ARTHRITIS & RHEUMATOLOGY
Vol. 69, No. 9, September 2017, pp 1840–1849
DOI 10.1002/art.40166

Enhanced Inflammasome Activity in Systemic Lupus Erythematosus Is Mediated via Type I Interferon–Induced Up-Regulation of Interferon Regulatory Factor 1

Jianhua Liu, Celine C. Berthier, and J. Michelle Kahlenberg

Objective. **The inflammasome complex is a driver of organ damage in patients with systemic lupus erythematosus (SLE). Although type I interferons (IFNs) are well established as mediators of SLE pathogenesis, their role in inflammasome activation in SLE has not been assessed. The aim of this study was to examine type I IFNs as regulators of the inflammasome.**

Methods. **SLE patients fulfilled ≥4 American College of Rheumatology criteria and were recruited from the University of Michigan Lupus Cohort. Primary monocytes were isolated from SLE patients or healthy controls by negative selection, treated with inflammasome activators in the presence or absence of IFNα, and IL-1β secretion was measured by enzyme-linked immunosorbent assay. Expression levels of IFN and inflammasome-related molecules were assessed by real-time polymerase chain reaction and Western blotting. IFN regulatory factor 1 (IRF-1) expression was specifically down-regulated by small interfering RNA (siRNA) transfection and a chemical inhibitor.**

Results. **Monocytes from patients with SLE exhibited increased expression and enhanced activation of the inflammasome by ATP when compared with control monocytes. Expression of inflammasome and IFN-regulated genes was significantly correlated in monocytes from SLE patients but not in control monocytes. Inflammasome activity was increased after prolonged exposure to IFNα. Reduction of IRF-1 expression via siRNA blocked caspase 1 up-regulation after treatment with IFNα. Importantly, hyperactivity of the inflammasome in the monocytes of SLE patients was significantly reduced after knockdown or inhibition of IRF-1.**

Conclusion. **Prolonged type I IFN exposure, as seen in SLE patients, primes monocytes for robust inflammasome activation in an IRF-1–dependent manner. IRF-1 inhibition may serve as a novel target for treatment of SLE-associated inflammation and organ damage.**

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by formation of autoantibodies, deposition of immune complexes, and pleotropic end-organ damage. Dysregulation of both the innate and adaptive immune responses is central to the development of SLE, and recent evidence from human and murine studies identifies the innate signaling complex termed the inflammasome as being dysregulated in SLE and a central contributor to the development of lupus nephritis and the associated organ damage (1–10).

Inflammasomes are multiprotein complexes that use a central scaffold and adaptor molecules to recruit and activate caspase 1. Active caspase 1 then cleaves the proinflammatory cytokines interleukin-1β (IL-1β) and IL-18 to their active forms. The best-characterized inflammasome is one that contains the nucleotide-binding oligomerization domain NLRP3, the adaptor molecule ASC protein that contains a caspase recruitment domain, and caspase 1. This inflammasome complex is hyperactivated in the macrophages of lupus patients (2), is activated by lupus-specific autoantibodies

Supported by the Mary Catherine Piazza Lupus Research Fund and the NIH (National Institute of Arthritis and Musculoskeletal and Skin Diseases [NIAMS] grant R03-AR-066337). Mrs. Liu's and Dr. Kahlenberg's work was supported by a grant to Dr. Kahlenberg from the Arthritis National Research Foundation. Dr. Kahlenberg's work was partially supported by the NIH (NIAMS grant K08-AR-063668).

Jianhua Liu, BS, MCS, Celine C. Berthier, PhD, J. Michelle Kahlenberg, MD, PhD: University of Michigan, Ann Arbor.

Dr. Kahlenberg has received consulting fees from Idera Pharmaceuticals (less than $10,000).

Address correspondence to J. Michelle Kahlenberg, MD, PhD, 5570A MSRB II, 1150 West Medical Center Drive, Ann Arbor, MI 48109. E-mail: mkahlenb@med.umich.edu.

Submitted for publication October 26, 2016; accepted in revised form May 25, 2017.

(1,6,7), and contributes to the development of nephritis (11,12).

Despite the plethora of research showing the importance of the inflammasome in SLE, the mechanisms that regulate inflammasome activation in this disease have not been well characterized. Experimental evidence collected in the past decade has identified type I interferons (IFNs), particularly IFNα, as being important contributors to the pathogenesis of SLE in humans and murine models (13–18). Many patients with SLE have elevated levels of IFN-induced gene expression, or the "IFN signature," which correlate with the presence of autoantibodies, nephritis, and disease activity (18). The effects of type I IFNs on inflammasome activity are unclear. Previous studies have identified type I IFN signaling as a negative regulator of inflammasome gene expression and activity in peripheral blood mononuclear cells (PBMCs) and bone marrow–derived macrophages (19,20). However, other studies have identified correlations between increased IFN-induced gene expression and activation of the inflammasome in SLE (4,21).

Monocytes are sentinels of the IFN signature in autoimmune diseases (22) and are also an important source of inflammasome activation. Thus, in this study we chose to clarify the effects of IFNα on inflammasome function in both control monocytes and those from lupus patients in order to better understand how an environment with persistently activated type I IFNs, as seen in SLE patients, changes inflammasome activity. We observed increased inflammasome activity in monocytes from SLE patients and identified the chronicity of IFNα exposure as a dichotomous regulator of this activity. Importantly, we demonstrated that IFNα-induced up-regulation of IFN regulatory factor 1 (IRF-1) is required for up-regulation of caspase 1 and enhanced IL-1β production by monocytes from patients with SLE. Our results identify an important intersection between type I IFNs and the inflammasome and may suggest an additional mechanism by which blockade of type I IFN signaling may result in improvement in SLE activity (23).

PATIENTS AND METHODS

Reagents and antibodies. Ficoll-Paque Plus and fetal bovine serum (FBS) were obtained from GE Healthcare. Lipopolysaccharide (LPS) was purchased from Sigma-Aldrich, RPMI 1640 was purchased from Lonza, recombinant IFNα was obtained from Schering, and 4,5,6,7-tetrabromobenzotriazole (TBB) was purchased from Tocris. Anti-human IRF-1 (D5E4), caspase 1 (D7F10), pSTAT-1 (Tyr^{701}) (D4A7), pSTAT-2 (Tyr^{690}) (D3P2P), STAT-1 (D1K9Y), STAT-2, and β-actin were purchased from Cell Signaling Technology. Horseradish peroxidase (HRP)–conjugated goat anti-rabbit IgG was obtained from Abcam.

Table 1. Characteristics of the 23 patients with SLE*

Age, mean ± SEM years	44.9 ± 2.3
Sex	
Female	91
Male	9
Medication	
Oral prednisone ≤10 mg/day	35
Oral prednisone >10 mg/day	0
Mycophenolate mofetil	30
Belimumab	9
Antimalarial agent	87
Methotrexate	0
Antibody status	
ANA positive, ever	78
Anti-DNA positive, at time of sampling	52
Titer, mean (range) IU/ml	63.6 (0–337)
SLEDAI score at time of sampling, mean (range)	2.7 (0–9)

* Among the 48 control subjects, the mean ± SEM age was 41.5 ± 1.5 years, 90% were female, and 10% were male. Except where indicated otherwise, values are the percent. ANA = antinuclear antibody; SLEDAI = Systemic Lupus Erythematosus Disease Activity Index.

