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Inflammasome Activation of IL-18 Results in Endothelial Progenitor Cell Dysfunction in Systemic Lupus Erythematosus

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Systemic lupus erythematosus (SLE) is an autoimmune disease with heterogeneous manifestations including severe organ damage and vascular dysfunction leading to premature atherosclerosis. IFN- α has been proposed to have an important role in the development of lupus and lupus-related cardiovascular disease, partly by repression of IL-1 pathways leading to impairments in vascular repair induced by endothelial progenitor cells (EPCs) and circulating angiogenic cells (CACs). Counterintuitively, SLE patients also display transcriptional upregulation of the IL-1 β /IL-18 processing machinery, the inflammasome. To understand this dichotomy and its impact on SLE-related cardiovascular disease, we examined cultures of human and murine control or lupus EPC/CACs to determine the role of the inflammasome in endothelial differentiation. We show that caspase-1 inhibition improves dysfunctional SLE EPC/CAC differentiation into mature endothelial cells and blocks IFN- α -mediated repression of this differentiation, implicating inflammasome activation as a crucial downstream pathway leading to aberrant vasculogenesis. Furthermore, serum IL-18 levels are elevated in SLE and correlate with EPC/CAC dysfunction. Exogenous IL-18 inhibits endothelial differentiation in control EPC/CACs and neutralization of IL-18 in SLE EPC/CAC cultures restores their capacity to differentiate into mature endothelial cells, supporting a deleterious effect of IL-18 on vascular repair in vivo. Upregulation of the inflammasome machinery was operational in vivo, as evidenced by gene array analysis of lupus nephritis biopsies. Thus, the effects of IFN- α are complex and contribute to an elevated risk of cardiovascular disease by suppression of IL-1 β pathways and by upregulation of the inflammasome machinery and potentiation of IL-18 activation. *The Journal of Immunology*, 2011, 187: 6143–6156.

Systemic lupus erythematosus (SLE) is an autoimmune disease with varied and often devastating organ involvement and a bimodal mortality pattern that stems from

immune mediated organ damage and from an up to 50-fold increase in atherosclerotic cardiovascular (CV) risk, when compared with age- and sex-matched controls. This CV risk cannot be fully explained by traditional Framingham risk factors (1). Furthermore, immunosuppression may decrease CV risk, suggesting that immune dysregulation characteristic of lupus may contribute to premature vascular damage in this disease (2).

Our group has proposed that one mechanism central to the development of premature CV disease (CVD) in SLE is a profound imbalance between vascular damage and vascular repair that leads to endothelial dysfunction and promotes plaque development. We have previously shown that the phenotype and function of bone marrow-derived endothelial progenitor cells (EPCs) and myeloid circulating angiogenic cells (CACs), both crucial cell subsets in vascular repair and maintenance of an intact endothelium (3, 4), are abnormal in SLE patients and in animal models of SLE (5, 6). Indeed, not only are lupus EPC/CAC numbers decreased, but these cells are unable to properly differentiate into mature endothelial cells (EC) in vitro when exposed to proangiogenic stimuli. Furthermore, lupus EPCs/CACs have impaired incorporation into formed vascular structures on a Matrigel assay and decreased capacity to synthesize crucial proangiogenic molecules such as vascular endothelial growth factor (VEGF). In addition, we have recently reported that decreased angiogenesis in SLE is present in vivo, as evidenced by vascular rarefaction and antiangiogenic signatures in lupus tissues (7).

The dysfunction of lupus EPC/CACs is mediated by type I IFN, as normal in vitro EPC/CAC function can be restored in this disease by blocking IFN- α signaling. Furthermore, healthy control EPCs/CACs exposed to IFN- α develop a similar abnormal phenotype to SLE cells (5). In addition, EPCs/CACs isolated from the lupus-prone New Zealand Black/New Zealand White (F₁) mouse,

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Abbreviations used in this article: ac-LDL, acetylated low-density lipoprotein; AIM2, absent in melanoma 2; ASC, apoptosis-associated speck-like protein containing a CARD; CAC, circulating angiogenic cell; CV, cardiovascular; CVD, cardiovascular disease; EC, endothelial cell; EPC, endothelial progenitor cell; INZM, IFN receptor knockout NZM mice; LD, living donor; LDL, low-density lipoprotein; LN, lupus nephritis; NET, neutrophil-associated extracellular trap; NZM, New Zealand mixed; SLE, systemic lupus erythematosus; SLEDAI, systemic lupus erythematosus disease activity index; UEA, ulexeuropus agglutinin; VEGF, vascular endothelial growth factor.

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a strain in which type I IFNs play a prominent role in disease development (8), also show a similar aberrant phenotype that correlates with impaired aortic endothelial function (6). Although it is clear that IFN- α hampers vascular repair in SLE, the pathways by which this cytokine exerts its antiangiogenic effects in progenitor cells have only recently begun to be characterized. Our group has reported that one of the mechanisms by which IFN- α mediates EPC/CAC dysfunction is through the downregulation of the proangiogenic molecules IL-1 β and VEGF and upregulation of the IL-1 receptor antagonist (IL1-RN). Indeed, addition of activated IL-1 β to SLE EPC/CAC cultures restores their capacity to differentiate into mature ECs (7).

Although IL-1 β is proangiogenic in SLE, less is known about the role of IL-18, another cytokine also activated via cleavage by caspase-1 (9). IL-18 is a constitutively expressed cytokine that acts as an important bridge between innate immune responses and the development of adaptive immunity. In addition, IL-18 may have an important role in lupus pathogenesis and organ damage. Serum levels of this cytokine are elevated in SLE patients, and this correlates with disease severity and the presence of lupus nephritis (LN) (10–12). Moreover, levels of IL-18 transcript are elevated in glomeruli from patients with LN. This correlates with increased recruitment of plasmacytoid dendritic cells, which express high levels of the IL-18R, to nephritic glomeruli (10).

The inflammasome is a multimolecular platform whose assembly results in rapid activation of caspase-1, the enzyme responsible for generation of the active forms of IL-1 β and IL-18. The central components of the inflammasome include a scaffold of the NLR family (NLRP1, NLRP3, or NLRC4) or absent in melanoma-2 (AIM2); an adaptor molecule, apoptosis-associated speck-like protein containing a CARD (ASC); and caspase-1 (13). Regulation of the inflammasome can occur at a transcriptional level, but the presence of an activation signal is the central switch to make this structure functional (13). Activation and assembly of the inflammasome occur downstream of varied stimuli, including cholesterol crystals, uric acid, intracellular bacteria and ATP (13–15).

Type I IFNs have been proposed to have a regulatory role in inflammasome activity, because the IFN- α responsive inflammasome scaffold absent in melanoma-2 (AIM2) activates caspase-1 in an ASC-dependent manner. AIM2 has recently been proposed to be a cytoplasmic receptor for dsDNA, and its mRNA levels are increased in leukocytes of SLE patients (16, 17). Caspase-1 also appears to be regulated by IFNs, because the IFN-regulated transcription factor IRF-1 is essential for caspase-1 transcription and activation of IL-18 in response to IL-12 administration (18, 19).

The role of the inflammasome and IL-18 in CVD has not been well characterized. Increased synthesis of IL-18 by an active inflammasome is seen in proatherosclerotic low-density lipoprotein (LDL) receptor-deficient mice (14). In addition, IL-18 levels are increased in patients with the metabolic syndrome and correlate with arterial stiffness (20). Importantly, endogenous IL-18 may play a major antiangiogenic role in postischemic injury and contribute to decreased EPC numbers in other conditions (21, 22).

Thus, in this study, we investigated the role of inflammasome activity in EPC/CAC dysfunction in SLE, a disease characterized by enhanced type I IFN synthesis and activity and specifically examined the role of inflammasome-driven IL-18 activation in the development of EPC/CAC dysfunction.

Materials and Methods

Patient selection

The University of Michigan institutional review board approved this study. Subjects gave informed consent in accordance with the Declaration of

Helsinki. To obtain peripheral blood, patients fulfilled the revised American College of Rheumatology criteria for SLE (23) and were enrolled from the University of Michigan outpatient Rheumatology clinic. Age- and gender-matched healthy controls were recruited by advertisement. Lupus disease activity was assessed by the SLE Disease Activity Index (SLEDAI) (24). Patient and control demographics and clinical variables for EPC/CAC studies are reported in Table I. For renal microarray analysis, human renal biopsies were collected by the European Renal cDNA Bank (25). A total of 15 pretransplant healthy living donors (LD) and 32 LN patients were processed for microarray analysis. Patient and control demographics and clinical variables for renal microarray studies are available in Supplemental Table I.

Cell isolation, EPC/CAC culture and fluorescent microscopy

Human PBMCs, known to contain both EPCs and CACs (3), were isolated and cultured under proangiogenic stimulation as described previously (7). To summarize, PBMCs contain bone marrow derived EPC and myelomonocytic CAC progenitors that will differentiate into EC when grown on extracellular matrix. These cells have been followed throughout development and have been found to differentiate from progenitors (CD34⁺, CD133⁺, VEGFR2⁺ for EPCs and CD14⁺, CD45⁺, CD115⁺ for CACs) to mature EC, which bind lectin, take up LDL, and express von Willebrand factor, caveolin, and NO synthetase (3, 26). EPC/CACs require the presence of other cells in culture for proper differentiation. Thus, culturing of PBMCs under proangiogenic conditions has become a standard for evaluating EPC/CAC function in vitro. As such, BMCs ($2.27 \times 10^6/\text{cm}^2$) were cultured in EC-specific enrichment medium (EBM; Cambrex, East Rutherford, NJ) on fibronectin-coated wells (BD Biosciences, Franklin Lakes, NJ). In some of the experiments, human rIFN- α 2b (Merck, Whitehouse Station, NJ), the caspase-1 inhibitor ac-YVAD-cmk (Enzo, Plymouth Meeting, PA), or the caspase-3 inhibitor ac-DEVD-cmk (CPC Scientific, Sunnyvale, CA) were added to the wells at the initiation of the culture, and medium was changed after 3 d and every 72 h thereafter, unless otherwise specified. Some of the molecules added to the culture were replaced with each media change. In experiments to assess the effects of IL-18 on EPCs/CACs, graded concentrations of IL-18 (BioVision, Mountain View, CA), IL-1 β (Biovision), or 1 mg/ml of a neutralizing IL-18 Ab (R&D Systems, Minneapolis, MN) were added at the time of cell isolation and left in culture for the first 3 d.

