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*J Immunol* 2010; 185:4457-4469; Prepublished online 30 August 2010;
doi: 10.4049/jimmunol.1001782

http://www.jimmunol.org/content/185/7/4457
The Detrimental Effects of IFN-α on Vasculogenesis in Lupus Are Mediated by Repression of IL-1 Pathways: Potential Role in Atherogenesis and Renal Vascular Rarefaction

Seth G. Thacker,*1 Celine C. Berthier,†1 Deborah Mattinzoli,‡ Maria Pia Rastaldi,‡ Matthias Kretzler,‡ and Mariana J. Kaplan*

Systemic lupus erythematosus (SLE) is characterized by increased vascular risk due to premature atherosclerosis independent of traditional risk factors. We previously proposed that IFN-α plays a crucial role in premature vascular damage in SLE. IFN-α alters the balance between endothelial cell apoptosis and vascular repair mediated by endothelial progenitor cells (EPCs) and myeloid circulating angiogenic cells (CACs). In this study, we demonstrate that IFN-α promotes an antiangiogenic signature in SLE and control EPCs/CACs, characterized by transcriptional repression of IL-1α and β, IL-1R1, and vascular endothelial growth factor A, and upregulation of IL-1R antagonist and the decoy receptor IL-1R2. IL-1β promotes significant improvement in the functional capacity of lupus EPCs/CACs, therefore abrogating the deleterious effects of IFN-α. The beneficial effects from IL-1 are mediated, at least in part, by increases in EPC/CAC proliferation, by decreases in EPC/CAC apoptosis, and by preventing the skewing of CACs toward nonangiogenic pathways. IFN-α induces STAT2 and 6 phosphorylation in EPCs/CACs, and JAK inhibition abrogates the transcriptional antiangiogenic changes induced by IFN-α in these cells. Immunohistochemistry of renal biopsies from patients with lupus nephritis, but not anti-neutrophil cytoplasmic Ab-positive vasculitis, showed this pathway to be operational in vivo, with increased IL-1R antagonist, downregulation of vascular endothelial growth factor A, and glomerular and blood vessel decreased capillary density, compared with controls. Our study introduces a novel putative pathway by which type I IFNs may interfere with vascular repair in SLE through repression of IL-1-dependent pathways. This could promote atherosclerosis and loss of renal function in this disease. The Journal of Immunology, 2010, 185: 4457–4469.

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ystemic lupus erythematosus (SLE) is an autoimmune disease that primarily affects women of childbearing age (1). A significant proportion of patients with lupus develops renal disease. In addition to inflammatory nephritis, renal involvement in lupus can manifest as a fibrotic, atrophic nephropathy with significant renal functional impairment and potential progression to end-stage disease. Although this manifestation can be the result of earlier unchecked inflammation, alternative mechanisms mediating progressive loss of renal function may be envisioned, and the precise relationship between acute inflammatory and chronic fibrotic nephropathy remains unclear (2, 3).

Furthermore, SLE is characterized by strikingly higher rates of premature atherosclerotic cardiovascular (CV) disease (4–7) not explained by Framingham risk factors (6, 8, 9). Whereas immune dysregulation may play the dominant role in atherogenesis (10), the exact mechanisms leading to enhanced CV risk in lupus remain to be determined. Our group previously reported that SLE patients without traditional CV risk factors display a striking imbalance between endothelial cell damage and repair. This is manifested by an increase in circulating apoptotic endothelial cells uncoupled from proper endothelial repair, as shown by a significant decrease in the numbers and function of bone marrow-derived endothelial progenitor cells (EPCs) and myeloid circulating angiogenic cells (CACs) (11, 12). High levels of circulating apoptotic endothelial cells in SLE strongly correlate with endothelial dysfunction (12), a surrogate marker of future atherosclerosis development (13). Additional studies have also reported aberrant phenotype and function of lupus EPCs/CACs in SLE patients (14).

Type I IFNs, particularly IFN-α, have been proposed to play major pathogenic roles in SLE (15, 16). However, the possibility that they may play a prominent role in premature vascular damage in SLE had not been systematically investigated. Our group previously reported that IFN-α induces EPC/CAC apoptosis and skew myeloid cells away from CACs and toward nonangiogenic phenotypes, including mature dendritic cells (DCs) (11). Importantly, neutralization of type I IFN pathways restores normal EPC/CAC phenotype and function in SLE (14). Furthermore, the New Zealand

Abbreviations used in this paper: ANCA, anti-neutrophil cytoplasmic Ab; CAC, circulating angiogenic cell; CV, cardiovascular; DC, dendritic cell; ELA V , embryonic lethal, abnormal vision; EPC, endothelial progenitor cell; HIF, hypoxia-inducible factor; IL-1RN, IL-1R antagonist; LDL, low-density lipoprotein; N/A, not expressed above the Affymetrix control baseline; SIGN, dendritic cell-specific intercellular adhesion molecule-3–grabbing nonintegrin; SLE, systemic lupus erythematosus; UEA-1, Ulex europaeus agglutinin-1; VEGF, vascular endothelial growth factor.

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Received for publication May 28, 2010. Accepted for publication July 30, 2010.

This work was supported by the Lupus Foundation of America, the National Institutes of Health through Public Health Service (Grant HL088419), and the Anthony S. Graner Fund in Inflammation Research. This work was also supported in part by the National Institutes of Health through the University of Michigan’s Cancer Center Support (Grant P30 CA46592), the Rheumatic Disease Core Center (Grant P30 AR48310), and the Applied Systems Biology Core in the O’Brien Renal Center (Grant P30 DK081943). C.C.B. was supported by a National Kidney Foundation Post-Doctoral Fellowship.

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The online version of this article contains supplemental material.

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Black/New Zealand White F1 murine model of lupus, a mouse strain in which type I IFNs are considered to play a prominent role in pathogenesis (17–20), is also characterized by endothelial dysfunction and aberrant EPC phenotype and function (21–23). All these observations support a potential role for type I IFNs in the development of premature atherosclerosis and altered vasculogenesis in SLE.

In murine and human systems, EPCs and CACs appear to be crucial in vasculogenesis and angiogenesis (24–28). Decreases in EPC/CAC numbers and function in disease states associated to enhanced vascular complications correlate with an increased risk of atherosclerosis and vascular events (29–31). Whereas type I IFNs have been implicated in the progression of SLE (32), in the severity of lupus nephritis, and in the alteration of endothelial cell repair (11, 14), the molecular pathways by which these mechanisms interfere with vasculogenesis in SLE.

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 (41) and were enrolled from the University of Michigan outpatient rheumatology clinic. Age- and gender-matched controls were recruited by advertisement. Lupus disease activity was assessed by the SLE disease activity index (42) (Supplemental Table 1).

