ-specific damage, and SLE-related antibodies were assessed.

Results: Median IFN-λ1 levels were 0 pg/mL (range, 0–510 pg/mL) and 0 pg/mL (0–171 pg/mL; $P = 0.814$) in SLE patients and control subjects, respectively. These figures were 0 pg/mL (0–28 pg/mL) and 0 pg/mL (0–43 pg/mL; $P = 0.659$) for IFN-λ2, as well as 83 pg/mL (0–965 pg/mL) and 42 pg/mL (0–520 pg/mL; $P = 0.002$) for IFN-λ3, respectively. According to the Systemic Lupus Erythematosus Disease Activity Index categories, IFN-λ3 levels were 44 pg/mL (0–158 pg/mL) in quiescent, 117 pg/mL (0–344 pg/mL) in mild, 79 pg/mL (0–965 pg/mL) in moderate, and 78 pg/mL (0–329 pg/mL) in severe disease, with the highest levels found in patients with serosal or cutaneous involvement. In line with this, IFN-λ3 levels were inversely correlated with C3 ($\rho = -0.44$; 95% confidence interval, −0.62 to −0.20; $P = 0.0003$) and C4 ($\rho = -0.40$; 95% confidence interval, −0.59 to −0.15; $P = 0.0001$) complement proteins. In addition, higher IFN-λ3 levels were found in patients positive for anti-Ro/SSA antibodies than in those negative for that antibody (122 pg/mL [0–965 pg/mL] vs. 0 pg/mL [0–165 pg/mL]; $P = 0.001$). The concentration of IFN-λ3 also was higher in patients receiving glucocorticoids (104 pg/mL [0–965 pg/mL] vs. 30 pg/mL [0–165 pg/mL]; $P = 0.009$), and a dose-related effect was observed.

Conclusions: Interferon λ3, a subtype of type III IFNs, is associated with the extent of lupus activity, in particular with active serosal and cutaneous disease. This association could be mechanistically related to anti-Ro/SSA antibodies.

Key Words: anti-Ro/SSA antibodies, interferons, systemic lupus erythematosus

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The authors declare no conflict of interest.

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Systemic lupus erythematosus (SLE) is an archetypal autoimmune disorder with a clinical spectrum ranging from subtle to life-threatening multiorgan involvement.[1] The production of antinuclear antibodies (ANAs) is nearly universal among patients with SLE. Common ANA specificities found in lupus patients include double-stranded DNA (dsDNA), extractable nuclear antigens (Sm, RNP, Ro/SSA, and La/SSB), histones, and chromatin.[2] Specific ANAs are associated with disease subsets and may play a direct role in the lupus pathogenesis, such as anti-Ro/SSA antibodies in subacute cutaneous and neonatal SLE and anti-dsDNA antibodies in renal disease; however, most autoantibody titers do not correlate with disease activity, with the notable exception of anti-dsDNA antibodies.[2,3] The etiology of SLE remains largely unknown, and the limited understanding of its pathogenic mechanisms had hampered the development of targeted therapies. However, there is emerging evidence that sheds new light on a number of key mechanisms involved in lupus-associated tissue injury.[4] In this regard, compelling advances have revealed direct implications of interferon α (IFN-α, the main element of type I IFN family) in the pathogenesis of SLE.[4] Indeed, gene expression studies showed several IFN-responsive genes inappropriately activated in peripheral blood mononuclear cells of patients with SLE,[5,6] and animal models have also demonstrated lupus hallmarks as highly correspondent to the manipulation of type I IFNs.[7–9] Isolated reports on the development of both SLE-related antibodies and clinically evident lupus in individuals receiving recombinant IFN-α as adjunct therapy for cancer or chronic hepatitis C virus infection have further strengthened this association.[10,11] Consequently, several treatment strategies aimed at controlling lupus activity by targeting the IFN-α signaling pathway have emerged as promising in animal models and in human disease.[4]

A new IFN family, type III or λ IFNs, has been characterized, which includes IFN-λ1 (also termed interleukin 29 [IL-29]), IFN-λ2 (IL-28A), and IFN-λ3 (IL-28B).[12,13] A variant upstream of the *IFNL3* gene was recently described and named *IFNL4* gene.[14] Although types I and III IFNs accomplish their effects by signaling through different heterodimeric receptors (IFN-αR1/IFN-αR2 and IFN-λR1/IL-10R2 complexes, respectively) on the cell surface, biological activities induced by one or the other IFN are nearly the same because of the existence of similar downstream signaling events in both receptor complexes.[12,13,15] Indeed, after being stimulated by its respective cytokine, each receptor activates the JAK/STAT pathway, an intricate intracellular signaling cascade resulting in the transcriptional activation of genes containing IFN-stimulated response elements in the promoter regions of IFN-stimulated genes, thus culminating in a similar gene transcription profile.[15]

