The Journal of Rheumatology 2022;49:388–97
doi:10.3899/jrheum.210391
First Release February 1 2022

The Journal of Rheumatology

High Systemic Type I Interferon Activity Is Associated With Active Class III/IV Lupus Nephritis

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ABSTRACT. ***Objective.*** Previous studies suggest a link between high serum type I interferon (IFN) and lupus nephritis (LN). We determined whether serum IFN activity is associated with subtypes of LN and studied renal tissues and cells to understand the effect of IFN in LN.

Methods. Two hundred and twenty-one patients with systemic lupus erythematosus were studied. Serum IFN activity was measured by WISH bioassay. mRNA in situ hybridization was used in renal tissue to measure expression of the representative IFN-induced gene, IFN-induced protein with tetratricopeptide repeats-1 (*IFIT1*), and the plasmacytoid dendritic cell (pDC) marker gene C-type lectin domain family-4 member C (*CLEC4C*). Podocyte cell line gene expression was measured by real-time PCR.

Results. Class III/IV LN prevalence was significantly increased in patients with high serum IFN compared with those with low IFN (odds ratio 5.40, $P = 0.009$). In multivariate regression models, type I IFN was a stronger predictor of class III/IV LN than complement C3 or anti-dsDNA antibody, and could account for the association of these variables with LN. *IFIT1* expression was increased in all classes of LN, but most in the glomerular areas of active class III/IV LN kidneys. *IFIT1* expression was not closely colocalized with pDCs. IFN directly activated podocyte cell lines to induce chemokines and proapoptotic molecules.

Conclusion. Systemic high IFN is involved in the pathogenesis of severe LN. We did not find colocalization of pDCs with IFN signature in renal tissue, and instead observed the greatest intensity of the IFN signature in glomerular areas, which could suggest a blood source of IFN.

Key Indexing Terms: interferon signature gene, lupus nephritis, podocyte injury type I interferon

This study was supported by grants to TBN from the Colton Center for Autoimmunity, National Institutes of Health (AR060861, AR057781, AR065964), the Lupus Research Foundation, and the Lupus Research Alliance.

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TBN has received research grants from EMD Serono and Janssen, and has consulted for Thermo Fisher, Progentec, and Inova, all unrelated to the current manuscript. The remaining authors declare no conflicts of interest relevant to this article.

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Accepted for publication November 3, 2021.

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease caused by dysregulation of the immune system and characterized by autoantibody production and immune complex formation with multiorgan system involvement.[1] It affects women 9 times more often than men, and is generally diagnosed in early adulthood.[2,3] It is also well known that genetic factors play an important role in the development of SLE.[1] Among the various organ manifestations of SLE, lupus nephritis (LN) represents one of the most serious and can progress to endstage renal disease, leading to increased morbidity and mortality. LN affects approximately 40% of patients with SLE throughout their lifetime.[3,4] Despite advances in treatment, patients with LN still have higher morbidity and mortality compared with those without LN.[4,5,6]

Type I interferon (IFN), in particular IFNα, plays a crucial role in the pathogenesis of SLE, having pleiotropic effects on various cell types and the potential to break immunologic self-tolerance.[7,8] IFNα induces dendritic cell differentiation to uptake self-antigens from dying cells and present them to CD4+ T cells, driving the autoimmune response.[9] IFNα also induces B cell activation and differentiation including autoreactive B cells promoting autoantibody production.[10] IFNα increases the

production of B cell survival factors, such as B lymphocyte stimulator, from dendritic cells.[10] Further, in vitro studies show that antinuclear antibody (ANA) immune complexes induce IFNα production in plasmacytoid dendritic cells (pDCs) through endosomal nucleic acid-sensing Toll-like receptors (TLRs) through their RNA or DNA component,[11,12] suggesting a feed-forward mechanism for ANAs in enhancing IFNα production in SLE.

High serum IFNα activity is a heritable risk factor for SLE, as familial aggregation of high serum IFNα activity is observed in SLE families.[13,14] Some SLE-associated genetic risk variants that function in the IFNα pathway are gain-of-function mutations in patients with SLE.[15,16,17] Serum IFNα activity is higher in patients with SLE compared with patients with other autoimmune diseases or healthy individuals.[18,19,20] High levels of serum IFNα activity and increased IFN-inducible gene expression in peripheral blood mononuclear cells (PBMCs) are also associated with more severe disease, as well as with the presence of SLE-associated autoantibodies.[19,20,21,22,23] Moreover, increased expression of IFNα-inducible genes in PBMCs is associated with LN, and the presence of ANAs including anti-dsDNA antibodies.[21,22,23,24] However, the relationship between IFN and LN International Society of Nephrology/Renal Pathology Society (ISN/RPS) histologic classes and activity/chronicity indices are less clear, and it is not known whether these relationships are independent of the strong association between type I IFN and autoantibodies such as anti-dsDNA. The source of IFN in renal disease is also unclear. While local production of IFN by pDCs in renal tissue would be possible, this has not been documented in previous single-cell sequencing studies,[25] despite a strong type I IFN signature in renal cells.

In this study, we determined whether serum type I IFN activity is associated with different classes of LN. We found that high serum type I IFN activity was significantly associated with class III/IV LN but not with other classes of LN, and this activity was independent of the presence of anti-dsDNA antibody and complement levels. We also found that the expression of the type I IFN-inducible gene, IFN-induced protein with tetratricopeptide repeats 1 (*IFIT1*) was significantly increased in class III/IV LN kidneys in a glomerular pattern that was not strongly colocalized with pDCs. Finally, in vitro experiments demonstrated type I IFN induced proapoptotic molecules and chemokines in human podocytes. In aggregate, these results suggest that systemic high type I IFN is involved in the pathogenesis of severe LN.