To assess the capacity of peripheral blood EPCs/CACs to differentiate into mature ECs, on day 14 of culture, cells were incubated with 1'-diodecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (diI)-acetylated LDL (ac-LDL; Biomedical Technologies, Stoughton, MA) and FITC-ulex europaeus agglutinin (UEA)-1 (Vector Laboratories, Burlingame, CA). To assess EC morphology and expression of endothelial markers, cells were analyzed by fluorescent microscopy using a Leica DMIRB fluorescent inverted microscope (Bannockburn, IL). Images were acquired with a $\times 100$ total magnification, at room temperature, using live cells in Ringer's buffer. A total of four random fields of view were acquired for every triplicate well and images were analyzed using the CellC program (<http://www.cs.tut.fi/sgn/csb/cellc/>) to quantify mature ECs, which were considered as those that coexpress UEA-1 and ac-LDL. The numeric aperture for the objective lens of the fluorescent microscope was 0.3. Images were acquired with an Olympus DP30BW camera (Olympus Corporation, Tokyo, Japan) using the acquisition software Olympus-BSW (Olympus). Final processing was done with Adobe Photoshop CS2 (San Jose, CA).

To inhibit IFN- α signaling, the pan-Janus Kinase (JAK) inhibitor 2-(1,1-dimethylethyl)-9-fluoro-3,6-dihydro-7H-benz[h]-imidaz[4,5-f]isoquinolin-7-one (Pyridone 6) or the PI3K inhibitor (5-(4-Fluoro-2-hydroxyphenyl) furan-2-ylmethylene)thiazolidine-2,4-dione (Calbiochem, Gibbstown, NJ) were used. In brief, human EPCs/CACs were cultured under proangiogenic stimulation for 72 h, followed by change of media containing 50 μM pyridone 6, 50 nM PI3K inhibitor, or vehicle (DMSO) for 1 h before addition of 1000 U/ml rIFN- α . EPC/CACs were incubated for 6 additional hours, and then, total RNA was isolated, and real-time PCR was completed.

Western blots

EPCs/CACs were treated with or without 1000 U/ml of IFN- α or 10 ng/ml LPS O26:B6 (Sigma-Aldrich) overnight, washed once with PBS and lysed in SDS-PAGE buffer. The equivalent of 1×10^6 cells were run on a 15% polyacrylamide gel and transferred to nitrocellulose. Membranes were incubated overnight with rabbit anti-human-caspase-1 (Cell Signaling Technology, Beverly, MA) at 1/1000 final concentration or with anti- β -actin (Cell Signaling Technology) at 1/5000 dilution, followed by HRP-conjugated goat anti-rabbit Ab (The Jackson Laboratory, Bar Harbor, ME) for 1 h at a dilution of 1/10,000. Ab detection was performed using

Western Lightening (PerkinElmer, Waltham, MA), following manufacturer's instructions.

RNA isolation

For EPC/CAC studies, total RNA was isolated with Tripure (Roche, Indianapolis, IN) following the manufacturer's recommendations. For human kidney tissues, cortical tissue segments were manually microdissected into glomeruli and tubulointerstitial compartments as previously published (27, 28). For microarray analysis, RNA was further purified and concentrated using an RNeasy micro kit and following the manufacturer's recommendations (Qiagen, Valencia, CA). RNA samples were processed on an Agilent 2100 BioAnalyzer (Agilent Technologies, Santa Clara, CA) to assess RNA integrity.

Microarray data processing, analysis, and pathway mapping

Microarray samples and data results from control and lupus EPCs/CACs in the presence or absence of IFN- α have been previously published by our group (7). The Affymetrix raw data are available in the Gene Expression Omnibus microarray data repository under the reference GSE23203 (<http://www.ncbi.nlm.nih.gov/geo>).

Gene expression profiling from microdissected human kidney biopsies was performed as previously published using the Human Genome U133A Affymetrix Genechip arrays (Santa Clara, CA). The CEL files were collected and processed using the GenePattern analysis pipeline (www.genepattern.com). CEL file normalization was performed with the Robust Multichip Average method using the mouse and human Entrez-Gene custom CDF annotation from Brain Array version 10 (<http://brainarray.mbi.med.umich.edu/Brainarray/default.asp>). The normalized files were \log_2 transformed. Of the 12,029 human genes, 11,285 and 11,429 were expressed above the 27 Poly-A Affymetrix control expression baseline (negative controls) in the glomerular and tubulointerstitial compartments respectively and were used for further analyses. Normalized data files are in the process of being uploaded on Gene Omnibus (<http://www.ncbi.nlm.nih.gov/geo/>).

Real-time quantitative PCR

Total RNA was transcribed into cDNA using oligodT and Moloney murine leukemia virus (Invitrogen, Carlsbad, CA) with 1 μ g RNA using a MyCycler thermocycler (Bio-Rad, Hercules, CA), and levels of expression of the following genes were measured: *caspase-1*, 5'-GGA CTC TCA GCA GCT CCT CAG GCA-3' (forward) and 5'-GCA AAG CTT GAC ATT CCC TTC TGA GCC-3' (reverse); *ASC*, 5'-CCT ACG GCG CCG AGC TCA C-3' (forward) and 5'-CTC CAG AGC CCT GGT GCG T-3' (reverse); *AIM2*, 5'-TGG GCA TGC TCT CCT GAG TCC T-3' (forward) and 5'-TCA GCC TCC TGA TCC CTG GGG C-3' (reverse); β -actin, 5'-CAT CAC GAT GCC AGT GGT ACG-3' (forward) and 5'-AAC CGC GAG AAG ATG ACC CAG-3' (reverse); *PRKR*, 5'-CTT CCA TCT GAC TCA GGT TT-3' (forward) and 5'-TGC TTC TGA CGG TAT GTA TTA-3' (reverse); *IFI44*, 5'-CTC GGT GGT TAG CAA TTA TTC CTC-3' (forward) and 5'-AGC CCA TAG CAT TCG TCT CAG-3' (reverse); and *IFIT*, 5'-CTC CTT GGG TTC GTC TAT AAA TTG-3' (forward) and 5'-AGT CAG CAG CCA GTC TCA G-3' (reverse).

Real-time PCR was carried out using an ABI PRISM 7900HT (Applied Biosystems) with the following cycling conditions: an initial denaturing/activation at 95°C for 10 min, followed by 40 cycles of denaturing at 95°C for 30 s, annealing at 52°C for 30 s, and elongation at 72°C for 30 s. Samples were normalized to the housekeeping gene β -actin, and fold change was expressed as IFN-treated versus untreated or SLE versus control samples.

Assessment of serum protein levels

Serum human IL-18 was quantified by ELISA (eBioscience, Vienna, Austria), following the manufacturer's instructions.

Assessment of SLE Ab profile

Patients' serum was analyzed for autoantibodies via binding to fluoromagnetic beads using the BioPlex 2200 System (Bio-Rad) following the manufacturer's instructions. Anti-dsDNA was quantified by Farr assay. Autoantibody quantification was performed at the University of Michigan Core Laboratories.

Mice and tissue harvesting

All animal protocols were reviewed and approved by the University of Michigan's committee on use and care of animals. To evaluate the role of caspase-1 inhibition in lupus-prone mice, 9 mo old New Zealand Mixed

(NZM) 2328 or NZM 2328 lacking the type I IFNR (INZM) (obtained initially as a gift from Dr. C. Jacob (University of Southern California, Los Angeles, CA) (29), and subsequently bred at the University of Michigan) were used. For IFN signaling studies, 15-20 wk old NZM (predisease onset) and BALB/c mice (The Jackson Laboratory) were used. Femurs and tibias were washed and flushed with ice-cold MACS buffer (Miltenyi Biotec, Auburn, CA). Mononuclear cells were then isolated on a Histo-paque 1083 density gradient (Sigma-Aldrich), and residual RBCs were lysed with RBC lysis buffer (BioLegend, San Diego, CA). Following subsequent wash in PBS, cells were plated onto fibronectin-coated plates (BD Biosciences) at a density of 1×10^6 cells/cm² in EGM-2 Bulletkit media (Lonza, Allendale, NJ) supplemented with 5% heat-inactivated FBS. At the time of plating, vehicle (DMSO) or ac-YVAD-cmk (Enzo, Plymouth Meeting, PA) were added to the media, which was replaced every 3 d. After 6 d in culture, live cells were incubated with FITC-conjugated Bandeiraea (Griffonia) Simplicifolia Lectin I (Isolectin B4) (BS-1; Vector Laboratories) and ac-LDL for 3 h. Cells were imaged as mentioned above for human studies. Mature ECs were designated as those cells costaining with BS-1 and ac-LDL and were quantified in triplicate in four random fields per well of a 48-well plate.

Statistical analysis

For Affymetrix microarray studies, the significance microarray analysis was used to define genes significantly differentially regulated between the studied groups; a *q*-value below 0.05 was considered significant. For correlation of EPC numbers with IL-18 and other variables, linear regression was performed. For correlation of the presence of autoantibodies with IL-18 levels, a Mann-Whitney *U* test was performed. For all other studies, a paired Student *t* test was performed. A *p* value <0.05 was considered significant.

