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Type I Interferons Produced by Resident Renal Cells May Promote End-Organ Disease in Autoantibody-Mediated Glomerulonephritis

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Increased Type I IFNs or IFN-I have been associated with human systemic lupus erythematosus. Interestingly augmenting or negating IFN-I activity in murine lupus not only modulates systemic autoimmunity, but also impacts lupus nephritis, suggesting that IFN-I may be acting at the level of the end-organ. We find resident renal cells to be a dominant source of IFN-I in an experimental model of autoantibody-induced nephritis. In this model, augmenting IFN-I amplified antibody-triggered nephritis, whereas ablating IFN-I activity ameliorated disease. One mechanism through which increased IFN-I drives immune-mediated nephritis might be operative through increased recruitment of inflammatory monocytes and neutrophils, though this hypothesis needs further validation. Collectively, these studies indicate that an important contribution of IFN-I toward the disease pathology seen in systemic autoimmunity may be exercised at the level of the end-organ. The Journal of Immunology, 2009, 183: 6831–6838.

The presence of self-reactive anti-nuclear Abs is a key characteristic in the development of the autoimmune disease, systemic lupus erythematosus (SLE). The deposition of anti-nuclear Abs, anti-glomerular Abs and related immune complexes within the end organs such as the kidney, play a significant role in the development of nephritis and death. Of the multiple cytokines implicated in disease progression, much research has focused on the key role of type I IFNs (IFN-I), particularly in human SLE. Early studies by Preble et al. (1, 2) demonstrated that IFNα was detectable in the serum of SLE patients. In addition, it has been shown that sera from patients with SLE can stimulate normal PBMCs to produce IFNα (3, 4). More recently, several groups have demonstrated the presence of an IFN-I gene expression signature in the peripheral blood of patients with SLE, with some suggesting a correlation with disease severity and end organ disease (5–9). Whether the presence of this signature is a cause or a consequence of disease is yet to be determined. In this context, murine studies have been particularly informative.

Materials and Methods

Reagents and mice

All mice were bred in the University of Texas Southwestern Medical Center. Breeding pairs for C57BL/6J (B6) mice were originally obtained from...
The Jackson Laboratory. B6.IFNAR−/− mice (lacking the common receptor for Type I IFNs) were obtained from Dr. Michel Aguet (ISREC Foundation, Epalinges, Switzerland) (24). The care and use of laboratory animals conformed to the National Institutes of Health guidelines and all experimental procedures conformed to an institutional animal care and use committee approved animal protocol.

**Anti-GBM serum and disease induction**

Anti-GBM serum was prepared as described previously (22). In brief, renal cortices of B6 mice were minced and then passed through sieves of decreasing pore size (150, 106, and 63 μm). Glomeruli were obtained from the finest sieve, washed in cold PBS and sonicated for 7 min. The glomerular sonicate was sent to the Lampire Laboratories for the generation of anti-GBM sera in rabbits. The specific binding of the immune rabbit sera to glomerular basement membrane was confirmed by direct immunofluorescence using frozen mouse kidney sections, and its efficacy in precipitating nephritis was tested in B6 and 129sv mice, as described previously (23). Mice were pre-sensitized with rabbit IgG (2.5 mg/ml with CFA, 100 μl/20g body weight) via the peritoneal cavity. Five days postinjection, mice were injected anti-GBM serum IV (170 μl/20g body weight). Blood and 24-h urine samples were collected before (day 0) and 14 days after induction of disease. In studies where this was combined with IFN-adenovirus (ADV), anti-GBM serum was administered IV at 130 μl/20g body weight to ensure the detection of any augmentation of disease. Prior trials with a lower dose of anti-GBM serum (100 μl/20g) did not have any effect with this combination.

**IFN-ADV and IFN**

IFN-I was deliberately administered to two cohorts of mice. In cohort 1, IFN-I (Universal type I IFN, no. 11200–2 PBL Laboratories) was injected i.p. at 10,000 U per mouse on days −4, 0, 4, 8, and 12 (where day 0 represents the day of rabbit Ig injection), as described previously (25). Mice in cohort II were administered IFN-I, using adenoviral delivery on D0 of the anti-GBM disease induction protocol. The IFN adenovirus (IFN-ADV) or control (ADV) was purchased from Q-Biogene. This was repeated twice and the data combined for analysis of renal disease. Ad5.CMV-mIFN-α expresses the murine IFN-α gene under the control of the Cytomegalovirus-IE promoter/enhancer. Ad5.Null contains an empty expression vector and was used as a control (ADV). One × 105 IFN-ADV or ADV particles were injected, as previously described (14).

