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TLR7-Mediated Lupus Nephritis Is Independent of Type I IFN Signaling

Sonya J. Wolf,*† Jonathan Theros,* Tammi J. Reed,* Jianhua Liu,* Irina L. Grigorova,‡ Giovanny Martínez-Colón,† Chaim O. Jacob,§ Jeffrey B. Hodgin,¶ and J. Michelle Kahlenberg*

Systemic lupus erythematosus is an autoimmune disease characterized by increased type I IFNs, autoantibodies, and inflammatory-mediated multiorgan damage. TLR7 activation is an important contributor to systemic lupus erythematosus pathogenesis, but the mechanisms by which type I IFNs participate in TLR7-driven pathologic conditions remain uncertain. In this study, we examined the requirement for type I IFNs in TLR7-stimulated lupus nephritis. Lupus-prone NZM2328, INZM (which lack a functional type I IFN receptor), and NZM2328 IL-1β−/− mice were treated at 10 wk of age on the right ear with R848 (TLR7 agonist) or control (DMSO). Autoantibody production and proteinuria were assessed throughout treatment. Multiorgan inflammation was assessed at the time of decline in health. Renal infiltrates and mRNA expression were also examined after 14 d of treatment. Both NZM2328 and INZM mice exhibited a decline in survival after 3–4 wk of R848 but not vehicle treatment. Development of splenomegaly and liver inflammation were dependent on type I IFN. Interestingly, autoantibody production, early renal infiltration of dendritic cells, upregulation of IL-1β, and lupus nephritis occurred independent of type I IFN signaling. Development of TLR7-driven lupus nephritis was not abolished by the deletion of IL-1β. Thus, although IFN-α is sufficient to induce nephritis acceleration, our data emphasize a critical role for IFN-independent signaling in TLR7-mediated lupus nephritis. Further, despite upregulation of IL-1β after TLR7 stimulation, deletion of IL-1β is not sufficient to reduce lupus nephritis development in this model. The Journal of Immunology, 2018, 201: 393–405.

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Address correspondence and reprint requests to Dr. J. Michelle Kahlenberg, University of Michigan, 5570A MSRB II, 1150 W. Medical Center Drive, Ann Arbor, MI 48109-5678. E-mail address: mkahlenb@med.umich.edu

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Abbreviations used in this article: alb, albumin; BMDM, bone marrow–derived macrophage; Cr, creatinine; DC, dendritic cell; dLN, draining LN; KO, knockout; LN, lymph node; PAS, periodic acid–Schiff; WT, wild-type.

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nephritis. These data support IFN-independent immune activation in the presence of robust TLR7 stimulation as sufficient for acceleration of lupus nephritis.

Materials and Methods

Mice

All mice were bred and housed at the University of Michigan in specific pathogen free (SPF) housing. All mice were treated according to the University of Michigan Institutional Animal Care and Use Committee-approved protocol. NZM2328 mice and INZM (lacking the α-chain of the IFN-α/β receptor) mice were a kind gift of Dr. C. Jacob, University of Southern California (12). NZM2328 IL-1β−/− mice were generated through the University of Michigan Transgenic Animal Model Core via CRISPR/Cas9 technology using IL-1β CRISPR/Cas9 knockout (KO) Plasmid sc-421097 from Santa Cruz Biotechnology (Santa Cruz, CA). An 8-bp deletion resulting in a frame shift mutation was confirmed via Sanger sequencing through the University of Michigan DNA Sequencing Core (Supplemental Fig. 1). NZM2328 IL-1β−/− mice were backcrossed onto the NZM2328 background for three generations to eliminate off-target effects of using the CRISPR system, followed by heterozygote crossing to develop mice homozygous for the IL-1β deletion. Female 10-wk-old mice were used for all experiments. The NZM mouse model has minimal autoantibody production and no nephritis at this age (12). Males were not used, as they do not achieve a lupus phenotype in this model, and lupus is predominantly a female disease (18).

TLR7 cutaneous stimulation

Female 10-wk-old mice were treated via epicutaneous application of 100 μg of the TLR7 agonist R848 (Enzo Life Sciences) dissolved in 8 μl of DMSO, or DMSO alone as a control, to the right ear three times weekly until euthanasia. DMSO and R848-treated mice were housed in separate cages to avoid cross-contamination. For some studies, mice were treated for only 2 wk before euthanasia (for evaluation of manifestations preproteinuria); others were treated until they developed proteinuria or became moribund (for survival studies), followed by euthanasia. Control mice were harvested with their paired R848-treated littermates.