METHODS

Patients, samples, and clinical data. Serum samples and clinical data of 221 European-American patients with SLE were obtained from the Rochester Mayo Clinic. Patients' ancestral background was determined by interview and self-report of the origins of the subjects' ancestors. Slides from kidney biopsy specimens for mRNA in situ hybridization (ISH) experiments were obtained from the New York University Medical Center. All patients fulfilled ≥ 4 of the American College of Rheumatology (ACR) revised criteria for the diagnosis of SLE.[26] Patients provided informed consent and the study was approved by the institutional review boards at the respective institutions (Mayo IRB# PR12-007618-01 and NYU IRB# S18-00489). Data regarding the presence or absence of ACR criteria for SLE diagnosis[26] and detailed clinical data were available. Samples were collected and studied at any point after the formal diagnosis of SLE. Patients with nonLN were defined as patients with SLE who never had kidney involvement, and patients with LN were defined as patients with SLE with kidney involvement based on renal biopsies who currently had LN or had a history of LN at the time of recruitment. Classes of LN were confirmed by renal biopsy review according to the classifications presented by the ISN/RPS.[27] Active LN was defined as involving ≥ 1 renal domain and including urine protein ≥ 0.5 g/24 h according to Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) renal criteria.[28]

Autoantibody measurement. ANAs were measured by indirect immunofluorescence methods. Anti-dsDNA antibodies were measured by ELISA. Anti-dsDNA antibodies < 30.0 IU/mL were considered negative and anti-dsDNA antibodies > 75.0 IU/mL were considered positive. Ranges between 30.0–75.0 IU/mL were considered as a borderline result and were re-analyzed using *Crithidia luciliae* immunofluorescence; the detectable fluorescence was considered positive. All the blood samples were assayed and analyzed in the same clinical laboratory at the Mayo Clinic, and standard clinical cutoffs were used to define a positive result.

Serum type I IFN activity measurement. Type I IFN activity in sera was measured by performing an IFN bioassay as described previously.[13,29] Briefly, reporter cells (WISH cells, ATCC #CCL-25) were used to measure the ability of sera to cause type I IFN-induced gene transcription. WISH cells were incubated with patient sera for 6 hours and then lysed and 3 canonical type I IFN-induced transcripts (ie, *IFIT1*, myxovirus resistance 1 [*MX1*], and protein kinase R [*PKR*]) were measured by reverse transcriptase PCR. Relative expression data from the 3 transcripts were then normalized by the healthy control data and presented as a type I IFN activity score.[13] Pretreatment of sera with anti-IFNα and anti-IFNβ antibodies completely abrogated the IFN-induced gene expression observed in the assay, confirming that type I IFN was driving the readout observed. The WISH cells did not express the type II IFN receptor or endosomal TLRs, and the assay output did not decrease in the presence of cycloheximide, ruling out autocrine loops and other parallel stimuli contributing to the gene expression observed.

mRNA ISH in kidney biopsy specimens. RNA ISH for the IFN-induced gene *IFIT1* and the pDC marker gene C-type lectin domain family 4 member C (*CLEC4C*, also known as *BDCA2*) was performed using the RNA scope 2.5 HD Red assay kit (Advanced Cell Diagnostics) and the RNA scope probe specific to the gene encoding Homo sapiens (Hs) *IFIT1* transcript variant 3 mRNA and Hs *CLEC4C* transcript variant 2 mRNA, according to the manufacturer's instructions. Briefly, 5-μm formalin-fixed paraffin-embedded biopsied kidney tissue sections were pretreated with heat and proteases prior to hybridization with the target oligo probes. Preamplifiers and amplifiers were sequentially hybridized to the target probe, and alkaline phosphatase-based amplifiers were then hybridized in the final steps followed by detection using chromogenic substrate. Samples were quality-controlled for RNA integrity with an RNA scope probe specific to Hs-PPIB RNA and for background signal using the bacterial negative control probe DapB. The specific RNA staining signal was identified as red, punctate dots. ISH for *IFIT1* mRNA and *CLEC4C* mRNA was done in serial sections for every sample. Quantitative measurement of mRNA expression (ie, *IFIT1* and *CLEC4C*) and heat map analysis was performed by Visiopharm Integrator System (Visiopharm).

Type I IFN-induced gene expression in human podocytes in vitro. A human immortalized podocyte cell line transformed with thermosensitive SV40 large T antigen (U19tsA58) and human telomerase (hTERT), was provided by Dr. Jeffrey B. Kopp (National Institutes of Health).[30] Briefly, the podocyte cell line was first cultured in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum, insulin-transferrin-selenium G supplement, and penicillin/streptomycin in type 1 collagen-coated dishes under growth permissive conditions (33°C) for cell expansion. Cells were then

cultured under growth-restricted condition (37°C) for 7 days for differentiation. After completing the differentiation step, cells were cultured in medium only or media with recombinant human (rh) IFNα (PBL Assay Science) 5000 U/ml or rhIFNβ (PBL Assay Science) 5000 U/ml for 24 hours. RNA extraction and cDNA synthesis were done according to the manufacturer's instructions using the mRNA micro kit (QIAGEN) and subsequent cDNA synthesis was done by PrimeScript II 1st strand cDNA synthesis kit (Takara/Clontech) according to the manufacturer's instructions. qPCR was performed using 40 cycles on a StepOnePlus Real-Time PCR System (Applied Biosystems). TaqMan probes and primers for glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*, Hs99999905_m1), BCL2-antagonist/killer 1 (*BAK1*, Hs00832876_g1) Bcl-2-associated X protein (*BAK1*, Hs00180269_m1), cathepsin L (*CTSL*, Hs00377632_m1), monocyte chemotactic protein-1 (*MCP1*, Hs00234140_m1), and C-X-C motif chemokine 10 (*CXCL 10*, Hs00171042_m1) were purchased from Applied Biosystems.

