Complement Component C5a Permits the Coexistence of Pathogenic Th17 Cells and Type I IFN in Lupus

Sudesh Pawaria, Kritika Ramani, Kelly Maers, Youhua Liu, Lawrence P. Kane, Marc C. Levesque and Partha S. Biswas

J Immunol published online 22 August 2014
http://www.jimmunol.org/content/early/2014/08/22/jimmunol.1401322

Supplementary Material
http://www.jimmunol.org/content/suppl/2014/08/22/jimmunol.1401322.2.DCSupplemental

Subscription
Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Complement Component C5a Permits the Coexistence of Pathogenic Th17 Cells and Type I IFN in Lupus

Sudesh Pawaria,* Kritika Ramani,* Kelly Maers,* Youhua Liu,† Lawrence P. Kane,‡ Marc C. Levesque,* and Partha S. Biswas*

Systemic lupus erythematosus (SLE) is a type I IFN (IFN-I)–driven autoimmune disorder characterized by exaggerated B cell response (1). In recent years, it has become clear that Th cells have a major impact on the development and propagation of SLE (1, 2). A subset of Th cells that produce proinflammatory cytokine IL-17 (Th17) play a central role in the pathogenesis of SLE (3–5). An increased frequency of Th17 cells was reported in SLE patients, and Th17 cell numbers correlated with disease activity (3–5). These observations were strengthened by numerous mouse model studies, in which lupus mice, deficient in Th17 cells or lacking IL-17 signaling components, were resistant to the development of lupus (5, 6). Although elevated levels of crucial Th17 differentiating cytokines have been documented in lupus (5), the underlying mechanisms in the generation of pathogenic Th17 cells are poorly defined.

SLE is also considered a type I IFN (IFN-I)–driven autoimmune disease (7, 8). Although the contribution of IFN-I in modulating the function of innate cells and B cells is known (9, 10), the role of IFN-I in the generation of pathogenic Th cells is poorly understood. IFN-I inhibits Th17 differentiation (11), and this is partly mediated by the induction of IL-27 (a heterodimeric cytokine consisting of p28 and EBi3 subunits) (12, 13). IL-27 suppresses Th17 differentiation through several mechanisms; IL-27 signaling inhibits RORc expression (14), and IL-27 induces a population of IL-10–producing Tr1 cells (15, 16). In addition, IL-27 priming of T cells controls IL-17 production in trans via induction of the ligand PD-L1 (17).

The mechanisms of IFN-I–mediated IL-27 production from innate cells to inflamed organs (23). C5a also modulates adaptive responses, including T cell proliferation/differentiation, and modulates the balance of the Th1, Th2, and Th17 cell subsets (23). C5a also modulates adaptive responses, including T cell proliferation/differentiation, and modulates the balance of the Th1, Th2, and Th17 cell subsets (23).

Activation of the complement cascade includes cleavage of the plasma glycoprotein C5 by C5 convertases, resulting in the generation of C5b and C5a (23). C5a binds to the G protein–coupled membrane receptors C5aR (CD88) and C5L2 (24). C5a is critically involved in the activation and chemotactic migration of innate cells to inflamed organs (23). C5a also modulates adaptive responses, including T cell proliferation/differentiation, and modulates the balance of the Th1, Th2, and Th17 cell subsets (23). A recent report indicated that C5a–C5aR interaction inhibited Th17 differentiation through diminished production of TGF-β, IL-6, and IL-23 from TLR2 ligand–activated DCs (25). Furthermore, DCs and macrophages deficient in C5aR or C5L2R signaling promoted Th17 cell differentiation (25, 26). Consequently, mice deficient in C5aR have increased numbers of Th17 cells.

Abbreviations used in this article: BMDC, bone marrow–derived DC; BMDM, bone marrow–derived macrophage; DC, dendritic cell; IFN-I, type I IFN; IRF-1, IFN regulatory factor 1; qPCR, quantitative PCR; SLE, systemic lupus erythematosus; WT, wild-type.