Analysis of anti-dsDNA and IgG serum levels

Serum was collected every 2 wk. Anti-dsDNA and IgG levels were analyzed with the use of ELISA kits (Alpha Diagnostic, San Antonio, TX, and Innovative Research, Novi, MI).

Analysis of proteinuria

Urine was collected weekly, and protein was screened via dipstick followed by albumin (alb) measurements via Albuwell kits (Exocell, Philadelphia, PA) and total creatinine (Cr) via commercial kit (BioAssay Systems, Hayward, CA). Urinary protein excretion was represented by the alb/Cr ratio.

Renal histopathology and immune complex deposition scoring

Glomerular inflammation (activity index) and scarring (chronicity index) of murine kidneys were quantified in a blinded fashion (by J.B. Hodgkin) on perfused kidneys fixed in 10% formalin followed by 3-μm sectioning and periodic acid–Schiff (PAS) staining as previously described by us and others (19, 20). In brief, a semiquantitative scoring system (0: no involvement; 0.5: minimal involvement [10% of section]; 1: mild involvement [10–30% of section]; 2: moderate involvement [31–60% of section]; 3: severe involvement [60% of section]) was used to assess 13 different parameters of activity and chronicity (mesangial hypercellularity, mesangial deposits, mesangial sclerosis, endocapillary cellular infiltrate, subepithelial and subendothelial deposits, capillary thrombi, capillary scarring, cellular or organized crescents, synechiae, tubular atrophy, and interstitial fibrosis). The chronicity and activity index were generated by compiling the scores from groups of related parameters (for activity: mesangial hypercellularity, mesangial deposits, and endocapillary cellular infiltrate; for chronicity: interstitial fibrosis, tubular atrophy, synechiae, organized crescents, and capillary scerosis). Glomerular immune complexes were quantified by immunofluorescence microscopy as previously described (19). Briefly, 6-μm frozen kidney sections were stained for 1 h at 4°C with Texas Red–conjugated anti-mouse IgG (Sigma-Aldrich) and FITC–conjugated anti-3′-CL (Immunology Consultants Laboratory, Portland, OR) followed by Hoechst (Invitrogen, Eugene, OR) counterstain to stain DNA. Quantification of immune complex staining in eight glomeruli per mouse was performed at the Center for Live-Cell Imaging at the University of Michigan using MetaMorph version 7.0.6 to calculate the mean fluorescence in a defined area for each stain. Glomeruli were identified based on DAPI staining and outlined to define the area for analysis. Both FITC and Texas Red staining were calculated and shown as staining per glomerulus.

Liver scoring

Inflammation of murine livers were quantified blindly by J.B. Hodgkin on livers fixed in 10% formalin followed by 3-μm sectioning and PAS staining as described by others (21). In brief, a scoring system (0: <0.5 inflammatory foci/field; 1: 0.5–1.0 foci/field; 2: 1.0–2.0 foci/field; 3: >2.0 foci/field) was used.

Microscopy

Images of H&E-stained sections and PAS-stained kidney and liver sections were captured using an Olympus BX41 microscope with a ×100 objective (total magnification ×1000). Images of kidney immune complex staining were captured at the Center for Live-Cell Imaging at the University of Michigan Medical School using an Olympus IX70 inverted microscope (Olympus, Center Valley, PA) with a ×40 objective.