Statistical analysis. Data are summarized as mean ± SD or as median and IQR as appropriate depending on the distribution of the data. Categorial analyses were performed using the chi-square or Fisher exact test, and quantitative analyses were performed using the 2-sample *t* test, Mann-Whitney *U* test, or Kruskal-Wallis test, again depending on the distribution of the data. Spearman rank order correlation analysis was performed to investigate the correlations among variables. A stepwise, multivariate logistic regression was performed to investigate predictor variables and type I IFN activity as the outcome variable. *P* values < 0.05 for variables in the multivariate analysis were considered significant.

RESULTS

High serum type I IFN activity is associated with a higher prevalence of active class III/IV LN. Clinical characteristics of 221 European-American patients with SLE stratified by low and high levels of serum type I IFN activity are shown in Table 1. Mean ages at disease onset and at recruitment were significantly younger in the patients with high levels of serum type I IFN (IFN score ≥ 2) than those with low levels of serum type I IFN (IFN score < 2; $P = 0.0005$ and $P = 0.0002$, respectively), which were consistent with previous reports.[14,20] The presence of anti-dsDNA antibodies was associated with a high type I IFN measurement in the same sample (60.4% positive anti-dsDNA antibody in high type I IFN vs 36.4% in low type I IFN; $P = 0.003$). Low complement C3 and C4 levels were also significantly associated with high levels of serum type I IFN in the patients with SLE (both $P = 0.03$).

Patients with SLE with high levels of serum type I IFN had a greater number of ACR clinical criteria and higher concurrent SLEDAI-2K scores than those with low levels of serum type I IFN (both $P < 0.0001$; Table 1). In addition, serum type I IFN levels and SLEDAI-2K scores were positively correlated in our cross-sectional evaluation ($r = 0.28$, $P < 0.0001$; Supplementary Figure 1, available with the online version of this article). Further, patients with SLE with high levels of serum type I IFN had a higher incidence of LN (ever or current; 56.5%) than those with low levels of serum type I IFN (26.3%; odds ratio [OR] 3.65, $P = 0.0002$; Table 1 and Table 2). Medications were not significantly different in either group, except that the dose of prednisolone was higher in the patients with high levels of serum type I IFN than those with low levels of serum type I IFN ($P = 0.003$).

As shown in Table 2, the risk of class III/IV LN was strikingly increased in the patients with high levels of serum type I IFN than in those with low levels of serum type I IFN (OR = 5.40,

Table 1. Clinical characteristics of European-American patients with SLE stratified by low and high levels of serum type I IFN activity.

	Total, n = 221	Low Type I IFN (IFN Score < 2), n = 175	High Type I IFN (IFN Score ≥ 2), n = 46	*P**
Female	188 (85.1)	149 (85.1)	39 (84.8)	NS
Age at recruitment, yrs, mean ± SD	47.7 ± 16.3	49.8 ± 16.4	39.9 ± 13.6	0.0002
Age of onset, yrs, mean ± SD	36.1 ± 15.8	37.8 ± 16.0	29.9 ± 13.4	0.0005
Disease duration, yrs, mean ± SD	11.6 ± 10.3	12.0 ± 10.7	10.0 ± 8.2	NS
ACR criteria[a], median (IQR)	6 (5–7)	6 (5–7)	7 (6–8)	< 0.0001
ANA	218 (98.6)	174 (99.4)	44 (95.7)	NS
Anti-dsDNA antibody	100 (41.7)	68 (36.4)	32 (60.4)	0.003
Low C3	29 (13.1)	18 (10.3)	11 (24.0)	0.03
Low C4	66 (29.9)	46 (26.3)	20 (43.5)	0.03
Serum creatinine, mg/dL, mean ± SD	0.93 ± 0.34	0.92 ± 0.31	0.98 ± 0.46	NS
LN[b]	72 (32.6)	46 (26.3)	26 (56.5)	0.0002
Total SLEDAI-2K score, (IQR)	2 (0–4)	2 (0–4)	4 (2–8)	< 0.0001
Current medications				
Hydroxychloroquine	171 (77.4)	140 (80.0)	31 (67.4)	NS
Cyclophosphamide	2 (0.9)	2 (1.1)	0 (0.0)	NS
Azathioprine	20 (9.0)	14 (8.0)	6 (13.0)	NS
Mycophenolate mofetil	55 (24.9)	43 (24.6)	12 (26.1)	NS
Methotrexate	24 (10.9)	20 (11.4)	4 (8.7)	NS
Prednisolone, mg/d (IQR)	0 (0–5)	0 (0–5)	5 (0–7.9)	0.003

Values are n (%) unless otherwise indicated. [a] No. of ACR criteria fulfilled by the time of recruitment. [b] LN was diagnosed on the basis of renal biopsy findings.* $P < 0.05$ were considered significant. ACR: American College of Rheumatology; ANA: antinuclear antibody; IFN: interferon; LN: lupus nephritis; NS: not significant; SLE: systemic lupus erythematosus; SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index 2000.

Table 2. Patients with SLE with high levels of type I IFN or with a positive result for anti-dsDNA antibody have a higher incidence of class III/IV LN.

Class of LN[a]	Low Type I IFN (IFN score < 2), n = 175	High Type I IFN (IFN score ≥ 2), n = 46	OR (95% CI)	*P**
LN (total)	46	26	3.65 (1.9–7.2)	1.8×10^{-4}
Class III/IV LN	27	23	5.40 (1.4–20.6)	0.009
Nonclass III/IV LN	19	3	0.54 (0.2–1.8)	0.42
Subtype of LN	Anti-dsDNA Antibody (–), n = 127	Anti-dsDNA Antibody (+)[b], n = 94	OR (95% CI)	*P*
LN (total)	34	38	1.86 (1.1–3.3)	0.04
Class III/IV LN	18	32	4.74 (1.6–6.0)	0.005
Nonclass III/IV LN	16	6	0.47 (0.2–1.3)	0.17
Subtype of LN	Low C3 (–), n = 192	Low C3 (+)[c], n = 29	OR (95% CI)	*P*
LN (total)	56	16	2.99 (1.3–6.6)	9.6×10^{-3}
Class III/IV LN	36	14	4.04 (1.8–9.1)	1.3×10^{-3}
Nonclass III/IV LN	20	2	0.64 (0.1–2.9)	0.75
Subtype of LN	Low C4 (–), n = 156	Low C4 (+)[c], n = 65	OR (95% CI)	*P*
LN (total)	47	25	1.45 (0.8–2.7)	0.27
Class III/IV LN	30	20	1.87 (0.9–3.6)	0.08
Nonclass III/IV LN	17	5	0.68 (0.2–1.9)	0.62