Copyright © 2014 by The American Association of Immunologists, Inc. 0022-1767/14/$16.00

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1401322
following house dust mite extract challenge (27). In sharp contrast, C5aR deficiency in SKG mice inhibited the differentiation of Th17 cells and consequently suppressed the development of arthritis (28). Fang et al. (29) demonstrated that C5a promotes the development of inflammatory Th17 cells through synergistic interaction with TLR4 signaling and IL-6 production. Similarly, C5a enhanced IL-17 production by human T cells, which is dependent on enhanced IL-6 and IL-1β expression from monocytes (30). Overall, these reports demonstrated that the effect of C5a on Th17 cell development is controversial.

In this article, we demonstrate the negative regulation of IFN-I–induced IL-27 production by the complement component C5a via the C5aR receptor on macrophages. Activation of C5aR on macrophages blocked IFN-I–mediated IL-27 production and permits the differentiation of Th17 cells. Consequently, C5aR−/− mice have increased IL-27 expression and reduced numbers of Th17 cells following pristane-induced lupus. In support of these mouse model studies, we found that C5a inhibited IFN-I–induced IL-27 production and the level of serum C5a correlated with Th17 frequency in the peripheral blood. Overall, our findings highlight a potential mechanism that explains how Th17 cells can develop despite strong IFN-I responses in SLE.

Materials and Methods

Mice

BALB/c, C5aR−/−, and MRL.Fas−/− mice were obtained from The Jackson Laboratory (Bar Harbor, ME). All mice were housed and bred under specific pathogen–free conditions. All animal protocols were approved by the University of Pittsburgh Institutional Animal Care and Use Committee and adhered to the guidelines in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

Bone marrow–derived macrophage and bone marrow–derived DC cultures

For bone marrow–derived macrophage (BMDM) and bone marrow–derived DC (BMDC) cultures, femoral and tibial bone marrow cells were extracted from mice and cultured in DMEM with L929 cell supernatants derived from secondary lymphoid organs of either BALB/c or MRL.Fas lpr mice, using conditioned medium from BMDMs treated with IFN-α (5 ng/ml), anti–IFN-γ (10 μg/ml), and MRL.Fas lpr mice were obtained from The Jackson Laboratory (Bar Harbor, ME). All mice were housed and bred under specific pathogen–free conditions.

Bone marrow–derived macrophage and bone marrow–derived DC cultures

For bone marrow–derived macrophage (BMDM) and bone marrow–derived DC (BMDC) cultures, femoral and tibial bone marrow cells were extracted from mice and cultured in DMEM with L929 cell supernatants and GM-CSF for 7 d, respectively. BMDMs and BMDCs were treated overnight in the presence or absence of IFN-α (25 U/ml and 250 U/ml) (PBL IFN source) or R848 (1 μg/ml) (InvivoGen) in the presence or absence of mouse C5a (0.1 μg/ml or 0.5 μg/ml) (R&D Systems). In some experiments, BMDMs were treated with anti–IL-27 (p28) Ab (10 μg/ml) (R&D Systems), Wortmannin (0.05 μM and 1 μM) (Cell Signaling Technology), or Akt inhibitor VIII (Akt1/2) (10 μM) (Calbiochem). The purity of the nuclear and cytoplasmic fractions was verified by probing with Abs against lamin B and α-tubulin (Santa Cruz Biotechnology).

Clinical samples

Blood samples were obtained from 45 SLE subjects followed at the University of Pittsburgh Medical Center Lupus Center of Excellence (Pittsburgh, PA), who fulfilled the American College of Rheumatology 1997 revised criteria for SLE. Institutional review board approval and informed consent for these studies were obtained from the University of Pittsburgh and the study subjects, respectively. The 45 SLE subjects had a mean ± SD age of 49.4 ± 14.8; sex ratio of (female/male): 38/7; racial background of 43 white and 2 African-American subjects; and a mean ± SD SLE disease activity index of 4.8 ± 3.2. All the patients were on low-prednisone therapy (<7.5 mg). Blood samples from age- and sex-matched 17 healthy individuals were used as control.

Statistical analyses

Results are expressed as mean ± SD. Differences between groups were calculated for statistical significance using two-tailed paired Student t tests. In some cases, one-way ANOVA has been used to account for multiple comparisons. Correlations in SLE subjects were determined using Spearman’s rank order correlation coefficients. A p value < 0.05 was considered significant.