Therefore, because type I IFNs are critical for the development of clinical and immunological disturbances in SLE, and because types I and III IFNs share a common post–receptor

signaling pathway, we have already hypothesized that type III IFNs may play a role in the course of the disease.[16] In this study, we sought to assess whether serum levels of type III IFNs are associated with overall disease activity or in the involvement of any organ system in patients with SLE. An association with several laboratory parameters was also evaluated.

MATERIALS AND METHODS

Consecutive adult patients fulfilling the American College of Rheumatology (ACR) updated classification criteria for SLE were recruited from an outpatient rheumatology practice at a university setting.[17] No patient was judged to have clinically apparent infections at the time of recruitment. Pregnant patients and those with a history of malignancy or antecedent of hepatitis B or C viruses and/or human immunodeficiency virus infection were not included. Patients with end-stage renal disease requiring dialysis were also excluded. In addition, control subjects were selected from healthy blood donors to match SLE patients in terms of age (±2 years) and sex.

The study protocol was approved by the local ethics committee (Comisión de Bioética del Instituto Nacional de Cardiología Ignacio Chávez, no. 12-771). At the time of enrollment, all individuals signed an informed consent and authorized the use of clinical data and biological specimens for investigations. All procedures were performed in accordance with the Declaration of Helsinki, its addenda, and local regulations.

On enrollment, all individuals underwent a detailed clinical examination and evaluation of medical charts by the same rheumatologist (L.M.A.-G.), and concomitant laboratory and radiographic reports were assessed for disease activity and organ-specific damage. The clinical manifestations were defined as follows: arthritis: 2 or more joints with pain and swelling or effusion confirmed by physical examination; renal involvement: ≥0.5 g/24-hour urine proteins, <50% glomerular filtration rate, >5 white or red blood cells per high-power field of unspun urine, or urinary casts; cutaneous involvement: malar rash, patchy or diffuse alopecia, oral or nasal ulceration, photosensitivity, or discoid rash; neuropsychiatric involvement: seizures, psychosis not associated with the use of glucocorticoids, acute confusional state, lupus migraine, cranial neuropathy, cognitive impairment, or transverse myelitis; vasculitis: ulceration, gangrene, evidence of periungual infarction or splinter hemorrhages on the dermatoscopic examination using noncontact, polarized light DermLite Photo dermatoscope (3Gen, San Juan Capistrano, CA); serositis: pleural or pericardial rubbing and effusion confirmed by chest radiograph or echocardiography, respectively.

The disease duration was calculated from the time of SLE diagnosis to the time of study entry. The Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) within 10 days before clinical evaluation was used to calculate disease activity.[18] The accumulated organ damage was assessed by the Systemic Lupus International Collaborating Clinics (SLICC)/ACR damage index.[19] Other covariates such as age, sex, current use and glucocorticoids dosing, antimalarial drugs, and immunosuppressant drugs, as well as comorbidities and other therapies, were also obtained.

Laboratory Studies

A fasting blood sample of 6 mL was used to detect ANA and anti-dsDNA antibodies by epifluorescence on HEp-2 cells and *Crithidia luciliae* substrate, respectively (Inova Diagnostics, San Diego, CA). Anti-Ro/SSA, anti-La/SSB, anti-Sm, and anti-RNP (Inova Diagnostics), as well as anticardiolipin and anti-β_2 glycoprotein 1 antibodies (The Binding Site, Birmingham, United Kingdom), were detected by enzyme-linked immunosorbent assay. Lupus anticoagulant was detected in agreement with the International Society of Thrombosis and Haemostasis guidelines.[20] High-sensitivity C-reactive protein and complement C3 and C4 proteins were measured by nephelometry (IMMAGE Beckman-Coulter, Brea, CA). Erythrocyte sedimentation rate and 50% hemolytic complement activity were assayed in fresh blood samples as previously described.[21] Other studies, such as complete blood count, blood urea nitrogen, serum creatinine, 24-hour urine protein, and creatinine clearance (Cockcroft-Gault equation), were performed within 3 days before enrollment.