Flow cytometry

Following euthanasia, the lymph nodes (LNs) (draining LN [dLN] from the cervical chain on the treated side and non-dLN from the inguinal chain) and spleen were removed, teased apart, and passed through a 70-μm filter to generate single-cell suspensions. One of the kidneys was also removed, minced, and digested as previously described (19) with 0.1 mg/ml Liberase (Roche), 200 U/ml DNase (Roche), and 2.4 mM CaCl2 in DMEM (Invitrogen) at 37°C in a humidified incubator for 1 h. The tissue was then passed through a 70-μm cell strainer, and RBCs were lysed with multi-species RBC Lysis Buffer (eBioscience). Live cells were counted via trypan blue exclusion. The cells were incubated in flow block (1% horse serum and 1% BSA in PBS) for 1 h, then stained for 1 h on ice using the following Abs: CD3-allophycocyanin, F4/80-PE, CD11b-allophycocyanin, CD11c-PE, CD4 allophycocyanin/Cy7, CD8 allophycocyanin/Cy7, B220 allophycocyanin (BioLegend, San Diego, CA), and Qdot 506 Streptavidin-Cy5 (Invitrogen), followed in some cases by permeabilization and intracellular staining for IgH+ A488 (Southern Biotech, Birmingham, AL). The flow data were collected using a BD LSR II flow cytometer and analyzed via FlowJo version 10.0.7 (Tree Star). The following gating strategy was used: live cells were gated for T cells: CD3+; macrophages: CD11c+CD11b+F4/80+; DCs: CD11c+CD11b+F4/80−; B cells: CD4+CD8+IgH+L2B20+; and plasma cells: CD4+CD8+IgH+L2B20−.

Bone marrow–derived macrophages

Bone marrow–derived macrophages (BMDM) were generated as previously described (22). Bone marrow was flushed from the tibiae and femurs of 10-wk-old NZM2328 mice and NZM IL-1β−/− mice and plated in macrophage differentiation medium (59% IMDM, 10% FBS, 30% L-cell supernatant, and 1% penicillin/streptomycin) for 7 d at 37°C in 5% CO2. BMDM were then plated 2 × 106 cells per well of a 6-well plate for detection of IL-1β activation.

IL-1β quantification

BMDM were incubated with or without 1 μg of LPS for 4 h followed by 1 h incubation with or without 5mM of ATP to activate the inflammatory core. Secreted IL-1β was measured via ELISA (DuoSet ELISA kit; R&D, Abingdon, U.K.). Cells were lysed with RIPA plus protease inhibitors for 10 min on ice. Ten micrograms of each lysate was then run on a 10% SDS–polyacrylamide gel and blotted on nitrocellulose membranes (GE Healthcare). The membranes were blocked in 4% milk/TBST, followed by probing with anti-murine IL-1β Ab 1:500 (Cell Signaling Technology) overnight. Then, the membrane was incubated with anti-rabbit IgG-HRP 1:1000 (Abcam). The pro–IL-1β band was detected using WesternBright Quantum (Advansta) and imaged using the Lum C system (Aplegen).

Real-time quantitative PCR analysis

Kidney tissue was homogenized in TriPure (Roche), and RNA was purified via Direct-zol RNA MiniPrep (Zymo Research). RNA (100 ng) was reverse-transcribed into cDNA, and quantitative real-time PCR analysis was completed on an ABI PRISM 7900HT (Applied Biosystems) by the DNA Sequencing Core at the University of Michigan. The primers used were as follows (all listed 5′→3′): C-C Motif Chemokine Ligand 2 (ccl2) 5′-AGTCCCTGTCATCCTGTGTC-3′ (forward), 5′-GGATCATCTTTGCTGGAAT-3′ (reverse); Myxovirus (influenza virus) resistance 1 (mx1)

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rise of dsDNA Abs in the serum was greater in NZM versus IgG production, both NZM2328 and INZM mice demonstrated NZM2328 mice (19). Importantly, although only NZM2328 mice shows that autoantibody production following TLR7 epicutaneous stimulation leads to accelerated development of murine lupus.

We next examined the development of systemic lupus characteristics following R848 stimulation. Using our assay, dsDNA Abs typically rise to ~100,000 U/ml during nephritis onset in NZM2328 mice (19). Importantly, although only NZM2328 mice treated with R848 demonstrated significant acceleration of total IgG production, both NZM2328 and INZM mice demonstrated increased dsDNA Abs in the serum (Fig. 2A, 2B); however, the rise of dsDNA Abs in the serum was greater in NZM versus INZM mice (p = 0.0167 NZM versus INZM at 2 wk). This suggests that autoantibody production following TLR7 epicutaneous stimulation is enhanced by but not dependent on type I IFN signaling. Consistent with previous literature (12, 25), splenomegaly was detected following R848 stimulation in NZM2328 but not INZM mice, supporting a role for IFNs in TLR7-driven splenomegaly (Fig. 2C). An increase in total splenic cells following R848 stimulation was detected in only NZM2328 mice; analysis of cell subsets identified no significant changes in T cells, B cells, macrophages (Fig. 2D, 2E). Further, NZM2328 mice treated with R848 demonstrated accelerated development of liver inflammation as indicated by necrotic hepatocytes and focal portal inflammation. INZM mice treated with R848 did not develop liver inflammation, indicating that type I IFNs are required for this manifestation following TLR7 activation (Fig. 2F, 2G).

