Proliferative lesions and metalloproteinase activity in murine lupus nephritis mediated by type I interferons and macrophages

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Glomerulonephritis is a major cause of morbidity in patients with systemic lupus erythematosus. Although substantial progress has been made in the identification of pathogenic triggers that result in autoantibody production, little is known about the pathogenesis of aggressive proliferative processes that lead directly to irreversible glomerular damage and compromise of renal function. In this study, we describe a model of polyinosinic: polycytidylic acid-accelerated lupus nephritis in NZB/W mice that is characterized by severe proliferative lesions with de novo crescent formation, findings that are linked with decreased survival and adverse outcomes in lupus. Proliferative glomerulonephritis was associated with infiltrating kidney macrophages and renal expression of IFN-inducible genes, matrix metalloproteinases (MMPs), and growth factors. Crescent formation and renal MMP and growth factor expression were dependent on renal macrophages that expressed Ifi10, MMPs, osteopontin, and growth factors, including Pdgfc and Hbegf. Infiltrating macrophages and renal MMP expression were induced by type I IFN. These findings reveal a role for type I IFNs and alternatively activated macrophages in aggressive proliferative lesions of lupus nephritis.

alternative macrophages | kidney disease | polyinosinic: polycytidylic acid | systemic lupus erythematosus | tissue repair

Renal involvement occurs in most patients with systemic lupus erythematosus (SLE); however, our understanding of the mechanisms that lead to irreversible renal injury is limited. The patterns of glomerular injury in human lupus nephritis are diverse. Mesangial and endothelial cell proliferation and leukocyte infiltration are frequent findings of active disease. Extracapillary epithelial cell proliferation, which leads to the formation of a cellular crescent, is a less-frequent finding in human lupus nephritis; however, it is characteristic of severe glomerular injury and an indicator of poor prognosis (1–3). The underlying mechanisms of crescent formation in lupus nephritis are largely unknown.

Type 1 interferons (IFNs) have been implicated in SLE pathogenesis and are thought to promote autoimmunity through activation of dendritic cells, T cells, and autoreactive B cells (4). Subsequent deposition of autoantibodies and immune complexes contributes to renal tissue damage (5). Lupus patients exhibit increased expression of IFN response genes (6, 7) and type I IFN receptor deficiency reduces autoimmunity in lupus-prone mice (8, 9). In addition, administration of an adenovirus encoding IFNα (AdIFNα), or the synthetic dsRNA mimic polyinosinic: polycytidylic acid [poly (I:C)], which induces an IFN response, accelerates the development of autoimmunity and clinical disease manifestations in several strains of lupus-prone mice, including NZB/W mice (10–13). NZB/W mice are a well-established model of SLE that develops nephritis associated with autoantibody deposition. Increased nephritis in poly (I:C)- and IFN-accelerated models of SLE, such as the NZB/W model, has been attributed to accelerated and increased autoantibody production and glomerular immune complex deposition. The direct effects of poly (I:C) and type I IFNs on glomerular disease are unknown.

Here we aimed to identify the effects of poly (I:C) and type I IFNs on the effector inflammatory phase of lupus nephritis that follows glomerular autoantibody deposition. To this end, we used poly (I:C) or AdIFNα to induce systemic IFN expression in NZB/W mice after the development of autoimmunity and at the early phase of autoantibody deposition, but before clinically penetrant nephritis. Poly (I:C) and IFN induced rapid onset of proteinuria and nephritis in NZB/W mice at 24 weeks of age. In contrast to spontaneous disease that develops in NZB/W mice at ∼40 weeks of age, IFN-accelerated nephritis was characterized by dramatically increased proliferative lesions with de novo crescent formation, and with increased renal expression of IFN response genes, matrix metalloproteinases (MMPs), and growth factors. Proliferative nephritis was mediated by infiltrating CD11b\textsuperscript{Gr1}+CD11c\textsuperscript{F4/80} macrophages that expressed Ifi10, osteopontin and growth factors, including Pdgfc and Hbegf. Our findings support a crucial role for type I IFNs and alternatively activated macrophages as mediators of aggressive proliferative lesions in murine lupus nephritis.

Results

Poly (I:C) Induces Proliferative Crescentic Glomerulonephritis in Lupus-Prone Mice. NZB/W mice are a well-characterized model of murine lupus nephritis, in which proteinuria and lupus nephritis develop spontaneously between 20 and 44 weeks of age (median 40 weeks of age, n = 10, our observations). Although there is significant heterogeneity in the time of onset of proteinuria, the course of glomerular disease in these mice is fairly uniform: after 2 weeks of proteinuria there is diffuse endocapillary proliferation, which subsequently progresses to glomerular sclerosis, fibrotic crescents, and death from end-stage renal disease by 36 to 54 weeks of age (14).