Measurement of Serum IFNs

Four milliliters of fasting blood was additionally obtained; centrifuged 600*g* for 15 minutes, 4°C; and stored in aliquots at −70°C until use. Stored sera were thawed under standard conditions, and the following cytokines were measured: LEGEND MAX IL-29 and IL-28A kits (BioLegend, San Diego, CA) were used to detect IFN-λ1 and IFN-λ2, respectively, whereas the DuoSet IL-28B kit (R&D Systems, Minneapolis, MN) was used to detect levels of IFN-λ3. Levels of IFN-α were detected with the Verikine IFN-α multisubtype kit (PBL InterferonSource, Piscataway, NJ). Laboratory procedures were performed according to the instructions provided by each manufacturer. Serum samples were assayed in duplicate, and mean values were used for calculations. Values below the minimum detectable concentration as described by each manufacturer were considered as "undetectable" and computed as "zero" for the calculations.

Statistical Analysis

Data distribution was assessed using the Kolmogorov-Smirnov test. Categorical data were described as proportions. Continuous variables were expressed as means with SD or medians with interquartile range (IQR) or minimum to maximum range, and differences were assessed using the unpaired *t* test with Welch correction or the Mann-Whitney *U* test, respectively. Differences between 3 or more groups were assessed using the Kruskal-Wallis test (Dunn multiple-comparisons posttest). Spearman ρ (r_s) coefficient with 95% confidence intervals was used to evaluate correlations.

Significance was set at $P < 0.05$, and the GraphPad Prism version 7.00 (GraphPad Software, La Jolla, CA) software was used for calculations.

RESULTS

A total of 93 patients with SLE (94% female; mean age, 37.3 ± 13.6 years) and 67 control subjects (93% female; mean age, 36.1 ± 9.5 years) were included. The main clinical and laboratory data are summarized in Table 1. As noted, disease activity varied widely from quiescence to a highly active disease, with SLEDAI-2K scores ranging from 0 to 26. Meanwhile, patients tended to have few accumulated organ damage with median SLICC/ACR score of 1 point, ranging from 0 to 12.

In serum analysis (Fig. 1), IFN-λ3 levels were higher in patients (median, 83 pg/mL; range, 0–965 pg/mL) than in control subjects (median, 42 pg/mL; range, 0–520 pg/mL; $P = 0.002$). In contrast, serum IFN-λ1 levels (median, 0 pg/mL [range, 0–510 pg/mL] vs. 0 pg/mL [range, 0–171 pg/mL]; $P = 0.814$) and IFN-λ2 levels (0 pg/mL [0–28 pg/mL] vs. 0 pg/mL [0–43 pg/mL]; $P = 0.659$) were similar between patients and control subjects, respectively. No differences were found in serum IFN-α levels between patients and control subjects.

Serum levels of any type III IFNs were not significantly affected by the presence of associated medical conditions, including

TABLE 1. Main Clinical and Laboratory Characteristics of 93 Patients With SLE

Female sex, n (%)	87 (94)
Age, y	37.3 ± 13.6
Disease duration, y	7.2 ± 6.9
Lupus medications	
• Antimalarial drugs, n (%)	68 (73)
• Glucocorticoids, n (%)	67 (72)
• Immunosuppressants, n (%)	42 (45)
Associated medical conditions	
• Antiphospholipid syndrome, n (%)	24 (26)
• Vascular thrombosis, n (%)	17 (71)
◦ Pregnancy morbidity, n (%)	5 (21)
◦ Thrombotic microangiopathy, n (%)	5 (21)
◦ Cardiac valve disease, n (%)	5 (21)
◦ Neurological manifestations n (%)	4 (17)
• Sjögren syndrome, n (%)	16 (17)
◦ Vasculitis, n (%)	4 (25)
◦ Tubulointerstitial nephritis, n (%)	2 (13)
◦ Peripheral neuropathy, n (%)	2 (13)
◦ Interstitial lung disease, n (%)	1 (6)
◦ Autoimmune hepatitis, n (%)	1 (6)
• Diabetes mellitus, n (%)	7 (8)
• Systemic hypertension, n (%)	19 (20)
• Cardiovascular disease, n (%)	22 (24)
Laboratory tests	
• Leukocytes, total/μL	5,675 ± 2,336
• Lymphocytes, total/μL	1,389 ± 651
• Hemoglobin, g/dL	12.8 ± 2.0
• Platelets, total/μL	250 ± 96
• Blood urea nitrogen, mg/dL	17.5 ± 12.5
• Serum creatinine, mg/dL	1.11 ± 0.77
• 24-h Urine protein, mg	733 ± 1544
• Creatinine clearance, mL/min	67.1 ± 26.5
Immunological tests	
• Erythrocyte sedimentation rate, mm/h	29.8 ± 36.4
• C-reactive protein, mg/L	9.39 ± 15.42
• C3 complement, mg/L	97.1 ± 69.7
• C4 complement, mg/L	14.6 ± 6.4
• CH50% activity, median (IQR)	160 (80–160)
• ANAs ≥1:160, + (%)	83 (89)
• Anti-dsDNA, + (%)	49 (53)
• Anti-Ro/SSA, + (%)	38 (41)
• Anti-La/SSB, + (%)	17 (18)
• Anti-Sm, + (%)	22 (24)
• Anti-RNP, + (%)	33 (35)
• Rheumatoid factor ≥20 IU/mL, + (%)	32 (34)
SLEDAI-2K score, median (IQR)	6 (2–10)
SLICC/ACR damage index, median (IQR)	1 (0–2)