Study subjects. All prospectively recruited patients and control subjects provided written, informed consent and were treated according to the Declaration of Helsinki. The study protocol was approved by the Institutional Review Board of the University of Michigan Medical School. The patients with SLE fulfilled ≥4 American College of Rheumatology criteria (24) and were recruited from the University of Michigan Lupus Cohort. We recruited 23 patients in whom SLE was diagnosed and treated at the University of Michigan Hospital clinic and 23 sex-, age-, and race-matched healthy control subjects for studies that compared monocytes from patients with SLE with control monocytes. An additional 25 control subjects were recruited for studies on control cells alone. Table 1 shows the characteristics of the patients. For the microarray study, 90 cases of discoid lupus erythematosus (DLE) and subacute cutaneous lupus erythematosus (SCLE) were identified via a Systematized Nomenclature of Medicine search of the University of Michigan Pathology Database using the search terms "lupus" and "cutaneous lupus." Patients who met both clinical and histologic criteria for DLE or SCLE were included in the study.

Human peripheral monocyte preparation. Heparinized blood (50 ml) was obtained from patients with SLE and control subjects. The buffy coat was obtained after density-gradient centrifugation on 15 ml Ficoll-Paque Plus (5:3 blood:Ficoll ratio). Red blood cells were removed via hypotonic lysis. CD14+CD16– monocytes were isolated using negative selection using an EasySep Human Monocyte Enrichment Kit (StemCell Technologies). Monocyte population purity was assessed by flow cytometry and judged to be 87% after negative selection.

Monocyte culture and treatment. Monocytes from control subjects and patients with SLE were plated onto 48-well or 96-well tissue culture plates at a density of 0.5×10^6 cells/well in 0.5 ml RPMI 1640 plus 10% FBS or 0.2×10^6 cells/well in 0.2 ml RPMI 1640 plus 10% FBS, respectively. Monocytes were cultured overnight in the presence or absence

of 1,000 units/ml human IFNα. In some cases, 50 μ*M* TBB was added 1 hour prior to IFN treatment. The next morning, the cells were stimulated with or without 100 ng/ml LPS or vehicle for 4 hours. This was followed by treatment with 5 m*M* ATP to stimulate inflammasome activation. In some experiments, IFNα was also used concurrently with LPS. To assess the effects of serum from SLE patients and serum from control subjects on inflammasome activity, monocytes were cultured in 50% serum overnight in the presence or absence of 50 μ*M* TBB prior to inflammasome activation with LPS and ATP.

Enzyme-linked immunosorbent assay (ELISA) and Western blotting. The supernatants from cultured monocytes were assayed for IL-1β using IL-1β human ELISA Ready-SET-Go kits (eBioscience) according to the instructions of the manufacturer.

For Western blot analysis, monocyte cultures were directly lysed in 2× protein sample buffer after supernatant removal. Protein from each sample (0.8×10^5 cells) was separated on 10% or 12% acrylamide gel and then transferred to Amersham Protran 0.2 μm NC nitrocellulose membranes (GE Healthcare). The membranes were blocked with 5% nonfat dry milk and incubated overnight at 4°C with primary antibodies (1:1,000 dilution) followed by HRP-conjugated anti-rabbit IgG. Protein expression bands were detected by chemiluminescence using WesternBright Quantum Western blot detection reagent (Advansta), and the protein bands were imaged using an Omega Lum C System (Gel Company). Expression quantification was completed using ImageJ software.

RNA isolation and real-time quantitative polymerase chain reaction (qPCR). Total RNA was isolated from human monocytes using a Direct-zol RNA MiniPrep kit (Zymo Research Corporation) according to the instructions of the manufacturer. One microgram of total RNA was transcribed into complementary DNA (cDNA) using Moloney murine leukemia virus reverse transcriptase and oligo(dT) primer (Invitrogen). Real-time qPCR was performed in triplicate using SYBR Green PCR Master Mix (Applied Biosystems) with the assistance of the University of Michigan DNA Sequencing Core. The primer sequences were as follows: *CASP1*, forward 5′-GGACTCTCAGCAG-CTCCTCAGGCA-3′, reverse 5′-GCAAAGCTTGACATTCCC-TTCTGAGCC-3′; *IRF1*, forward 5′-CTGTGCGAGTGTA-CCGGATG-3′, reverse 5′-ATCCCCACATGACTTCCTCTT-3′; *NLRP1*, forward 5′-GAGTTGGGAAAACAACCTTGGT-3′, reverse 5′-ACTTAAACCTCTGCTGGTAGAGA-3′; *NLRP3*, forward 5′-GATCTTCGCTGCGATCAACAG-3′, reverse 5′-CGTGCATTATCTGAACCCCAC-3′; *AIM2*, forward 5′-TG-GGCATGCTCTCCTGAGTCCT-3′, reverse 5′-TCAGCCTC-CTGATCCCTGGGGC-3′; *PRKR*, forward 5′-CTTCCATC-TGACTCAGGTTT-3′, reverse 5′-TGCTTCTGACGGTATGT-ATTA-3′; *MX1*, forward, 5′-TACCAGGACTACGAGATTG-3′, reverse 5′-TGCCAGGAAGGTCTATTAG-3′; *ASC*, forward 5′-CCTACGGCGCCGAGCTCAC-3′, reverse 5′-CTCCAGAGC-CCTGGTGCGT-3′; *IFI44*, forward 5′-GGTGGGCACTAATA-CAACTGG-3′, reverse 5′-CACACAGAATAAACGGCAGG-TA-3′; *IL1b*, forward 5′-ATGTCTGGAACTTTGGCCATC-TT-3′, reverse 5′-AGACAATTACAAAAGGCGAAGAAGA-CT-3′; *IL18*, forward 5′-CCCTTTGCTCCCCTGGCGAC-3′, reverse 5′-AGACTGCAGCAGGTGGCAGC-3′; *IL6*, forward 5′-ACTCACCTCTTCAGAACGAATTG-3′, reverse 5′-CCA-TCTTTGGAAGGTTCAGGTTG-3′; *TNF*, forward 5′-TCCTT-CAGACACCCTCAACC-3′, reverse 5′-AGGCCCCAGTTTG-AATTCTT-3′; β-actin, forward 5′-CATCACGATGCCAGTG-GTACG-3′, reverse 5′-AACCGCGAGAAGATGACCCAG-3′.

Gene expression was normalized to that of β-actin, relative gene expression to normal controls was calculated by the comparative threshold cycle (C_t) method, and fold change was expressed as $2^{-\Delta\Delta C_t}$ (25). IFN scores were calculated as previously described (26,27) for *IFI44*, *MXA*, and *PRKR*. Briefly, the mean fold change in samples from healthy control subjects was calculated, and the SLE IFN gene score was calculated as (SLE [gene] fold change – control [mean] fold change)/SD control. The 3 gene scores were then summed to provide a total SLE IFN gene score. Control scores were calculated similarly.

Small interfering RNA (siRNA) and transfection. Small interfering RNA targeting human IRF-1 or AllStars Negative Control siRNA (scramble) was purchased from Qiagen. The siRNA target sequence for IRF-1 is CTGGCTAGAGATG-CAGATTAA. Electroporation of human IRF-1 siRNA or scramble siRNA into monocytes was performed with a 4D-Nucleofector System (Lonza) using a P3 Primary Cell 4D-Nucleofector X Kit S (Lonza). Briefly, 1×10^6 monocytes were resuspended in 20 μl of P3 Primary Cell 4D-Nucleofector X Solution containing 250 n*M* human IRF-1 siRNA or scramble siRNA, transferred into Nucleocuvette Vessels, and electroporation was performed using a standard program optimized for human monocytes. Immediately after electroporation, 0.5×10^6 cells from each sample were diluted into 500 μl RPMI 1640 with 5% FBS, plated onto a 48-well plate, and cultured overnight. The cells were then treated with IFNα, LPS, and ATP, as described above.