Results

Inflammasome components are upregulated in EPCs/CACs by IFN- α and in SLE patients

All patient and control demographics are listed in Table I. Our group has demonstrated that EPCs/CACs isolated from SLE patients, or the exposure of healthy control EPCs/CACs to recombinant IFN- α , results in their increased apoptosis and decreased ability to differentiate into mature ECs (5). These abnormalities are mediated in part by transcriptional repression of IL-1 β and transcriptional upregulation of IL1-RN (7). However, additional analysis of previously published microarray data (7) indicates that, while IFN- α represses IL-1 β levels, mRNA of various inflammasome components are significantly upregulated by this cytokine (Table II) in SLE and control patients. With few exceptions, real-time PCR confirmed the microarray analysis. Up

Table I. Demographic and clinical characteristics of healthy controls and SLE patients used for EPC studies

Variables	Controls (n = 37)	SLE (n = 43)	<i>p</i> Value
Females, %	97.3	93.1	0.46
Age (SEM)	39 (2.0)	41 (1.9)	0.7
Disease Activity by SLEDAI, Mean (SEM)	—	3.9 (0.6)	
SLEDAI < 2, %	—	26	
SLEDAI \geq 2, %	—	75	
Medications, %			
Antimalarials	—	91	
Methotrexate	—	9.3	
Azathioprine	—	7	
Mycophenolate Mofetil	—	32.5	
Cyclophosphamide	—	2.3	
Prednisone	—		
None		46.5	
<10 mg daily		39.5	
\geq 10 mg daily		14	
Past or current LN, %	—	25.5	
Positive antiphospholipid Abs, %	—	18.6	

—, not applicable.

Table II. EPC/CACs from healthy controls or SLE patients with and without IFN- α treatment

Gene Symbol	Gene Name	Entrez Gene ID	IFN- α -Treated Compared with Nontreated Healthy Control Peripheral Blood EPCs/CACs	IFN- α -Treated Compared with Nontreated SLE Peripheral Blood EPCs/CACs	Nontreated SLE Compared with Nontreated Healthy Control Peripheral Blood EPCs/CACs	IFN- α -Treated SLE Compared with IFN- α -Treated Healthy Control Peripheral Blood EPCs/CACs
AIM2	Absent in melanoma 2	9447	1.93*	5.81*	0.50*	1.66*
PYCARD (ASC)	PYD and CARD domain containing	29108	1.18*	<i>1.01</i>	1.41*	<i>1.21</i>
CASP1	Caspase 1, apoptosis-related cysteine peptidase (IL-1, β , convertase)	834	1.27*	1.57*	<i>0.99</i>	<i>1.13</i>

Results in italics suggest no significant regulation.

* $q < 0.05$ was considered significant.

to a 5-fold increase of *AIM2* mRNA and 3-fold increase in *CASP1* mRNA was detected in healthy control or SLE EPC/CACs exposed to recombinant IFN- α (Fig. 1A). In contrast to some of the microarray data, a 3-fold increase in *AIM2*, *PYCARD* (ASC), and *CASP1* transcripts was observed in untreated EPC/CACs from SLE patients when compared with untreated control cells (Fig. 1B). These increases correlated with the type I IFN signature in each patient analyzed, suggesting that SLE patients with higher basal levels of IFN- α have greater upregulation of both caspase-1 and *AIM2* in their EPCs (Fig. 1C). Thus, despite our previous observations that IFN- α treatment of EPC/CACs results in repression of IL-1 β pathways, *AIM2* and caspase-1 are upregulated. This suggests that inflammasome activity may contribute to IFN- α -mediated effects on EPC/CAC dysfunction. Confirmation of increased induction of caspase-1 at the protein level upon IFN- α exposure was confirmed by Western blot analysis on control early EPCs/CACs (Fig. 1E). A less robust upregulation of caspase-1 in IFN- α -treated lupus EPCs/CACs was noted, which may reflect already increased procaspase-1 protein levels in SLE-untreated lysates, when compared with untreated controls (Fig. 1D). These results indicate that the inflammasome machinery is upregulated in SLE patients and that this phenomenon is mediated, at least in part, by their enhanced exposure to type I IFNs.

The upregulation of inflammasome components by IFN- α is mediated by JAK signaling

We previously reported that repression of IL-1 β and upregulation of IL-1 receptor antagonist by IFN- α requires signaling through JAK/STATs, but not through PI3K, downstream of the type I IFN receptor (7). To assess whether the same signaling pathways are involved in upregulation of inflammasome components by type I IFNs, EPCs/CACs from both controls and SLE patients were preincubated with a pan-JAK or PI3K inhibitors prior to stimulation with IFN- α . Real time PCR analysis demonstrated that loss of JAK signaling downstream of type-I IFN receptor completely abrogated the transcriptional upregulation of the inflammasome components *AIM2* and *CASP1* by IFN- α . In contrast, PI3K inhibition did not alter *AIM2* mRNA levels but there was a non-significant trend toward partial inhibition of caspase-1 transcript upregulation (Fig. 1E). These results indicate that the regulation of *AIM2* and *CASP1* transcripts are a direct downstream result of type I IFN receptor activation and JAK/STAT signaling and that *CASP1* transcripts may also be influenced by PI3K activation.

Inhibition of caspase-1 results in improved EPC/CAC differentiation in SLE and blocks IFN- α mediated EPC/CAC dysfunction

To understand whether the upregulation of caspase-1 in SLE negatively impacts EPC/CAC differentiation into mature ECs,

control or lupus EPCs/CACs were cultured in the presence of the caspase-1 inhibitor ac-YVAD-cmk (YVAD) or vehicle for 2 wk under proangiogenic conditions. This inhibitor is able to block formation of the active p20 fragment of caspase-1 (Fig. 2D). As previously reported by us (5), SLE patients display significantly fewer mature ECs after two weeks of culture (Fig. 2A), when compared with healthy controls. Although YVAD treatment did not alter the number of mature ECs in control samples, it significantly increased cell numbers in SLE cultures (Fig. 2A, 2C). These results indicate that inhibition of the central inflammasome enzyme caspase-1 improves the ability of SLE EPCs/CACs to differentiate into mature ECs in culture. In contrast, incubation of control or SLE cells with the caspase-3 inhibitor ac-DEVD-cmk (DEVD) did not improve differentiation (Fig. 2B). These results indicate that inhibition of caspases involved in apoptosis does not significantly impact endothelial differentiation.

As caspase-1 is upregulated by type I IFNs, the requirement for functional type I IFN signaling for the beneficial effects of caspase-1 blockade on these cells was examined. To this effect, lupus prone mice, which have previously been shown to have dysfunctional EPCs, were examined (6). EPC-containing bone marrow cells from lupus prone NZM 2328 mice or from NZM 2328 mice lacking a functional type I IFN receptor (IZNM) were cultured in the presence or absence of YVAD during their proangiogenic differentiation in vitro. As shown in Fig. 2E, YVAD treatment of male and female bone marrow from NZM mice improved their capacity to differentiate into mature ECs, while this effect was not observed in female and male INZM mice (Fig. 2E). These results suggest that only EPCs with functional type I IFN signaling can improve their differentiation capacity through caspase-1 inhibition, suggesting that caspase-1 effects on EPCs lie downstream of IFN- α . The effect of YVAD was not as robust in the mouse bone marrow EPCs as compared with the human samples; this may reflect the fact that the bone marrow compartment could lack CACs which are aberrant in SLE and have been shown to contribute to endothelial differentiation (26).

As the murine experiments indicated that the effect of caspase-1 inhibition in human and murine SLE is dependent on the presence of functional IFN signaling, we examined whether YVAD could inhibit IFN-mediated EPC dysfunction. Control and SLE EPCs/CACs were incubated with IFN- α in the presence or absence of YVAD. As previously reported by our group, IFN- α treatment resulted in ~50% inhibition of EC numbers in control and SLE cultures (5). Although IFN- α treatment did not result in detectable active caspase-1 p20, as measured by Western blot (data not shown), the addition of YVAD abrogated the effects of IFN- α on EPC/CAC differentiation (Fig. 3A). In contrast, the caspase-3 inhibitor DEVD did not alter the IFN-mediated EPC/CAC dysfunction (data not shown). Similar findings were obtained when analyzing bone marrow-derived EPCs isolated from non-lupus-