[a] Subtypes of LN were confirmed by renal biopsy review according to the classification of the International Society of Nephrology/Renal Pathology Society guidelines.[27] [b] Positivity of anti-dsDNA antibody is defined as described in the Methods. [c] Low complement C3 or C4 levels were defined as described in the Methods. * *P* values were calculated by the chi-square or Fisher exact test. $P < 0.05$ was considered significant. IFN: interferon; LN: lupus nephritis; SLE: systemic lupus erythematosus.

$P = 0.009$; Table 2). Specifically, 88.5% of patients with high IFN had proliferative class III/IV LN compared to 58.7% with low IFN (Table 2).

Positivity of anti-dsDNA antibody at the time of the blood draw was also significantly associated with a diagnosis of LN (OR 1.86) and more strongly associated with class III/IV LN (OR 4.74, $P = 0.005$) in univariate analyses (Table 2). Similarly, low levels of complement C3 were significantly associated with a diagnosis of overall LN (OR 2.99), and more strongly associated with class III/IV nephritis (OR 4.04, $P = 1.3 \times 10^{-3}$). Complement C4 levels were not associated with overall LN or class.

Given that type I IFN, anti-dsDNA positivity, and low complement C3 are all correlated with each other,[20] and are each associated with class III/IV LN in our univariate analysis above, we performed a multivariate regression to determine independent predictors of class III/IV nephritis. In a logistic regression model, we found that high levels of serum type I IFN was the independent predictor strongly associated with active class III/IV LN ($P = 0.04$, OR 3.65, stepwise logistic regression; Supplementary Table 1, available with the online version of this article). In contrast, anti-dsDNA antibody positivity and low complement C3 levels were not significantly associated with active class III/IV LN when type I IFN was included in the multivariate regression. This suggests that type I IFN could account for a large portion of the association of these variables with class III/IV LN in univariate analyses. While anti-dsDNA antibody positivity and low complement C3 were not completely redundant and overlapping with high type I IFN, it is striking that type I IFN was a stronger overall predictor than either of the other 2 more traditional biomarkers of LN.

Type I IFN-inducible gene expression is increased in active class III/IV LN. To look for the effects of type I IFN in the affected tissue, we next performed mRNA ISH to detect the expression levels of *IFIT1*, one of the major type I IFN-inducible genes, and *CLEC4C* (also known as *BDCA2*), a unique marker of pDCs, in kidney biopsy samples obtained from healthy controls and patients with LN. Target mRNA expression was normalized by cell counts as assessed by nuclei. *IFIT1* mRNA expression was significantly increased in class III/IV LN kidneys compared to healthy kidneys ($P < 0.05$; Figure 1A [red dots] and Figure 1B). However, there were no statistical differences in *IFIT1* expression between class III/IV LN and nonclass III/IV (Figure 1B). *IFIT1* expression was observed in both glomerular lesions and interstitial lesions in class III/IV LN kidneys (Figure 1A), but the glomerulus demonstrated greater staining when tissue was examined in overview heat maps (Figure 2). *CLEC4C* gene expression levels in tissue did not differ between LN classes (data not shown). While infiltrating pDCs were present in all LN classes, these cells were scarce and tended to be localized in the interstitial areas. Proximity analyses did not detect any significant proximity of pDCs to *IFIT1* staining density (Figure 3).

Type I IFN induces mRNA expression of proapoptotic molecules and chemokines in human podocytes. Based on the prominent expression of *IFIT1* mRNA in the glomeruli of class III/IV LN (Figure 1A) and previous reports indicating that podocyte injury is a pathologic feature of class III/IV LN,[31,32] we next determined