RNA isolation and microarray procedures. Formalin-fixed paraffin-embedded blocks of skin biopsy specimens were obtained, and 5 sections (10 μm) were cut with a microtome. RNA was extracted using an E.N.Z.A. FFPE RNA Kit (Omega Bio-Tek) according to the instructions of the manufacturer. Complementary DNA was prepared, according to the NuGEN Ovation PicoSL WTA System V2 Manual, P/N M01226 v4, from ~30 ng total RNA. Biotinylated cDNA (2.5 μg) was prepared using a NuGEN Encore Biotin Module (Encore Biotin Module Manual, P/N M01111 v6). A Poly-A RNA Control Kit was used according to the routine procedure at the University of Michigan Micro Array Core Lab. Labeled cDNA was hybridized at 48°C to Affymetrix Human Gene ST 2.1 array plates, which were then washed, stained, and scanned using an Affymetrix GeneTitan system (software version 3.2.4.1515) with the assistance of personnel at the University of Michigan DNA Sequencing Core. Quality control and Robust Multi-array Average (28) normalization of CEL files were performed in R version 3.1.3, using custom CDF version 19 and modified Affymetrix_1.44.1 packages from BrainArray (http://brainarray.mbni.med.umich.edu/brainarray/default.asp). Log_2-transformed expression values were batch-corrected using ComBat implemented in GenePattern (http://www.broadinstitute.org/cancer/software/genepattern/). Baseline expression was defined as minimum plus 1SD of the median of all genes. A variance filter with a cutoff value of 80% was then applied. Of the 25,582 unique genes represented on the Human ST2.1 chip, a total of 20,410 genes passed the defined criteria. The normalized data file was uploaded to the Gene Expression Omnibus web site (http://www.ncbi.nlm.nih.gov/geo/) under accession no. GSE81071.

Statistical analysis. Data are presented as the mean ± SEM. Student's unpaired 2-tailed *t*-test was used for analysis of differences between simultaneously cultured

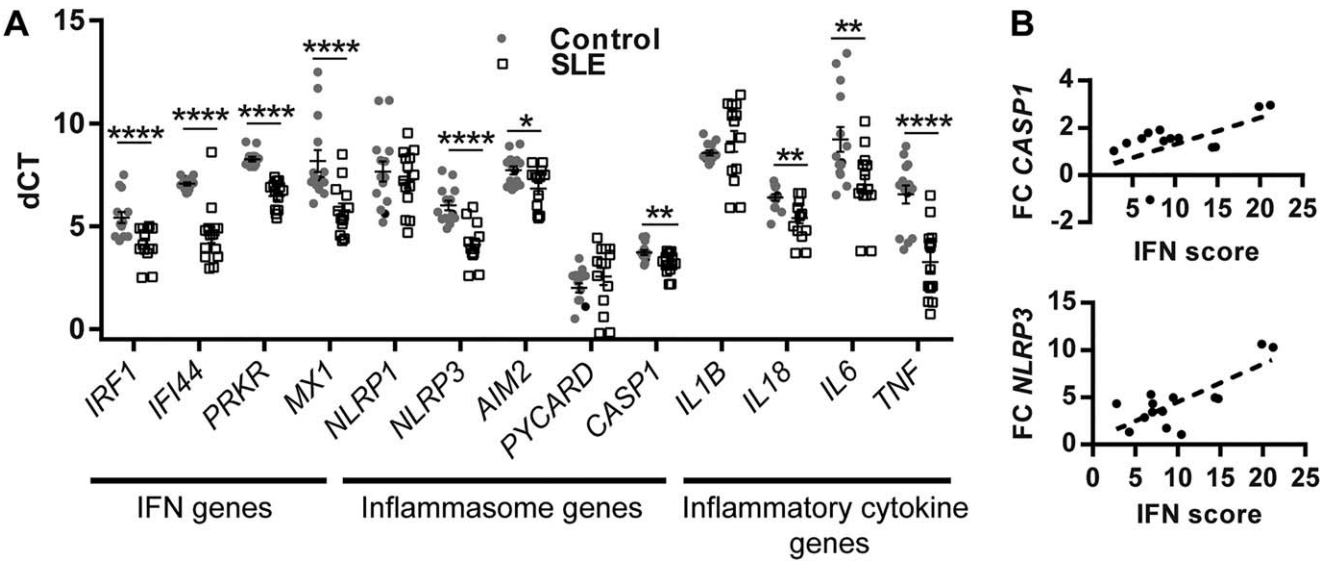

Figure 1. Inflammasome expression is increased in the monocytes of patients with systemic lupus erythematosus (SLE) and correlates with type I interferon (IFN) gene expression. Total RNA was collected from the freshly isolated monocytes of SLE patients (n = 14) and control subjects (n = 14). **A,** Levels of mRNA for type I IFN, inflammasome, and inflammatory cytokine genes, as measured by real-time quantitative polymerase chain reaction, are expressed as the threshold cycle (ΔC_t) value versus β-actin. Each symbol represents an individual sample. Horizontal lines with error bars show the mean ± SEM of triplicate determinations for each tested gene. **B,** Fold change (FC) in expression of the indicated genes versus the IFN score in each patient. $* = P < 0.05$; $** = P < 0.01$; $**** = P < 0.0001$.