Cell isolation and culture and fluorescent microscopy

Human PBMCs, known to contain both EPCs and CACs (43–45), were isolated and cultured under proangiogenic stimulation, as previously described (11), with a few modifications. Briefly, PBMCs (2.27 × 10^6/cm²) were cultured in endothelial cell-specific enrichment medium (EBM2; Cambrex, East Rutherford, NJ) on fibronectin-coated wells (BD Biosciences, Franklin Lakes, NJ). Typically, after 1–3 wk in culture, these cells differentiate into mature endothelial cells that display typical mature endothelial cell markers and display functional characteristics of vascular cells (43–45). For one of the experiments (gene expression studies), media was changed after 72 h and fresh media was added without human rIFN-α 2b (Scheringer-Pough, Kenilworth, NJ) at a final concentration of 1000 IU/ml. Cells were incubated in the presence or absence of IFN-α for 6 h prior to RNA harvesting. This dose was chosen due to its effects in inhibiting vasculogenesis in control EPC/CAC cultures (11). Similar experiments were performed with EPCs directly obtained from control bone marrow. In brief, CD133+ cells obtained from healthy control bone marrows (AllCells, Emeryville, CA) were thawed following manufacturer’s recommendations, and next cultured in StemSpan SFEM (StemCell Technologies, Vancouver, British Columbia, Canada) for 48 h to allow for cell recovery and expansion, then under similar conditions as peripheral blood EPCs/CACs, but at a density of 5 × 10^5 cells/ml.

To assess the capacity of peripheral blood EPCs/CACs to differentiate into mature endothelial cells, media was changed 120 h after plating, then every 3 d. On days 14–21, cells were incubated with markers of mature endothelial cells, including 1–diododecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate-acetylated low-density lipoprotein (LDL; Biomedical Technologies, Stoughton, MA), and FITC Ulex europaeus agglutinin-1 (UEA-1; Vector Laboratories, Burlingame, CA). In experiments to assess the effect of type I IFNs on SLE and control EPCs/CAC function, IL-1β (10 ng/ml), IL-6 (10 ng/ml), TNF-α (10 ng/ml; PeproTech, Rocky Hill, NJ), or vascular endothelial growth factor (VEGF; 1 µg/ml; National Cancer Institute, Bethesda, MD) was added to EPC/CAC cultures and replenished every 48 h.

To assess endothelial cell morphology and expression of endothelial cell markers, cells were analyzed by peroxidase microscopy using a Leica (Bannockburn, IL) DMIRB fluorescent inverted microscope. Images were acquired at room temperature using live cells in PBS without mounting media. A total of eight random fields of view was acquired, and images were analyzed using the CellC program (http://www.cs.tut.fi/sgn/csb/cellc/) to quantify mature endothelial cells, which were considered as those that coexpress UEA-1 and acetylated LDL. Images were acquired with an original total magnification ×100. The numeric aperture of the fluorescent microscope was 0.3. Images were acquired with an Olympus DP30BW camera (Olympus, Tokyo, Japan) using the acquisition software Olympus-BSW (Olympus). Final processing was done with Adobe Photoshop CS2 (San Jose, CA).

Phosphorylated STAT detection and inhibition of IFN-α signaling pathways

EPCs/CACs were cultured under proangiogenic stimulation, as stated above for 60 h, and then media was harvested and changed to endothelial cell growth medium without 20% FBS. After overnight incubation in reduced media, cells were stimulated with 1000 IU IFN-α for 30 min, followed by fixation with 4% paraformaldehyde. Cells were permeabilized with 90% methanol and incubated with anti-pSTAT2 FITC (R&D Systems, Minneapolis, MN), and anti-pSTAT6 FITC (Cell Signaling Technology, Danvers, MA). Immunofluorescence was acquired using ACSF/Caliburn (BD Biosciences, Bedford, MA), followed by analysis with FlowJo (Tree Star, Ashland, OR).

To inhibit IFN-α signaling, the pan-JAK inhibitor 2-(1,1-dimethylthyl)-9-fluoro-3,6-dihydro-7H-benz[b]imidaz[4,5-f]isoquinolin-7-1 (pyridone 6) or the PI3K inhibitor (5'-4-fluoro-2-hydroxyphenyl)furan-2-ylmethylene thiazolidine-2,4-dione were used (Calbiochem, Gibbstown, NJ). 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 IFN-α. EPCs/CACs were incubated for additional 6 h, and then total RNA was isolated.

RNA isolation

Total RNA was isolated with TriPure (Roche, Indianapolis, IN), following the manufacturer’s recommendations. For microarray analysis, RNA was further purified and concentrated using an RNAeasy 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

Affymetrix Human U133 Plus 2.0 Genechips (Affymetrix, Santa Clara, CA) were processed at the University of Michigan Microarray Core Facility following the manufacturer’s instructions (46). The samples analyzed and compared were as follows: untreated and IFN-α–treated peripheral blood EPCs/CACs from healthy control and lupus patients (n = 6 in each group), as well as untreated and IFN-α–treated bone marrow EPCs from healthy controls (n = 5 in each group). The CEL files were normalized in GenePattern (http://www.GenePattern.org) using the RMA (Robust MultiChip Average) method and the Human Entrez Gene custom CDF annotation version 10 (http://brainarray.mbi.ucsf.edu/Brainarray/de-fault.lap). Of the 17,527 gene IDs (corresponding to the 54,675 Affymetrix probesets), the number of genes expressed above the Poly-A Affymetrix control expression baseline (negative controls) and used for further analyses were, respectively, 15,700, 15,186, and 15,909 in the healthy control peripheral blood cells (n = 12), the SLE peripheral blood cells (n = 12), and the healthy bone marrow EPCs (n = 10). Normalized data files are available on Gene Omnibus Web site, under the reference number GSE23203 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE23203). Statistical paired analyses were performed using significance analysis of microarrays comparing control IFN-α–treated with untreated peripheral blood cells, lupus IFN-α–treated with untreated peripheral blood cells, and healthy IFN-α–treated with untreated bone marrow EPCs. The significantly regulated genes between the groups (q < 0.05 depicting the false discovery rate) were analyzed by building biological literature-based networks using Genomatix Bibliothek Pathway Edition software (Genomatix, Munich, Germany; http://www.genomatix.de) (47, 48). Canonical pathways were analyzed using the Ingenuity Pathway Analysis Software (http://www.ingenuity.com).