Results

C5a inhibits IFN-I–induced IL-27 production from macrophages

BMDMs from BALB/c mice treated with different concentrations of IFN-α produced IL-27(p28) in a dose-dependent manner (Supplemental Fig. 1A), confirming the previous report (12). When BMDCs were treated with IFN-α, the concentration of IL-27(p28) was 3- to 4-fold less in comparison with BMDMs treated with IFN-α. The expression of C5a was strongly up-regulated by IFN-I in BMDMs and BMDCs treated with IFN-α (Supplemental Fig. 1A), confirming the previous report (12).

Flow cytometry

Single-cell suspensions from spleen and lymph nodes were surface stained with fluorochrome-conjugated CD4, CD8, CD3ε, C5aR (CD88), and F4/80 (eBioscience) and then analyzed by FACS. For human macrophages, cells were stained with fluorochrome-conjugated human anti-CD14 Ab (BD Pharmingen). Data were analyzed using FlowJo (TreeStar) software.

Real-time quantitative PCR analyses

Expression of the gene of interest was measured by real-time quantitative PCR (qPCR) using a PerfeCTa SYBR Green FastMix (Quanta BioSciences) on an Applied Biosystems instrument. The primers for mouse and human GAPDH, IRF-1, and IL-27(p28) and mouse EBId were purchased from QuantiTect Primer Assays, QIAGEN.

ELISA

Cell culture supernatants were assayed for cytokine production by ELISA kits: IL-27(p28), TNF-α, IL-6, IL-17A, IL-10 (eBioscience). Serum levels of total mouse IgG, IgG2a, and IgG1 anti-dsDNA autoantibody levels were measured by ELISA (Alpha Diagnostic International). Serum levels of human IL-27(p28) and C5adesArg were measured by ELISA (Neogen and BD Pharmingen, respectively).

Transfection of BMDMs

BMDMs of MRL.Fas−/− mice (3 × 106 cells) were transfected with either ca-Akt (MSCV2.2-Myc-Akt) or control vector (MSCV2.2-MCS-IREG-GFP) (10 μg DNA) using the AMEXA system. For intracellular staining of IL-27(p28), macrophages were permeabilized with BD Cytofix/Cytoperm buffer (BD Pharmingen) followed by intracellular staining for IL-27(p28), using mouse anti–IL-27(p28)–PE Ab (eBioscience).

Cell extracts and Western blotting

BMDMs from MRL.Fas−/− mice were lysed in RIPA (radioimmuno-precipitation assay) buffer (Sigma-Aldrich) containing a Halt protease inhibitor mixture (Thermo Scientific) for whole-cell lysates or used for nuclear and cytoplasmic extractions using a Pierce NE-PER Nuclear and Cytoplasmic Extraction Reagent kit, according to the manufacturer’s instructions (Thermo Scientific). IRF-1, pAkt and total Akt, and β-actin Abs (Cell Signaling) were used to probe the blots according to the manufacturer’s instructions. The purity of the nuclear and cytoplasmic fractions was verified by probing with Abs against lamin B and β-tubulin (Santa Cruz Biotechnology).
with IFN-α under identical experimental conditions (Fig. 1A). These results indicated that BMDMs appear to be the primary source of IL-27 during IFN-I stimulation. Therefore, we used BMDMs as a cellular source of IFN-I–mediated IL-27 production for subsequent experiments.

Previous studies have suggested that C5aR activation on macrophages promoted the development of Th17 cells in autoimmune-prone mice (28). To test whether C5a has any impact on IFN-I–induced IL-27 production, BMDMs from BALB/c mice were treated with IFN-α ± C5a. As shown in Fig. 1B and 1C, IFN-α–treated BMDMs expressed higher levels of IL-27(p28) mRNA and protein compared with untreated controls. The expression of EBi3 mRNA was not affected in the presence of IFN-α (Fig. 1B). Of interest, the presence of C5a significantly reduced the IFN-I–induced IL-27 (p28) production in a dose-dependent manner. However, EBi3 mRNA expression was unaltered in the presence of C5a. The number of live BMDMs recovered > 12 h post treatment was comparable between the C5a-treated and untreated groups (Supplemental Fig. 1B). In addition, intracellular cytokine staining revealed an elevated expression of IL-27(p28) in F4/80+C5aR+ BMDMs treated with IFN-α, an effect clearly inhibited by C5a (Fig. 1D). Confirming the previous report, we also found that C5a-treated BMDMs produced significantly higher levels of IL-6 compared with untreated controls (Fig. 1E) (28). However, the level of TNF-α was comparable between the groups (data not shown). Overall, these data clearly suggested that C5a–C5aR interaction specifically inhibited IFN-I–mediated IL-27 production from macrophages.