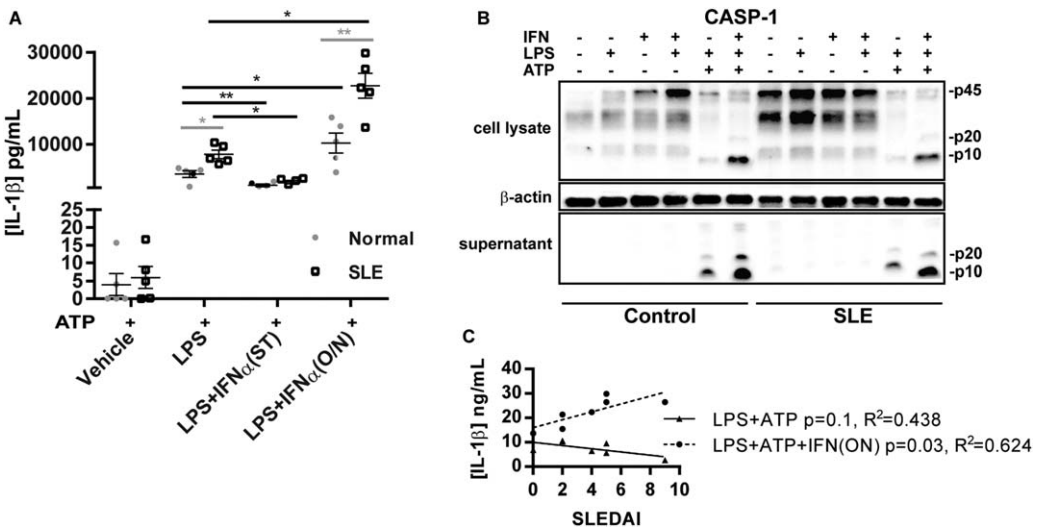

Figure 2. Monocytes from patients with SLE demonstrate increased inflammasome activation that is enhanced by pre-exposure to type I IFNs. **A,** Release of interleukin-1β (IL-1β) by control monocytes and SLE patient monocytes under different treatment conditions. Monocytes from 5 SLE patients and 5 control subjects were treated with or without IFNα either 1 night before (O/N) or simultaneously (ST) with lipopolysaccharide (LPS) treatment. ATP (5 m*M*) was added 2 hours before harvesting to activate the NLRP3 inflammasome and release mature IL-1β. Supernatants were collected, and IL-1β production was measured by enzyme-linked immunosorbent assay. Shaded bars represent comparisons between control monocytes and SLE patient monocytes; differences were determined by Student's unpaired *t*-test. Solid bars represent comparisons between control monocytes and SLE patient monocytes treated with or without IFN; differences were determined by Student's paired *t*-test. Each symbol represents a single sample. Horizontal lines with error bars show the mean ± SEM. $* = P < 0.05$; $** = P < 0.01$. **B,** Representative Western blot for caspase 1 (Casp-1) expression in cell lysate and supernatant (extracellular media) from the samples in **A**. **C,** Correlation between SLE Disease Activity Index (SLEDAI) scores and IL-1β production, as determined by linear regression analysis, in SLE patient monocytes treated in duplicate with or without IFNα overnight prior to inflammasome activation. See Figure 1 for other definitions.

monocytes from the control and SLE groups. For studies examining the effects on samples with multiple treatment conditions, Student's paired 2-tailed *t*-test was used to analyze differences between the untreated and treated groups. Linear regression analysis was used for comparison of the monocyte IFN score with inflammasome and inflammatory genes and for comparison of *IRF1* with *CASP1* from microarray data. Both data sets were normally distributed. *P* values less than 0.05 were considered significant.

RESULTS

Increased inflammasome gene expression and IFN signature in monocytes from SLE patients. In order to develop an understanding of the relationship between the inflammasome and type I IFN exposure, we first examined inflammasome- and IFN-regulated gene expression in monocytes from SLE patients and those from normal control subjects. Real-time qPCR on freshly isolated monocytes demonstrated that levels of messenger RNA (mRNA) for inflammasome genes such as *NLRP3, AIM2*, and *CASP1* were elevated in monocytes from lupus patients compared with those from healthy control subjects (Figure 1A). Levels of mRNA for genes downstream of type I IFN signaling (*IRF1, PRKR, IFI44*, and *MX1*) as well as the proinflammatory cytokines *IL6* and *TNF* were also significantly higher in monocytes from SLE patients (Figure 1A).

Linear regression analysis was performed to evaluate expression of IFN-regulated genes, expressed as an IFN score (18), compared with inflammasome genes. A strong positive correlation of *CASP1* and *NLRP3* with the IFN score in SLE patients, but not in control subjects, was noted (Figure 1B). In contrast to inflammasome genes, the proinflammatory gene *TNFA* was not significantly correlated with the IFN-regulated genes in monocytes from SLE patients. This suggests that the IFN–inflammasome correlation is not a generalized inflammatory effect. These data suggest the hypothesis that long-term exposure to type I IFNs may result in up-regulation of inflammasome components and facilitate inflammasome activation.

Prolonged, but not short-term, exposure to IFNα enhances inflammasome activity in monocytes from SLE patients and control subjects. To determine whether IFNα promotes inflammasome activation in monocytes, monocytes from SLE patients and controls were primed with LPS in the presence or absence of simultaneous addition of IFNα followed by activation of the NLRP3 inflammasome with ATP (29,30). We observed that at baseline, monocytes from SLE patients had significantly enhanced IL-1β production when compared with control monocytes (Figure 2A). Consistent with previous macrophage studies (20), inhibition of IL-1β release was detected when cells were treated simultaneously with IFNα (Figure 2A). However, because it is known that SLE patients are exposed to extended IFNα

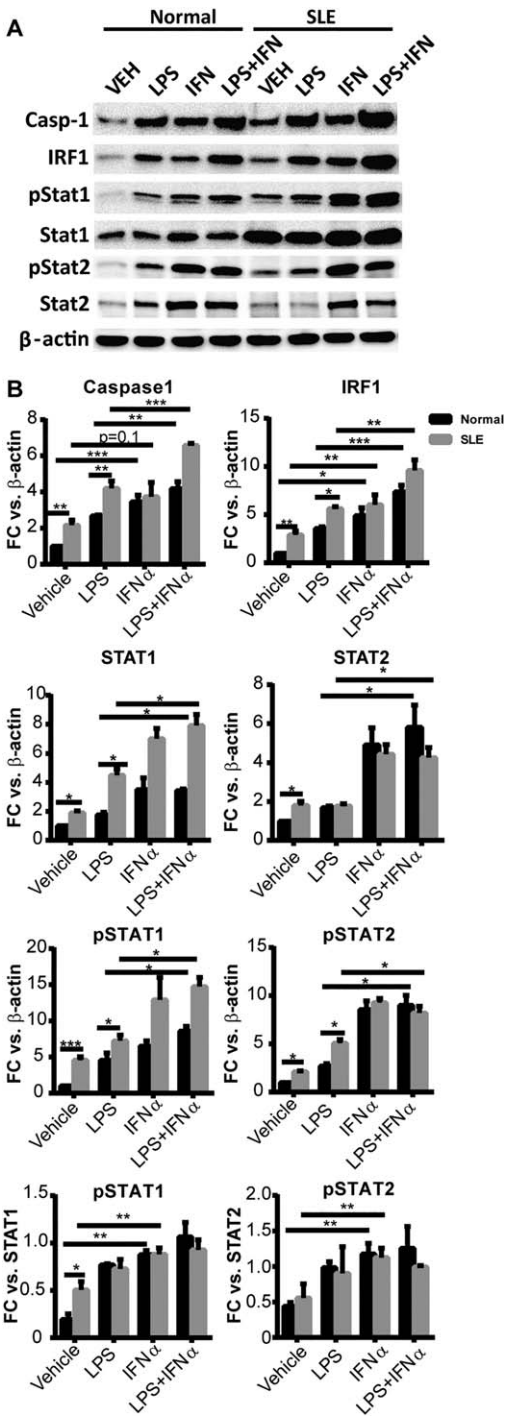

Figure 3. Type I IFN enhances caspase 1 (Casp-1; 45 pro form) expression. **A,** Representative Western blot for expression of antibodies in lipopolysaccharide (LPS)– and/or IFNα-treated monocytes. Cell lysates were collected, separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and blotted against the indicated antibodies. **B,** Protein band density of caspase 1, IFN regulatory factor 1 (IRF-1), STAT-1, pSTAT-1, STAT-2, and pSTAT-2, as digitally quantified by ImageJ. Values are the mean ± SEM fold change versus vehicle (veh)–treated control monocytes normalized to β-actin or STAT-1/STAT-2, as indicated (n = 5 control samples and 5 SLE patient samples). $* = P < 0.05$; $** = P < 0.01$; $*** = P < 0.001$, by Student's paired *t*-test. See Figure 1 for other definitions.