Real-time quantitative PCR

Total RNA was transcribed into cDNA using oligo(dT) and Moloney murine leukemia virus (Invitrogen, Carlsbad, CA) with 1 µg RNA using a MyCycler thermocycler (Bio-Rad, Hercules, CA), and levels of expression of the following genes were measured: IL-1β, 5′-ATG TCT GTA ACT TGT GGC ATC TTC-3′ (forward) and 5′-AGA CAA TTA CAA AAG GCG AAG GAG CTC-3′ (reverse); IL-1α, 5′-CCA GAA GAA GAG GAG GAT GTC C-3′ (forward) and 5′-TCC CAC AAT AAG AAC AAG ACC AAC-3′ (reverse); IL-1RN, 5′-CCT CAG ATG GAT GCT GTC AAG AAC-3′ (forward) and 5′-ATG CTG ACT CAA AGG AGA AGA TC-3′ (reverse);
IL-1RI, 5′-GAA GCC TGA TGG TTC TCT GAC TAA AAT GA-3′ (forward) and 5′-AGG TCT GAG AAG AGT GTG ATG ATA AGC-3′ (reverse); IL-1R2, 5′-AAA ATT TGC GGG TAT GAG AAG ACC G-3′ (forward) and 5′-AAG TCT GCA CTA CTA GAA ATG CTG CT-3′ (reverse); VEGF-A, 5′-GTT CTC TGT TGG ATG GCA GTA G-3′ (forward) and 5′-CAC CCA TGG CAG AAG GAG GA-3′ (reverse); MX-1, 5′-TACCGAG-GACTCAGGAT-3′ (forward) and 5′-TGGCAAGAGTCTTATAG-3′ (reverse); and β-actin, 5′-CAT CAC GAT GCC AOT GGT AGC-3′ (forward) and 5′-AAC GCC GAG AAG ATG ACC CAG-3′ (reverse).

Real-time PCR was carried out using an ABI PRISM 7900HT (Applied Biosystems, Foster City, CA) with the following cycling conditions: an initial denaturing/activation at 95˚C for 10 min, followed by 40 cycles of 92˚C for 15 s and 72˚C for 30 s.

Assessment of serum protein levels, EPC/CAC proliferation, and apoptosis

Serum IL-1R antagonist (IL-1RN) and IL-1β were quantified by ELISA (R&D Systems), following the manufacturer’s instructions. EPC/CAC proliferation and caspase 3/7 activation were assessed after 1 d in proangiogenic culture using the XTT cell proliferation kit (Cayman Chemical, Ann Arbor, MI) and the Apo-ONE caspase 3/7 assay (Promega, Madison, WI), respectively, following the manufacturers’ instructions.

Assessment of myeloid cell phenotype

After 5 d in proangiogenic culture in the presence or absence of 10 ng/ml IL-1β, cells were harvested using cell dissociation buffer (Invitrogen) and incubated with the following fluorochrome-conjugated anti-human mAbs: anti-CD14, anti-CD11c, anti-class II MHC (Ancell, Bayport, MN), and anti-CD68 (BD Biosciences). Immunofluorescence was quantified by FACS with a FACS Calibur, followed by analysis with FlowJo.

Human kidney tissue and immunohistochemistry

Kidney tissue was obtained from 25 renal biopsies from subjects with clinical and histological diagnosis of lupus nephritis [5 with class II, 5 with class III, 5 with class IV, and 5 with class V, according to the new SLE nephropathy classification (29)]; anti-neutrophil cytoplasmic Ab (ANCA)-associated vasculitis (n = 5); and, for comparison, normal samples from 5 control kidneys from tumor nephrectomies. Relevant clinicohistological parameters are given in Supplemental Table II.

Tissue samples for light microscopy were fixed in 10 mM citrate buffer (pH 6.0), treated with microwave irradiation for 30 s, followed by 3 cycles of 92˚C for 10 min, and then cooled to room temperature. After incubation with 0.5% avidin (Sigma-Aldrich, Gallarate, Milan, Italy) and 0.01% biotin (Sigma-Aldrich), to suppress endogenous avidin-binding activity, 3% H2O2 solution was applied to block endogenous peroxidase. After washing, sections were sequentially incubated with the primary Abs CD31, IL-1β, and IL-1RN (Abcam, Cambridge, U.K.), anti-CD14, anti-CD11c, and anti-class II MHC (AnCell, Bayport, MN), and anti-CD68 (BD Biosciences). DAPI staining was performed (a Mann-Whitney test or, in the case of the ELISA analysis, a Mann-Whitney t test was performed to determine differences; a p value <0.05 was considered significant.

Results

IFN-α induces an antiangiogenic signature in control and lupus EPCs/CACs

The demographic and clinical characteristics of the patients and controls studied are included in Supplemental Table I. The effect of IFN-α on peripheral blood EPCs/CACs and on bone marrow EPCs exposed to proangiogenic stimulation on steady state mRNA levels was assessed with Affymetrix genechip microarrays. IFN-α-treated versus untreated control and lupus cells exposed to angiogenic stimulation showed no differential expression in IFN-α mRNA, but did show a significant up-regulation of type I IFN-inducible genes (Table I).

For the healthy control EPCs/CACs, a total of 2850 genes (q <0.01) was found to be differentially regulated by IFN-α (Supplemental Table III). Assessing the association of these transcripts with canonical pathways using ingenuity pathway analysis identified IFN signaling as the top regulated pathway in IFN-α–treated cells compared with nontreated (p = 3.98E-05; Supplemental Table IV). Transcriptional network analysis integrating differentially regulated mRNAs with literature mining and automated promoter analysis (Biosophware software suite; Genomatrix) highlighted IL-1β and VEGF-A among important regulatory nodes (Supplemental Fig. 1).

IL-1β and VEGF-A were significantly downregulated in IFN-α–treated control EPCs/CACs when compared with untreated cells (0.7-fold for both genes, q < 0.05; Table I).

Based on these results, we verified whether IL-1β was also regulated in lupus EPCs/CACs after IFN-α exposure. Affymetrix microarray analysis defined 1631 genes significantly up- and downregulated by exogenous IFN-α, with a fold-change ≥0.15 between the untreated and treated lupus cells (q < 0.05; Supplemental Table V). IFN signaling also appeared as the top regulated pathway (p = 5.75E-07; Supplemental Table V). Similar to the IFN-α–treated control cells, the literature-based network analysis of those genes highlighted a major IL-1β node. This IL-1β node and the regulated gene nodes with a direct edge to IL-1β (201 genes in total) are displayed in Fig. I and listed in Supplemental Table VI. IL-1β was downregulated 0.6-fold in IFN-α–treated SLE cells when compared with untreated SLE cells (Table I). IL-1RN is a member of the IL-1 family that binds to IL-1Rs without inducing a cellular response, thereby antagonizing the effects of IL-1α and IL-1β (50, 51). IL-1RN mRNA expression was upregulated 11-fold in SLE cells after treatment with IFN-α, whereas IL-1α was downregulated (0.43-fold) in the IFN-treated cells (Table I; q < 0.05). The expression of IL-1RN gene was doubled in SLE compared with the healthy control EPCs/CACs (fold-change 11.0 versus fold-change 4.2, respectively; Table I). Similar to what was found in the control cells, treatment with IFN-α led to decreased expression of the proangiogenic molecule VEGF-A (52) (fold-change 0.78) in the lupus cells (Table I). Thus, microarray analysis of EPCs/CACs from control and SLE patients exposed to proangiogenic stimuli identified an antiangiogenic signature secondary to type I IFN treatment and a more pronounced antiangiogenic response in SLE than in control cells in response to this cytokine.