C5a inhibition of IFN-I–mediated suppression of Th17 differentiation

Because IL-27 can inhibit Th17 differentiation (13–17), we next asked whether C5a could support the differentiation of Th17 cells by suppressing IFN-I–mediated IL-27 production from macrophages. Supernatants of BMDMs treated with IFN-α ± C5a or IFN-α ± anti–IL-27 Ab (conditioned medium) were used to differentiate naive CD4+ T cells in the presence of Th17-skewing conditions (IL-6 + TGF-β). Because C5a-treated BMDMs produced higher levels of IL-6 in comparison with the untreated group, the addition of exogenous IL-6 negated any effect on Th17 differentiation owing to the difference in IL-6 levels between the C5a-treated and untreated groups. As indicated in Fig. 2A, naive CD4+ T cells differentiated in the presence of conditioned medium from IFN-α–treated BMDMs showed a significantly diminished number of Th17 cells compared with untreated BMDMs. Of interest, the presence of C5a or anti–IL-27 Ab in the conditioned medium abrogated the suppressive effect of IFN-α on Th17 differentiation. No significant difference was observed in IFN-γ+ cells between the groups (Fig. 2A). Collectively, these data clearly indicated that C5a–C5aR interaction inhibited IFN-I–dependent IL-27 production from macrophages and permitted the differentiation of Th17 cells.

We next determined whether the increase in the number of Th17 cells in the presence of C5a was dependent on a direct effect of C5a on CD4+ T cells or on macrophages. We performed a reciprocal experiment in which conditioned medium from IFN-α ± C5a–treated wild-type (WT) or C5aR−/− BMDMs were used to differentiate naive CD4+ T cells from C5aR−/− or WT mice, respectively. Th17 differentiation was inhibited when macrophages, but not CD4+ T cells, lacked C5aR (Fig. 2B). Because macrophages express both C5aR and C5L2 (23), these data also suggested that binding of C5a to C5aR, and not C5L2, on macrophages was responsible for the observed effects.

Association of reduced numbers of Th17 cells and protection of C5aR−/− mice from nephritis

To assess the in vivo consequence of C5aR activation on Th17 responses, we used the well-characterized pristane-induced lupus model. In this model, pristane elicits nephritis, IFN-I production, and autoantibody production through a TLR7-dependent pathway (31, 32). Thus, this model provides a valuable tool to study the
interrelationship between IFN-I, C5a, and Th17 cells in pathological settings. To this end, C5aR−/− and BALB/c (WT) mice were injected with pristane and evaluated for a period of >6 mo for the development of nephritis. Whereas 80% of the WT mice developed proteinuria and demonstrated overt renal disease, C5aR−/− mice were completely protected from the development of nephritis (Fig. 3A). Whereas WT mice developed diffuse proliferative glomerulonephritis, C5aR−/− mice showed no evidence of any form of glomerulonephritis (Fig. 3A). Moreover, tubular inflammation was significantly reduced in C5aR−/− mice compared with WT mice (Fig. 3B). Both C5aR−/− and WT mice showed comparable serum total IgG, IgG2a, and IgG1 anti-dsDNA Ab levels (Fig. 3C). Interestingly, C5aR−/− mice, compared with WT mice, demonstrated significantly higher expression of IL-27 in the kidney (Fig. 3E–G). Moreover, purified CD4+ T cells from secondary lymphoid organs of C5aR−/− mice were associated with diminished frequency of Th17 cells in the spleen, mesenteric lymph nodes, and kidney (Fig. 3E–G). Moreover, purified CD4+ T cells from secondary lymphoid organs of C5aR−/− mice produced significantly reduced levels of IL-17A compared with WT mice following anti-CD3 and anti-CD28 stimulation (Fig. 3E). Nevertheless, IFN-γ and IL-4 levels were comparable between the groups (data not shown). Overall, these results clearly indicated that the protective phenotype in C5aR−/− mice was associated with increased expression of IL-27 and reduced number of Th17 cells in secondary lymphoid organs and kidney.