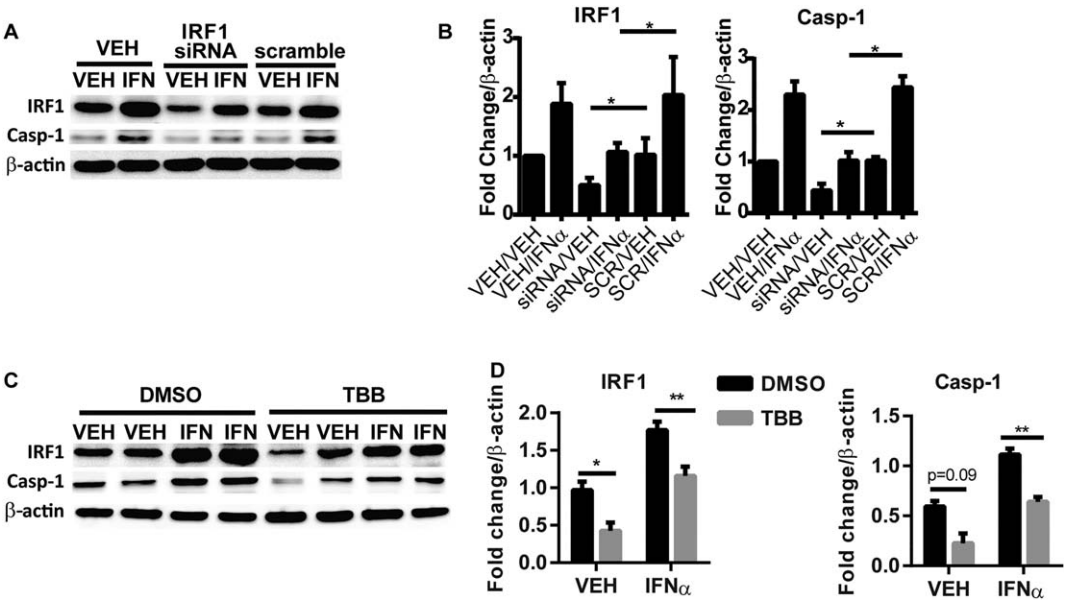

Figure 4. Inhibition of interferon (IFN) regulatory factor 1 expression prevents IFNα-induced up-regulation of caspase 1. **A,** Western blot for caspase 1 and IRF-1 expression in control monocytes transfected with human IRF-1 small interfering RNA (siRNA) or scramble (SCR) by electroporation and then treated with vehicle or IFNα for 6 hours. **B,** Densitometric analysis of the caspase 1 and IRF-1 protein expression shown in **A**. **C,** Western blot for caspase 1 and IRF-1 expression in control monocytes incubated with 50 μM 4,5,6,7-tetrabromobenzotriazole (TBB), an inhibitor of IRF-1 regulator casein kinase II, prior to 6-hour stimulation with IFNα. **D**, Densitometric analysis of the caspase 1 and IRF-1 expression shown in **C**. Values are the mean ± SEM fold change versus vehicle-treated control (n = 6 experiments in **A** and **B**; n = 4 experiments in **C** and **D**). $* = P < 0.05$; $** = P < 0.01$. See Figure 3 for other definitions.

signaling, we examined whether there were differential effects of long-term IFNα exposure on inflammasome activation. Thus, monocytes from SLE patients and control subjects were treated with or without IFNα overnight prior to LPS priming and inflammasome activation with ATP treatment.

As shown in Figure 2A, release of IL-1β was significantly enhanced by overnight exposure to IFNα in control monocytes and to a greater extent in the monocytes of SLE patients. This effect was seen at IFNα doses as low as 100 IU, which is estimated to be equivalent to IFN concentrations in SLE plasma (31). Interestingly, IL-1β production correlated with the SLE Disease Activity Index score (32) following long-term IFN exposure but not at baseline (Figure 2C). These results suggested that prolonged exposure to type I IFNs, prior to inflammasome priming with LPS, promotes inflammasome activity, and that patients with more active disease may be more susceptible to the effect of type I IFNs.

In order to examine the effects of prolonged type I IFN exposure on expression of IFN-regulated and inflammasome proteins, Western blot analysis of control monocytes and monocytes from SLE patients was performed. Evidence of long-term type I IFN activation of monocytes from SLE patients at baseline was observed as reflected by increased pSTAT-1 and IRF-1 expression (Figure 3A). Importantly, expression of the p45 pro form of caspase 1 was also increased in monocytes from SLE patients compared with control monocytes at baseline (Figure 3B). Overnight exposure to IFNα alone significantly up-regulated phosphorylation of STAT-1 and STAT-2 and up-regulated IRF-1 and caspase 1 expression in control monocytes. IFNα induced moderate, but not significant, increases in caspase 1 expression over baseline levels in monocytes from SLE patients. The addition of LPS after overnight exposure to IFNα resulted in even greater expression of caspase 1 and IRF-1 in monocytes from SLE patients and control subjects (Figure 3B), implying a synergistic effect of Toll-like receptor (TLR) and IFN signaling in caspase 1 expression. Taken together, these data suggested that exposure to type I IFNs prior to priming and activation of the inflammasome results in enhanced caspase 1 expression and increased inflammasome activity, whereas if TLR-4 and IFN signals are received simultaneously, inflammasome activity is inhibited.

Knockdown of IRF-1 effectively attenuates IFNα-mediated caspase 1 expression and inflammasome hyperactivation in monocytes from SLE patients. IRF-1 is a transcription factor that is activated by type I IFN signaling and is important for the development (33)