Data are expressed as the mean ± 1 SD unless otherwise specified.
CH50% indicates 50% hemolytic complement activity of serum.

Sjögren syndrome, antiphospholipid syndrome, diabetes mellitus, systemic hypertension, or cardiovascular disease (data not shown). In contrast, the concentration of IFN-λ3 but no other type III IFNs was strongly influenced by the use of steroids, because patients receiving glucocorticoids had serum IFN-λ3 levels 3 times higher than those who did not receive them (104 pg/mL [0–965 pg/mL] vs. 30 pg/mL [0–165 pg/mL], respectively; $P = 0.009$). In addition, a dose-related effect was observed, with the highest levels of IFN-λ3 found in patients receiving prednisone (or equivalent) at a dosage greater than 10 mg/d, intermediate levels in those receiving prednisone 10 mg/d or less, and the lowest levels in those who did not receive steroids (111 pg/mL [0–965 pg/mL] vs. 101 pg/mL [0–344 pg/mL] vs. 30 pg/mL [0–165 pg/mL], respectively; $P = 0.038$). The use of immunosuppressants, hydroxychloroquine, or statins was not associated with significant changes in serum cytokine levels.

Patients were categorized according to the SLEDAI-2K index in quiescent (score = 0, n = 12), mild (score 1–5, n = 34), moderate (score 6–12, n = 31), and severe (score >12, n = 16) disease activity. As shown in Figure 1, significant differences were observed in median IFN-λ3 levels between patients with quiescence and those with active disease, although we could not find a correlation between IFN-λ3 levels and disease activity when the SLEDAI-2K score was computed as a continuous variable ($r_s = -0.012$; −0.303 to 0.280; $P = 0.931$), whereas no differences were observed in the serum levels of IFN-λ1, IFN-λ2, and IFN-α (Fig. 1). Potential associations between serum levels of type III IFN and activity in selected organ systems were then evaluated (Table 2). Higher IFN-λ3 levels were found in the sera from patients with serositis (135 pg/mL [83–329 pg/mL] vs. 40 pg/mL [0–965 pg/mL]; $P < 0.01$) or cutaneous involvement (66 pg/mL [0–965 pg/mL] vs. 40 pg/mL [0–329 pg/mL]; $P < 0.05$) than in the sera of patients without the given manifestation. No differences were observed in other clinical manifestations. Neither serum IFN-λ1 nor IFN-λ2 levels had an association with lupus activity in any organ system. In addition, no associations were found between serum levels of type III IFNs and accrued lupus damage, as assessed by the SLICC/ACR damage index (Table 3).

The association of serum IFNs levels with circulating antibodies and complement proteins was also assessed. Serum IFN-λ3 levels were higher in patients positive for anti-Ro/SSA antibodies (122 pg/mL [0–965 pg/mL] vs. 0 pg/mL [0–165 pg/mL]; $P = 0.001$) and to a lesser extent in those positive for anti-dsDNA antibodies (93 pg/mL [0–965 pg/mL] vs. 0 pg/mL [0–344 pg/mL]; $P = 0.021$) compared with patients who tested negative. In addition, an inverse correlation was found between serum levels of IFN-λ3 and the concentration of C3 and C4 complement proteins (Fig. 2A). It should be noted that serum levels of IFN-λ1 and IFN-λ2 were not associated with circulating antibodies or complement protein levels (data not shown). Subsequently, bivariate analyses were performed to identify potential confounders, and an association between high levels of serum IFN-λ3 and anti-Ro/SSA–positive antibodies was observed regardless of the presence of cutaneous involvement (Fig. 2B). In support to this, patients with cutaneous involvement and positive anti-Ro/SSA antibodies had lower C3 (81.2 ± 19.8 mg/L vs. 95.2 ± 21.6 mg/L; $P = 0.022$) and C4 (11.9 ± 4.5 mg/L vs. 17.4 ± 7.1 mg/L; $P = 0.009$) complement levels than did patients without cutaneous involvement who were also negative for anti-Ro/SSA antibodies. Despite the previously mentioned association with anti-Ro/SSA antibodies, serum levels of IFN-λ3 were similar in patients with SLE irrespectively of the presence of secondary Sjögren syndrome, a clinical condition closely related to anti-Ro/SSA antibodies. We found no statistical significance for other associations, including the combination of active nephritis and anti-dsDNA antibodies (Fig. 2B).