**Assessment of renal disease**

Mice were caged in metabolic cages and urine was collected over a 24 h period. Protein was measured using the Coomassie Plus Protein Assay kit (Pierce) as per the manufacturer’s instructions. BSA (Pierce) was used as a standard. Blood urea nitrogen (BUN) was assessed using the QuantiChrom Urea Assay Kit (BioAssay). Following euthanasia, kidneys were fixed in formalin and embedded in paraffin for blinded analysis by an Animal Committee approved animal protocol.

Cell preparation and flow cytometry

Kidneys were dissected out, chopped finely with a razor blade, and suspended in PBS. Kidney cells were spun to a pellet at 1100 rpm at 4°C for 6 min. The supernatant, enriched in interstitial fluid or “renal plasma”, was stored frozen at −20°C in aliquots until analysis. IFN-I protein was detected using an ELISA kit from PBL as per the manufacturer’s protocol. The kit measures the following subtypes: IFNα, IFNγ, IFNβ, IFNα5, IFNα6, and IFNγ9, with a detection range between 12.5–5000 pg/ml. RNA was analyzed using Applied Biosystems Taqman Gene Expression Assays using primers for CD45 (Mm00833969_s1), IFNα5 (Mm00833976_s1), IFNβ1 (Mm00439546_s1), B2M (Mm00437762_m1), and CD45 (Mm01293570_m1, specific for a region outside the extra-cellular receptor splicing area). Absolute MAX RQT-PCR mix from Thermo Scientific was used for amplification, as per the manufacturer’s instructions. An ABI 7300 Real Time System, using Applied Biosystems Sequence Detection Software, Version 1.2.3 was used for amplification and analysis. The message levels of IFN-I genes were expressed after normalization to corresponding CD45 and B2M expression levels.

**Cytokine multiplex analysis**

The supernatants from kidneys were analyzed using 22-plex murine cytokine assay kits (Bio-Rad) as per the manufacturer’s protocol at BHR Luminex Core, Dallas.

**Assaying IFN-I protein and message**

Kidneys were dissected out, chopped finely with a razor blade, and suspended in PBS. Kidney cells were spun to a pellet at 1100 rpm at 4°C for 6 min. The supernatant, enriched in interstitial fluid or “renal plasma”, was stored frozen at −20°C in aliquots until analysis. IFN-I protein was detected using an ELISA kit from PBL as per the manufacturer’s protocol. The kit measures the following subtypes: IFNαA, IFNα1, IFNα4, IFNα5, IFNα6, and IFNα9, with a detection range between 12.5–5000 pg/ml. RNA was analyzed using Applied Biosystems Taqman Gene Expression Assays using primers for IFNα4, IFNα5, and B2M genes. The supernatants from kidney were analyzed using 22-plex murine cytokine assay kits (Bio-Rad) as per the manufacturer’s protocol at BHR Luminex Core, Dallas.

**Statistical analyses**

Results are expressed as the arithmetic mean ± SEM (SE). Study groups were analyzed for normal distribution using Kolmogorov-Smirnov test. Comparisons between two groups passing normality were assessed using the parametric Student t test, those that did not pass the normality test were assessed using the Mann-Whitney U test. Groups were compared using 1 or 2 way ANOVA with either Bonferroni or Dunn’s post hoc analyses for parametric and nonparametric data respectively. Correlations between two variables (Fig. 1, B and C) were calculated using Spearman’s rank nonparametric 2-way analyses, with rho, p and the probability, p, shown within the graph. Analyses were completed using Instat version 3.0 and Prism 5.0 for Windows (GraphPad Software) or Microsoft Excel.

**Results**

Resident renal cells may be the predominant source of IFN-I in immune nephritis

Nonautoimmune prone B6 mice were injected with rabbit anti-GBM Abs. As reported previously, B6 mice exhibit modest disease following anti-GBM Ab challenge (22). To determine whether disease was associated with increased IFN-I expression, kidneys from these mice were extracted and assessed for levels of soluble IFN-I in the renal interstitial fluid or “plasma” 14 days later. Although the secreted levels were low, the anti-GBM Ab assay was associated with a significant increase in IFN-I (Fig. 1A) and the levels of this cytokine trended with the levels of proteinuria and azotemia (Fig. 1, B and C), although this did not reach statistical significance, (p = 0.08 and p = 0.11, respectively). Because the elevated IFN-I may have been derived from infiltrating leukocytes or resident renal cells, and because the ELISA detects a number of IFN-I subtypes, we next analyzed IFN-I message levels in infiltrating vs resident kidney cells using real time PCR.