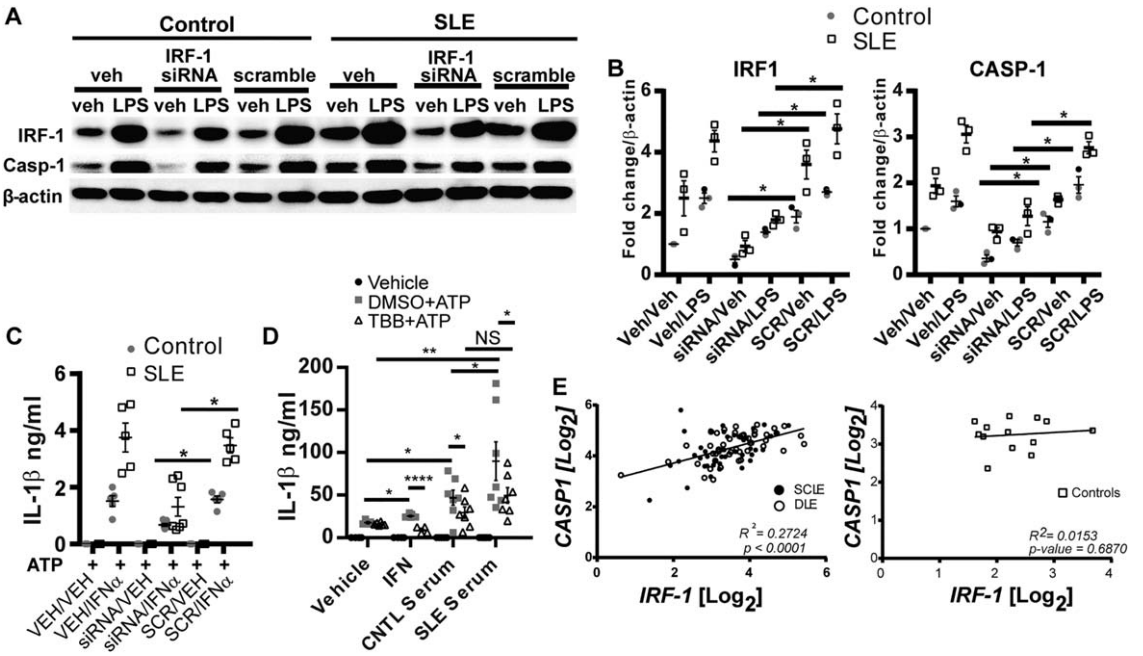

Figure 5. Interferon (IFN) regulatory factor 1 regulates caspase 1 (p45 pro form) expression and interleukin-1β (IL-1β) production in the monocytes of patients with systemic lupus erythematosus (SLE). **A,** Western blot for expression of the indicated proteins in cell lysates. Monocytes from lupus patients and control (CNTL) subjects were transfected with human IRF-1 small interfering RNA (siRNA) or scramble (SCR) and treated with vehicle or LPS for 4 hours. **B,** Densitometric measurement of data shown in **A**. Values are the fold change versus β-actin (n = 3). Each symbol represents an individual sample. Horizontal lines with error bars show the mean ± SEM. **C,** IL-1β production in monocytes treated as described in **A**, as measured by enzyme-linked immunosorbent assay (ELISA). Treatment was followed by stimulation of the inflammasome with 5 m*M* ATP. Each symbol represents the average of duplicate experiments in 5 SLE patients and 5 control subjects. Horizontal lines with error bars show the mean ± SEM. **D,** IL-1β production in monocytes, as measured by ELISA. Monocytes were stimulated overnight with IFNα or 50% control or SLE patient serum in the presence or absence of DMSO (vehicle) or 50 μ*M* 4,5,6,7-tetrabromobenzotriazole (TBB) followed by LPS priming and inflammasome activation with 5 m*M* ATP. Each symbol represents the average of duplicate experiments in 7 control serum samples and 7 SLE patient samples. Horizontal lines with error bars show the mean ± SEM. **E,** Linear regression analysis showing the correlation between *CASP1* and *IRF1* $\log_2$-transformed mRNA expression values (determined using microarray data) in biopsy specimens obtained from the lesional skin of patients with discoid lupus erythematosus (DLE) and patients with subacute cutaneous lupus erythematosus (SCLE) (left), and from control skin (right). $* = P < 0.05$; $** = P < 0.01$; $**** = P < 0.0001$ by Student's paired *t*-test. NS = not significant (see Figure 3 for other definitions).

and prediction (34) of a treatment response in lupus nephritis (34). Importantly, IRF-1 has been identified as a central regulator of target genes in monocytes from lupus patients (35) and has been reported as a transcription factor of caspase 1 (36,37). Our data support parallel regulation of IRF-1 and caspase 1 downstream of IFNα. To determine whether an IFNα-mediated increase in caspase 1 expression required IRF-1, we knocked down the expression of IRF-1 by targeting human IRF-1 in control monocytes with siRNA followed by treatment with or without IFNα. As shown in Figures 4A and B, we were able to achieve a 50% reduction in IRF-1 expression when this method was used. Importantly, knockdown of IRF-1 significantly inhibited basal and IFNα-induced expression of caspase 1 (Figures 4A and B). Inhibition of IRF-1 transcriptional activity with TBB, an inhibitor of casein kinase II, an upstream positive regulator of IRF-1 (38), also gave similar results (Figures 4C and D).

In order to determine whether inhibition of IRF-1 was sufficient to normalize caspase 1 overexpression and inflammasome hyperactivation in the monocytes of SLE patients, which have prolonged exposure to type I IFNs, monocytes from SLE patients and control monocytes were then transfected with scramble or human IRF-1 siRNA followed by priming of the inflammasome with LPS and activation with ATP. A reduction in IRF-1 expression significantly down-regulated baseline and LPS-induced caspase 1 expression in monocytes from patients with SLE and control monocytes (Figures 5A and B). Importantly, IRF-1 inhibition normalized IL-1β activation and secretion following LPS priming and ATP-mediated inflammasome activation in monocytes from SLE patients to levels similar to those in control monocytes and had further inhibitory effects on IL-1β production in control cells (Figure 5C). Inhibition of IRF-1 with TBB also inhibited IFNα-mediated

enhancement of IL-1β release after inflammasome activation (Figure 5D). These data suggest that type I IFN–mediated enhanced IRF-1 activity in monocytes from patients with SLE is driving amplified inflammasome activity.

To investigate whether IFN activity in the serum of SLE patients can enhance inflammasome activation in control monocytes, we incubated control monocytes with IFNα or serum from controls or SLE patients overnight prior to inflammasome activation with LPS and ATP. We detected a robust increase in IL-1β release from monocytes that were preincubated overnight with control serum prior to inflammasome stimulation, and this effect was amplified with the use of serum from SLE patients (Figure 5D). Importantly, we were able to significantly diminish the enhanced effect of serum from SLE patients on inflammasome activation via IRF-1 inhibition with TBB, returning IL-1β release to levels obtained with control serum. Taken together, these data suggest that IFNs present in the serum of lupus patients can enhance inflammasome activation in an IRF-1–dependent manner.

Cutaneous lupus lesions are marked by strong type I IFN signatures (39,40) and monocyte infiltration (41,42). In order to confirm the relevance of caspase 1 and IRF-1 dysregulation in an SLE disease phenotype, expression of both genes was assessed in lesional skin biopsy specimens obtained from 47 patients with DLE and 43 patients with SCLE and biopsy specimens obtained from 13 normal control subjects. As shown in Figure 5E, there was a strongly significant correlation between *IRF1* and *CASP1* expression in both DLE and SCLE. This correlation was also observed when each disease subtype was considered separately: for *CASP1* versus *IRF1* in DLE, $R^2 = 0.3315$ and $P \leq 0.0001$; for *CASP1* versus *IRF1* in SCLE, $R^2 = 0.2129$ and $P = 0.002$. No correlation between *IRF1* and *CASP1* expression was noted in control skin. Taken together, these data suggest that chronic type I IFN exposure drives enhanced inflammasome activity in SLE in an IRF-1–dependent manner.

DISCUSSION

Data from recent studies have supported the role of the inflammasome in contributing to the pathogenesis of SLE (1,4–7,9,10,43). In the current study, we provide mechanistic data on the intersection of inflammasome activation with another SLE-relevant innate immune pathway, long-term type I IFN exposure. Indeed, we demonstrate that prolonged exposure to IFNα promotes inflammasome activation in the monocytes of SLE patients in an IRF-1–dependent manner.

The mechanisms by which the inflammasome impacts SLE pathogenesis are being defined. Up-regulation of inflammasome gene expression and inflammasome activity has been identified in both human and murine lupus (1,2,4,6,7,44). Inhibition of caspase 1– or NLRP3-mediated inflammasome signaling in murine models demonstrated that inflammasome pathways contribute to disease pathogenesis via promotion of autoantibody production, endothelial dysfunction, and nephritis (5,9,10,12). Activation of the inflammasome occurs downstream of SLE-specific factors, including autoantibodies and dysregulated neutrophil extracellular traps (1,2,6,7), which may implicate the inflammasome as a regulatory switch between autoimmunity and inflammation-mediated organ damage.

Links between type I IFN and the inflammasome in SLE have been less studied. Bisphenol A, a chemical that up-regulates both type I IFN production and inflammasome activity in monocytes (21), may be linked with autoimmunity, but the question of whether the 2 pathways intersected was not examined. A study by Yang et al examining human PBMC populations demonstrated decreased expression of NLRP3 in samples from SLE patients versus control samples (19). However, our study, which utilized purified monocyte populations, clearly shows increased expression of NLRP3 and NLRP3-mediated inflammasome activity in monocytes from SLE patients compared with control monocytes. It is possible that analysis of a mixed cell population diluted the expression changes in monocytes from SLE patients in the study by Yang and colleagues. Another study suggested that IFNβ is repressive to inflammasome activation through production of IL-10 in bone marrow–derived murine macrophages (20). Consistent with that study, we demonstrated that short-term exposure to IFNα represses inflammasome activation, but that prolonged exposure, as seen in SLE, promotes inflammasome activation. This concept is supported by the strong correlation between NLRP3 and type I IFN–regulated genes in monocytes from patients with SLE but not in control monocytes and in cutaneous lupus lesions.