We next examined changes in B cells and Ab-secreting cells in the spleen and LNs. As shown in Fig. 3, both total B cells (B220+) and Ab-secreting cells (IgH+Lhigh) increased in the spleen of NZM2328 mice, but only Ab-secreting cells increased in INZM mice after R848 stimulation (Fig. 3A–C), suggesting that amplification of splenic Ab production is independent of type I IFNs. We then examined the LN populations to determine if IFN-independent B cell activation was also occurring there. As shown in Fig. 3D, stimulation with R848 resulted in increased total number of cells in the cervical (draining) LN in both NZM2328 and INZM mice. In contrast, increased cell numbers were noted in inguinal (nondraining) LN in only NZM mice after R848 treatment. The total number of B cells (B220+) in both NZM2328 and INZM mice was increased in dLN but not the non-dLN following R848 stimulation (Fig. 3E). NZM2328 mice showed an increase in the Ab-secreting cell population in the dLN and non-dLN, but INZM mice only demonstrated an increase in the dLN (Fig. 3F). Of note, the overall numbers of B cells and Ab-secreting cells were significantly smaller in the INZM mice. These data indicate that TLR7 stimulation amplifies local B cell and Ab production responses in the absence of type I IFN signaling, but the responses may be diminished. However, systemic amplification of Ab-secreting cells in the non-dLN required the presence of type I IFN signaling. Together, this suggests that the accelerated autoantibody production seen after TLR7 cutaneous stimulation is partially type I IFN–independent and that the Ab-secreting cells in the dLN and spleen may serve as a site for autoantibody production in this model.
FIGURE 2. TLR7 stimulation leads to IFN-independent elevated autoantibody production and IFN-dependent splenomegaly and liver inflammation. Ten-week-old NZM2328 and INZM mice treated with R848 or DMSO control were analyzed. (A) Total IgG in serum of NZM2328 and INZM mice after 2 wk of treatment. Each dot represents an individual mouse. (B) dsDNA IgG in serum at 0, 2, and 4 wk of treatment (n = 13 NZM R848; n = 14 NZM DMSO; n = 9 INZM DMSO; n = 11 INZM R848). (C) Spleen weight of NZM2328 and INZM mice from survival studies. Spleens were harvested when mice were moribund (around 20–40 d of treatment). Littermate DMSO controls were harvested at the same times as the moribund mice. Each dot represents an individual mouse. Representative photographs of DMSO and R848 NZM2328 spleens shown in inset. (D) Total number of splenocytes for each treatment group is shown. (E) Immune cell populations in the spleen were evaluated by flow cytometry after 2 wk of R848 or DMSO treatment (n = 13 NZM R848; n = 11 NZM control; n = 8 INZM control; n = 9 INZM R848). (F and G) Ten-week-old NZM2328 and INZM mice treated with R848 or DMSO control were treated until moribund and analyzed for development of liver inflammation. (F) Representative photo of the portal vein (via 100× objective). (G) Graph represents liver inflammation scoring of NZM2328 and INZM mice. Each symbol represents one mouse.
FIGURE 3. TLR7 stimulation leads to IFN-independent increases in secreting cells in the dLN and the spleen. Immune cell populations in the spleen were evaluated by flow cytometry after 2 wk of R848 or DMSO treatment (n = 5 NZM DMSO; n = 5 NZM R848; n = 5 INZM DMSO; n = 5 INZM R848). (A) Gating strategy for Ab-secreting cells. (B and C) Graphs displaying changes in B cells: CD4<sup>+</sup>CD8<sup>+</sup>IgH<sup>+</sup>IgL<sup>B220<sup>+</sup></sup> [shown in (B)] and Ab-secreting cells: CD4<sup>+</sup>CD8<sup>-</sup>IgH<sup>+</sup>IgL<sup>high</sup> [shown in (C)]. (D) Total number of cells isolated from indicated LNs for DMSO or R848-treated mice. (E and F) Graphs displaying changes in B cells: CD4<sup>+</sup>CD8<sup>+</sup>IgH<sup>+</sup>IgL<sup>B220<sup>+</sup></sup> [shown in (E)] and Ab-secreting cells: CD4<sup>+</sup>CD8<sup>-</sup>IgH<sup>+</sup>IgL<sup>high</sup> [shown in (F)] for dLN (cervical) and non-dLN (inguinal). Data are displayed as mean ± SD. Each symbol represents one mouse.
TLR7 epicutaneous stimulation leads to accelerated development of lupus nephritis in a type I IFN–independent manner