Resident kidney cells were magnetic bead sorted away from infiltrating leukocytes with 99% purity, using CD45 beads. Message from the CD45+ enriched and CD45− cell populations were standardized to both CD45 and B2M message levels for mRNA analysis. We specifically assayed the message levels of IFN-I, IFNα4, and IFNα5, because highly specific PCR primers for
These IFN-I genes could readily be designed. Interestingly, increased IFNα4 (p = 0.007) but not IFNα5 or IFNβ1 message was noted in CD45"renal" cells following anti-GBM challenge. In contrast, analysis of mRNA in CD45" renal" leukocytes did not reveal any increase in IFN-I message (Fig. 1, G–I). Moreover, a significant decrease in the level of IFNα4 was observed in the CD45" renal" leukocyte population following anti-GBM challenge (Fig. 1G). This was repeated in a separate experiment using 129 mice. Interestingly, while IFNα4 mRNA was increased in CD45" renal" cells following treatment with anti-GBM, consistent with the results from the B6 mice, there was no detectable change in expression in the infiltrating CD45" renal" leukocyte population following treatment (data not shown). Collectively, these findings indicate that intrarenal IFN-I is increased in anti-GBM disease, and arises predominantly from resident (CD45" renal") cells, rather than the infiltrating (CD45" renal") leukocytes.

**Systemic IFNα administration amplifies experimental immune nephritis if administered chronically**

To examine the effect of exogenous IFNα on anti-GBM induced disease, a first cohort of mice were injected at 4 day intervals with recombinant IFNα, over the 14 day anti-GBM challenge/follow-up period. Analysis of proteinuria and BUN following anti-GBM assault revealed a modest increase in the IFN-I injected mice, although this was not significant (Fig. 2, A and B). Examination of renal pathology also demonstrated that recombinant IFN-I administered intermittently had no effect on anti-GBM induced disease (Fig. 2C). The grade of tubular and interstitial nephritis and the percentage of glomerular crescents were also unaffected by intermittent treatment with IFN-I (data not shown). Examination of serum at the end of the study (day 14) showed that IFN levels were rather low following intermittent administration (ranging from undetectable to 25 pg/ml).

Because the levels of serum IFNα following intermittent administration of recombinant IFN-I were low, we next used an adenoviral vector system for sustained delivery of IFNα. Serum levels at the end of the study using this system averaged 240 pg/ml for IFN-ADV alone, and 380 pg/ml for IFN-ADV plus anti-GBM (Fig. 3A). Interestingly, a combination of the control ADV vector and anti-GBM Ab resulted in low, but detectable levels of serum IFNα. Analysis of protein in the urine demonstrated a significant increase following combined exposure to IFN-ADV and anti-GBM Ab (Fig. 3B), compared with mice receiving either alone. This was also accompanied by a visible increase in BUN (Fig. 3C), though not significant.

Blinded analysis of renal histology by an independent pathologist demonstrated a significant increase in glomerulonephritis, tubulo-interstitial nephritis, and glomerular crescent formation after anti-GBM/IFN-ADV, compared with the controls (Fig. 3, D–F). No other single or combination treatment group demonstrated a significant increase in disease.

Next, leukocyte infiltration into the kidney was assessed using flow cytometry. Analyses demonstrated that a combination of sustained IFNα administration and anti-GBM Ab injection resulted in a significant increase in the percentage of CD45" leukocytes within the kidney (9.65 ± 2.34, IFN-ADV plus anti-GBM; Fig. 4A, top). The overall numbers of leukocytes within the kidney showed a moderate, but nonsignificant increase, which suggests a loss of renal cells possibly due to the disease (Fig. 4A, bottom). An alternative

![FIGURE 1](https://example.com/figure1.png)