C5aR activation on lupus-prone macrophages inhibited IRF-1–mediated expression of IL-27(p28)

In lupus, IFN-I is primarily produced by innate cells in response to ssRNA and dsDNA in a TLR7- and TLR9-dependent manner, respectively (7, 8). To mimic these in vivo conditions, BMDMs from MRL.FasLy mice were stimulated with TLR7/8 agonist (R848) in the presence or absence of different concentrations of C5a. Because TLR9 has been shown to play a protective role in MRL.FasLy mice, we have decided to use TLR7/8 agonist for this study (33). R848-treated MRL.FasLy BMDMs expressed higher levels of IL-27(p28) protein in comparison with untreated controls, an effect clearly inhibited by C5a in a dose-dependent manner (Fig. 4A). Naive CD4+ T cells differentiated with conditioned medium from R848-treated BMDMs exhibited a significantly diminished number of Th17 cells compared with untreated BMDMs (Fig. 4B). Addition of C5a or depletion of IL-27 in the R848-treated BMDM–conditioned medium rescued the frequency of Th17 cells. No significant difference was observed in IFN-γ+ cells between the groups (Fig. 4B).

Given that transcription factor IRF-1 is critical for the activation of the IL-27(p28) gene in response to IFN-I (18, 19), we assessed the expression of IRF-1 in MRL.FasLy macrophages in response to IFN-α or R848 stimulation. BMDMs treated with R848 expressed higher levels of IRF-1 mRNA and protein (both whole-cell and nuclear extract) compared with untreated BMDMs, which were inhibited by C5a (Fig. 4C, 4D).

The most commonly known signaling pathway for C5aR activation is mediated by PI3K/Akt (23). To evaluate the involvement of C5aR-mediated activation of the PI3K/Akt pathway in IL-27 and IRF-1 expression, we used the PI3K/Akt-specific inhibitor wortmannin to block the signaling pathway downstream of C5aR. As expected, the upregulation of IL-27 (p28) and IRF-1 transcripts in the presence of R848 was significantly inhibited in the presence of C5a (Fig. 5A, 5B). Of note, wortmannin reversed the inhibition caused by C5a in a dose-dependent manner. We also observed diminished pAkt levels in cells treated with wortmannin by immunoblot analysis (data not shown). Furthermore, we performed similar experiments with Akt-specific inhibitor (Akt inhibitor VII) that selectively targets Akt1/2. Akt inhibitor VII blocked C5a-mediated suppression of IFN-I–induced IL-27 production (Fig. 5C). Consequently, ectopic expression of constitutively active Akt (ca-Akt) suppressed IRF-1–induced transcription of IL-27(p28) gene expression in a PI3K/Akt-dependent manner.

C5a negatively regulates the expression of IL-27 in human macrophages from lupus patients

On the basis of our lupus-prone mouse model studies, we next tested the hypothesis that C5a in lupus subjects suppresses IFN-I–
mediated upregulation of IL-27 and thereby permits the generation of Th17 cells. We collected peripheral blood from 45 lupus subjects and 17 healthy controls and measured serum C5adesArg and IL-27(p28) levels by ELISA and number of Th17 cells by intracellular staining. Because C5a is rapidly cleaved to C5adesArg (34), the quantitation of C5adesArg in serum yields a reliable measurement of the level of C5a. We observed significant increase in the number of Th17 cells and diminished level of serum IL-27 (p28) protein in the lupus subjects compared with healthy controls (Fig. 6A, 6B). In addition, serum C5adesArg levels were increased in lupus subjects compared with healthy controls (Fig. 6C). As shown in Fig. 6D–F, a statistically significant inverse correlation was found between serum C5adesArg and IL-27(p28) levels (r = −0.6; p = 0.008) and between IL-27 and the percentage of Th17 cells in the peripheral blood (r = −0.56; p = 0.01); and a significant direct correlation was found between serum C5adesArg and the percentage of Th17 cells in the peripheral blood (r = 0.58; p = 0.006) of lupus subjects. To further validate our mouse model studies in human subjects with SLE, we next determined whether C5a could inhibit IFN-λ-induced IL-27 production in human macrophages. Macrophages from SLE subjects treated with IFN-λ alone had higher levels of both IL-27(p28) and IRF-1 mRNA in comparison with untreated macrophages (Fig. 6G, 6H). The presence of C5a inhibited the IFN-λ–mediated transcript expression of IL-27(p28) and IRF-1 (Fig. 6G, 6H). Overall, these data indicated that C5a can suppress IFN-λ–mediated upregulation of IL-27 from macrophages of lupus patients, and C5a levels correlated with the amount of IL-27 in the serum of these patients.