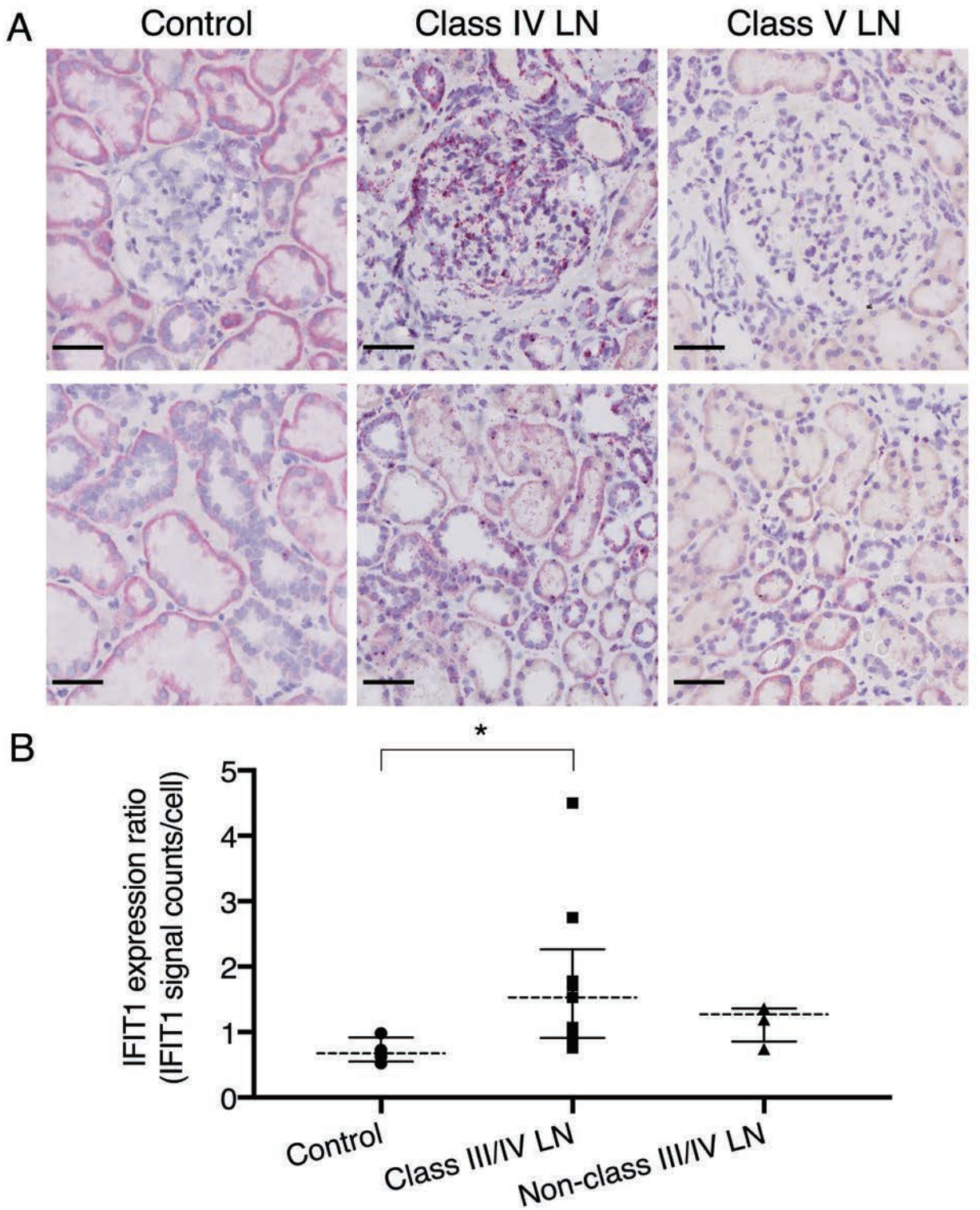

Figure 1. Expression of type I IFN-inducible gene *IFIT1* is increased in class III/IV LN kidneys. mRNA ISH was performed. Briefly, 5-μm formalin-fixed paraffin-embedded biopsied kidney tissue sections were pretreated with heat and proteases prior to hybridization with the target oligo probes. Preamplifiers and amplifiers were sequentially hybridized to the target probe, and alkaline phosphatase-based amplifiers were then hybridized in final steps followed by specific RNA detection using chromogenic substrate. (A) mRNA ISH for *IFIT1* was performed using biopsied kidney samples. Pathological LN classes were classified according to the International Society of Nephrology/Renal Pathology Society 2003 classification of LN. Representative photomicrographs of mRNA ISH of glomerular lesions (upper panels) and interstitial lesions (lower panels) of the kidneys are shown for class IV LN and class V LN. Kidney specimens obtained from the noncancerous lesion dissected from kidney clear cell carcinoma were used as controls. Each red dot shows the expression of *IFIT1* mRNA. Bars indicate 50 μm. (B) Scatter plot of *IFIT1* mRNA expression ratio (normalized by the number of nucleus) in control kidneys, class III/IV LN kidneys, and nonclass III/IV LN kidneys are shown (n = 4, 9, and 4, respectively). Data are expressed as median with IQR. * $P < 0.05$. *IFIT1*: interferon-induced protein with tetratricopeptide repeats 1; IFN: interferon; ISH: in situ hybridization; LN: lupus nephritis.