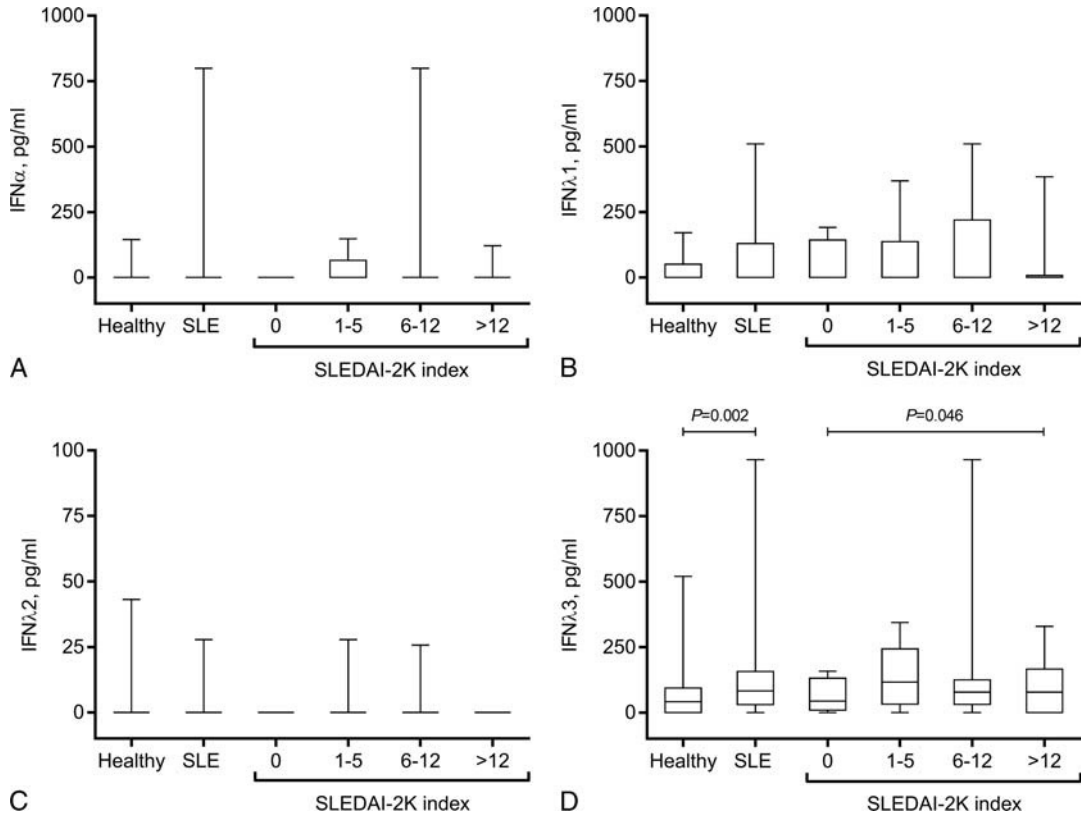

FIGURE 1. Levels of IFN-α (A), IFN-λ1 (B), IFN-λ2 (C), and IFN-λ3 (D) in sera from lupus patients according to the disease activity and in control subjects. No differences were found with the exception of IFN-λ3, whose levels were significantly higher in lupus patients than in control subjects, as well as in patients with lupus activity (categorized by the SLEDAI-2K score) compared with those with quiescent disease.

DISCUSSION

This study was performed to evaluate the serum levels of type III IFNs in patients with SLE and to identify their major clinical and laboratory associations. Our results suggest that IFN-λ3 is pathologically associated with the extent of lupus activity, specifically with cutaneous disease, as well as pleuritis and pericarditis. In addition, an unprecedented association was observed between high serum levels of IFN-λ3 and the presence of anti-Ro/SSA antibodies.