Discussion
The goal of this study was to explain how Th17 cells are generated and expanded in the face of robust IFN-λ signaling in SLE. Given the pathogenic role of IL-17 in the development and propagation of lupus, identifying the factor or factors that inhibit the suppressive effect of IFN-λ on Th17 cells represents a topic of fundamental and translational interest. In this report, our data position C5a as a new innate factor that inhibits IFN-λ–mediated suppression of Th17 cells. Multiple lines of evidence suggest that complement activation by exogenous or endogenous stimulation can initiate Th17 cell differentiation in certain autoimmune diseases and presumably in microbial infections (28). To date, the mechanisms by which C5a augments Th17 differentiation are poorly understood. In this article, we demonstrated that in addition to its direct role in inducing Th17-differentiating cytokines from innate cells, C5a can also permit the differentiation of Th17 cells indirectly by inhibiting the factor or factors that negatively regulate Th17 differentiation. The pathological significance of these findings was verified in a mouse model of lupus. Interestingly, the protective phenotype observed in C5aR−/− mice was associated with increased IL-27 expression and reduced number of Th17 cells in the secondary lymphoid organs and kidney. In such a scenario, chronic inflammation in SLE leads to accumulation of active C5a. Subsequent activation of C5aR on macrophages would not only induce production of Th17-differentiating cytokines but would also inhibit the suppressive effect of IFN-λ by blocking production of IL-27.

Given the inhibitory role of IFN-λ in Th17 differentiation, it would be reasonable to speculate that anti–IFN-λ treatment in lupus patients may lead to exaggerated Th17 responses and more severe disease. However, IFN-α/β receptor–deficient IFNAR−/− mice were protected from developing nephritis, and phase I clinical trials with anti–IFN-λ Abs in SLE patients demonstrated encouraging results (35). A number of reasons can account for this dichotomy. First, IFN-λ is a potent systemic inducer of many proinflammatory cytokines, including IL-6, IL-1, and IL-23, which are absolutely required for the differentiation of Th17 cells (7–10). Second, in the absence of IFN-λ–induced kidney inflammation, chemotactic signals may not reach the required threshold to drive the influx of Th17 cells in the renal parenchyma. Lajoie et al. (27) showed that, in sharp contrast to autoimmune diseases, C5a–C5aR interaction negatively regulated Th17 cells in experimental allergic asthma. Specifically, Ab blockade or genetic disruption of C5aR or C5 enhanced Th17 cell differentiation following house dust mite extract challenge. In this setting, C5a stimulates IL-10 production from DCs, resulting in inhibition of IL-23 production. Currently, the reasons for the discrepancy