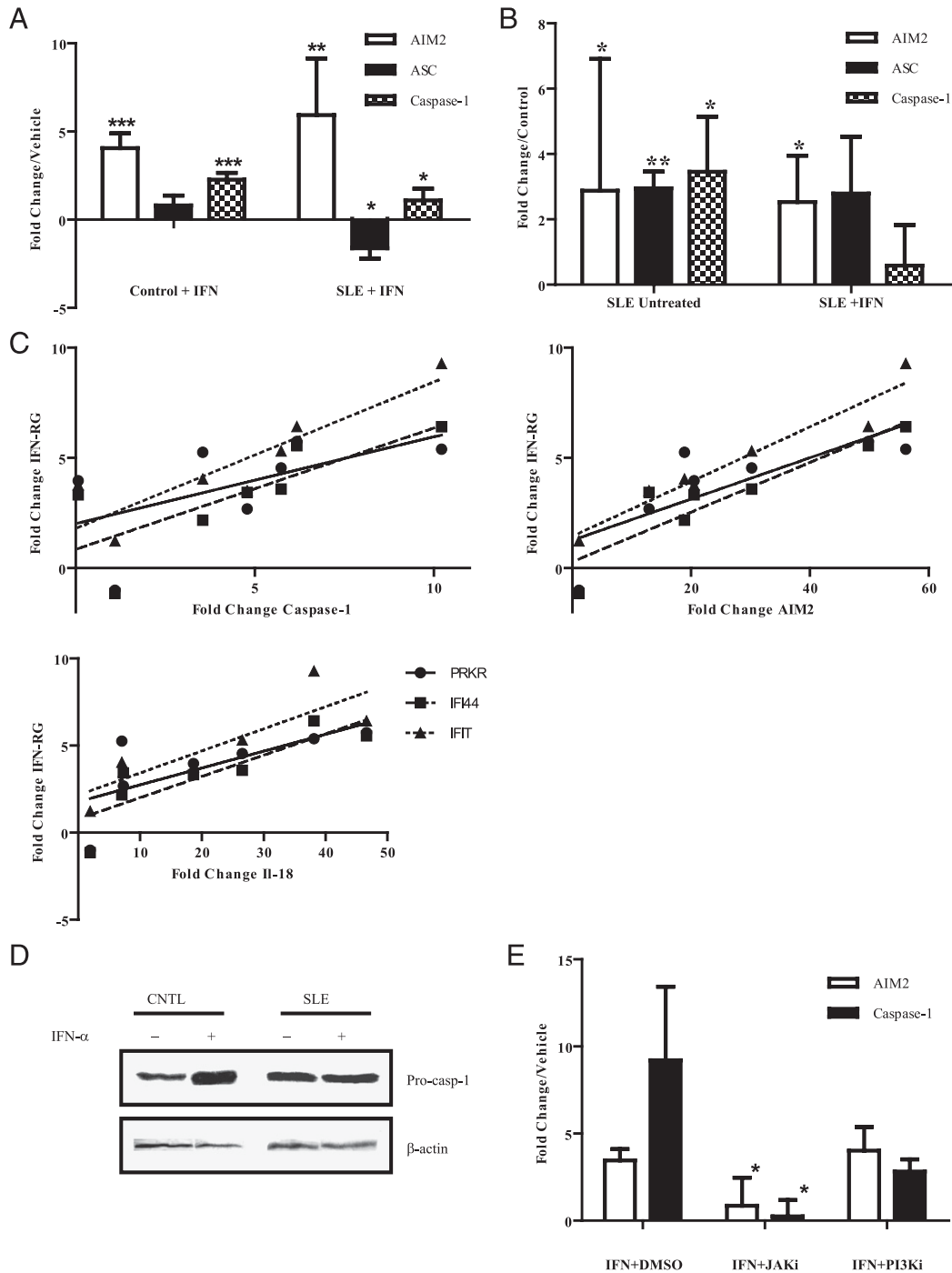


FIGURE 1. Inflammation upregulation occurs in SLE EPCs/CACs by IFN- α signaling in a JAK-dependent manner. Real-time PCR was performed in triplicate on mRNA from control and SLE EPC/CACs ($n = 11$ /group) cultured under proangiogenic stimulation for 72 h, followed by culture in the presence or absence of 1000 U/ml rIFN- α for 6 h. **A**, IFN- α significantly upregulates mRNA of inflammasome components in control and lupus EPCs. Control (*left*) and SLE (*right*) EPCs/CACs treated with IFN- α were compared with untreated control or untreated SLE cells respectively. **B**, Inflammasome component transcripts in untreated or IFN- α -treated SLE EPCs/CACs are increased when compared with control EPCs/CACs, and their upregulation correlates with type I IFN exposure. **B**, Untreated SLE (*left*) cells were compared with untreated control cells and IFN- α -treated SLE (*right*) were compared with IFN- α -treated control cells ($n = 11$ /group). **C**, Association of type I IFN-regulated genes (IFN-RG) in SLE patients ($n = 7$) with various inflammasome components. *Left panel*, *IFI44* and *IFIT* levels significantly correlated with caspase-1 ($p = 0.0459$ and 0.008 , respectively), whereas *PRKR* did not reach statistical significance ($p = 0.184$). *Right panel*, IFN-RGs significantly correlated with AIM2 levels: *PRKR* $p = 0.036$, *IFI44* $p = 0.0042$, *IFIT* $p = 0.0004$. *Bottom panel*, *IFI44* ($p = 0.02$) and *IFIT* ($p = 0.02$) significantly correlated with IL-18 levels, whereas *PRKR* did not reach statistical significance ($p = 0.08$). Levels of IFN-RGs in SLE are reported as their increase over levels in healthy controls ($n = 5$). **D**, Western blot of procaspase-1 (*top*) or β -actin (*bottom*) as a loading control. Blots are representative of three controls and three SLE patients. **E**, Control and SLE EPC/CACs were preincubated with either vehicle (DMSO) or inhibitors of JAK (50 μ M) or PI3K (50 nM) for 30 min prior to addition of rIFN- α as above. Fold change was expressed as compared with vehicle-treated cells ($n = 5$). Results represent mean \pm SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

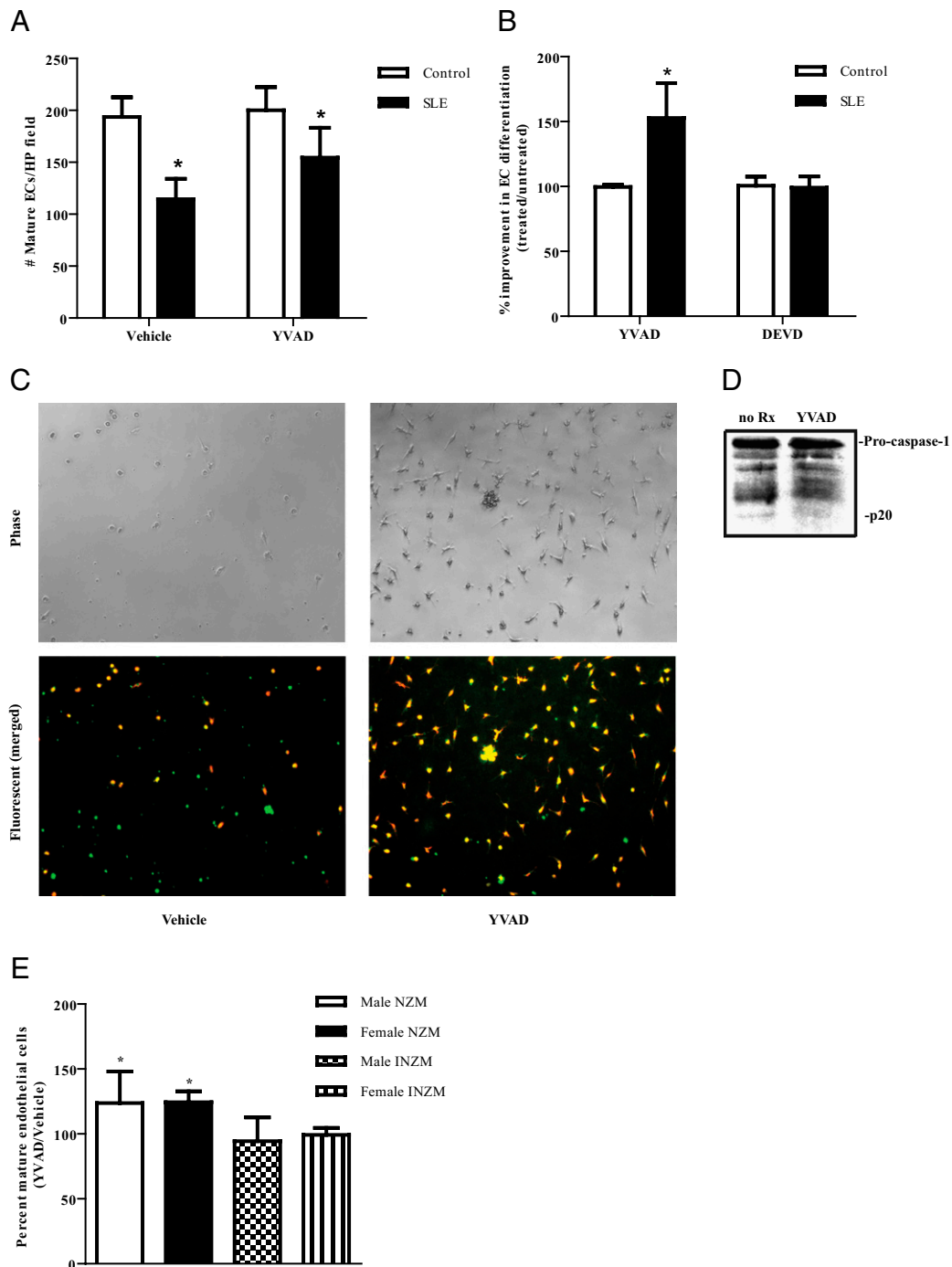


FIGURE 2. Inhibition of caspase-1 improves the capacity of human and murine lupus EPCs/CACs to differentiate into mature ECs in an IFN- α -dependent manner. *A–C*, EPCs/CACs from controls ($n = 7$) or SLE patients ($n = 10$) were incubated with either vehicle (DMSO), 10 μ M of the caspase-1 inhibitor ac-YVAD-cmk (*A*, *B*), or 1 μ M of the caspase-3 inhibitor ac-DEVD-cmk (*B*) in proangiogenic media for 14 d. ECs were quantified as those displaying dual uptake of ac-LDL (red) and UEA-1-lectin (green). Photomicrographs represent phase contrast (*top*) or fluorescent (*bottom*) images (original magnification $\times 100$) (*C*). *D*, PBMCs were treated overnight with 10 ng/ml LPS to activate caspase-1 in the presence of absence of 10 μ M ac-YVAD-cmk. Cells were then lysed in SDS-page buffer and subjected to Western blot analysis using an anti-caspase-1 Ab that recognizes both the pro (inactive) and p20 (active) forms. *E*, EPC-containing bone marrow cells from lupus prone NZM2328 (male, $n = 7$; female, $n = 5$), and NZM2328 lacking a functional INZM (male, $n = 5$; female, $n = 4$) were cultured in proangiogenic media in the presence of vehicle or 10 μ M ac-YVAD-cmk for 7 d. ECs were quantified as those displaying dual expression of ac-LDL (red) and BS-1-lectin (green). Cells were visualized by fluorescent microscopy. Results are expressed as percent improvement in EC differentiation in the presence of YVAD/vehicle alone. Bar graphs represent mean \pm SEM of individual cultures performed in triplicate for each patient or mouse. * $p < 0.05$.

prone Balb/c or SLE-prone NZM2328 mice. In both strains, preincubation with YVAD significantly blocked the ability of rIFN- α to disrupt differentiation of EPCs in culture (Fig. 3*B*). These results indicate that inhibition of caspase-1 disrupts IFN- α -me-

diated EPC/CAC dysfunction in murine and human systems. Furthermore, although IFN- α does not directly activate caspase-1, its effects require downstream caspase-1 activation for inhibition of EPC/CAC differentiation.