Healthy CD133+ bone marrow EPCs were found to have 710 genes (q < 0.05; Supplemental Table VII) regulated by IFN-α. Again the IFN signaling pathway was identified as the top regulated one (p = 6.76E-10, ingenuity pathway analysis; Supplemental Table VII). Although the IFN-α and β mRNA expression were not modified, the IFN-inducible genes showed significant upreg-
Table 1. Affymetrix microarray expression data of studied genes in peripheral blood EPCs/CACs and bone marrow EPCs

<table>
<thead>
<tr>
<th>Entrez Gene ID</th>
<th>Gene Symbol</th>
<th>Gene Name</th>
<th>IFN-α-Treated Compared with Nontreated Healthy Control Peripheral Blood EPCs/CACs</th>
<th>IFN-α-Treated Compared with Nontreated SLE Peripheral Blood EPCs/CACs</th>
<th>IFN-α-Treated Compared with Nontreated Healthy Control Bone Marrow EPCs</th>
</tr>
</thead>
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<tr>
<td>3439</td>
<td>IFN-α1</td>
<td>IFN-α1</td>
<td>1.29 0.243</td>
<td>1.08 0.242</td>
<td>1.06 0.999</td>
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<td>3440</td>
<td>IFN-α2</td>
<td>IFN-α2</td>
<td>N/A N/A</td>
<td>N/A N/A</td>
<td>N/A N/A</td>
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<tr>
<td>3454</td>
<td>IFNAR1</td>
<td>IFN (α, β, and ω) receptor 1</td>
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<td>0.79 0.002*</td>
<td>0.80 0.144</td>
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<tr>
<td>3455</td>
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<td>4599</td>
<td>MX1</td>
<td>Myxovirus resistance 1, IFN-inducible protein p78</td>
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<td>6.00 0.000*</td>
<td>6.09 0.003*</td>
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<tr>
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<tr>
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</tr>
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<td>3554</td>
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<td>2.56 0.000*</td>
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<td>VEGFA</td>
<td>VEGF-A</td>
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<td>0.78 0.002*</td>
<td>1.04 0.999</td>
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<tr>
<td>5175</td>
<td>PECAM1</td>
<td>Platelet/endothelial cell adhesion molecule</td>
<td>1.10 0.091</td>
<td>1.05 0.242</td>
<td>0.88 0.098</td>
</tr>
</tbody>
</table>

* q < 0.05 was considered significant.
N/A, not expressed above the Affymetrix control baseline.

ululation in IFN-α–treated compared with untreated bone marrow cells (fold-changes from 2.35 to 9.84; Table I). As observed in the healthy control and lupus PBMCs, IL-1RN mRNA was 3.4-fold increased when cells were exposed to IFN-α compared with non-exposed cells.

Analysis by real-time PCR confirmed that IL-1β, IL-1R1, and VEGF-A mRNA levels were significantly downregulated and IL-1RN and the decoy receptor IL-1R2 significantly upregulated in IFN-α–treated control and lupus peripheral blood EPCs/CACs when compared with untreated cells (p < 0.05; Fig. 2A). Because higher levels and/or increased sensitivity to type I IFNs have been previously reported in SLE (53, 54), we then assessed whether similar changes in IL-1–related molecules were seen at the protein level in vivo. A significant increase in circulating IL-1RN levels could be detected in the serum of SLE patients compared with healthy controls (p < 0.02; Fig. 2B). Both circulating IL-1β and IL-1α protein levels were below the limit of detection in SLE and control serum (data not shown). We had previously reported that SLE patients have decreased serum levels of VEGF-A compared with healthy controls, further supporting the microarray and real-time PCR data (11).

**IL-1β restores the capacity of lupus EPCs/CACs to differentiate into mature endothelial cells**

We had previously reported that lupus EPCs/CACs, in contrast to control cells, fail to effectively differentiate into endothelial-like cells. Lupus EPCs/CACs cannot form a mature endothelial monolayer when cultured under proangiogenic stimulation on fibronectin-coated wells. We previously showed that IFN-α blockade restores the functional capacity of lupus EPCs/CACs (11). Given that IFN-α downmodulated IL-1 and VEGF pathways, we next tested whether addition of IL-1β and/or VEGF-A to lupus EPC/CAC cultures would restore the capacity of these cells to differentiate into a mature endothelium. To this end, IL-1β and/or VEGF-A were added to proangiogenic media, and the number of mature endothelial cells was counted as determined by the uptake of acetylated LDL and the binding of the UEA-1 lectin in control and SLE cells after 14–21 d in culture. We again confirmed a significant decrease in the capacity of SLE EPCs/CACs to differentiate to mature endothelial cells when compared with control samples. Addition of IL-1β significantly increased the number of mature endothelial cells by 3-fold in lupus cell cultures when compared with media alone (p = 0.010; Fig. 3A, 3B). VEGF-A also increased the number of mature endothelial cells by 2-fold (p = 0.045; Fig. 3A, 3B). The specificity of the response to molecules regulated by IFN-α was demonstrated, as treatment with other proangiogenic factors (IL-6 and TNF) (55–57) not regulated by IFN-α treatment in the gene expression analysis (data not shown) did not result in any improvement in EPC/CAC function (Fig. 3A, 3B). In addition, the beneficial effect of IL-1 and VEGF-A was only seen in SLE samples, but not in controls (Fig. 3A, 3B).

**IL-1β abrogates the defects in proliferation and viability of lupus EPCs/CACs**

Various mechanisms may lead to improvements in lupus EPC/CAC function by addition of IL-1β, including increased proliferation or increased viability, including modulation of caspase activity. IL-1β has been shown to increase proliferation of murine EPCs (58), rat bone marrow cells (59), and human T (60–62) and B cells (61, 63). IL-1β is also able to inhibit apoptosis and promote survival of monocytes (64) and granulocytes (65). With evidence for increased
proliferation and viability possibly playing a role in the improvement in EPC/CAC function, we tested whether supplementation with IL-1β and/or VEGF-A promoted increases in proliferation and/or protection from apoptosis. SLE and control cells were cultured with proangiogenic media in the presence or absence of IL-1β and/or VEGF-A for 24 h. Lupus EPC/CAC proliferation was significantly increased by the addition of rIL-1β ($p = 0.023$) or VEGF-A ($p = 0.019$) when compared with cells cultured with media alone (Fig. 4A). Treatment with a combination of IL-1β and VEGF-A did not show a synergistic or additive effect on proliferation compared with cells treated with IL-1β or VEGF-A only (Fig. 4A). When control EPCs/CACs were treated with IL-1β, there was a trend of increased proliferation ($p = 0.084$). Control cells treated with VEGF-A alone did not show any increase in proliferation, but the combination of IL-1β and VEGF-A did significantly ($p = 0.016$) improve proliferative capacity (Fig. 4A).