FIGURE 3. C5aR−/− mice have reduced numbers of Th17 cells following pristane-induced nephritis. Age- and sex-matched BALB/c (WT) and C5aR−/− mice (n = 5) were injected i.p. with pristane and evaluated for the development of lupus nephritis over a period >6 mo. Mice were evaluated for (A) kidney pathology by H&E and periodic acid–Schiff staining; (B) tubule–interstitial inflammation score (as assessed and scored by a renal pathologist); (C) serum total IgG, IgG2a, and IgG1 anti-dsDNA antibodies by ELISA; (D) IL-27(p28) transcript level in the spleen by qPCR; and (E–G) frequencies of IL-17–producing CD4+ T cells in the spleen, mesenteric lymph nodes (MLN), and kidney by intracellular staining following restimulation with PMA/ionomycin (gated on CD4+ T cells) (H). Purified CD4+ T cells from secondary lymphoid organs were restimulated with anti-CD3 and anti-CD28 mAbs and 17 healthy controls and measured serum C5adesArg and IL-27(p28) levels by ELISA. Each dot represents an individual mouse, and the horizontal bar indicates the mean for each group. **p < 0.01. Scale bars in (A), 200 μm.
Sorted naive CD4+ T cells from MRL.Faslpr mice were tested for IL-27(p28) by ELISA. (B) Sorted naive CD4+ T cells from MRL.Faslpr mice were differentiated in the presence of conditioned media from R848 ± C5a ± anti–IL-27Ab (10 μg/ml)–treated MRL.Faslpr macrophages in the presence of Th17-skewing cytokines (IL-6 + TGF-β) for 5 d. Cells were stained for intracellular IL-17 and IFN-γ and analyzed by FACS (gated on CD4+ T cells). BMDMs from MRL.Faslpr mice were stimulated with R848 (1 μg/ml) ± C5a (0.1 and 0.5 μg/ml) for 12 h and analyzed by immunoblotting in whole-cell extracts and nuclear extracts. Purity of the nuclear extracts was checked by evaluating the expression of lamin B and β-tubulin. The error bars represent mean ± SD. For (A) and (B), the data are pooled from three independent experiments. For (B) and (D), the data are representative of three independent experiments with similar results. **p < 0.01.

between this finding and ours are poorly understood. From these findings, it is apparent that the actions of C5a are context dependent and may be dependent on the target cell and the copresence of other activators or regulators such as inflammatory mediators and the nature of TLR activation. Thus, it is possible that C5a modifies inflammatory cytokine production by DCs and macrophages in a manner that can inhibit or support the generation of Th17 cells. Future in-depth studies are needed to shed light on the role of C5a in modulating Th17 response in various inflammatory conditions.

The data presented in this report demonstrated the negative regulation of IFN-γ–induced IL-27 expression by the complement activation product C5a. Complement component C5a blocked IFN-γ–induced IL-27 production in mouse and human macrophages, suggesting that this regulatory mechanism is conserved across species. However, these results differ from recent observations made by Batten et al. (36), who demonstrated that IL-27 was required for lupus pathogenesis. This report directly argues against what has been known about the role of IL-27 in autoantibody production in lupus-prone models. For example, in MRL. Faslpr mice, overexpression of IL-27Rα resulted in diminished titers of autoantibodies and reduced skin disease (37). In addition, deletion of EBI3 in MRL.Faslpr mice resulted in increased titers of autoantibodies but, surprisingly, improved disease scores (38). We also showed that, consistent with these studies, in the absence of C5aR, and hence increased IL-27 production, mice developed diminished lupus nephritis despite unchanged autoantibody levels. The reasons for the inconsistency between our results and the phenotype observed in IL-27R−/− mice are unclear at present. Recently, Stunhofer et al. (39) showed that IL-27( p28), independent of EBI3, antagonized cytokine signaling through gp130- and C5aR, and hence increased IL-27 production, mice developed diminished lupus nephritis despite unchanged autoantibody levels. The reasons for the inconsistency between our results and the phenotype observed in IL-27R−/− mice are unclear at present. Recently, Stunhofer et al. (39) showed that IL-27(p28), independent of EBI3, antagonized cytokine signaling through gp130- and Il17a–/− mice are unclear at present. Recently, Stunhofer et al. (39) showed that IL-27(p28), independent of EBI3, antagonized cytokine signaling through gp130- and Il17a–/− mice are unclear at present. Recently, Stunhofer et al. (39) showed that IL-27(p28), independent of EBI3, antagonized cytokine signaling through gp130- and Il17a–/− mice are unclear at present. Recently, Stunhofer et al. (39) showed that IL-27(p28), independent of EBI3, antagonized cytokine signaling through gp130- and Il17a–/− mice are unclear at present. Recently, Stunhofer et al. (39) showed that IL-27(p28), independent of EBI3, antagonized cytokine signaling through gp130- and Il17a–/− mice are unclear at present. Recently, Stunhofer et al. (39) showed that IL-27(p28), independent of EBI3, antagonized cytokine signaling through gp130- and Il17a–/− mice are unclear at present. Recently, Stunhofer et al. (39) showed that IL-27(p28), independent of EBI3, antagonized cytokine signaling through gp130- and Il17a–/− mice are unclear at present. Recently, Stunhofer et al. (39) showed that IL-27(p28), independent of EBI3, antagonized cytokine signaling through gp130- and Il17a–/− mice are unclear at present. Recently, Stunhofer et al. (39) showed that IL-27(p28), independent of EBI3, antagonized cytokine signaling through gp130- and Il17a–/− mice are unclear at present. Recently, Stunhofer et al. (39) showed that IL-27(p28), independent of EBI3, antagonized cytokine signaling through gp130- and Il17a–/− mice are unclear at present. Recently, Stunhofer et al. (39) showed that IL-27(p28), independent of EBI3, antagonized cytokine signaling through gp130- and Il17a–/− mice are unclear at present. Recently, Stunhofer et al. (39) showed that IL-27(p28), independent of EBI3, antagonized cytokine signaling through gp130- and Il17a–/− mice are unclear at present. Recently, Stunhofer et al. (39) showed that IL-27(p28), independent of EBI3, antagonized cytokine signaling through gp130- and Il17a–/− mice are unclear at present. Recently, Stunhofer et al. (39) showed that IL-27(p28), independent of EBI3, antagonized cytokine signaling through gp130- and Il17a–/− mice are unclear at present. Recently, Stunhofer et al. (39) showed that IL-27(p28), independent of EBI3, antagonized cytokine signaling through gp130- and Il17a–/− mice are unclear at present. Recently, Stunhofer et al. (39) showed that IL-27(p28), independent of EBI3, antagonized cytokine signaling through gp130- and Il17a–/− mice are unclear at present. Recently, Stunhofer et al. (39) showed that IL-27(p28), independent of EBI3, antagonized cytokine signaling through gp130- and Il17a–/− mice are unclear at present.
is possible that in the absence of IL-27Rα, IL-27(p28) can independently antagonize IL-6R signaling in these mice. Given the role of IL-6 in the generation of follicular T cells, IL-21 production, and autoantibody response, it is thus not surprising to see diminished follicular and humoral response in IL-27Rα−/− mice. Further elucidation of the molecular mechanisms underlying the role of IL-27 in renal disease and detailed dissection of the situations in which IL-27 differentially exerts its opposite roles will help fill the gap in our knowledge pertinent to lupus pathogenesis.