Poly (I:C) is a potent inducer of systemic type I IFN production in vivo and is used to accelerate autoimmunity in lupus models (13). To study more direct effects of poly (I:C) on lupus nephritis, we injected NZB/W mice with poly (I:C) for 4 weeks between 19 and 23 weeks of age, when autoantibody deposits are starting to develop (Fig. S1, Left) but renal disease is not apparent (Fig. L4, Left) (15). We used NZB/W mice with spontaneous renal disease as disease controls. We monitored mice daily for proteinuria and killed them 1, 4, and 14 days after the onset of proteinuria. After 2


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weeks of proteinuria, poly (I:C)-treated mice manifested both mesangial and endocapillary cellular proliferation and extensive crescent formation, while spontaneously proteinuric mice manifested mesangial expansion and endocapillary cellular proliferation without significant formation of crescents (Fig. 1A). This was of great interest because crescent formation in human membranoproliferative lupus nephritis is indicative of severe glomerular injury and associated with decreased survival and adverse outcomes (1–3). Poly (I:C)-treated mice displayed significantly higher histological glomerular and interstitial scores, with crescent formation in 60% of glomeruli; crescents were not observed in glomeruli from spontaneously proteinuric mice (Fig. 1B). Poly (I:C) did not induce any notable changes in the amount or distribution of glomerular autoantibody deposition relative to spontaneously nephritic mice (Fig. S1), suggesting that the observed effects may be secondary to mechanisms distinct from immune complex deposition. Poly (I:C) treatment resulted in clinically evident nephrotic syndrome and dramatically increased serum blood urea nitrogen levels (mean > 100 mg/dL, P < 0.001) within 2 weeks after the onset of proteinuria (Fig. S2). We conclude that poly (I:C) in older NZB/W mice induces nephritis with a distinct pathology from that occurring in spontaneous nephritis, thus providing an opportunity to investigate the pathogenesis of the proliferative and crescentic components of lupus nephritis.

**Poly (I:C) Induces a Bimodal IFN Response in Nephritic Kidneys.** We examined whether crescent formation was associated with induction of IFN-response genes in the kidneys in response to poly (I:C) treatment. As expected, poly (I:C) induced a high peak of IFN response gene expression 6 h following poly (I:C) injection, which was resolved by 24 h (Fig. 1C). Interestingly, poly (I:C) treatment induced a second peak of IFN-response gene expression that became apparent 2 weeks after the onset of proteinuria, in contrast to spontaneously proteinuric mice (Fig. 1C and Fig. S3). Thus, poly (I:C)-induced proliferative glomerulonephritis was associated with an increased IFN response in renal tissue.

**Infiltration of Kidneys of Poly (I:C)-Treated Mice by CD11b<sup>-</sup>Gr1<sup>-/hi</sup>F4/80<sup>-</sup> Macrophages.** Severe renal injury can be mediated by infiltrating proinflammatory leukocyte populations. FACS analysis of cells extracted from kidneys of poly (I:C)-treated mice showed a time-dependent increase of mononuclear CD11b<sup>-</sup>Gr1<sup>-</sup>F4/80<sup>-</sup> myeloid cells, which, after 2 weeks of proteinuria, were mostly F4/80<sup>-</sup> (Fig. 2A). This was in contrast to the low numbers of kidney neutrophils, T cells, B cells, and dendritic cells (Fig. 2B); CD11b<sup>-</sup>Gr1<sup>-</sup> neutrophils were a minor population and increased from 0.035 to 0.35% of extracted kidney cells after poly (I:C) treatment. Compared to spontaneous disease, the number of infiltrating macrophages (defined as CD11b<sup>-</sup>Gr1<sup>-/hi</sup>F4/80<sup>-</sup>) in poly (I:C)-induced

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**Fig. 1.** Poly (I:C) induces severe proliferative glomerulonephritis with crescents. Untreated nonproteinuric, untreated spontaneously proteinuric, and poly (I:C)-treated proteinuric NZB/W mice, were analyzed after 14 days of proteinuria. (A) Paraffin-embedded kidney sections stained for basement membrane and matrix constituents with periodic acid shiff stain (PAS). (B) Glomerular and interstitial histological scores. Each symbol indicates an individual mouse (n = 4–11 mice per group). **, P < 0.01. (C) Mice were treated with poly (I:C) for the indicated duration of treatment and analyzed after the indicated duration of proteinuria; kidney cortex mRNA was analyzed by real-time RT-PCR for ISG15 and STAT1; values are relative to expression of the gene encoding GAPDH (n = 4–6 mice per group). *, P < 0.05.