Caspases 3 and 7 play a central role in the execution phase of apoptosis (66), and activation of these molecules is used as a marker of programmed cell death. The mRNA levels of these caspases were significantly upregulated by IFN-α treatment (Table 1), and caspase 3 was also a subnode in the literature-based Bibliosphere network (Fig. 1). Untreated lupus EPCs/CACs displayed significant increases in caspase 3/7 activation in the presence of media alone, when compared with control cells (Fig. 4B), indicating increased apoptosis. Treatment of lupus EPC/CAC cultures with rIL-1β significantly inhibited caspase 3/7 activation ($p = 0.045$; Fig. 4B), whereas treatment with VEGF-A did not have any effect. A combination of rIL-1β and VEGF-A decreased caspase 3/7 activity to a similar level as IL-1β treatment alone. These results indicate that both IL-1β and VEGF-A can improve EPC/CAC proliferation, but only IL-1β has an effect in inhibiting enhanced apoptosis on these cells. In contrast, neither IL-1β nor VEGF-A addition had any effect on healthy control EPC/CAC apoptosis (Fig. 4B).

IL-1β decreases lupus DC differentiation

We previously reported that, in EPC/CAC cultures, IFN-α alters the expression of surface developmental markers on myelomonocytic cells, consistent with skewing the differentiation of these cells.
cells from angiogenic CACs to IFN-α–derived DCs. Indeed, IFN-α induces a significant reduction in DC-dendritic cell-specific intercellular adhesion molecule-3–grabbing nonintegrin (SIGN)+ CD14+ cells, CD86 upregulation, and overall downregulation of CD14 (11). To address whether addition of IL-1β could inhibit this phenomenon and allow myeloid lupus cells to favor proangiogenic differentiation rather than DC differentiation, surface markers of mature DCs were examined in 5-d cultures of lupus PBMCs subjected to proangiogenic stimulation in the presence or absence of IL-1β. Addition of IL-1β led to a significant downregulation of the mature DC markers CD86 and MHC class II on SLE patients, and a similar trend was observed in healthy controls (Table II). There was also a decrease in CD11c+ CD14+ cell population, indicating a decrease in total DCs. Additionally, expression of CD14 was increased in cultures, suggesting an increase in myeloid CACs. These results indicate that the skewing of myeloid cells into nonangiogenic pathways by IFN-α is mediated, at least in part, by downregulation of IL-1 pathways.

FIGURE 3. IL-1β abrogates the abnormal phenotype of lupus EPCs/CACs. A, Addition of rIL-1β to lupus EPC/CAC cultures improves the ability of these cells to differentiate into mature endothelial cells to levels comparable to those of healthy controls (*p < 0.01; n = 24), whereas addition of rVEGF-A resulted in a more modest, but significant improvement (*p < 0.05; n = 24). Addition of IL-6 or TNF-α to the same cultures had no beneficial effect when compared with untreated cultures (n = 9). Control cells showed no significant improvement in their capacity to differentiate into mature endothelial cells with any of the treatments mentioned above (n = 7, n = 5, n = 3, and n = 3, respectively). Results are expressed as a ratio of the number of endothelial cells/high power field to the number of untreated lupus endothelial cells/high power field (*p < 0.05). B, Representative images displaying the effects of these proteins on EPC/CAC cultures from two SLE patients at 2–3 wk. Original magnification ×100; scale bar, 200 μm. Mature endothelial cells show coexpression of FITC UEA-1 (green) and uptake of 1’-dioctadecyl-3,3’,3’-tetramethylindocarbocyanine perchlorate-acetylated LDL (red).
VEGF-A supplementation. Results represent mean duplicate wells.

A significant decrease of HIF-1α in SLE EPCs/CACs, have an ischemia response element to IFN-γ, a ubiquitously expressed RNA-binding protein (ELAV), a major substrate for tyrosine phosphorylation subsequent to IFN binding. The mechanism for VEGF-A downregulation in EPCs/CACs.

Various pathways potentially involved in the regulation of IL-1β family members and VEGF were investigated. Hypoxia-inducible factor (HIF)-1α is the main regulator of VEGF-A expression, mainly by posttranslational regulation via proteasomal degradation and stabilization by von Hippel-Lindau factor (67). HIF-1α is a transcription factor, and 117 genes, including IL-1β and VEGF-A, of the 2850 in SLE EPCs/CACs, have an ischemia response element contained in their promoter. A significant decrease of HIF-1α mRNA expression was detected in lupus EPCs/CACs treated with IFN-α; the significance was not reached in control cells (Table III). However, there was no significant correlation between mRNA levels of HIF-1α and VEGF-A in each group studied (data not shown), indicating that it is unlikely that the changes in HIF-1α steady state mRNA levels induced by IFN-α were the main determinants of VEGF-A downregulation in EPCs/CACs.

Regulation of VEGF-A occurs not only at the transcriptional level, but also posttranscriptionally (68), and includes mRNA stabilization by the HuR protein, a ubiquitously expressed RNA-binding protein (68). To determine whether HuR mRNA stabilization may play a role in VEGF-A changes observed secondary to IFN-α, we analyzed mRNA expression of known HuR targets (69) in the microarray expression profiles. Of the 14 HuR targets examined, only 4 (including VEGF-A mRNA) were significantly reduced in lupus EPCs/CACs treated with IFN-α, whereas 3 were reduced in the IFN-treated control EPCs/CACs (Table III). Whereas HuR mRNA was significantly reduced in both control and SLE EPCs/CACs upon exposure to IFN-α, several HuR targets were either unchanged or increased after IFN-α treatment. Overall, these results suggest that HuR-dependent RNA degradation is unlikely to be an important mechanism for VEGF-A reduction induced by IFN-α.

Furthermore, control bone marrow CD133+ EPCs did not show any significant HIF1A or HuR mRNA expression changes in response to IFN-α (Table III).