Past work has shown type I IFNs are sufficient to induce renal flares in lupus-prone mice and are required for lupus nephritis development in several murine models (12, 15, 16, 26). We next examined whether TLR7 epicutaneous stimulation led to accelerated lupus nephritis development and whether it was dependent on type I IFNs. Surprisingly, both NZM2328 and INZM mice treated with R848 demonstrated a rise in urinary alb/Cr ratio, supportive of glomerular damage (Fig. 4A, 4B). Histopathologic scoring demonstrated a significant increase in renal activity score when mice were treated with R848 until moribund (Fig. 4C, 4D). A strong positive correlation was detected between renal activity score and urinary alb/Cr ratio for both NZM2328 and INZM mice (Fig. 4E), which supports renal inflammation in R848-treated mice. No significant increase in renal chronicity index score for prolonged exposure in NZM2328 or INZM mice was detected (Fig. 4F). As immune complex deposition is a hallmark of lupus nephritis, this was also assessed. R848 treatment of the NZM2328 and INZM mice led to a significant increase in both IgG and C3 deposition within the kidney (Fig. 5A–C). To examine whether TLR7-induced lupus created similar transcriptional changes to naturally occurring lupus nephritis found in older, untreated NZM2328 mice, we examined transcriptional signatures of the kidneys of both. As shown in Supplemental Fig. 2, identical upregulation of various inflammatory and type I IFN–associated genes was noted in both R848-induced and naturally occurring nephritis. Together, these parameters support development of accelerated lupus nephritis following cutaneous stimulation with a TLR7 agonist in a type I IFN–independent manner.

Discussion

In this paper, we examine a novel model of lupus flare in which accelerated lupus nephritis is induced in genetically prone mice after 3–4 wk of epicutaneous application of a TLR7 agonist, R848. R848 treatment led to accelerated development of autoantibodies, splenomegaly, liver inflammation, and lupus nephritis. With the use of NZM mice lacking the type I IFN receptor, we demonstrated that TLR7-mediated splenomegaly and liver inflammation were dependent on type I IFN signaling. Surprisingly, however, renal injury was independent of type I IFNs. Indeed, INZM mice demonstrated proteinuria, increased renal immune complex formation, upregulation of NF-κB–regulated cytokines, and infiltration of CD11b+CD11c+Foxp3+ DCs in the kidney, similar to NZM mice.