**Fig. 2.** Poly (I:C) promotes kidney infiltration by CD11b<sup>-/hi</sup>Gr1<sup>-/hi</sup>F4/80<sup>-</sup> macrophages. Untreated nonproteinuric, untreated spontaneously proteinuric and poly (I:C)-treated proteinuric NZB/W mice, were analyzed after 1, 4, and 14 days of proteinuria. (A) Whole-kidney single-cell suspensions were analyzed by FACS for expression of myeloid cell markers Gr1, CD11b, and F4/80. Representative dot plots from individual mice are shown. Percentages represent means ± SD (n = 3–6 mice per group). (B) Relative percentage of B cells, T cells, granulocytes, macrophages, and dendritic cells, per total kidney cells. Percentages represent means ± SD from three independent experiments (n = 3–9 mice per group). (C) Localization of CD11b<sup>-</sup> cells in poly (I:C)-treated kidneys after 14 days of proteinuria. Representative of three independent experiments.
Macrophages were located predominantly in periglomerular and intraglomerular regions and were also scattered throughout the intestium of poly (I:C)-treated mouse kidneys (Fig. 2C). Thus, poly (I:C)-induced kidney macrophages were both numerous and strategically located at the interface of active disease. This finding led us to investigate the effects of kidney macrophage depletion in poly (I:C)-treated mice.

**Macrophage Depletion Suppresses Proliferative Lesions and Down-Regulates IFN Response Gene Expression in Poly (I:C)-Treated Mice.** Macrophage depletion was achieved using the well-established approach of i.v. injection of clodronate liposomes (16, 17). This method depletes phagocytic monocytes, with effective depletion of bone marrow and circulating monocytes (thus effectively suppressing monocyte trafficking into inflammatory sites) and more variable depletion of tissue macrophages. Clodronate liposome treatment effectively diminished poly (I:C)-induced accumulation of kidney macrophages (Fig. 3A). Strikingly, depletion of infiltrating kidney macrophages resulted in dramatically diminished poly (I:C)-induced intraglomerular proliferative lesions and crescent formation was abrogated (Fig. 3A and B). Furthermore, macrophage depletion in poly (I:C)-treated mice suppressed the renal expression of IFN-response genes (Fig. 3C). These data collectively indicated that macrophages mediate both glomerular proliferative lesions and drive the delayed renal IFN response in poly (I:C) nephritis.

**Macrophage-Mediated Induction of Renal MMP Activity.** Increased MMP expression and activity have been reported in several forms of human glomerulonephritis, including lupus nephritis (18–20), and attenuation of nephritis by MMP inhibitors has implicated these proteases in pathogenesis (20, 21). In particular, the gelatinases Mmp2 and Mmp9 cleave basement-membrane components, and mostly collagen IV, a major event in induction, progression, and repair of kidney disease (22). We therefore tested whether poly (I:C) increases MMP expression and activity in proteinuric kidneys. mRNA expression of Mmp2, Mmp9, Mmp14 and the tissue inhibitor of metalloproteinase 1 was up-regulated in poly (I:C)-induced nephritis (Fig. 4A). Homogenates from poly (I:C)-treated kidneys had increased enzymatic activity of MMP9 and Mmp2 compared to nonproteinuric kidneys (Fig. 4B and Fig. S4). We verified by Western blot the expression of MMP9 and Mmp2 in cortical kidney lysates from poly (I:C)-treated mice and compared them with spontaneously proteinuric and nonproteinuric controls.
(Fig. 4C). There was a striking up-regulation of Mmp2 in poly (I:C)-treated proteinuric kidneys, compared to spontaneously proteinuric and nonproteinuric controls (Fig. 4C and Fig. S4). Because MMP14 is known to activate Mmp2 (23), we tested MMP14 expression by Western blot. Similarly to Mmp2, MMP14 expression was significantly up-regulated in poly (I:C)-treated proteinuric kidneys, compared to spontaneously proteinuric mice and non-proteinuric controls (Fig. 4C and Fig. S4).

We next determined the location of MMP gelatinolytic activity in the kidneys by in situ zymography (Figs. 4D). Gelatinolytic activity was highly induced in the glomeruli of poly (I:C)-nephritic kidneys, while lower levels of activity were detected in spontaneously proteinuric mice and controls.