Patients with SLE usually show an increased expression of IFN-α–induced genes in peripheral blood mononuclear cells.[5] This enhanced response is central to the lupus pathogenesis via

TABLE 2. Serum Type III IFN Levels and the Presence of Concurrent Organ Involvement in Patients With SLE

		IFN-λ1, pg/mL	IFN-λ2, pg/mL	IFN-λ3, pg/mL
Neuropsychiatric	Present (n = 5)	0 (0–0)	0 (0–0)	0 (0–234)
	Absent (n = 88)	0 (0–510)	0 (0–28)	0 (0–965)
Vasculitis	Present (n = 6)	0 (0–0)	0 (0–0)	0 (0–0)
	Absent (n = 87)	0 (0–510)	0 (0–28)	0 (0–965)
Arthritis	Present (n = 16)	0 (0–219)	0 (0–22)	40 (0–122)
	Absent (n = 77)	0 (0–510)	0 (0–28)	58 (0–965)
Renal	Present (n = 35)	0 (0–510)	0 (0–26)	58 (0–344)
	Absent (n = 58)	0 (0–369)	0 (0–28)	46 (0–965)
Cutaneous	Present (n = 29)	0 (0–510)	0 (0–25)	66 (0–965)[a]
	Absent (n = 64)	0 (0–384)	0 (0–28)	40 (0–329)
Serositis	Present (n = 4)	0 (0–31)	0 (0–0)	135 (83–329)[a]
	Absent (n = 89)	0 (0–510)	0 (0–28)	40 (0–965)
Hematologic	Present (n = 11)	0 (0–271)	0 (0–11)	74 (0–119)
	Absent (n = 82)	0 (0–510)	0 (0–28)	46 (0–965)

Data are expressed as median (range).

[a]$P < 0.05$.

TABLE 3. Serum Type III IFN Levels and the Presence of Accumulated Organ Damage in Patients With SLE

		IFN-λ1, pg/mL	IFN-λ2, pg/mL	IFN-λ3, pg/mL
Ocular	Present (n = 9)	0 (0–0)	0 (0–0)	84 (0–225)
	Absent (n = 84)	0 (0–510)	0 (0–28)	46 (0–965)
Neuropsychiatric	Present (n = 20)	0 (0–257)	0 (0–26)	0 (0–235)
	Absent (n = 73)	0 (0–510)	0 (0–28)	53 (0–965)
Renal	Present (n = 24)	0 (0–219)	0 (0–0)	50 (0–319)
	Absent (n = 69)	0 (0–510)	0 (0–28)	52 (0–965)
Pulmonary	Present (n = 22)	0 (0–369)	0 (0–0)	76 (0–329)
	Absent (n = 71)	0 (0–510)	0 (0–28)	40 (0–965)
Cardiovascular	Present (n = 22)	0 (0–365)	0 (0–22)	83 (0–965)
	Absent (n = 71)	0 (0–510)	0 (0–28)	39 (0–344)
Peripheral vascular	Present (n = 14)	0 (0–0)	0 (0–0)	97 (0–215)
	Absent (n = 79)	0 (0–510)	0 (0–28)	40 (0–965)
Gastrointestinal	Present (n = 1)	0 (0–0)	0 (0–0)	0 (0–0)
	Absent (n = 92)	0 (0–510)	0 (0–28)	52 (0–965)
Musculoskeletal	Present (n = 8)	0 (0–196)	0 (0–0)	29 (0–155)
	Absent (n = 85)	0 (0–510)	0 (0–28)	64 (0–965)
Skin	Present (n = 5)	0 (0–0)	0 (0–0)	0 (0–0)
	Absent (n = 88)	0 (0–510)	0 (0–28)	52 (0–965)
Diabetes	Present (n = 7)	0 (0–510)	0 (0–25)	75 (0–344)
	Absent (n = 86)	0 (0–384)	0 (0–28)	51 (0–965)

Data are expressed as median (range).