The large family of IFN-αβ proteins all bind to a single type of receptor, which is composed of two chains: IFNAR1 and IFNAR2. The intracellular domain of IFNAR1 associates with a member of the JAK kinase family, Tyk2, whereas IFNAR2 associates with Jak1. The major substrates for tyrosine phosphorylation subsequent to IFN binding are members of the STAT family of transcription factors. These proteins are normally latent and reside in the cytoplasm in unstimulated cells. Once phosphorylated, STAT1 and STAT2 dimerize and assemble with IFN regulatory factor-9 to form the multimeric transcription factor, latent cytotoxic transcription factor. Latent cytotoxic transcription factor binds to the IFN-stimulated response element of IFN-stimulated genes in the nucleus and activates their transcription (70). From the microarray analyses, the JAK/STAT pathway was one of the most significantly regulated canonical pathways in control and SLE EPCs/CACs after IFN-α treatment (p = 0.023 and 0.017, respectively). Indeed, SLE peripheral blood EPCs/CACs exposed to IFN-α significantly upregulated STAT1, 2, 3, 4, 5A, and 6, whereas control EPCs/CACs significantly upregulated STAT2, 4, 5A, and 6 (Table III) and control bone marrow CD133+ EPCs significantly upregulated STAT1 and 2 in response to IFN-α treatment (Table III). Confirming the array data, when control and SLE peripheral blood EPCs/CACs allowed to differentiate for 3 d were stimulated with IFN-α for 30 min, there were significant increases in phosphorylation of both STAT2 and 6 (p < 0.05; Fig. 5). We then performed subanalysis on EPCs and CACs; in the EPCs, STAT2 and 6 were significantly activated upon IFN-α stimulation (p = 0.04 and 0.02, respectively; Fig. 5A), whereas in CACs there was a significant activation of STAT2 (p < 0.05; Fig. 5B) and a trend toward STAT6 activation. We then tested whether JAK inhibition would abrogate the downregulation of proangiogenic molecules seen in IFN-α–treated cells. Indeed, the pan-JAK inhibitor pyridone 6 induced significant upregulation of IL-1α, IL-1β, IL-1R1, and VEGF-A (p = 0.02, 0.001, 0.01, and 0.01, respectively; Fig. 5C) and downregulation of IL-1RN and the canonical type I IFN-inducible gene MX1 (p = 0.06

### Table II. Effect of IL-1β on myelomonocytic cell differentiation

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls</th>
<th>Lupus</th>
<th>p Value</th>
<th>Healthy controls</th>
<th>Lupus</th>
<th>p Value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Untreated</td>
<td>+IL-1β</td>
<td></td>
<td>Untreated</td>
<td>+IL-1β</td>
<td></td>
</tr>
<tr>
<td>CD14</td>
<td>61.4 ± 6.23</td>
<td>75.9 ± 6.63</td>
<td>0.018</td>
<td>51.7 ± 6.82</td>
<td>75.3 ± 4.68</td>
<td>0.002</td>
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<tr>
<td>CD11c</td>
<td>51.8 ± 4.70</td>
<td>50.3 ± 6.56</td>
<td>0.779</td>
<td>46.6 ± 6.64</td>
<td>41.7 ± 7.61</td>
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<td>DC-SIGN</td>
<td>21.7 ± 0.96</td>
<td>12.2 ± 1.87</td>
<td>0.318</td>
<td>20.9 ± 6.68</td>
<td>5.8 ± 1.82</td>
<td>0.033</td>
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<tr>
<td>CD86</td>
<td>57.5 ± 3.29</td>
<td>36.1 ± 7.21</td>
<td>0.117</td>
<td>40.5 ± 5.93</td>
<td>28.4 ± 5.62</td>
<td>0.079</td>
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<tr>
<td>MHC class II</td>
<td>55.2 ± 12.25</td>
<td>35.7 ± 16.28</td>
<td>0.037</td>
<td>22.0 ± 4.75</td>
<td>7.7 ± 3.16</td>
<td>0.001</td>
</tr>
<tr>
<td>CD11c/CD86&lt;sup&gt;+&lt;/sup&gt;</td>
<td>44.8 ± 4.16</td>
<td>19.9 ± 1.72</td>
<td>0.011</td>
<td>32.1 ± 4.93</td>
<td>14.1 ± 3.52</td>
<td>0.006</td>
</tr>
<tr>
<td>CD14&lt;sup&gt;+&lt;/sup&gt;DC&lt;sup&gt;-&lt;/sup&gt;SIGN&lt;sup&gt;+&lt;/sup&gt;</td>
<td>20.3 ± 6.65</td>
<td>11.1 ± 0.81</td>
<td>0.282</td>
<td>18.8 ± 7.22</td>
<td>5.7 ± 1.98</td>
<td>0.065</td>
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<tr>
<td>CD11c/CD14&lt;sup&gt;-&lt;/sup&gt;</td>
<td>1.03 ± 0.31</td>
<td>0.18 ± 0.11</td>
<td>0.024</td>
<td>1.8 ± 0.46</td>
<td>0.6 ± 0.28</td>
<td>0.002</td>
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</table>

Numbers indicate mean percentage of positive cells ± SEM.

p < 0.05 is considered significant.
and 0.05, respectively; Fig. 5D) in both IFN-α–treated control and lupus EPCs/CACs. In contrast, there was no change in level of expression of IL-1R2 mRNA after JAK inhibition. The PI3K pathway is also activated by IFN-α in various cell types, and PI3K interacts with the JAK-STAT signaling pathway and aids in the activation of a limited number of genes (71). To assess whether PI3K pathway was also involved in the induction of an antiangiogenic signature in IFN-treated EPCs/CACs, we performed similar experiments using a PI3K inhibitor. In contrast to what was observed with a JAK inhibitor, PI3K inhibition did not induce significant changes in any of the genes examined. Overall, these results indicate that the antiangiogenic signature induced by IFN-α on EPCs/CACs is signaled through JAK-STAT pathways.

**Decreased endothelial density and altered IL-1 pathways are observed in SLE in vivo**

Whereas our group and others have suggested decreased vasculogenesis in human SLE (11, 14) and murine lupus models also show similar abnormalities in vivo (23), there was no previous evidence on the occurrence of this phenomenon in vivo in humans. To this end, we proceeded to examine the glomeruli and renal blood vessels of lupus nephritis biopsies and compared blood vessel density and protein expression of IL-1RN and VEGF-A in these compartments with those of control kidneys as well as renal biopsies from patients with another immune-mediated disease, ANCA-positive vasculitis (Supplemental Table II). Immunohistochemistry analysis revealed that the glomerular compartment of patients with lupus nephritis had a significant decrease in VEGF-A expression when compared with control biopsies or biopsies from patients with vasculitis and similar degree of kidney function. This was observed in class II–V lupus nephritis, but was most pronounced in classes IV and V (Fig. 6). Confirming the in vitro data on IFN-treated EPCs/CACs and the serum findings in SLE, IL-1RN was detected in the renal blood vessels of patients with lupus nephritis (particularly class V), but not in the blood vessels of control patients or patients with ANCA-positive vasculitis. IL-1β was not detected by immunohistochemistry in any of the tissues examined. The density of the peritubular and interstitial vessels was evaluated. CD31 expression, used as a marker of endothelial cell density, was significantly decreased in the glomerular compartment of lupus nephritis biopsies (particularly class V) when compared with control biopsies, and the levels of this molecule in the renal blood vessels were significantly decreased in all subsets of lupus nephritis compared with control or ANCA-positive vasculitis.