Anti-GBM administration stimulates the production of IFN-I within the kidneys. B6 mice were injected with anti-GBM sera on day 5 (after presensitization with rabbit IgG on day 0). On day 14, total renal cells were extracted into a single cell suspension in PBS. The resulting supernatant from the untreated and anti-GBM challenged mice was assessed for IFNα protein, by ELISA (A). Shown in B and C are the correlation profiles of renal IFN-I levels with 24-h proteinuria or BUN levels in both groups of mice. The experiment was repeated, with the data combined in Fig. 1, A–C, with a final mouse number of 16 per group. In a separate study, CD45" resident renal cells were isolated using magnetic bead sorting and assessed for type I IFN mRNA (IFNa4, IFNa5, IFNb1) expression by real time PCR (D–F). CD45" leukocytes were also assessed for IFN message expression (G–I, IFNa4, IFNa5, IFNb1). Units are fold change relative to the normalized expression level observed in control untreated mice. Each dot represents data obtained from an individual mouse.
reason for the disparity between the percentage infiltrate and total cell numbers is that the processing procedures of the kidney (collagenase and percoll, see Materials and Methods) may have created variability between samples. However, leukocyte infiltration does appear to be real, because additional assays on IFN-ADV treated B6 mice published previously have shown minimal infiltration following ADV or IFN-ADV treatment alone (18).

Further analyses on the CD45<sup>+</sup> population suggested a moderate increase in B and T lymphocyte infiltration and a significant increase in the myeloid population in the IFN-ADV plus anti-GBM group (Fig. 4B). Gating strategies for analyzing the myeloid lineage are shown in Fig. 4C. Cells were gated on CD11b<sup>+</sup> (CD19<sup>-</sup>/H11002 CD3<sup>-</sup>/H11002) and initially through Neu7/4 and Gr1 which showed that both Gr1<sup>+</sup> inflammatory monocytes and neutrophils increased in response to the combination of IFN-ADV and anti-GBM (11- and 3-fold greater numbers than anti-GBM alone for Gr1<sup>+</sup> monocytes and neutrophils respectively, data not shown and Fig. 4D). The Gr1<sup>-</sup> CD11b<sup>+</sup> population was further subgated using CD11c and F4/80 as described in the Materials and Methods (Fig. 4C, bottom). The CD11c<sup>-</sup> F4/80<sup>low</sup> population, together with the CD11c<sup>+</sup> F4/80<sup>low</sup> population showed a significant expansion in response to anti-GBM plus IFN-ADV (Fig. 4E). Analyses of surface receptor expression showed a surprising decrease in MHC II expression in response to IFN-ADV. This was an IFN-specific response because the IFN-ADV alone had a similar effect (Fig. 4F). However, surface CD86 expression was increased following a combination of IFN-ADV and anti-GBM in both the CD11c<sup>-</sup> and CD11c<sup>+</sup> F4/80<sup>low</sup> populations which was not due to IFN-ADV (Fig. 4G).

Analysis of CD69 positivity in multiple cell types suggested trends in activation following IFN<sub>α</sub> administration independent of an anti-GBM insult (Fig. 4H). This may not be a surprise because CD69 is expressed very early on lymphocytes following activation from multiple challenges, including IFN<sub>α</sub> (30–32). Further analysis of CXCR4 expression, a molecule we have found to be important in the pathogenesis of lupus nephritis (33), also demonstrated an increase in activation in multiple cell types following the administration of IFN-ADV and anti-GBM Abs (Fig. 4I). Overall, this data demonstrates that while IFN<sub>α</sub> can mediate activation...
across multiple cell types, these effects are not necessarily associated with the development of disease. In particular, increased expression of CXCR4 and CD86 occur preferentially in mice exposed to anti-GBM and IFN-ADV, which develop nephritis.

Analyses of the renal interstitial fluid or plasma using a multiplex cytokine luminex assay demonstrated small nonsignificant increases in the myeloid attractant chemokines MCP-1 and keratinocyte chemoattractant (KC, Groα, CXCL1) (Fig. 5A) and the proinflammatory cytokines IL-12p40 and G-CSF (Fig. 5B) following IFN-ADV treatment alone. The elevation in these specific mediators was significantly higher in mice treated with a combination of anti-GBM and IFN-ADV (Fig. 5, A and B). Other cytokines and chemokines that were detected in the kidney, but exhibited no change following treatment were IL-1α, IL-1β, IL-6, IL-9, IL-13, and eotaxin (data not shown). In addition, MIP-1α, MIP-1β, IL-10, IL-2, IL-3, IL-4, IL-5, IFN-γ, GM-CSF, IL-17, and IL-12p70 were expressed at low or undetectable levels and did not increase following any treatment (data not shown). Examination of IFNα in the renal interstitial fluid demonstrated that although there is detectable IFNα following ADV plus anti-GBM, this is no different from the levels in the anti-GBM alone mice, in contrast to the observations in the sera (Fig. 5C). In addition, there does not seem to be any effect of ADV alone in any other measure of renal disease.