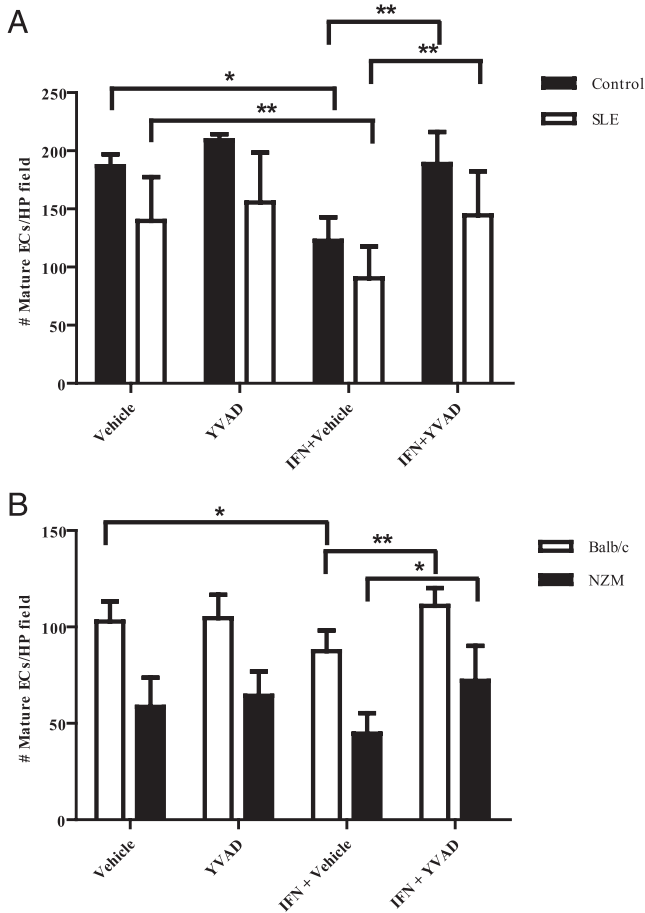


FIGURE 3. Caspase-1 blockade abrogates the inhibition of EPC/CAC differentiation induced by exogenous IFN- α . *A*, Control ($n = 3$) or SLE ($n = 6$) EPCs/CACs were cultured in proangiogenic media in the presence of vehicle or 10 μ M YVAD for 30 min and then treated with 1000 U/ml recombinant human IFN- α . Media were changed after 3 d, followed by 11 more days of growth in proangiogenic media without additional inhibitors or IFN- α . On day 14 of culture, ECs were quantified as in Fig. 2. *B*, Bone marrow cells from NZM ($n = 7$) and BALB/c ($n = 8$) mice were isolated and cultured in proangiogenic media under similar conditions as in Fig. 3*A* with or without 1000 U/ml recombinant murine IFN- α . ECs were quantified as in Fig. 2. Results represent mean \pm SEM of experiments completed in triplicate for each patient or mouse. * $p < 0.05$, ** $p < 0.01$.

IL-18 is elevated in SLE serum and mediates EPC/CAC dysfunction

We have previously shown that IL-1 β is proangiogenic and is transcriptionally repressed by type I IFNs and in SLE patients. Because inhibition of caspase-1 has positive effects on SLE EPC/CAC differentiation, we hypothesized that this improvement occurred through inhibition of IL-18 production, as previous reports have indicated that IL-18 levels are increased in SLE serum and tissues (12). IL-18 transcripts, assessed by real-time PCR, were upregulated in SLE EPC/CAC. Furthermore, IL-18 levels correlated with type I IFN signatures in each patient (Fig. 1*C*). These results indicate that, unlike IL-1 β levels, which are repressed by exposure to IFN- α (7, 30), IL-18 levels are upregulated by type I IFNs.

Quantification of serum IL-18 in SLE patients demonstrated that the presence of detectable IL-18 was significantly associated with decreased endothelial differentiation capacity of EPCs/CACs (Fig. 4*A*). In addition, serum IL-18 protein levels significantly associated with the degree of EPC/CAC dysfunction, as assessed by their differentiation into ECs (Fig. 4*B*). In contrast, IL-18 was not

detected in any of the healthy control serum samples analyzed (not shown). IL-18 serum levels or EPC/CAC dysfunction were not associated with lupus disease activity, as assessed by SLEDAI (Fig. 4*C*, 4*D*). These results indicate that IL-18 may have detrimental effects on endothelial function, possibly by promoting dysfunction of EPC/CAC differentiation, and this impact of IL-18 on the endothelium is independent of disease activity.

To evaluate whether IL-18 could directly impair EPC/CAC differentiation, healthy control EPC/CAC cultures were incubated with recombinant IL-18. In a dose-dependent manner, IL-18 significantly impaired endothelial differentiation by $\sim 50\%$ when concentrations equivalent to those seen in SLE serum were used (Fig. 5*A*, 5*B*). No further inhibition of endothelial differentiation was detected when higher concentrations of IL-18 were tested (data not shown). In addition, the levels of IL-18 in lupus serum significantly correlated with the ability of caspase-1 inhibition by YVAD to improve endothelial differentiation in EPC/CAC cultures (Fig. 4*E*). Taken together, these results indicate that inflammasome activation and enhanced synthesis of IL-18 results in EPC/CAC dysfunction.

As IL-18 levels correlate with a decreased differentiation capacity of EPCs/CACs and exogenous IL-18 interferes with differentiation of control EPC/CACs, we determined whether endogenous production of this cytokine in SLE contributes to dysfunction of these cells. By ELISA, IL-18 was not detected in the supernatants of EPC/CAC cultures (data not shown). Thus, a neutralizing Ab to IL-18 was used to disrupt paracrine or low-level production that could be contributing to EPC/CAC dysfunction. Addition of an anti-IL-18 neutralizing Ab, but not an isotype control, to SLE EPCs/CACs for the first 72 h of culture restored normal endothelial differentiation (Fig. 6*A*, 6*B*). This improvement was equivalent to improvement seen with neutralization of IFN- α (Fig. 6*A*), as has been previously demonstrated by our group (5). These results suggest that production of IL-18 by lupus EPC/CAC cultures contributes to their poor differentiation into mature ECs. In contrast, when similar experiments were performed using healthy control EPCs/CACs, IL-18 neutralization did not modify their differentiation capacity (Fig. 6*A*). These results indicate that upregulation of the inflammasome and IL-18 production in EPC/CAC cultures is a hallmark of SLE cells.

To understand the relationship between the effects of IL-18 versus IL-1 β on EPC differentiation, healthy control EPCs/CACs were incubated with rIL-18 in the presence or absence of rIL-1 β . Treatment with a combination of both cytokines was able to overcome the inhibitory effects of IL-18 (Fig. 6*C*). In addition, as mentioned above and as previously reported (7), SLE EPCs/CACs displayed improvement in their differentiation capacity with both IL-1 β stimulation and IL-18 blockade, and this effect was increased when a combination of IL-1 β and IL-18 neutralization was tested (Fig. 6*D*). These results suggest that both blockade of IL-18 pathways and activation of IL-1 β pathways enhance endothelial differentiation. The effects of both cytokines likely lie on convergent pathways, because IL-1 β can overcome the detrimental effects of IL-18. Furthermore, divergent pathways may also be involved, given the enhanced differentiation of SLE EPCs/CACs observed in the presence of IL-18 blockade and concomitant IL-1 β stimulation. In SLE, however, the dominant effect appears to be that of inhibitory IL-18 signaling, because IL-1 β pathways are repressed (7).