Surprisingly, we did not observe a correlation between *AIM2*, an inflammasome scaffold that is activated by double-stranded DNA and known to be up-regulated by type I IFNs (45), and monocyte IFN scores. This may reflect increased levels of circulating BAFF in SLE patients, which is known to repress absent in melanoma (Aim2) expression (46). The role of Aim2 in SLE

is multifaceted, and Aim2 may have inhibitory and promoting effects on autoimmunity (for review, see ref. 47). No correlation between another inflammasome scaffold, NLRP1, and IFN scores was noted. NLRP1 has genetic links to SLE (48), and transient type I IFN exposure has been reported to down-regulate NLRP1 inflammasomes (20); however, the role of NLRP1 in SLE remains undefined.

Our data show that inhibition of IRF-1 in the monocytes of SLE patients normalizes inflammasome hyperactivity. IRF-1 is increasingly becoming a target of investigation in SLE pathogenesis. Deletion of IRF-1 is protective in the MRL/*lpr* model of lupus (49). Expression of IRF-1–regulated genes is increased in SLE by an average of 7.8%, secondary to increased IRF-1 binding to H3K4me3 sites (35). Furthermore, increased IRF-1 expression is observed in biopsy specimens from patients with lupus nephritis (33), DLE, and SCLE and in monocytes from patients with SLE (35). Increased expression of IRF-1 facilitates type I IFN–accelerated lupus nephritis in (NZB/NZW)F1 mice, and its down-regulation correlates with a reduction in lupus nephritis activity (33). Importantly, IRF-1 expression in the kidney may also be predictive of a treatment response in lupus nephritis (34).

The addition of serum to monocytes has been shown to facilitate inflammasome activation in part via immune complexes and stimulation of Fcγ receptors in the context of TLR signaling (1,6,7,50). We detected similar enhancement of inflammasome activation when control monocytes were exposed to serum from SLE patients and control serum, although the effect was greater in serum from SLE patients.

Interestingly, the enhanced effects of SLE patient serum on IL-1β release returned to the level in control serum following inhibition of IRF-1 via TBB. This suggests that increased IFN activity in the serum of SLE patients is a significant contributor to inflammasome activation in an IRF-1–dependent manner. The residual enhancement of inflammasome activity in the presence of TBB is likely secondary to immunoglobulin effects.

Interest in the inflammasome as a promoter of SLE organ damage is growing. Our work supports a role for long-term type I IFN exposure as a mechanism by which the inflammasome is hyperactivated in SLE patients and identifies IRF-1 as an important regulator of this process. These data will inform development of novel therapeutic targets in SLE.

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. Kahlenberg 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. Liu, Kahlenberg.

Acquisition of data. Liu, Berthier.

Analysis and interpretation of data. Liu, Berthier, Kahlenberg.

REFERENCES

1. Zhang H, Fu R, Guo C, Huang Y, Wang H, Wang S, et al. Anti-dsDNA antibodies bind to TLR4 and activate NLRP3 inflammasome in lupus monocytes/macrophages. J Transl Med 2016;14:156.
2. Kahlenberg JM, Carmona-Rivera C, Smith CK, Kaplan MJ. Neutrophil extracellular trap-associated protein activation of the NLRP3 inflammasome is enhanced in lupus macrophages. J Immunol 2013;190:1217–26.
3. Kahlenberg JM, Kaplan MJ. The inflammasome and lupus: another innate immune mechanism contributing to disease pathogenesis? Curr Opin Rheumatol 2014;26:475–81.
4. Kahlenberg JM, Thacker SG, Berthier CC, Cohen CD, Kretzler M, Kaplan MJ. Inflammasome activation of IL-18 results in endothelial progenitor cell dysfunction in systemic lupus erythematosus. J Immunol 2011;187:6143–56.
5. Kahlenberg JM, Yalavarthi S, Zhao W, Hodgin JB, Reed TJ, Tsuji NM, et al. An essential role of caspase 1 in the induction of murine lupus and its associated vascular damage. Arthritis Rheumatol 2014;66:152–62.
6. Shin MS, Kang Y, Lee N, Kim SH, Kang KS, Lazova R, et al. U1-small nuclear ribonucleoprotein activates the NLRP3 inflammasome in human monocytes. J Immunol 2012;188:4769–75.
7. Shin MS, Kang Y, Lee N, Wahl ER, Kim SH, Kang KS, et al. Self double-stranded (ds)DNA induces IL-1β production from human monocytes by activating NLRP3 inflammasome in the presence of anti-dsDNA antibodies. J Immunol 2013;190:1407–15.
8. Vilaysane A, Chun J, Seamone ME, Wang W, Chin R, Hirota S, et al. The NLRP3 inflammasome promotes renal inflammation and contributes to CKD. J Am Soc Nephrol 2010;21:1732–44.
9. Zhao J, Wang H, Dai C, Wang H, Zhang H, Huang Y, et al. $P2X_7$ blockade attenuates murine lupus nephritis by inhibiting activation of the NLRP3/ASC/caspase 1 pathway. Arthritis Rheum 2013;65:3176–85.
10. Zhao J, Zhang H, Huang Y, Wang H, Wang S, Zhao C, et al. Bay11–7082 attenuates murine lupus nephritis via inhibiting NLRP3 inflammasome and NF-κB activation. Int Immunopharmacol 2013; 17:116–22.
11. Ka SM, Lin JC, Lin TJ, Liu FC, Chao LK, Ho CL, et al. Citral alleviates an accelerated and severe lupus nephritis model by inhibiting the activation signal of NLRP3 inflammasome and enhancing Nrf2 activation. Arthritis Res Ther 2015;17:331.
12. Li M, Shi X, Qian T, Li J, Tian Z, Ni B, et al. A20 overexpression alleviates pristine-induced lupus nephritis by inhibiting the NF-κB and NLRP3 inflammasome activation in macrophages of mice. Int J Clin Exp Med 2015;8:17430–40.
13. Eloranta ML, Ronnblom L. Cause and consequences of the activated type I interferon system in SLE. J Mol Med (Berl) 2016; 94:1103–10.
14. Crow MK. Type I interferon in the pathogenesis of lupus. J Immunol 2014;192:5459–68.
15. Agrawal H, Jacob N, Carreras E, Bajana S, Putterman C, Turner S, et al. Deficiency of type I IFN receptor in lupus-prone New Zealand mixed 2328 mice decreases dendritic cell numbers and activation and protects from disease. J Immunol 2009;183:6021–9.
16. Liu Z, Bethunaickan R, Huang W, Lodhi U, Solano I, Madaio MP, et al. Interferon-α accelerates murine systemic lupus erythematosus in a T cell–dependent manner. Arthritis Rheum 2011; 63:219–29.
17. Thacker SG, Zhao W, Smith CK, Luo W, Wang H, Vivekanandan-Giri A, et al. Type I interferons modulate vascular