Human genetics support a role for TLR7 in lupus (4, 5), and murine data has further supported this (9, 10, 11). BXSB male mice develop lupus secondary to the Yaa locus that contains a duplication of the TLR7 gene (23). The role of TLR7 has also been demonstrated in transgenic mice overexpressing TLR7 (9) and in lupus inducible models such as the pristane model (10). A recent paper also demonstrated that TLR7 epicutaneous stimulation of WT mice led to development of mild lupus characteristics after long-term (13–15 wk) treatment (11). Our model furthers these observations and demonstrates that TLR7 stimulation rapidly (in 3–4 wk) accelerates lupus development in young, lupus-prone mice. Further, we show that mice that are otherwise protected from lupus development in the absence of functional IFN signaling (12) are similarly susceptible to the nephritis-inducing effects of TLR7. Others have shown that overexpression of IFN-α...
FIGURE 4. TLR7-mediated lupus nephritis occurs in an IFN-independent manner. Ten-week-old NZM2328 and INZM mice treated with R848 or DMSO control were analyzed for development of lupus nephritis. (A) Urine Alb/Cr ratio was measured serially in NZM2328 treated mice (n = 12 NZM R848; n = 15 NZM DMSO). (B) Urine Alb/Cr ratio was measured serially in INZM treated mice (n = 5 INZM DMSO; n = 6 INZM R848). (C) Representative photo of the glomeruli in the kidney of NZM2328 and INZM mice following treatment until moribund. Littermate control mice were harvested at the time of illness in R848-treated mice. Scale bar, 20 μm. (D) Renal activity score for NZM2328 and INZM mice after 2 wk of treatment or when moribund from R848 treatment (long-term treatment). (E) The moribund renal activity score for NZM2328 and INZM mice treated with R848 and DMSO was plotted versus the Alb/Cr ratio at euthanasia and analyzed via Pearson correlation. (F) Renal chronicity index for NZM2328 and INZM mice after 2 wk of treatment and when moribund (long-term treatment).
is sufficient to stimulate lupus nephritis in a similar time frame to TLR7 treatment (15). However, in the presence of TLR7 agonist, type I IFN signaling is not required. This observation may reflect duplicative roles of other inflammatory cytokines induced by TLR7.

Recently, the role of type I IFN has been explored in other murine models of lupus. Treatment of mice with HgCl₂ is able to induce autoantibody production in a lysosomal TLR–dependent but type I IFN–independent fashion (30), similar to the data we see in the R848 NZM model. Development of renal disease and direct contribution of TLR7 in this xenobiotic model were not assessed (30). In a different model, exacerbation of autoimmunity via deletion of TLR9 in the MRL/lpr mouse was found to be type I IFN dependent (31). In this model, IFNs are required for development of renal disease but not anti-nucleosome Ab production. HepG2–positive anti-nuclear Abs and anti-RNA Abs were dependent on type I IFN signaling (31). Combined with our findings, these data

![FIGURE 5. Immune complex deposition in the kidney is IFN independent. Ten-week-old NZM2328 and INZM mice treated with R848 or DMSO until moribund were analyzed for immune complex deposition. (A) Representative immunofluorescence microscopy of glomeruli (outlined by white dashed line), Texas Red, IgG; green, C3; blue, DAPI. (B and C) Quantification of immune complex staining/area was completed. Littermate DMSO controls were harvested when littermates were ill. (B) Quantification of IgG/area. (C) Quantification of C3/area. Each dot represents the average fluorescence of eight glomeruli from a single mouse.](image_url)
FIGURE 6. TLR7-mediated upregulation of IL-1β is not required for lupus nephritis. (A–C) RNA was isolated from the kidney of NZM or INZM mice treated for 2 wk with DMSO control or R848. Real-time PCR was completed using primers for the genes listed. Graphs display the mean ± SD for each gene as compared with the average of β-actin. Each dot represents an individual mouse. (D) Ten-week-old NZM IL-1β KO mice were treated with R848 or DMSO control, and survival was plotted (n = 9 NZM IL-1β−/− DMSO; n = 8 NZM IL-1β−/− R848). (E) Anti-dsDNA IgG in serum at 2 wk of treatment (n = 8 NZM IL-1β−/− R848; n = 8 NZM IL-1β−/− DMSO). (F) Representative photomicrograph of the glomeruli in the kidney of moribund NZM IL-1β−/− mice treated with R848 or DMSO. Littermate controls were harvested when R848-treated mice were moribund. Scale bar, 20 μm. (G) Renal activity score for NZM IL-1β−/− mice when moribund. (H) Renal chronicity index for mice in (G).
suggest that the role for IFNs in lupus development may vary depending on the stimuli. Autoantibody production may be independent of IFNs when the trigger is strong environmental exposure (such as R848 or HgCl2). Conversely, lupus that develops based on genetic factors without the need for external triggers (such as TLR9-deficient MRL/lpr or NZM2328 mice) may require IFN signaling for autoantibody and nephritis development.