We tested whether increased MMP activity in poly(I:C)-induced nephritis was dependent on infiltrating macrophages. Macrophage depletion down-regulated MMP mRNA expression (Fig. 4A), MMP activity measured by zymography (Fig. 4B and Fig. S4), suppressed the expression of Mmp2, MMP9, and MMP14 proteins as measured by Western blot (Fig. 4C and Fig. S4), and reversed the poly (I:C)-induced gelatinolytic activity in proteinuric glomeruli (Fig. 4D). All together, these data indicated that poly (I:C)-induced kidney macrophages are crucial for the Mmp2- and MMP14-mediated basement membrane remodeling, which is central in the pathogenesis of proliferative nephritis.

**Poly (I:C) Increases Renal Growth Factor Expression and Collagen IV Deposition in a Macrophage-Dependent Fashion.** The response to tissue injury can be cellular proliferation or fibrotic processes, both of which usually involve up-regulation of growth factors. We tested the expression of several growth factor genes in poly (I:C)-treated kidney cortices. Tgfβ, Hbegf, Pdgfβ, and osteopontin expression were up-regulated in poly (I:C) nephritis compared to spontaneous disease or nonproteinuric kidneys (Fig. 5).

To determine whether the poly (I:C)-induced proliferative changes had a fibrotic component, we used trichrome staining, which stains collagens and connective tissue blue, as well as immunohistochemistry to detect collagen IV. Trichrome staining was negative both in the area of crescents and the renal interstitium (Fig. S5A). Collagen IV deposition was weakly positive selectively in crescents of poly (I:C) nephritic kidneys after 2 weeks of proteinuria, with a focal pattern (Fig. S5B).

Macrophage depletion down-regulated poly (I:C)-induced renal expression of growth factors (Fig. 5), as well as crescents and crescent-associated collagen IV deposition (Fig. S5B). Collectively, these data indicate that poly (I:C), via macrophages, induced growth factor expression associated with acute crescent formation and early deposition of collagen IV, suggestive of early stage fibrosis.

**Renal Macrophages Express Il10, MMPs, Osteopontin, and Growth Factors in Poly (I:C)-Induced Nephritis.** Macrophages can be classified on a functional continuum ranging from classically activated macrophages that produce inflammatory cytokines, regulatory macrophages, and “alternatively activated” macrophages that participate in tissue remodeling (24, 25). The current paradigm implicates classically activated macrophages in the pathogenesis of inflammatory diseases, such as lupus nephritis (26). We analyzed the functional phenotype of renal mononuclear phagocytes in poly (I:C)-induced nephritis using FACS and mRNA expression analysis of isolated renal macrophages. Purified renal macrophages were B220–CD4–CD8–CD49b–CD11b+CD11c+IFN-γ+ cells (Fig. 6A). Contrary to expectations, renal macrophages in poly (I:C)-induced nephritis did not express substantially elevated Tnfα, Il12, or Nos2 mRNA relative to macrophages from control or spontaneously nephritic NZB/W mice (Fig. 6B and Fig. S6). Instead, we found striking increases in expression of Il10, Mmp2, osteopontin, and the growth factors Pdgfβ and Hbegf in poly (I:C)-induced nephritic macrophages compared to spontaneous disease-associated or resident macrophages (Fig. 6B). Mmp14, on the other hand, was up-regulated in the macrophages from poly (I:C)-induced as well as from spontaneously proteinuric kidneys. These results show that macrophages directly contribute to the elevated Mmp2 and Mmp14 expression described above. Il10, osteopontin, Pdgfβ, and Hbegf are expressed by alternatively activated macrophages and are associated with tissue remodeling, proliferation, and scar formation (25, 27). Thus, the results suggest that poly (I:C)-induced lesions were caused by alternatively activated macrophages expressing a dysregulated “tissue repair” program.

**Type I IFN Mediated Induction of Proliferative Crescentic Nephritis.** The accelerating effects of poly (I:C) on SLE autoimmunity have been attributed to induction of type I IFNs (12), although it is possible that other poly (I:C)-induced cytokines or direct activation of cells by poly (I:C) may contribute. To explore the role of IFNs in our system, we administered AdIFNα to 20-week-old NZB/W mice, thus mimicking the time course of IFN production induced by poly (I:C) in the above-described experiments.