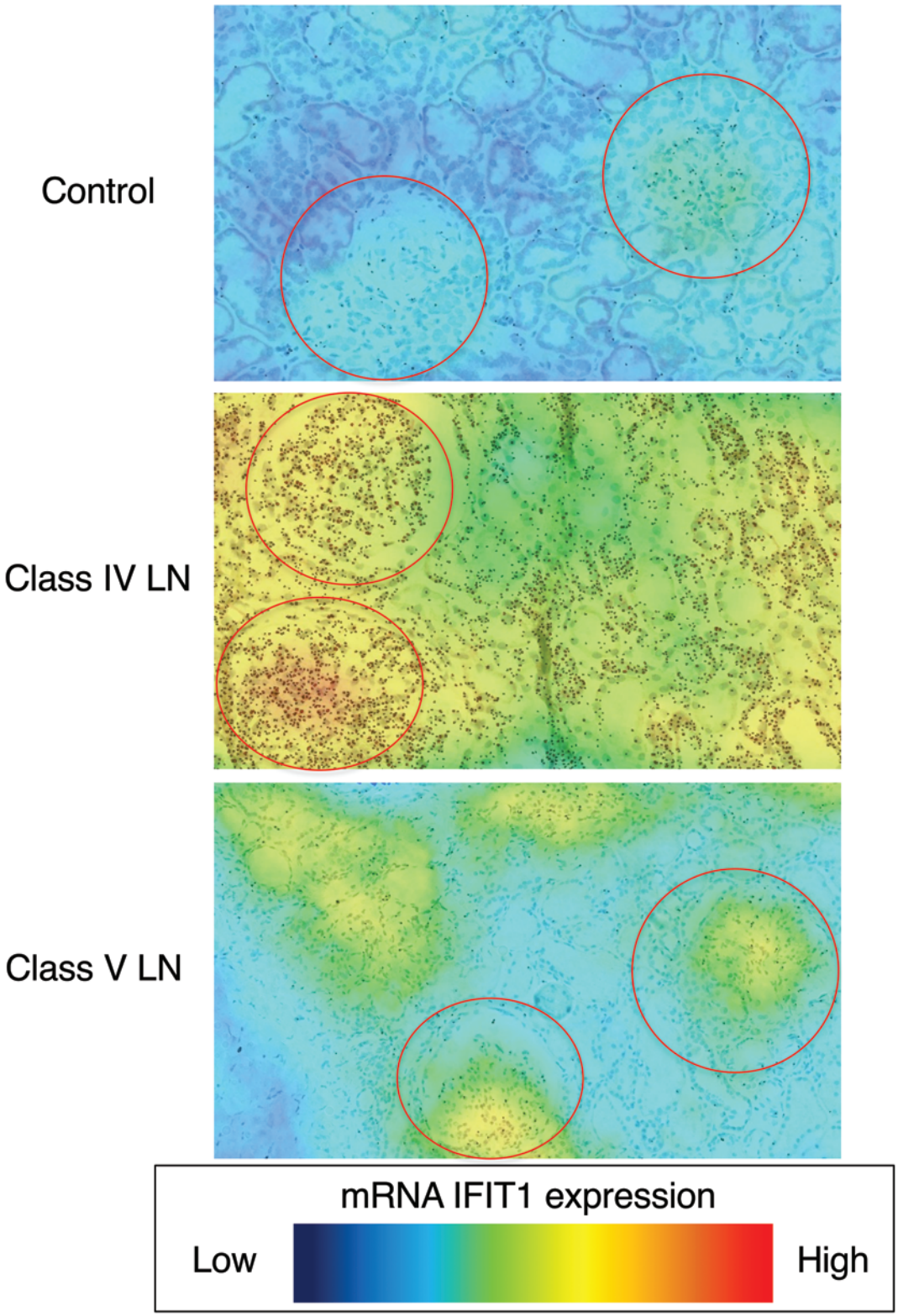

Figure 2. IFIT1 mRNA expression in LN kidney is higher in glomerulus lesion compared to that in interstitial lesion. Quantitative measurement and heat map analysis of mRNA *IFIT1* expression in LN and control mRNA ISH samples was performed by Visiopharm Integrator System (Visiopharm). Representative figures of each LN subtype and control are shown. Heat map analysis results are representative of 4 control kidneys, 9 class III/IV LN kidneys, and 4 nonclass III/IV LN kidneys. Red circle indicates glomerulus lesion. *IFIT1*: interferon-induced protein with tetratricopeptide repeats 1; ISH: in situ hybridization; LN: lupus nephritis.