**IFNAR elimination ameliorates anti-GBM disease**

Because intrarenal IFN-I was up-regulated in anti-GBM disease (Fig. 1), we next asked whether IFN-I was playing an essential role in mediating anti-GBM disease. To test this, we used an IFNAR-deficient strain lacking the common receptor for Type I IFN, on the B6 background (B6.**IFNAR−/−**). Following challenge with the anti-GBM Abs, the significant increase in proteinuria observed in the B6 control strain was absent in the knockout mice (Fig. 6A). Anti-GBM challenge increased BUN in both B6 and B6.**IFNAR−/−** mice (Fig. 6B). However, the induced level of BUN was significantly less in the knockout animals compared with the levels in the B6 controls. Likewise, the degree of GN and glomerular crescent formation in IFNAR−/− mice following anti-GBM challenge was lower in the knockouts compared with the B6 controls (Fig. 6, C and D). These initial findings were reproduced in an additional study, as detailed in the legend. Overall, the deficiency of IFNAR ameliorated all aspects of the renal disease following the immune challenge, indicating that the up-regulated IFN-I during the course of anti-GBM induced nephritis was playing an essential role in the pathogenesis of this disease.

**Ablating IFN-I activity does not appear to dampen systemic immune responses**

We reasoned that the reduced anti-GBM-induced renal disease observed in B6.**IFNAR−/−** mice may relate either to reduced systemic xenogenic immune response to the administered rabbit Ig or to reduced local inflammatory events within the kidneys. To distinguish between these possibilities, we next examined the serum levels of anti-rabbit xenogenic immune response in the anti-GBM challenged B6.**IFNAR−/−** mice and B6 controls. B6.**IFNAR−/−**
Discussion

Several studies in human SLE have pointed to the strong association of elevated IFN-I with disease. In addition, the published studies reveal that elevated IFN-I is often associated or correlated with various end-organ disease manifestations in SLE, including nephritis and cutaneous symptoms (34–36). Reports have also demonstrated a pivotal role for IFNα in pure cutaneous lupus where the disease is limited to the skin (37). Other studies have shown that IFNα is detectable in the CSF of SLE patients with neuropsychiatric syndromes (38). Moreover, hepatitis and malignant melanoma patients treated with IFN-I have a tendency to down-regulate their IFNα mRNA expression in this strain, perhaps in a compensatory role to the inflammatory environment. Inferring leukocytes appear to down-regulate their IFNα mRNA expression in this strain, perhaps in a compensatory role to the inflammatory environment.

Primary candidate receptors responsible for the production of type I IFN include antiviral immune receptors, RIG-I and Mda-5, and the intracellular TLRs. Although TLR7 and TLR9 are not expressed by renal cells, Anders and colleagues (39–41) have shown that TLR3 is expressed by multiple cells within the kidney, and it is increased in response to an inflammatory challenge. Furthermore, this group has also demonstrated that viral triphosphate RNA (3-P RNA), a non-TLR agonist, aggravates lupus nephritis in MRL/lpr mice, increasing systemic IFN-I expression. These findings were reproduced in an additional study in which mice did not exhibit reduced anti-rabbit alloimmune response, indicating that the reduced disease in these mice is not simply the consequence of reduced systemic adaptive immune responsiveness to the administered anti-GBM Abs (data not shown).

genetic models of lupus (10–12), deliberate administration of IFN-I or TLR ligands that elicit IFN-I production, invariably augment disease (11, 14–18). Again, in all of these studies, it is not clear whether the observed change in renal disease is due to the direct impact of IFN-I on the end-organs, or they simply follow as a consequence of changes in autoantibody titers and/or leukocyte function. The present study contributes a novel perspective to this important question. This is the first demonstration that the bulk of disease (11, 14–18) is generated by resident cells in the organ, as opposed to infiltrating leukocytes. Moreover, infiltrating leukocytes appear to down-regulate their IFNα mRNA expression in this strain, perhaps in a compensatory role to the inflammatory environment.

Primary candidate receptors responsible for the production of type I IFN include antiviral immune receptors, RIG-I and Mda-5, and the intracellular TLRs. Although TLR7 and TLR9 are not expressed by renal cells, Anders and colleagues (39–41) have shown that TLR3 is expressed by multiple cells within the kidney, and it is increased in response to an inflammatory challenge. Furthermore, this group has also demonstrated that viral triphosphate RNA (3-P RNA), a non-TLR agonist, aggravates lupus nephritis in MRL/lpr mice, increasing systemic IFNα expression. These findings were reproduced in an additional study in which mice did not exhibit reduced anti-rabbit alloimmune response, indicating that the reduced disease in these mice is not simply the consequence of reduced systemic adaptive immune responsiveness to the administered anti-GBM Abs (data not shown).
ascertain whether this is also true in spontaneous lupus nephritis. Determining whether resident renal cells are the major source of IFN-I in lupus nephritis could have important diagnostic and therapeutic implications.