Specific autoantibody profiles in SLE are associated with IL-18 levels and EPC/CAC dysfunction

Recent reports indicate that the presence of anti-Ro and anti-dsDNA Abs positively correlates with increased serum IFN- α

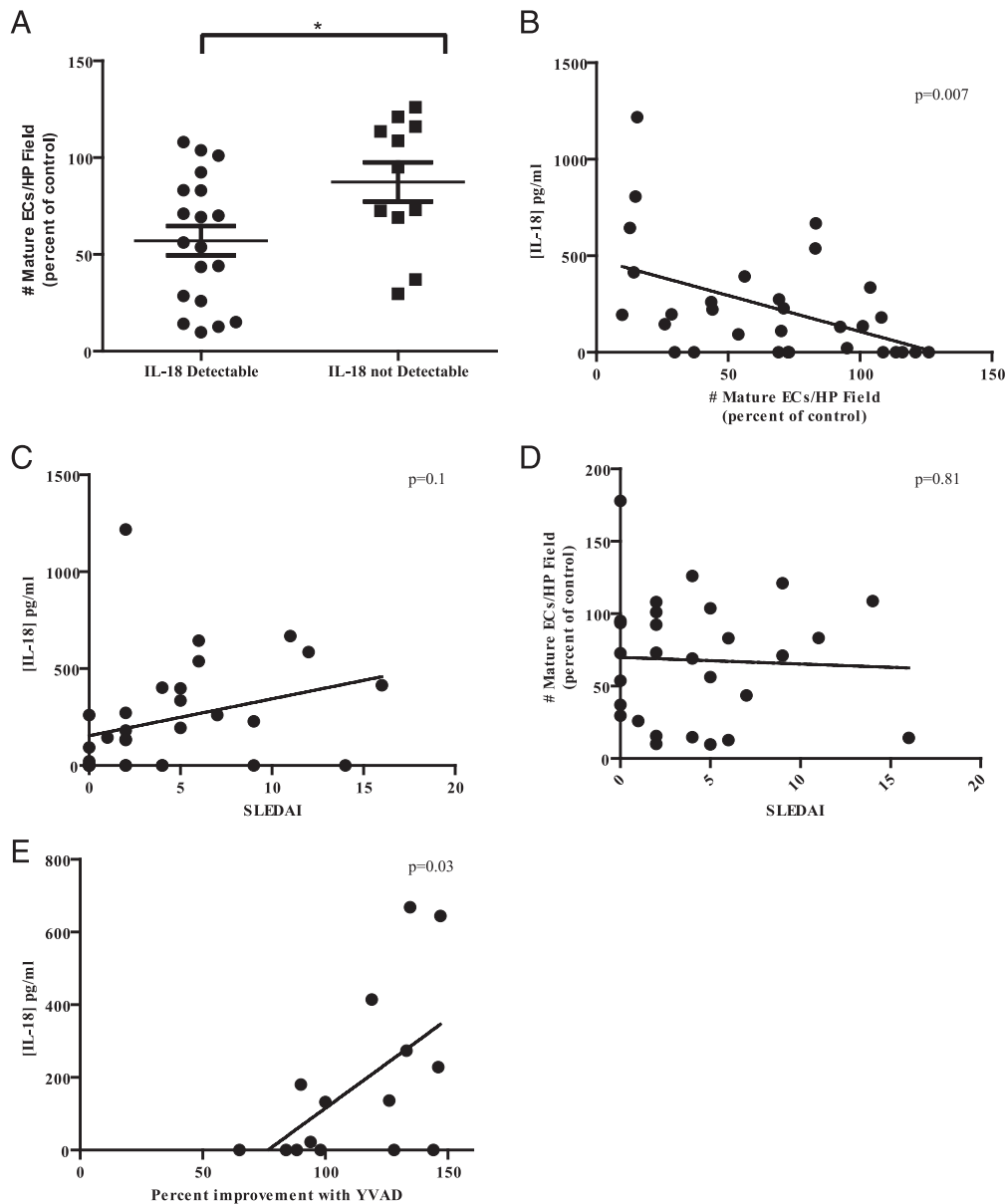


FIGURE 4. Circulating IL-18 levels correlate with EPC/CAC dysfunction in SLE. *A*, The percentage of lupus mature ECs as compared with control mature ECs obtained after 14 d of EPC/CAC culture is plotted versus the presence or absence of detectable IL-18 in SLE serum ($n = 30$). *B*, Levels of IL-18 in SLE serum ($n = 32$) were plotted against the ratio of SLE ECs after 14 d of culture as compared with age- and gender-matched controls; $r^2 = 0.226$. *C*, Serum IL-18 concentrations were plotted against SLEDAI disease activity scores determined on the day of blood draw; $r^2 = 0.09$. *D*, The average number of lupus mature ECs per power field after 14 d of EPC/CAC culture is plotted against SLEDAI disease activity scores determined on the day of blood draw; $r^2 = 0.002$. *E*, Lupus serum IL-18 levels are plotted against the percentage of improvement in EC differentiation (number of mature ECs in the presence of YVAD per field/number of mature ECs in the presence of vehicle per field) observed after caspase-1 inhibition with YVAD; $r^2 = 0.3103$; $n = 15$. * $p < 0.05$.

activity in SLE patients (31). Nucleoprotein-containing immune complexes can stimulate IFN- α production via TLR7 and TLR9 signaling, both of which can serve as priming signals for inflammasome activation (15, 32, 33). As such, we examined whether specific autoantibody production correlated with increased serum IL-18 levels. As shown in Fig. 7A, the presence of an anti-Ro Ab significantly correlated with increased IL-18 levels, whereas other nucleoprotein-containing autoantibodies, such as anti-Smith and anti-RNP did not. Neither the presence (Fig. 7A) nor titers of anti-dsDNA (data not shown) were significantly associated with IL-18 levels. Additionally, the presence of anti-Ro Abs negatively correlated with lupus EPC/CAC differentiation, whereas the presence of anti-Sm and anti-RNP did not, suggesting a putative link among anti-Ro, IL-18 levels, and EPC dysfunction (Fig. 7B).

Aberrant inflammasome activity is operational in vivo in SLE

Given the above findings, evidence for aberrant inflammasome activity was sought in vivo. Others have reported elevated IL-18 protein in class IV and V LN specimens (11). Using microdissected specimens from kidneys from control and LN patients (see Supplemental Table I for patient demographics), microarray analyses was completed and inflammasome components were examined. We observed significant upregulation of *PYCARD* (*ASC*), *CASP1*, and *IL-18* transcripts in both glomerular and tubulointerstitial compartments of LN specimens. In addition, glomeruli from nephritis patients also demonstrated significant *IL-18R* transcript upregulation (Table III). The transcriptional upregulation of *PYCARD* and *CASP1* was more pronounced in World Health Organization classes III and IV nephritis biopsies

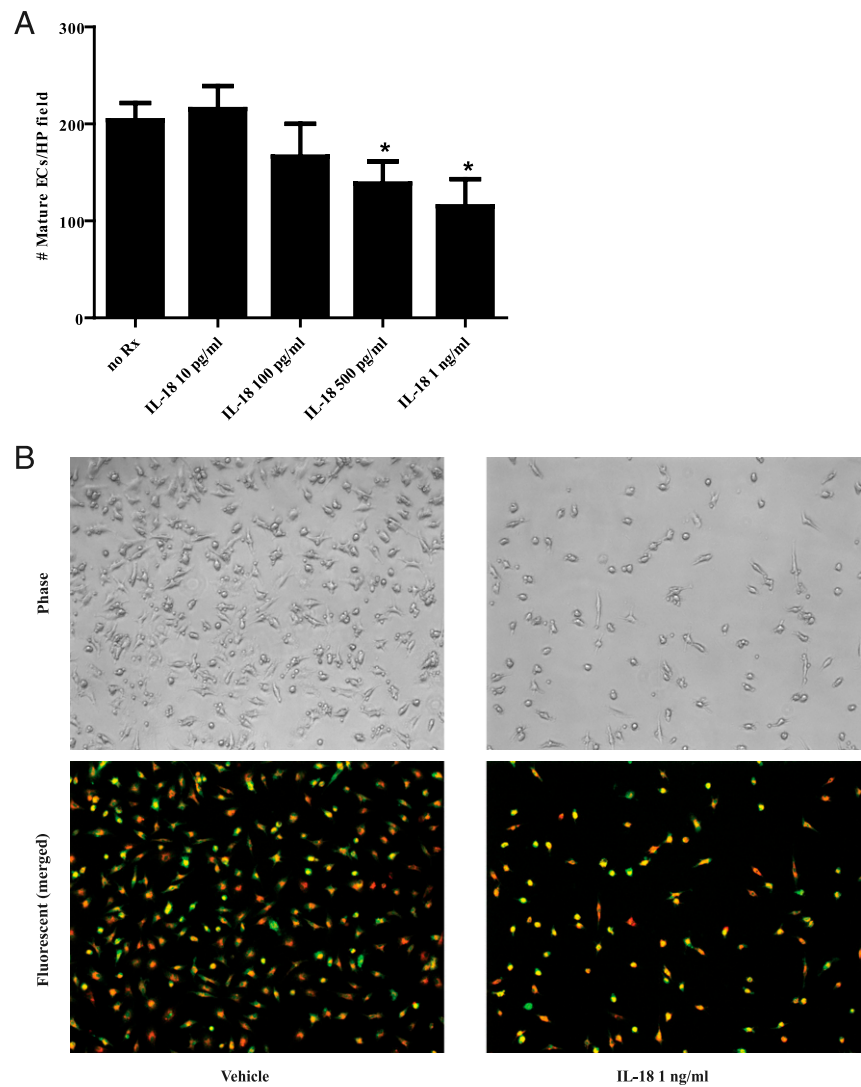


FIGURE 5. Exogenous IL-18 inhibits the capacity of control EPCs/CACs to differentiate into mature ECs. *A*, Graded concentrations of rIL-18 were added in triplicate to healthy control EPCs/CACs ($n = 6$) and cultured under proangiogenic stimulation. Media were changed after 3 d, followed by 11 d of growth in proangiogenic medium without further addition of IL-18. On day 14 of culture, ECs were quantified as those binding both ac-LDL (red) and UEA-lectin (green). Results represent mean \pm SEM of mature ECs/high power field. *B*, Representative images from Fig. 5*A* showing decreased endothelial differentiation in healthy control EPCs/CACs in the presence of 1 ng/ml IL-18. Photomicrographs represent phase contrast (*top*) or fluorescent (*bottom*) images (original magnification $\times 100$). * $p < 0.05$.

(data not shown) as compared with class II, suggesting a possible link with more aggressive disease. As we had previously noted vascular rarefaction in glomeruli from LN biopsies (7), and given our findings of the effects of IL-18 on EPC/CAC differentiation, these data suggest that elevated IL-18 in LN may contribute to decreased vascular density *in vivo* in this disease.