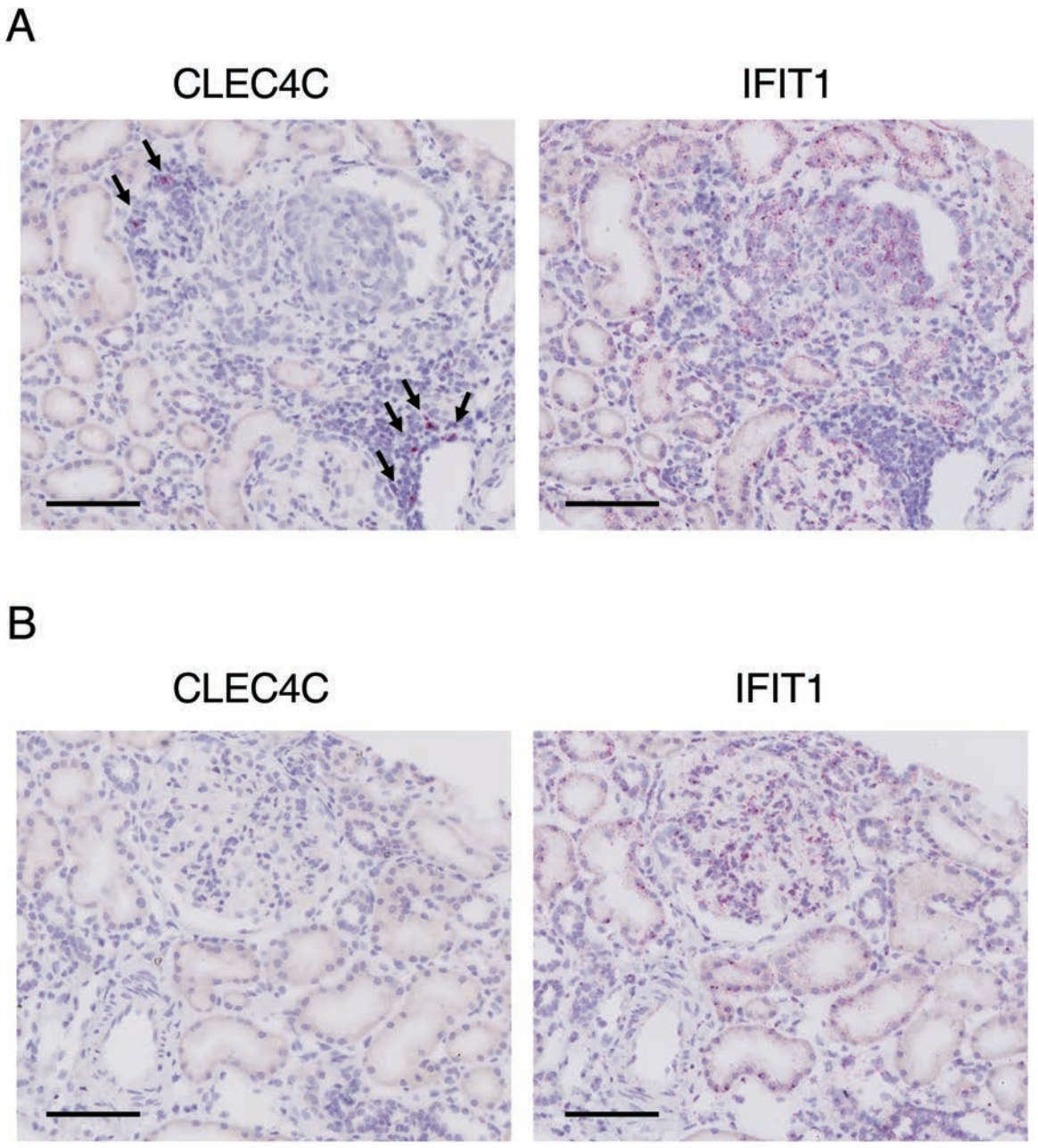

Figure 3. No significant colocalization of pDCs with *IFIT1* expression was observed in the renal tissue. mRNA ISH for *CLEC4C* and *IFIT1* mRNA was performed in class III/IV LN kidneys and nonclass III/IV LN kidneys (n = 4 and 9, respectively). Representative figures of *CLEC4C* and *IFIT1* mRNA expression density are shown in class IV LN kidneys. In each sample, both *CLEC4C* and *IFIT1* mRNA ISH are done in serial section. Each red dot indicates mRNA expression as indicated above. (A) Lesion with pDC (*CLEC4C*) infiltration. (B) Lesion without pDC (*CLEC4C*) infiltration. Black arrows indicate *CLEC4C* mRNA expression. Bars indicate 50 μm. *CLEC4C*: C-type lectin domain family 4 member C; *IFIT1*: interferon-induced protein with tetratricopeptide repeats 1; ISH: in situ hybridization; LN: lupus nephritis; pDC: plasmacytoid dendritic cell.

the direct effect of type I IFN on human podocyte cell lines. IFNα and IFNβ both significantly induced mRNA expression of proapoptotic genes *BAK1* and *BAX* compared to unstimulated podocytes at 24 hours (Figure 4), with IFNβ inducing greater mRNA expression of *BAK1* than IFNα. IFNα and IFNβ also significantly induced *CTSL* mRNA expression (Figure 4), which degrades cytoskeleton-related proteins such as CD2-associated protein during podocyte injury.[33] Further, IFNβ induced *MCP1* and *CXCL10*, which are indispensable for recruiting inflammatory cells (monocytes/macrophages and pDCs) to inflamed tissues,[34] whereas, IFNα induced *CXCL 10* alone (Figure 4). These results indicate that type I IFN induces gene expression patterns that characterize podocyte injury in LN as well as chemokines that can result in inflammatory cell recruitment.

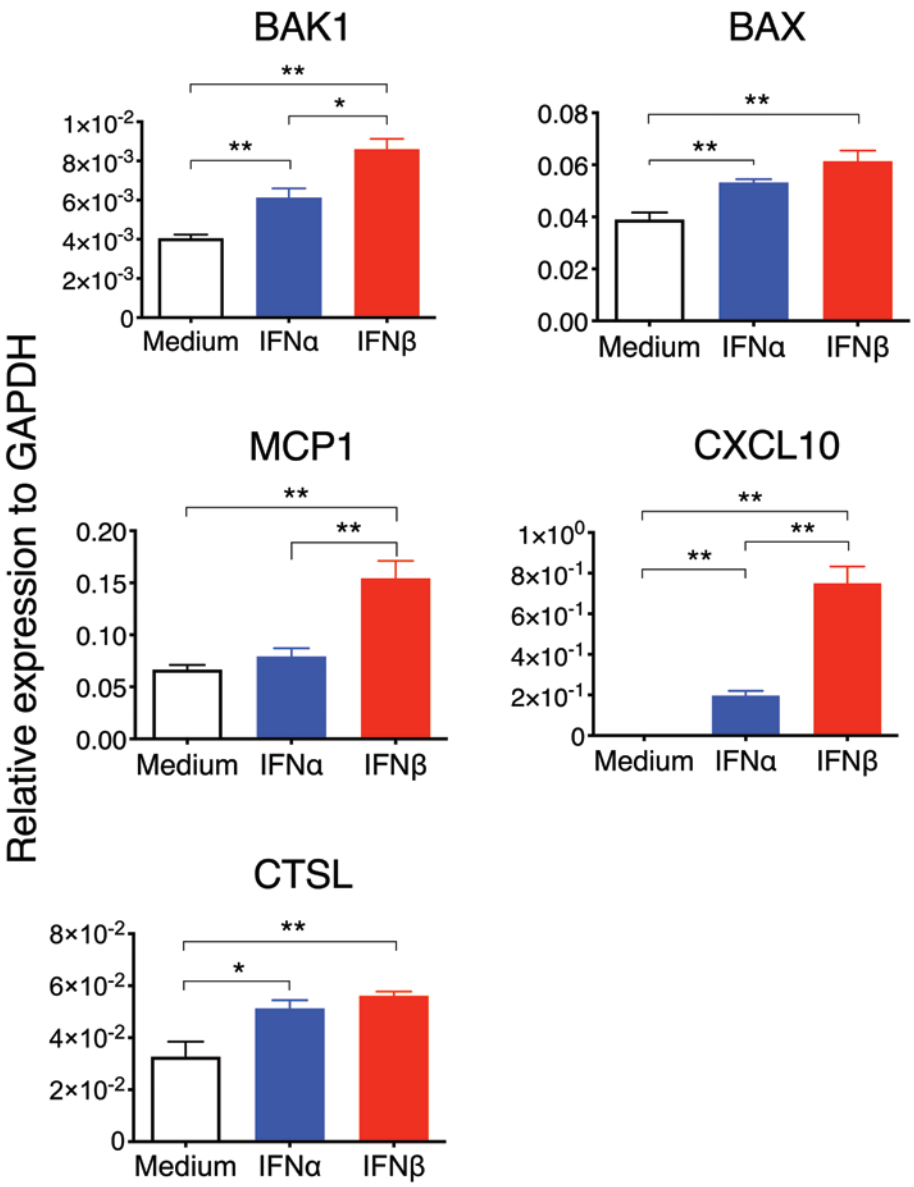

Figure 4. Type I IFN induces mRNA expressions of proapoptotic molecules, chemokines, and *CTSL* in human podocytes. Human podocyte cell lines were first cultured under growth restricted conditions (37˚C) for 7 days for differentiation as described in the Methods. Cells were then stimulated with either rhIFNα (5000 units/mL), rhIFNβ (5000 units/mL), or medium alone for 24 hours and reverse transcriptase qPCR was performed to measure mRNA expressions for proapoptotic molecules *BAK1* and *BAX*, chemokines *CXCL10* and *MCP1*, and *CTSL*. mRNA expression results are representative of 3 independent experiments. Data are expressed as means ± standard error of the mean (n = 3 experiments). $P < 0.05$ was considered significant. * $P < 0.05$ and ** $P < 0.01$. *CTSL*: cathepsin L; IFN: interferon; rh: recombinant human.