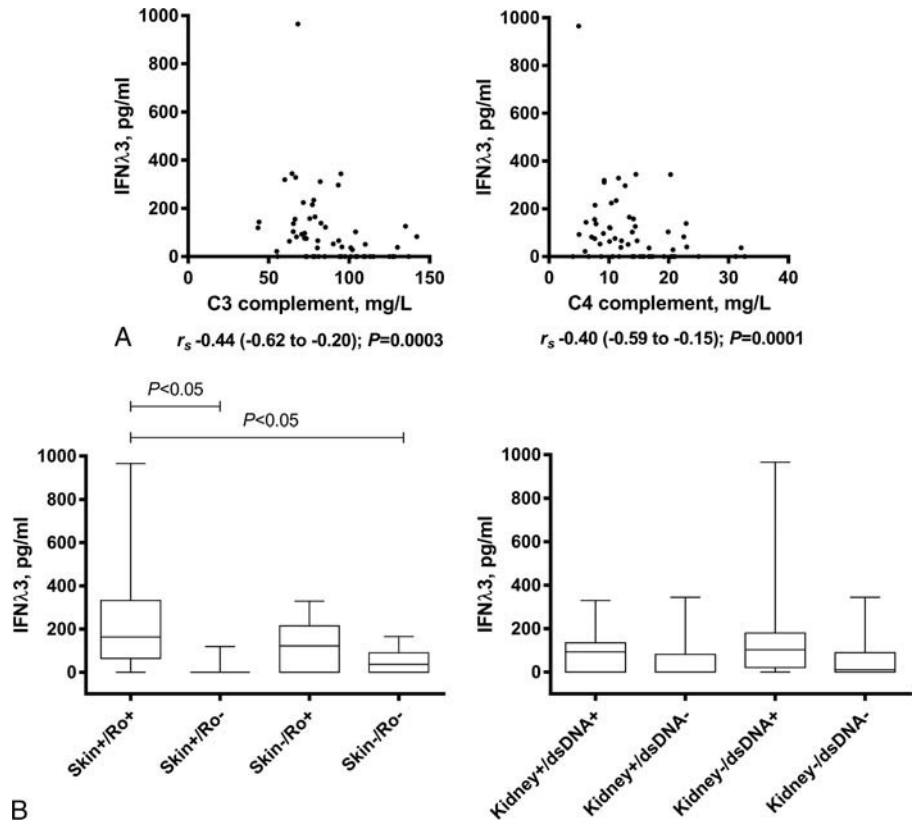

FIGURE 2. Spearman correlations (r_s) between the levels of IFN-λ3 and the concentration of C3 and C4 complement proteins in the serum of patients with lupus (A). In addition, an association was observed between high serum IFN-λ3 levels and anti-Ro/SSA antibodies, regardless of the presence of cutaneous disease (B), whereas no differences were found in the levels of IFN-λ3 for renal activity and anti-dsDNA antibodies.

the differentiation of monocytes into dendritic cells with high antigen-presenting properties.[22] It also regulates B-cell functioning by facilitating the isotype switching to high-affinity immunoglobulin G antibodies.[23] The induction of IFN-stimulated genes by IFN-α may also modulate the expression and signaling of pattern-recognition receptors, chemokines, and other molecules by leukocytes and endothelial cells.[4,24] Accordingly, a number of drugs targeting type I IFN–related pathways have recently emerged, including monoclonal antibodies that neutralize IFN-α or its receptor, anti–IFN-α antibody–inducing vaccines, and inhibitors of Toll-like receptors.[25] Phase I trials using anti–IFN-α antibodies demonstrated effective inhibition of IFN-α signature, and preliminary analyses suggested that inhibition of IFN-α could be associated with clinical benefits in SLE.[26,27] Unfortunately, this initial excitement has been overshadowed by convincing evidence showing no significant difference in the extent of disease activity between anti–IFN-α monoclonal antibodies and placebo, despite a noticeable improvement in a number of laboratory parameters.[28,29] Therefore, additional contributing mechanisms may also need interference to produce clinically significant changes.

The main clinical novelty in our study is the finding of high levels of IFN-λ3 in sera from patients with serositis and cutaneous disease. Previously, Wu et al.[30] found IFN-λ1 mRNA and serum protein elevated in patients with SLE, especially in subjects with concurrent arthritis or renal disease. In addition, Lin et al.[31] found elevated levels of IFN-λ2 in serum and in transcripts from activated $CD4^+$ T cells of SLE patients compared with healthy individuals. More recently, Zickert et al.[32] described higher IFN-λ levels in patients with active lupus nephritis than in control subjects, and persistently increased IFN-λ levels were associated to an unfavorable response to treatment. Here, we have shown IFN-λ3 levels to be increased in sera from active lupus patients, especially in those with concurrent serosal or cutaneous disease. This coincides with the notion that type III IFNs act mainly on epithelial cells, constituting an important epithelial antiviral defense system.[33] Indeed, IFN-λ treatment is more effective in preventing herpes virus replication in the vaginal mucosa than IFN-α therapy,[34] and the stimulated keratinocytes produce large amounts of IFN-λ but not IFN-α after infection with the vesicular stomatitis virus.[35] In addition, whereas type I IFN receptors are widely expressed in many cell types, IFN-λR1 demonstrates a much more restricted expression pattern, limiting type III IFN–dependent responses primarily to epithelial-like tissues.[36–38] A recent study provided evidence that IFN-λ's and its receptor are strongly expressed in the epidermis of cutaneous lesions of lupus, with IFN-λ–stimulated keratinocytes driving the recruitment of immune cells into the skin via an increased expression of IFN-λ–inducible chemokines.[35] Together, these data support that the functioning of type III IFNs is enhanced in SLE.