A second novel contribution of this study is the demonstration that altering IFN-I levels can directly alter the degree of renal disease following an anti-glomerular Ab-mediated assault. Whereas ablating IFN-I activity altogether ameliorates immune nephritis, the deliberate administration of IFN-I amplifies end-organ disease. Moreover, following a combination of systemic IFN-I and anti-GBM serum (Fig. 4), we observed a leukocyte infiltration pattern in the assaulted kidney akin to our previous observations in two different spontaneous models of SLE (18, 26). Specifically, an increase in the numbers of inflammatory monocytes, neutrophils and activated CD4⁺ T cells within the kidney appears to be a key feature of severe nephritis associated with elevated IFN-I. Moreover, following treatment with IFNα and anti-GBM, we detected a significant increase in the chemokine MCP-1, which is known to be associated with disease pathogenesis in multiple murine models and in human disease (44–49). Increased levels of MCP-1 mRNA and protein have been detected within the NZB/W strains and inhibition of MCP-1 ameliorates the disease progression in both the NZB/W and MRL+/− SLE strains, decreasing the myeloid recruitment into the kidney (44–46, 48, 50). In addition MCP-1 is increased in sera and kidney of patients with SLE, correlating with disease activity manifestations (47, 49). In addition, both keratinocyte chemotactant and G-CSF were significantly higher within the kidney following combined treatment with anti-GBM and IFNα. These molecules are primarily responsible for the recruitment of neutrophils into the site of inflammation. In addition, G-CSF has been found to increase nephritis upon low dose administration in the MRL+/− strain, and it may drive the differentiation of monocytes into dendritic cells; both of these findings thus place it as an important mediator in disease progression (51, 52). IL-12p40 was also increased in diseased mice treated with the combination of IFNα and anti-GBM. We and others have previously demonstrated an elevated production of IL-12, which is important for T cell activation and proliferation, across multiple murine lupus models (53–57).

Importantly, all of these changes appear to be occurring at the level of the end-organ, because parallel changes were not observed in the degree of systemic immune responsiveness to the administered rabbit Ig (data not shown). Given the local expression of IFN-I within the end-organs (rather than infiltrating leukocytes), and its apparent association with disease severity in immune nephritis, it is important to carefully evaluate the role of renal-expressed IFN-I in spontaneous lupus nephritis.

We propose that the anti-GBM model of experimentally induced nephritis offers an accessible snapshot into the pathogenesis of spontaneous lupus nephritis, offering us the means to assess the initiation and progression of disease within a defined time frame. Specifically, in the 2-wk experimental model of nephritis, the anti-GBM challenge is targeting the kidney directly via exogenous kidney-specific autoantibodies. The immune response, which leads to nephritis, appears to parallel events that take place in spontaneous models, including a requirement for T cells and myeloid infiltration. However, eventually the inflammation is resolved in nonautoimmune prone strains, which is likely due to the clearance of the immune complexes within the kidney, in the absence of an additional challenge. This is unlike the nephritis in spontaneous models, where there is a continuous cycle of inflammatory challenge, with the endogenous Abs and immune complexes persisting far beyond a 2 wk period.

We have previously described a multistep model in which at least three genetically determined pathways lead to the development of SLE (58–60). The first stage of disease development results in a loss of tolerance to nuclear Ags and the production of autoantibodies, as exemplified by Sle1. This is not sufficient to drive full disease pathology. The second pathway or checkpoint involves the peripheral dysregulation in innate and/or adaptive immunity, including myeloid cell hyperactivity or up-regulated TLR expression, as exemplified by Sle3 and Yaa. The third pathway or checkpoint dictates the severity of end organ damage, once pathogenic autoantibodies are formed.

The reported findings indicate that at least the third pathway or checkpoint that is operative within the end organs is susceptible to IFN-I driven amplification. Whether IFN-I has the capacity to drive any of the other checkpoints in lupus development warrants further investigation. The suggestion that locally produced IFN-I could be critical in driving end-organ pathology in autoimmunity could have profound implications toward our understanding of how IFN-I contributes to SLE, and how we manage it clinically.

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Disclosures

The authors have no financial conflict of interest.

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