DISCUSSION

In the present study, the large cohort allowed us to analyze detailed SLE patient clinical data within a single ancestral background, reducing the complexity as different ancestral backgrounds have a different prevalence of both clinical manifestations and high type I IFN.[20] We show that high serum type I IFN activity is significantly associated with class III/IV LN but not with nonclass III/IV LN in European-American patients with SLE. We also demonstrate that the expression of type I IFN-inducible gene signature (ie, *IFIT1*) is significantly increased in class III/IV LN kidneys. While pDCs were observed in the tissue, they did not correspond spatially to the type I IFN signature in tissue. Type I IFN induced mRNA expression of chemokines and molecules related to apoptosis and cytoskeleton function in human podocytes. Together, these results suggest a pathogenic role for systemic high type I IFN in severe class III/IV LN in patients with SLE.

Our results are novel as we can separate the association of high serum type I IFN activity with active class III/IV LN in SLE as independent from anti-dsDNA antibody positivity or

complement levels in regression models. This is important, as anti-dsDNA antibodies and low complement C3 are associated with LN,[35,36,37] and are also associated with increased expression of type I IFN-inducible genes in PBMCs[21,22,23] and serum IFNα activity.[20] In multivariate logistic regression, we found that the association between serum type I IFN activity and class III/IV LN is primary, and not secondary because of the presence of positive anti-dsDNA antibodies or low complement C3. Thus, our results suggest the importance of high type I IFN activity over anti-dsDNA antibodies in the pathogenesis of class III/IV LN.

Similar to previous single-cell gene expression studies, we find increased type I IFN-inducible gene expression in the kidneys of patients with LN. Using mRNA ISH, we could spatially map the type I IFN signature in the tissue, providing the novel finding that the IFN signature was most prominent in glomerular areas of the kidney. *IFIT1* mRNA was universally expressed in the glomeruli of class III/IV LN: in the mesangium, endothelium, epithelium, and infiltrating inflammatory cells. Together with the association of serum type I IFN activity with the presence of class III/IV LN, this suggests that high systemic type I IFN in SLE plays a role in the pathogenesis of proliferative nephritis. Podocyte injury is a pathologic feature of class III/IV LN.[31,32] We found type I IFN induced expression of *MCP1*, *CXCL10*, and other molecules related to apoptosis and inflammation in human podocytes. Interestingly, IFNβ had a greater effect on some transcripts in the podocyte cell lines than did IFNα, and this could be important pathologically. The relative contributions of IFNα vs IFNβ in human SLE are not currently well understood, but may contribute to the observed heterogeneity in patient manifestations. Because all of the glomerular cell types demonstrate an IFN signature in our study, future studies are needed to investigate the direct effect of type I IFN on other resident glomerular cells (ie, mesangial cells, tubular, and endothelial cells in LN).

We investigated pDC infiltration in the biopsies because pDCs are a primary type I IFN-producing cell.[34] Interestingly, we did not find any significant differences in pDC infiltration in the kidneys among any of the LN classes. However, *IFIT1* expression in the glomeruli was significantly increased only in class III/IV LN but not in the other classes. There was no significant colocalization of pDCs with *IFIT1* expression in the renal tissue. Thus, while pDCs can produce massive amounts of type I IFN after TLRs are ligated by nucleic acid-containing immune complexes,[11] these data suggest that the initial source of the IFN signature in the kidney may not be the pDC. Other previous studies have also suggested alternate sources of the type I IFN signature in SLE.[38] pDCs can play a number of other roles in immunity and inflammation, including inflammatory cytokine and chemokine production as well as antigen presentation and cross-presentation to CD4+ T cells,[34] and it is possible that they could be functioning this way in LN.

Limitations of this study include the cross-sectional design, which could not assess the fluctuation of variables over time. While patients were receiving various immunosuppressive agents, previous studies have shown that most of the immunosuppressive agents (ie, hydroxychloroquine, azathioprine, and mycophenolate mofetil) have not reduced type I IFN-induced gene expression in PBMCs when compared to patients with SLE who were not receiving these agents.[22] Further negating concerns regarding the influence of treatment, we did not find any association between commonly used medications and circulating type I IFN except for the well-described reduction associated with corticosteroids.

In conclusion, we have shown that high serum type I IFN activity is associated with active class III/IV LN in European-American patients with SLE, which is independent of anti-dsDNA antibody status and complement levels. Second, we have shown that type I IFN-inducible gene expression is increased in class III/IV LN kidneys and not clearly related to pDC infiltration, and that type I IFN directly stimulates podocytes to induce chemokines and molecules related to podocyte injury. Taken together, these results suggest that systemic high type I IFN in SLE contributes to severe kidney involvement. While type I IFN receptor blockade has been shown to improve human SLE overall,[39] we await trials of anti-IFN agents focused specifically on LN.[40]

ACKNOWLEDGMENT

We thank Dr. Jeffrey B. Kopp (National Institutes of Health) for his generous gift of human immortalized podocyte cell lines.

ONLINE SUPPLEMENT

Supplementary material accompanies the online version of this article.

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