With regard to elevated levels of IFN-λ3 in SLE patients with serositis, we did not find any mechanistic explanation to support such association. However, sporadic cases on the development of pleuritis and pleural effusion in individuals receiving recombinant IFN-α as a treatment for chronic hepatitis C virus infection suggest the existence of unknown but plausible mechanisms for the development of clinically evident serositis after activation of the common IFN-α/IFN-λ post–receptor signaling pathway.[39,40] The concentration of IFN-λ3 was also markedly higher in patients receiving glucocorticoids in a dose-related manner, supporting that steroids might induce the production of type III IFNs as previously suggested.[41] However, steroid-treated patients, with some exceptions, are mainly more severe diseased, thus leaving open the possibility that the higher values observed in steroid-treated patients are related to a more severe disease. Indeed, in the bivariate analyses, it was observed that the aforementioned association depended on the degree of disease activity because steroid-treated patients showed a significantly higher SLEDAI-2K score than did patients who did not receive glucocorticoids (median, 6 [0–26] vs. 2 [0–16]; $P < 0.0001$). In addition, it was remarkable that serum IFN-α levels were virtually undetectable in patients with SLE and control subjects. However, serum concentrations of IFN-α molecules themselves are usually very low and difficult to detect. In fact, this is a major reason why the evaluation of type I IFN activity using IFN-induced genes is used instead.[25]

Another important novelty in our study is the association between high levels of IFN-λ3 and circulating anti-Ro/SSA antibodies. Ro proteins are normal constituents of several fetal tissues such as the myocardium and epidermis, although they are also found in cells of the normal adult skin.[42] Ro antigens consist of 2 unrelated proteins, namely, Ro60 kDa and Ro52 kDa, which are the major autoantigens targeted by commercially available anti-Ro/SSA enzyme-linked immunosorbent assays.[43] It has recently been shown that the Ro52 protein, an E3 ligase with antiproliferative and proapoptotic properties via the ubiquitination of IRF-8, is initially up regulated by IFN-α or IFN-γ and then prompted to be translocated to the nucleus. Therefore, the Ro52 antigen is now considered as an important IFN-inducible protein that contributes to the elicitation of innate immunity.[44,45] In this vein, high levels of IFN-λ3 found in patients positive for anti-Ro/SSA antibodies suggest that IFN-λ3 may lead to cutaneous lupus damage with the consequent release of large amounts of Ro antigens. Alternatively, the appearance of anti-Ro/SSA antibodies could intrinsically regulate IFN-λ3 expression in keratinocytes and other skin cells.

We are aware that the small number of patients with serositis may limit our results. In addition, although the role of Sjögren syndrome was evaluated, there was no evidence of differentiated antibody testing against the Ro52 and Ro60 antigens. Finally, experiments and procedures to differentiate between downstream signaling due to activation of the type I IFN receptor from those resulting from stimulation of the type III IFN receptor are not yet available; consequently, dissection of the exact function of each IFN in lupus activity remains a pending task.

As already described for type I IFNs, to our knowledge, we are the first to hypothesize the existence of a feed-forward loop between IFN-λ3, anti-Ro/SSA antibodies, and cutaneous lupus disease. Although additional in vitro and in vivo assays are needed to fully understand the role of IFN-λ3 in lupus, the apparent pathological relationship between IFN-λ3 and anti-Ro/SSA antibodies results in novel evidence in favor of the aforementioned association and uncovers a possible new therapeutic target for patients with SLE in the family of type III IFNs.

KEY POINTS

- Interferon λ3 is associated with lupus activity especially in serosal and cutaneous disease, as well as with circulating anti-Ro/SSA antibodies.
- Type III IFNs could be new therapeutic targets in SLE.

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