### Discussion

Whereas it is widely accepted that SLE patients exhibit greater propensity to develop CV complications (5, 72), the mechanisms leading to this enhanced risk remain unclear. Our group and others have proposed that IFN-α may play a prominent role in vascular

### Table III. Affymetrix microarray expression data of STAT, HIF1A, HuR, and HuR target genes in peripheral blood EPCs/CACs and bone marrow EPCs

<table>
<thead>
<tr>
<th>Entrez Gene ID</th>
<th>Gene Symbol</th>
<th>Gene Name</th>
<th>Fold-Change</th>
<th>q Value</th>
<th>Fold-Change</th>
<th>q Value</th>
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<td>STAT1</td>
<td>STAT1</td>
<td>1.06</td>
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<td>1.45</td>
<td>0.031*</td>
<td>2.61</td>
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<td>6773</td>
<td>STAT2</td>
<td>STAT2</td>
<td>1.43</td>
<td>0.000*</td>
<td>1.69</td>
<td>0.000*</td>
<td>1.86</td>
<td>0.000*</td>
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<td>6774</td>
<td>STAT3</td>
<td>STAT3</td>
<td>1.02</td>
<td>0.480</td>
<td>1.54</td>
<td>0.001*</td>
<td>1.45</td>
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<td>STAT4</td>
<td>1.10</td>
<td>0.025*</td>
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<td>0.036*</td>
<td>1.23</td>
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<td>STAT5A</td>
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<td>0.003*</td>
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<td>ELAVL1</td>
<td>ELAV (Drosophila)-like 1, Hu Ag R</td>
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<tr>
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<td>FBJ murine osteosarcoma viral oncogene homolog</td>
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<td>0.009*</td>
<td>0.67</td>
<td>0.006*</td>
<td>1.16</td>
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<tr>
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<td>V-myc myelocystoma-tosis viral oncogene homolog</td>
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<td>V-myc myelocystoma-tosis viral-related oncogene, neuroblastoma derived</td>
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<td>0.201</td>
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<tr>
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<td>VEGF-A</td>
<td>0.66</td>
<td>0.002*</td>
<td>0.78</td>
<td>0.002*</td>
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<tr>
<td>2596</td>
<td>GAP43</td>
<td>Growth-associated protein 43</td>
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<td>N/A</td>
<td>1.03</td>
<td>0.211</td>
<td>0.97</td>
<td>0.548</td>
</tr>
</tbody>
</table>

*q < 0.05 was considered significant.

N/A, not expressed above the Affymetrix control baseline.
damage in SLE, at least in part, by interfering in the balance between blood vessel damage and repair, leading to a dysfunctional endothelium (11, 14). Indeed, abnormalities in vasculogenesis have been proposed to play an important role in the development of atherosclerosis and organ damage (including renal failure) in various diseases (31, 73–76).

Whereas IFN-α is a potent antiangiogenic factor (77–79), the exact pathways by which this molecule regulates EPC/CAC gene expression had not been characterized (39, 80). In this study, we demonstrate that IFN-α exerts its antiangiogenic effects on EPCs/CACs through the modulation of molecules relevant to IL-1 function and signaling and downregulation of VEGF-A. Furthermore, these IFN-induced pathways are operational in vivo in serum and tissue of SLE patients. A previous study reported high levels of IL-1RN in SLE serum and correlation with lupus disease activity (81). Lupus PBMCs are also less capable of releasing IL-1β in response to stimulation (82). However, a link between these abnormalities and IFN-α in SLE and their potential role in vascular damage in this disease had not been reported. Our results also support studies performed in other populations, in which exogenous type I IFNs induce IL-1 downregulation and IL-1RN increases in patients with viral hepatitis C, as well as previous reports of the

![Image](http://www.jimmunol.org/)

**FIGURE 5.** IFN-α activates STAT2 and 6 on EPCs/CACs, and JAK inhibition leads to downregulation of the antiangiogenic signature in SLE. Phosphorylation of STAT2 and STAT6 was detected by FACS in EPCs and CACs. Stimulation with IFN-α leads to STAT2 and 6 activation in EPCs (A) and STAT2 activation in CACs. B, Results represent mean percentage ± SEM of cells positive for STAT phosphorylation in seven SLE and four control subjects. C, JAK inhibition induces upregulation of IL-1α, IL-1β, IL-1R1, and VEGF-A mRNA and decreases mRNA levels of IL-1RN and MX1 (D). Results represent average fold-change ± SEM over vehicle-treated cells. SLE, n = 10; control, n = 5; p < 0.05.

![Image](http://www.jimmunol.org/)

**FIGURE 6.** Kidneys from patients with lupus nephritis display decreased VEGF-A, increased IL-1RN, and decreased glomerular and capillary density. A and D, Representative photographs show that expression of IL-1RN is negative in the endothelium and vessel wall of a control kidney biopsy. In contrast, the vascular endothelium is clearly stained in a case of class V SLE nephritis, and numerous positive cells are present in the vessel wall, particularly in the external layers. A mild vascular positivity for IL-1RN can be detected in a medium-sized vessel from a case of renal ANCA+ vasculitis (immunoperoxidase, original magnification ×400). B and E, Evident loss of podocyte staining for VEGF-A in glomeruli from class IV lupus nephritis, as compared with control kidneys and ANCA+ vasculitis (immunoperoxidase, original magnification ×200). C and F, Compared with glomeruli from a control kidney and a case of vasculitis, glomerular expression of CD31 is significantly reduced in a case of SLE class III lupus nephritis (immunoperoxidase, original magnification ×400).
effect of IFN-α on healthy control myeloid cells in vitro, in which IL-1RN is upregulated without inducing concomitant increases in IL-1 (83–85). Supporting the hypothesis that other type I IFNs may promote similar modulation, IFN-β also has effects on IL-1 family members, an indirect effect by inhibiting the ability of T cells to stimulate monocytes to synthesize IL-1β and a direct effect by upregulating IL-1RN synthesis in the latter (86, 87).

Previous studies on hepatocytes report that exposure to type I IFNs leads to STAT activation and stimulation of the formation of STAT2:STAT6 complexes, subsequently leading to enhanced IL-1RN synthesis (88). In this study, we confirmed that IFN-α led to STAT2 and 6 phosphorylation in control and lupus EPCs/CACs, and that inhibition of JAK-STAT signaling leads to abrogation of the antiangiogenic responses. The effects of JAK inhibition on IL-1 and VEGF-A mRNA expression are similar to what has been seen in other angiogenesis models (89) and in different cell types, including macrophages (90).