![Fig. 5.](image)

Poly (I:C) increases growth factor expression in a macrophage-dependent fashion. Kidney cortex mRNA was analyzed by real-time PCR for Tgfβ, Hbegf, Pdgfβ, and osteopontin; values are relative to expression of the gene encoding GAPDH (n = 4–6 mice per group). *, P < 0.05; **, P < 0.01; ***, P < 0.001.

![Fig. 6.](image)

Poly (I:C)-induced kidney macrophages have a dysregulated tissue repair phenotype. (A) Whole kidney-cell suspensions were labeled with antibodies against lineage markers (B220, CD4, CD8, CD5, CD49b), as well as myeloid cell markers (CD11b, F4/80, CD11c) and analyzed by FACS. Lineage (B220–CD4–CD8–CD49b–)CD11b+CD11c+IFN-γ+ cells were sorted. (B) Total RNA from sorted kidney macrophages was analyzed by real-time PCR for Il10, Tnfα, Mmp2, Mmp14, osteopontin, Hbegf, and Pdgfβ; values are relative to expression of the gene encoding GAPDH (n = 4–5 mice per group). *, P < 0.05.
Administration of adenovirus LacZ (AdLacZ) has been previously reported to have no effect on renal disease in NZB/W mice (10, 11), as we also verified. Similarly to poly (I:C) treatment, administration of AdIFNα not only precipitated nephritis, but also resulted in increased proliferative lesions (Fig. 7 A and B), with crescent formation and significantly higher numbers of infiltrating renal macrophages than in spontaneously nephritic mice (Fig. 7C). Furthermore, similar to the effects of poly (I:C), gelatinolytic activity was increased in glomeruli of AdIFNα-injected mice and this activity was suppressed by macrophage depletion in parallel with a significant decrease in glomerulonephritis scores (Fig. 7A, B, and D). These data show that IFNα is sufficient to induce the key aspects of poly (I:C)-induced proliferative nephritis, although it remains possible that additional poly (I:C)-induced responses contribute to proliferative nephritis.

**Discussion**

The most severe forms of lupus nephritis are characterized by inflammatory and proliferative glomerular lesions that lead to fibrosis, scarring, and loss of renal function. Pathogenic mechanisms underlying proliferative glomerulonephritis in lupus, and mechanisms linking inflammation and immune cell infiltration with proliferation and fibrosis are not known. The present study describes a mouse model of lupus nephritis induced by poly (I:C) or AdIFNα and characterized by increased MMP activation, glomerular proliferation, and rapid crescent formation (as summarized in Fig. S7). MMP activation and proliferative nephritis were mediated by infiltrating macrophages that exhibited characteristics of alternative activation, including expression of Il10, MMPs, osteopontin, and growth factors. This model is suitable for the study of mechanisms of proliferative and crescentic glomerular lesions that lead to fibrosis, and identify a pathogenic role for alternatively activated macrophages in murine lupus nephritis.

Emerging evidence has demonstrated an association between macrophage infiltration of kidneys and nephritis in murine and human lupus nephritis (3, 15, 29–31), but the role of these macrophages in the disease process was not known. We previously reported that renal macrophages are markers of disease onset in spontaneous NZB/W nephritis (15), and others have shown macrophage infiltration of kidneys in IFN-accelerated models of lupus nephritis (28, 31). It has been previously suggested that classically activated macrophages that produce inflammatory cytokines may contribute to inflammation in lupus nephritis (26). In contrast, a striking finding of this study was that the dominant kidney macrophage population in poly (I:C)- or IFN-treated mice did not express markers of classical activation, such as Tnfα, Nos2, or Il12. Instead, they showed evidence of alternative activation and expressed Il10, MMPs, osteopontin, and growth factors, including Pdgfc and Hbegf (Fig. 6B). Alternatively activated kidney macrophages and their products have been implicated in tissue remodeling, proliferation, and fibrosis in other systems. PDGF and Hbegf play a major role in proliferative nephritis in nonautoimmune models, MMPs have been linked to lupus nephritis pathogenesis (32), and osteopontin is associated with glomerular crescent formation and ensuing fibrosis and glomerular sclerosis (33). Thus, our results implicate alternatively activated macrophages in the proliferative and crescentic components of lupus nephritis that are dominant in our model. Relative levels of infiltration by classically and alternatively activated macrophages can vary over time during inflammatory processes (34, 35), and these macrophage subtypes can coexist at sites of inflammation, including nephritis (26). Thus, it is likely that macrophages can contribute to both inflammatory and proliferative components of lupus nephritis, depending on the stage of disease and the type of pathology.