NF-κB activation occurs downstream of TLR7 stimulation, and activation of this pathway is important for stimulation of lupus nephritis (32). Thus, TLR7 activation of NF-κB pathways may be a critical step in driving lupus flares. Mutations in A20 that drive NF-κB activation in WT mice led to lupus nephritis (33, 34), possibly through decreased regulation of inflammasome activity (35). Although roles for TNF-α and IL-6 in lupus development...
have been suggested (36), and IL-6 can exacerbate TLR7-driven lupus (37), we do not see these cytokines significantly upregulated in the kidney in an IFN-independent manner in our TLR7 stimulation model. Intriguingly, we see an upregulation of IL-1β in the kidney prior to the onset of lupus nephritis in both NZM2328 and INZM mice. This finding coincides with data that support a potential role for IL-1β and inflammasome activation in lupus development (reviewed in Ref. 38). However, deletion of IL-1β in NZM2328 mice did not reduce the development of lupus nephritis after TLR7 exposure. Given that TLR7 activates pleotropic pathways, generation of double or triple KOs may be required to hinder lupus nephritis development in the presence of such a strong inflammatory activator.

Similar to others (12), we found that splenomegaly following TLR7 stimulation was type I IFN dependent. Interestingly, the massive R848-induced splenomegaly was out of proportion to the small increases in B and T cell populations in the spleen that were identified by flow. This supports a possible role for extra medul- lary hematopoiesis resulting in R848-mediated splenomegaly, consistent with findings by other groups (39, 40).

TLR7 epicutaneous stimulation can lead to development of autoantibody production in the absence of type I IFN signaling. We were able to see an increase in dsDNA Abs in NZM2328 and INZM mice following R848 stimulation, although the increase in anti-dsDNA levels was less robust in INZM mice. Increases in B (B220+) cell numbers in the spleen were noted in only the NZM mice, which suggests that expansion of B cell populations in the spleen may require type I IFNs following TLR7 exposure. This would be consistent with a recently described role for splenic follicular DC production of type I IFN in promotion of autoreactive B cell populations (41, 42). Type I IFNs also enhance the TLR7 signaling response, allowing for autoantibody production (43), and they support survival of transitional stage B cells in the spleen (44). Thus, the more extensive systemic immune activation seen in NZM mice may be secondary to these effects of type I IFN. Overexpression of TLR7 stimulation has also been shown to lead to expansion of transitional stage B cell populations in a type I IFN–independent manner (45). Given that we see a rise in Ab-secreting cells in the spleen and dLN but not in the non-dLN of INZM mice, this may indicate that a certain threshold of TLR7 stimulation, and possibly other cytokines, is needed to generate Ab-secreting cell expansion in a type I IFN independent manner.

TLR7 stimulation results in the recruitment of CD11b⁺CD11c⁺ F4/80⁺ DCs in the kidney prior to proteinuria onset without significant changes in the T cell or B cell population. Interestingly, this recruitment occurs in a type I IFN–independent manner. The DC chemoattractant MCP-1/CCL2 was increased in the kidney in both NZM and INZM mice, which supports its potential role for instigating nephritis downstream of TLR7 activation. Intriguingly, CCL2 has been shown to rise during human lupus nephritis development (27) and is proposed as a urinary biomarker for disease (46). Blockade of CCL2 demonstrates some efficacy in murine lupus models (47). Macrophages, which also are recruited by CCL2, were not increased in the kidney after R848 treatment, which may reflect differential upregulation of CCL2’s receptor, CCR2, on DCs and macrophages in this model.
Overall, consideration of the role of CCL2 downstream of TLR7-driven lupus nephritis should be made in future studies. In summary, we have demonstrated a novel role for TLR7 epicitaneous stimulation in mediating lupus flare in lupus-prone mice. Following stimulation, splenomegaly and liver inflammation occur in a type I IFN–dependent manner. Importantly, autoantibody production and lupus nephritis occur independent of IFN signaling. We demonstrate that IL-1β is not required for development of TLR7-activated lupus nephritis. Future studies should address the role of CCL2 in TLR7-mediated lupus nephritis.

In addition, our data lend a note of caution to ongoing trials using type I IFN blockade in lupus nephritis; consideration of the upstream drivers of nephritis (which may vary in individual patients) may be important for identifying effective treatment modalities. In particular, our data would suggest that blockade of type I IFN signaling may not be effective if TLR7 is driving the phenotype.

**Disclosures**

The authors have no financial conflicts of interest.

**References**


altered transitional B cell signaling and function in systemic lupus erythematous. J. Autoimmun. 58: 100–110.