In contrast to the changes observed in peripheral blood EPC/CAC cultures, bone marrow EPCs responded to IFN-α primarily by significantly upregulating IL-1RN expression, but displayed no significant changes in other IL-1-related molecules or in VEGF-A, although a similar trend was observed. These results indicate that part of the changes seen in peripheral blood were secondary to direct alterations in the myeloid CAC compartment and/or that EPCs present in peripheral blood represent a distinct subset responding in different ways to exogenous signals than those EPCs that have not left the bone marrow. Future studies should investigate this possibility.

Whereas IL-1α and IL-1β are considered predominantly proinflammatory molecules produced in response to infection or cellular damage, they also have strong proangiogenic properties and effects on murine and human EPCs (58, 91, 92, 93). Furthermore, sIL-1RN (anakinra) has been used as antiangiogenic therapy in various diseases. Part of the effects of IL-1β on angiogenesis are mediated through the regulation of various proangiogenic factors, including VEGF-A (94, 95). IL-1 can induce increased VEGF-A synthesis (96), and genes in a cluster of IL-1–related molecules, including IL-1RN, may regulate VEGF-induced angiogenesis (97). This may explain, at least in part, why the strongest responses in improvement of EPC/CAC function in the SLE cultures were observed with addition of IL-1β, as this molecule may have promoted upregulation of endogenous VEGF-A expression. IL-1β also plays a key role in ischemia-induced neovascularization by mobilizing endothelial precursor cells in a VEGF-dependent manner, as well as by upregulating VEGF and VEGFR-2 expression on endothelial cells (98).

The role of proinflammatory stimuli on the viability and function of EPCs is conflicting. Some studies have reported enhanced EPC death in inflammatory conditions (99), whereas others report increased endothelial differentiation (59). Our study does not address the question of the effects of IL-1β modulation in a proinflammatory setting, but, rather, in a proangiogenic setting in the absence of exogenous proinflammatory mediators. In this case, IL-1β (but not other proinflammatory cytokines) can induce substantial improvements in EPC/CAC differentiation and survival (55). In addition, IL-1β inhibited the skewing of myeloid cells present in the proangiogenic culture from CACs to mature DCs (11, 100), a phenomenon previously reported to be induced by IFN-α. Whereas this may seem counterintuitive, as IL-1β is widely used to promote DC maturation, it is generally used in combination with other proinflammatory cytokines (101–104). Previous studies have reported that IL-1β can impair maturation in DCs treated with rapamycin (105), but, to our knowledge, no other study had examined the isolated effects of IL-1β treatment on DCs or differentiating monocytes.

Whereas decreased IL-1 and increased IL-1RN may theoretically support a phenotype that protects the vasculature, given the anti-inflammatory effects, it is reasonable to speculate that in a disease like SLE in which there is ongoing endothelial cell damage and death (12), a cytokine profile that promotes an antiangiogenic response would be overall deleterious and could accelerate atherosclerosis development. A variety of vascular insults in SLE that lead to disease flares (106–108) in conjunction with increased levels of type I IFNs may lead to periods of endothelial damage, followed by aberrant repair due to decreased IL-1 and VEGF-A and increased IL-1RN. This could allow the initiation and expansion of vascular lesions during these flares.

We have also addressed whether these findings are operational in vivo in SLE, potentially due to increased circulating and/or tissue IFN-α levels as well as increased sensitivity to this molecule by SLE cells (53, 109). Kidneys from SLE patients display repression of VEGF-A and induction of IL-1RN, and this correlates with decreased renal vascular density and vascular rarefaction. These results are consistent with our hypothesis of impaired renal vasculogenesis in SLE and may help to increase our understanding on the mechanisms leading to loss of renal function in this disease. Progressive renal disease is characterized by glomerulosclerosis and interstitial fibrosis, and although many studies have focused on the mechanisms underlying excessive deposition of extracellular matrix, there is increasing evidence of a vascular component as a key driver in the pathogenesis of renal scarring (110, 111). Loss of glomerular and peritubular capillaries is strongly associated with the progression of chronic kidney disease to end-stage renal disease in other populations and animal models (112). VEGF-A is constitutively expressed in human and rodent kidneys (113, 114), and rodent models of progressive renal failure show loss in glomerular and tubular VEGF-A coinciding with loss of capillaries and the subsequent development of glomerulosclerosis and interstitial fibrosis (115). In humans, pharmacologic inhibition of VEGF-A during cancer treatment can lead to proteinuria and worsening kidney function (116). Glomerular and peritubular capillary rarefaction is an important feature of disease progression in other conditions, including diabetic nephropathy (117). Recently, a role for VEGF-A contributing to the maintenance of glomerular or peritubular capillaries and renal tissue survival has been proposed in diabetes (118). Interestingly, other renal diseases, including minimal change disease or idiopathic membranous nephritis, have not shown the reduction of tubulointerstitial VEGF-A, indicating that renal damage or proteinuria per se does not lead to downregulation of this molecule (118). This was confirmed in our study, in which patients with ANCA-positive vasculitis and renal damage did not display downregulation of VEGF-A or renal capillary dropout. Supporting our data, a recent study shows that decreased VEGF in SLE renal tissue may predict short-term loss of kidney function (119).

Various studies have also linked progression of renal disease to certain IL-1RN polymorphisms that are associated to higher IL-1RN levels in the general population (120, 121). However, the exact mechanisms linking renal failure progression to high IL-1RN had not been characterized. Given the observations made in our study, it is possible that the antiangiogenic effect of increased IL-1RN further promotes a reduction in renal capillaries and hampers renal perfusion. Therefore, reduced VEGF-A and IL-1RN (potentially secondary to enhanced exposure to type I IFN levels in the kidney) may contribute to decreased endothelial survival and angiogenesis and to progression of kidney damage. This hypothesis is supported by recent observations that type I IFNs produced by resident renal cells promote end-organ disease in autoantibody-mediated glomerulonephritis (122), and that systemic...
administration of IFN-α to lupus murine models worsens nephritis (123). Whereas these effects are most likely multifactorial, the potential role of this cytokine in leading to microvascular rarefaction and progression of renal failure may play an important role.

IL-1RN has been used in various inflammatory conditions, including rheumatoid arthritis and various autoinflammatory diseases (124, 125). The exact role that exogenous IL-1RN could play in overall CV risk in these diseases is unclear. Whereas small numbers of SLE patients have been treated with rIL-1RN without major adverse effects (126, 127), no studies have addressed the effect of this compound on vascular risk in SLE or other systemic autoimmune diseases. Our study adds a note of caution that should be further investigated with regard to the role of IL-1 blockade in conditions in which vascular repair is already impaired, including SLE and rheumatoid arthritis (11, 74).

Overall, we have identified the mechanisms by which IFN-α interferes with EPC/CAC function and may lead to abnormal vascular repair, atherosclerosis progression, and loss of function in SLE. Future studies should also investigate whether similar abnormalities are also present in individuals with other autoimmune diseases associated to increased type I IFN signatures (128–133).

Disclosures

The authors have no financial conflicts of interest.

References

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