Consistent with a dysregulated tissue repair function, macrophage depletion after the onset of poly (I:C)-induced glomerulonephritis suppressed extracapillary crescentic proliferation (Fig. 3A and B), Mmp2 and MMP14 expression (Fig. 4), growth factor expression, and early deposition of collagen IV (Fig. 5 and Fig. S5B). Previous studies have implied that MMP activation in injured myocardium is mediated by proinflammatory macrophages with scavenger functions (35). Interestingly, in our nephritis model this function is instead mediated by alternatively activated macrophages. The increased expression of Mmp2, and its activator MMP14, which were induced by alternative tissue repair macrophages in our model, is consistent with the key role of Mmp2 in the glomerular remodeling that takes place during crescent formation and involves cleavage of basement membrane components and enhancement of cell proliferation (22).

Depletion of renal infiltrating macrophages was achieved using liposomal clodronate. This method is well established (16, 17) and, compared to the CD11b-DTR method of depletion, offers the advantages that it preferentially depletes phagocytic monocytes and avoids issues related to complexity of genetic background and backcrossing transgenes onto the NZB/W strain. Liposomes cannot cross capillary walls, therefore they are known to deplete phagocytic monocytes in the blood, as well as macrophages in the liver and spleen, where macrophages are in contact with the blood but not interstitial macrophages (16). This finding suggests that the infiltrating kidney macrophages likely originated from extravasated blood monocytes that differentiated in situ into alternatively activated macrophages. The dominant renal myeloid cells observed in our study were monocytic B220-CD4-CD8-CD49b-GR1-CD11bhiF4/80+ cells, which
expressed intermediate levels of CD11c (Figs. 2B and 6D). These cells were likely macrophages, given their expression of the macrophage marker F4/80 and our finding of a distinct population of CD11c<sup>+</sup>CD11b<sup>+</sup> cells (Fig. 6G,15), which likely represent myeloid dendritic cells; however, it remains possible that some of these CD11b<sup>+</sup>CD11c<sup>+</sup> cells may correspond to renal dendritic cells. The alternatively activated phenotype of these macrophages is most likely induced at least in part by simultaneous exposure to immune complexes and TLR ligands, as has been described in other systems (25). It is also possible that the activated renal parenchyma contributes to alternative macrophage differentiation through expression of factors such as IL-33 (Fig. S8) (36), and this possibility will be investigated in future work.

Our model differs from previously described models of TLR ligand- and IFN-activated SLE where TLR ligands or IFN are administered to young lupus-prone mice to accelerate autoimmunity, autoantibody production and subsequent autoantibody-mediated glomerulonephritis (10, 11, 33). In these other models, direct effects of TLR ligands and TLR-induced type I IFNs have predominated, and the induction of nephritis is entirely dependent on the effects of IFNs on autoantibody deposition. Instead, in this model, polycl (IC) and IFNs act on renal cells during development of nephritis and in this setting promote a dominant proliferative and crescentic pathology. Interestingly, IFN alone was sufficient to induce nephritis (Fig. 7), although it is possible that direct activation of immune or kidney cells by polycl (IC) contributed to the observed pathology. These findings suggest a pathogenic role for IFNs in the kidney and in promoting monocytic infiltration, which is consistent with a pathogenic effect of IFNs on the renal parenchyma in other systems (28).

Renal injury and repair is a balance between cell loss and proliferation, which when dysregulated leads to fibrosis and glomerular sclerosis. Our study provides evidence that, in the setting of subclinical autoimmunity in NZB/W mice, polycl (IC), and IFN greatly increase matrix metalloproteinase activation and growth factor expression to promote glomerular cell proliferation and early fibrosis. This process was mediated by alternatively activated macrophages expressing a dysregulated tissue repair program. These findings suggest that manipulating kidney macrophage programming in lupus nephritis may represent a fruitful therapeutic approach to attenuating proliferative nephritis and associated irreversible renal cell loss and deterioration of function in SLE.

Materials and Methods

Mice. Nineteen-week-old female NZB/NZW F1 (Jackson Laboratory) mice were treated i.p. with 200-μg poly IC (Invivogen) three times per week for 4 weeks, or i.v. with a single injection of AdIFNα or AdLacZ or PBS, as previously described (12).

Macrophage Depletion. Macrophages were depleted by i.v. injections of mice with liposomes containing dichloromethylene bisphosphonate (clodronate liposomes) (17) on days 0, 3, 7, and 11 after the onset of ≥300 mg/dL proteinuria. See SI Materials and Methods and Table S1 for more detailed information.

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