function, repair, thrombosis, and plaque progression in murine models of lupus and atherosclerosis. Arthritis Rheum 2012;64: 2975–85.
18. Kirou K, Lee C, George S, Louca K, Peterson MG, Crow M. Activation of the interferon-α pathway identifies a subgroup of systemic lupus erythematosus patients with distinct serologic features and active disease. Arthritis Rheum 2005;52:1491–503.
19. Yang Q, Yu C, Yang Z, Wei Q, Mu K, Zhang Y, et al. Deregulated NLRP3 and NLRP1 inflammasomes and their correlations with disease activity in systemic lupus erythematosus. J Rheumatol 2014;41:444–52.
20. Guarda G, Braun M, Staehli F, Tardivel A, Mattmann C, Förster I, et al. Type I interferon inhibits interleukin-1 production and inflammasome activation. Immunity 2011;34:213–23.
21. Panchanathan R, Liu H, Leung YK, Ho SM, Choubey D. Bisphenol A (BPA) stimulates the interferon signaling and activates the inflammasome activity in myeloid cells. Mol Cell Endocrinol 2015;415:45–55.
22. Kyogoku C, Smiljanovic B, Grun JR, Biesen R, Schulte-Wrede U, Haupl T, et al. Cell-specific type I IFN signatures in autoimmunity and viral infection: what makes the difference? PLoS One 2013;8:e83776.
23. Furie R, Merrill JT, Werth VP, Khamashta M, Kalunian K, Brohawn P, et al. Anifrolumab, an anti–interferon α receptor monoclonal antibody, in moderate-to-severe systemic lupus erythematosus. Arthritis Rheumatol 2017;69:376–86.
24. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus [letter]. Arthritis Rheum 1997;40:1725.
25. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C}{}_{T}$ method. Methods 2001;25:402–8.
26. Kirou KA, Lee C, George S, Louca K, Papagiannis IG, Peterson MG, et al. Coordinate overexpression of interferon-α–induced genes in systemic lupus erythematosus. Arthritis Rheum 2004;50: 3958–67.
27. Ekholm L, Kahlenberg JM, Barbasso Helmers S, Tjarnlund A, Yalavarthi S, Zhao W, et al. Dysfunction of endothelial progenitor cells is associated with the type I IFN pathway in patients with polymyositis and dermatomyositis. Rheumatology (Oxford) 2016;55:1987–92.
28. Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, et al. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics 2003;4:249–64.
29. Asgari E, Le Friec G, Yamamoto H, Perucha E, Sacks SS, Kohl J, et al. C3a modulates IL-1β secretion in human monocytes by regulating ATP efflux and subsequent NLRP3 inflammasome activation. Blood 2013;122:3473–81.
30. Qu Y, Ramachandra L, Mohr S, Franchi L, Harding CV, Nunez G, et al. P2X7 receptor-stimulated secretion of MHC class II-containing exosomes requires the ASC/NLRP3 inflammasome but is independent of caspase-1. J Immunol 2009;182:5052–62.
31. Hua J, Kirou K, Lee C, Crow MK. Functional assay of type I interferon in systemic lupus erythematosus plasma and association with anti–RNA binding protein autoantibodies. Arthritis Rheum 2006;54:1906–16.
32. Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang DH, and the Committee on Prognosis Studies in SLE. Derivation of the SLEDAI: a disease activity index for lupus patients. Arthritis Rheum 1992;35:630–40.
33. Han X, Wang Y, Zhang X, Qin Y, Qu B, Wu L, et al. MicroRNA-130b ameliorates murine lupus nephritis through targeting the type I interferon pathway on renal mesangial cells. Arthritis Rheumatol 2016;68:2232–43.
34. Parikh SV, Malvar A, Song H, Alberton V, Lococo B, Vance J, et al. Characterising the immune profile of the kidney biopsy at lupus nephritis flare differentiates early treatment responders from non-responders. Lupus Sci Med 2015;2:e000112.
35. Zhang Z, Shi L, Song L, Ephrem E, Petri M, Sullivan KE. Interferon regulatory factor 1 marks activated genes and can induce target gene expression in systemic lupus erythematosus. Arthritis Rheumatol 2015;67:785–96.
36. Fantuzzi G, Reed D, Qi M, Scully S, Dinarello CA, Senaldi G. Role of interferon regulatory factor-1 in the regulation of IL-18 production and activity. Eur J Immunol 2001;31:369–75.
37. Karlsen AE, Pavlovic D, Nielsen K, Jensen J, Andersen HU, Pociot F, et al. Interferon-γ induces interleukin-1 converting enzyme expression in pancreatic islets by an interferon regulatory factor-1-dependent mechanism. J Clin Endocrinol Metab 2000;85:830–6.
38. Lin R, Hiscott J. A role for casein kinase II phosphorylation in the regulation of IRF-1 transcriptional activity. Mol Cell Biochem 1999;191:169–80.
39. Meller S, Winterberg F, Gilliet M, Muller A, Lauceviciute I, Rieker J, et al. Ultraviolet radiation–induced injury, chemokines, and leukocyte recruitment: an amplification cycle triggering cutaneous lupus erythematosus. Arthritis Rheum 2005;52:1504–16.
40. Stannard JN, Reed TJ, Myers E, Lowe L, Sarkar MK, Xing X, et al. Lupus skin is primed for IL-6 inflammatory responses through a keratinocyte-mediated autocrine type I interferon loop. J Invest Dermatol 2017;137:115–22.
41. Kennedy Crispin M, Fuentes-Duculan J, Gulati N, Johnson-Huang LM, Lentini T, Sullivan-Whalen M, et al. Gene profiling of narrowband UVB-induced skin injury defines cellular and molecular innate immune responses. J Invest Dermatol 2013; 133:692–701.
42. Deng GM, Liu L, Kyttaris VC, Tsokos GC. Lupus serum IgG induces skin inflammation through the TNFR1 signaling pathway. J Immunol 2010;184:7154–61.
43. Kahlenberg JM. Activation of caspase-1 signaling complexes by the P2X7 receptor requires intracellular potassium efflux and protein synthesis induced by priming with Toll-like receptor ligands [thesis]. Cleveland: Case Western Reserve University; 2004.
44. Clark KL, Reed TJ, Wolf SJ, Lowe L, Hodgin JB, Kahlenberg JM. Epidermal injury promotes nephritis flare in lupus-prone mice. J Autoimmun 2015;65:38–48.
45. DeYoung KL, Ray ME, Su YA, Anzick SL, Johnstone RW, Trapani JA, et al. Cloning a novel member of the human interferon-inducible gene family associated with control of tumorigenicity in a model of human melanoma. Oncogene 1997;15:453–7.
46. Panchanathan R, Choubey D. Murine BAFF expression is upregulated by estrogen and interferons: implications for sex bias in the development of autoimmunity. Mol Immunol 2013;53:15–23.
47. Choubey D, Panchanathan R. Absent in melanoma 2 proteins in SLE. Clin Immunol 2017;176:42–8.
48. Pontillo A, Girardelli M, Kamada A, Pancotto JA, Donadi EA, Crovella S, et al. Polimorphisms in inflammasome genes are involved in the predisposition to systemic lupus erythematosus. Autoimmunity 2012;45:271–8.
49. Reilly CM, Olgun S, Goodwin D, Gogal RM Jr, Santo A, Romesburg JW, et al. Interferon regulatory factor-1 gene deletion decreases glomerulonephritis in MRL/lpr mice. Eur J Immunol 2006;36:1296–308.
50. Vogelpoel LT, Hansen IS, Rispens T, Muller FJ, van Capel TM, Turina MC, et al. Fc γ receptor-TLR cross-talk elicits pro-inflammatory cytokine production by human M2 macrophages. Nat Commun 2014;5:5444.