Discussion

The experiments reported above demonstrate for the first time, to our knowledge, a potential role for inflammasome activation in the development of type I IFN-mediated EPC/CAC dysfunction in SLE. As such, the inflammasome may play a crucial role in the development of aberrant vascular repair and increased CV risk in this disease. Caspase-1 and the inflammasome scaffold AIM2 are upregulated by IFN- α . Specific inhibition of caspase-1 improves EPC/CAC differentiation in murine and human SLE, suggesting that ongoing activation of this inflammasome-associated enzyme contributes to dysfunction of cells that are crucial in vascular repair. It appears that the deleterious effects of caspase-1 activation in EPC/CAC function are mediated by enhanced IL-18 activity, as neutralization of this cytokine restores lupus EPC/CAC differentiation capacity. This is further confirmed by the observed induction of healthy control EPC/CAC dysfunction by exogenous IL-18, as well as by the association of increased circulating levels of this cytokine with EPC dysfunction in SLE. Overall, these

observations indicate a model in which the effects of IFN- α on EPC/CACs are complex and result in skewing of inflammatory cytokine production to favor increased IL-18 activation and repression of IL-1 β , which may lead to profound dysfunction in vasculogenesis (Fig. 8). Given the important role of EPCs/CACs in vascular repair and CV disease prevention (reviewed in Ref. 3), these abnormalities may be crucial in the development of premature vascular disease in SLE.

The regulation of caspase-1 and AIM2 by functional JAK activation is consistent with current dogma regarding activation of the type I IFNR (34). In addition, the trend of inhibition of caspase-1 activation by IFN- α in the presence of PI3K inhibitors suggests an additional role for the antiviral IRS-dependent regulation of caspase-1.

IL-18 is an inflammatory cytokine with an emerging role in endothelial dysfunction and atherosclerosis. Its levels have previously been shown to correlate with decreased circulating EPC numbers in Graves' disease (22). Additionally, elevated levels of IL-18 correlate with increased vascular stiffness and intima media thickness in men without evidence of coronary artery disease, indicating that this cytokine may play a role in early stages of atherosclerosis development (35). Further, IL-18 levels correlate with instability of atherosclerotic plaque and strongly serve as a predictor of CV death in patients with subclinical atherosclerosis (36, 37). Certainly, its effects on ECs could be proposed to be

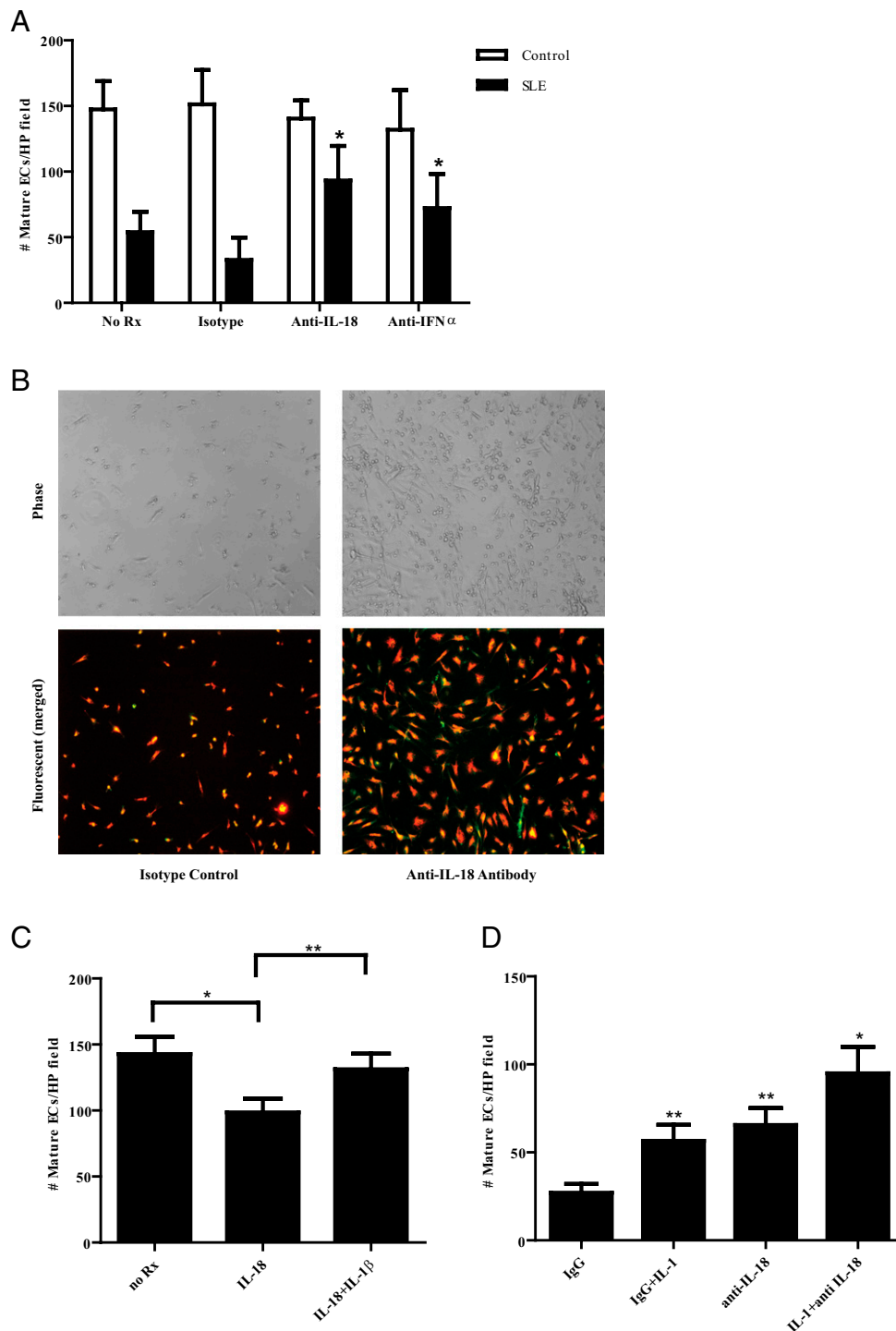


FIGURE 6. Neutralization of IL-18 in SLE EPC/CAC cultures restores endothelial differentiation, and this is enhanced with addition of IL-1 β . **A**, EPCs/CACs from control ($n = 8$) or SLE ($n = 6$) patients were cultured under proangiogenic stimuli in the presence of neutralizing anti-IL-18 (1 $\mu\text{g/ml}$), anti-IFN- α (2 $\mu\text{g/ml}$), or IgG1 isotype control (1 $\mu\text{g/ml}$) Abs. Media were changed after 3 d, followed by 11 more days of culture in proangiogenic media without additional Abs. On day 14 of culture, ECs were quantified as those which dually bound ac-LDL (red) and UEA-lectin (green). Bar graph represents mean \pm SEM. * $p < 0.05$. All patient or control samples were treated in triplicate for each experimental variable. **B**, Representative image of an SLE EPC/CAC culture after treatment with isotype or anti-IL-18 Abs as mentioned above. Photomicrographs represent phase contrast (*top panels*) or fluorescent (*bottom panels*) images (original magnification $\times 100$). **C**, EPCs/CACs from healthy controls ($n = 5$) were cultured under proangiogenic stimuli in the presence of 1 ng/ml IL-18 with or without 1 ng/ml IL-1 β . Media were changed on day 3, and mature ECs were quantified on day 14 as in **A**. **D**, EPCs/CACs from SLE patients ($n = 9$) were cultured under proangiogenic stimuli in the presence of neutralizing anti-IL-18 (1 $\mu\text{g/ml}$) or same concentration of IgG1 isotype control, both in the presence or absence of 1 ng/ml IL-1 β . Media were changed on day 3, and mature ECs were quantified on day 14 as in **A**. * $p < 0.05$, ** $p < 0.01$.

proatherosclerotic and proinflammatory, as this cytokine also upregulates IL-6, IL-8, and adhesion molecules including ICAM (9). Thus, we propose that in SLE, chronic dysregulation of

inflammasome activity, possibly induced by increased circulating IFN- α levels, results in elevated IL-18 that leads to downstream detrimental effects on vascular repair.

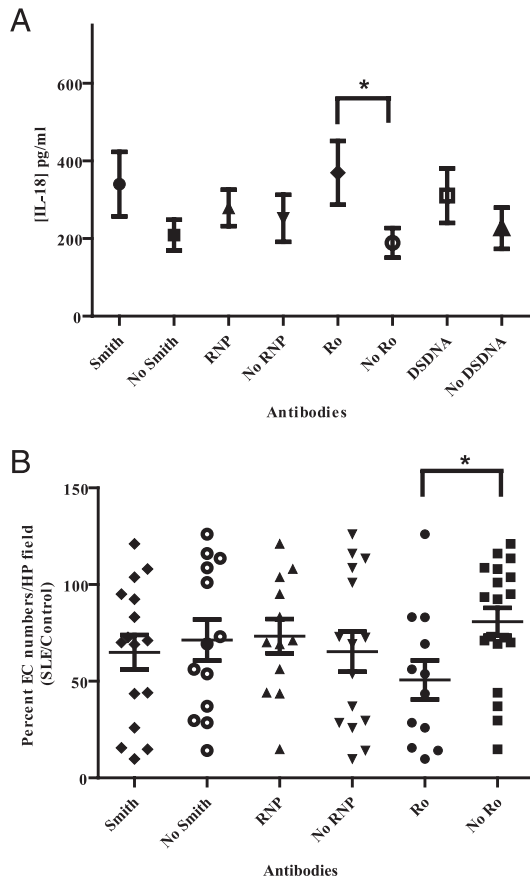


FIGURE 7. The presence of anti-Ro Abs in SLE serum correlates with circulating IL-18 levels and is negatively associated with lupus EPC/CAC differentiation capacity. *A*, Lupus serum IL-18 levels ($n = 69$) are plotted according to the presence or absence of specific autoantibodies in autologous sera. *B*, Endothelial differentiation of lupus EPC/CACs was quantified as in other figures, and the percentage of mature endothelial numbers, compared with those of age-matched healthy controls, was plotted in relationship to the presence ($n = 16$) or absence ($n = 13$) of anti-Sm, presence ($n = 12$) or absence ($n = 13$) of anti-RNP or presence ($n = 12$) or absence ($n = 19$) of anti-Ro Abs. * $p < 0.05$.