The studies described above have potential implications for maintaining normal immune homeostasis, particularly in the case of viral infections in which IFN-I plays a critical role. In such a scenario, IFN-I-induced IL-27 will not only favor the development of Th1 cells necessary for antiviral immunity but also suppress the tissue-damaging Th17 response to prevent undesirable immunopathology. This immunoregulatory pathway is disrupted in the case of SLE and other IFN-I–dominated autoinflammatory conditions, in which chronic activation of C5a will suppress IFN-I-induced IL-27 production and permit the generation of pathogenic Th17 cells, resulting in breakdown of tolerance and subsequently end-organ damage.

In addition, these studies have important implications for the treatment of patients with SLE. Our studies suggest that strategies designed to reduce the levels of C5a, by neutralizing it or preventing its cleavage from C5, and/or strategies to downregulate C5aR expression or signaling may have beneficial effects for SLE patients. Several therapeutics have been developed that block the effects of C5a, and some have been tested in patients with autoimmune disorders. One such therapy, eculizumab, prevents C5 from being cleaved to form C5a and is currently FDA approved for use in paroxysmal nocturnal hemoglobinuria (40). Another agent, PMX53, is a small cyclic peptide that blocks C5aR and has been tested in rheumatoid arthritis patients (41). Two other agents, MEDI7814 and NNC0215-0384, which block the C5a–C5aR interaction and C5aR signaling, are both undergoing phase 1 testing in RA (clinicaltrials.gov).

Acknowledgments
We thank the clinicians of the Lupus Center of Excellence, Drs. Moreland, Liang, Naoiseh, Vina, and D'Amico, for contributions in providing lupus biospecimens for this research. We also thank Drs. Sarah Gaffen and Mandy McGee for critical reading of the manuscript and helpful discussions.

Disclosures
The authors have no financial conflicts of interest.

References