In other model systems, such as inflamed synovium, IL-18 has a proangiogenic role (38). These discrepancies of IL-18 effects on the vasculature are not necessarily contradictory. Indeed, many of the cytokines upregulated by IL-18 can in turn be proangiogenic in highly inflammatory environments, increase inflammatory cell migration and alter endothelial repair, which may also promote accelerated atherosclerosis development.

The cellular source of IL-18 in SLE remains unknown, but likely candidates would include monocytes, macrophages, and other myeloid cells that represent the classic source of inflammasome-produced cytokines (13). In EPC/CAC cultures, myeloid cells are present, especially early on, and may be the source of continued IL-18 production in vitro. This hypothesis would agree with previous findings that active IL-18 colocalizes with mononuclear phagocytes in atherosclerotic plaques, while the endothelium is rich in the IL-18R (39).

Our observations suggest that an environment characterized by enhanced type I IFN levels and activity, such as seen in SLE, skews the inflammasome machinery toward IL-18 over IL-1 β activation, through alteration of substrates available to the inflammasome. First, IL-1 β is tightly regulated and IFN- α exposure leads to significant transcriptional downregulation of this cytokine in human and murine systems (7, 30, 40). Second, IL-18 is more constitutively expressed than IL-1 β (9), and we have shown that IL-18 transcript levels positively correlate with type I IFN signatures in SLE patients. Thus, we can propose a model where, under the influence of type I IFNs, IL-1 β transcript and protein are repressed while IL-18 is upregulated. In this environment, activation of caspase-1 will result in preferential processing of IL-18 as the cytokine most available to the inflammasome machinery. Although others have proposed that alternative proteases can also cleave and activate IL-18 (41–43), our data suggest that caspase-1 inhibition improves differentiation of EPC/CAC cultures, supporting the inflammasome as the trigger of enhanced production of this cytokine in SLE. If the cellular source of IL-18 is indeed monocytes, a cell subset known to have constitutively active caspase-1 (44), then it is possible that upregulation of this enzyme by IFN- α may contribute significantly to processing and activation of IL-18 in SLE.

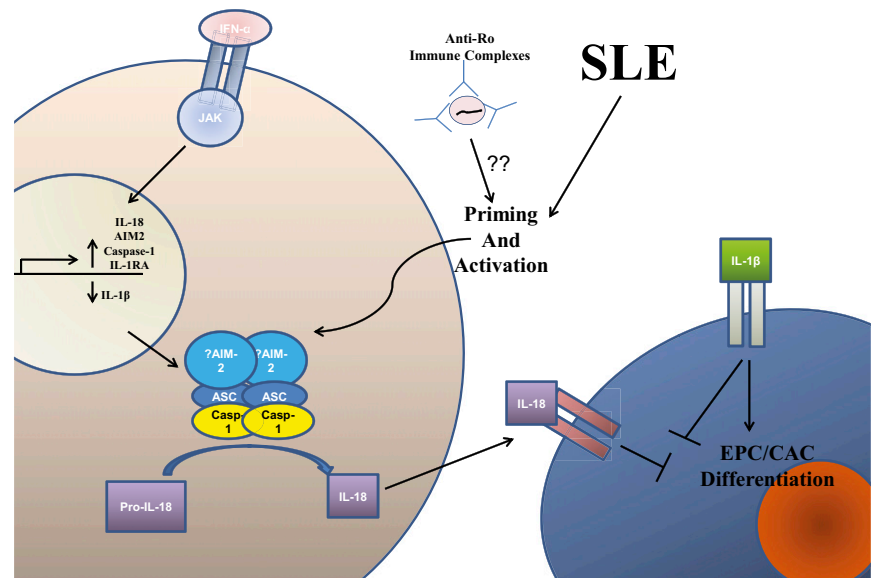
Additional steps may also contribute to the activation of the inflammasome in SLE. One possibility for inflammasome activation may be the production of neutrophil-associated extracellular traps (NETs). Lupus neutrophils are capable of releasing NETs containing DNA/antimicrobial peptide complexes that enter plasmacytoid dendritic cells and stimulate TLR9 (45). In addition, low-density granulocytes, which produce abundant NETs in vivo (46) and are found in PBMC fractions in SLE, but not in control patients (47), externalize the cathelicidin LL37 and other immunostimulatory molecules through NETosis. LL37 can stimulate inflammasome activation via induction of the P2X7 receptor (48). Priming of the inflammasome in SLE could occur via signaling through TLR7 or TLR9 via immune complex uptake (15, 33). TLR7 is typically expressed in plasmacytoid dendritic cells, but its expression is inducible in monocytes (49). As such, signaling through TLR7 could contribute to enhanced IL-18 production in

Table III. Microdissected renal biopsies from pretransplant LD and LN patients

Gene Symbol	Gene Name	Entrez Gene ID	Glomerular Compartment: LN Compared with LD Controls	Tubulointerstitial Compartment: LN Compared with Living Donor Controls
AIM2	Absent in melanoma 2	9447	<i>1.37</i>	<i>1.07</i>
PYCARD (ASC)	PYD and CARD domain containing	29108	4.14*	1.56*
CASP1	Caspase 1, apoptosis-related cysteine peptidase (IL 1 β convertase)	834	3.45*	2.27*
IL-18	IL 18 (IFN- γ -inducing factor)	3606	1.32*	1.18*
IL-18R	IL 18 receptor 1	8809	1.49*	1.00

Results in italics suggest no significant regulation. * $q < 0.05$ was considered significant.

FIGURE 8. IFN- α mediates EPC/CAC dysfunction via inflammasome upregulation and skewing toward IL-18 production. Exposure of EPCs/CACs to IFN- α results in repression of IL-1 β but upregulation of IL-18, caspase-1, and AIM2. In SLE, yet undefined priming and activation steps possibly influenced by the presence of anti-Ro immune complexes, result in activation of the inflammasome and processing of IL-18 into its mature and active form. IL-18 then exerts inhibitory effects on endothelial differentiation leading to decreased vascular repair and an increased risk of atherosclerotic disease. The inhibitory effects of IL-18 can be overcome by the presence of IL-1 β .



the presence of immune complexes, both via induction of IFN- α synthesis in plasmacytoid dendritic cells and inflammasome priming in monocytes. In addition, as the presence of IFN- α increases neutrophil netting in response to autoantibodies (50), this could explain the increased association with elevated IL-18 levels in the presence of anti-Ro Abs. Given the link we report between the presence of anti-Ro Abs, IL-18 levels and EPC/CAC dysfunction, it is possible that Ro-containing immune complexes prime monocytes to activate the inflammasome. Further research into the mechanisms involved in this potential phenomenon should be performed.

In addition, the identification of the inflammasome adaptors involved in EPC dysfunction needs to be characterized. Others have shown that the NLRP3 inflammasome is repressed by type I IFNs in bone marrow-derived macrophages, in an IL-10-dependent fashion (30). Thus, in environments with high type I IFN levels, such as in SLE, the AIM2 inflammasome may be the more relevant scaffold molecule. AIM2 is upregulated by type I IFNs and is activated by dsDNA. Others have shown increased AIM2 transcript in peripheral leukocytes from SLE patients, but no increase was found in nephritis biopsies (16). We have now confirmed that IFN- α regulates AIM2 transcript in EPC/CAC cultures. This suggests that the AIM2 inflammasome may be relevant to increased IL-18 production in SLE. Future studies will address the role of various inflammasome components in SLE-related vascular disease.

The upregulation of inflammasome components in LN biopsies suggests that inflammasome activation of IL-18 in the kidney may play a pathogenic role. Little is known about the role of caspase-1 in LN, but IL-18 has been shown to possibly play a pathogenic role in both humans and mice (11, 12, 51, 52). Vascular rarefaction, which is demonstrated in SLE but not in ANCA-associated vasculitis nephritis biopsies (7), and has been associated with progression of renal disease (53), may be reflective of aberrant vasculogenesis in LN. Given our data showing the role of caspase-1 in EPC/CAC dysfunction in SLE and the detrimental effects of IL-18 on endothelial differentiation, we speculate that inflammasome activity in LN may contribute to vascular rarefaction at the level of glomeruli and renal blood vessels and contribute to progressive renal failure. Future studies should address the role of the inflammasome components in mediating LN, and given our data on caspase-1 regulation by IFN- α , further consideration should be given to models in which type I IFNs play a role.

In conclusion, we have demonstrated a novel role for the inflammasome in contributing to EPC/CAC dysfunction, and potentially to premature vascular damage, in SLE. Enhanced levels or sensitivity to type-I IFNs may skew the inflammasome toward production of IL-18 by repression of IL-1 β and upregulation of caspase-1 and AIM2. Given its effects on EPC/CAC function, enhanced IL-18 may play a deleterious role in vascular repair mechanisms in SLE and contribute to accelerated CVD, progression of kidney damage and premature mortality in this disease.

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Disclosures

The authors have no financial conflicts of interest.

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