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Anti-IFNAR Antibody Treatment Ameliorates Disease in Lupus-Predisposed Mice

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Abstract

The demonstration in humans and mice that nucleic acid-sensing Toll-like receptors (TLRs) and type I interferons (IFNs) are essential disease mediators is a milestone in delineating the mechanisms of lupus pathogenesis. Here, we show that *Ifnb* gene deletion does not modify disease progression in NZB mice, thereby strongly implicating IFN-α subtypes as the principal pathogenic effectors. We further document that long-term treatment of male BXSB mice with an anti-IFNAR antibody of mouse origin reduced serologic, cellular and histologic disease manifestations and extended survival, suggesting that disease acceleration by the *Tlr7* gene duplication in this model is mediated by type I IFN signaling. The efficacy of this treatment in BXSB mice was clearly evident when applied early in the disease process, but only partial reductions in some disease characteristics were observed when treatment was initiated at later stages. A transient therapeutic effect was also noted in the MRL-Fas^{lpr} model, although overall mortality was unaffected. The combined findings suggest that IFNAR blockade, particularly when started at early disease stages, may be a useful treatment approach for human SLE and other autoimmune syndromes.

Introduction

Type I IFNs, particularly the IFN-αs and IFN-β, have received prominent attention for their role in the pathogenesis of systemic lupus erythematosus (SLE) and other autoimmune and inflammatory syndromes (1, 2). By signaling through a common receptor (IFNAR), these pleiotropic cytokines affect almost every aspect of innate and adaptive immune responses, including upregulation of MHC and costimulatory molecules, and production of B cell survival factors (BAFF, April) by antigen-presenting cells, culminating in the engagement and expansion of autoreactive T and B cells (1, 2). Of particular relevance to lupus pathogenesis is the induction of type I IFNs under sterile conditions through the engagement of endosomal Toll-like receptors (TLRs) by self-nucleic acids (3–6). This systemic autoimmunity-inducing pathway has been well documented by studies showing reduced disease in predisposed mice lacking expression of endosomal TLRs (7), IFNAR (8, 9), or Unc93b1 (10), a molecule that acts as a transporter of TLRs 3, 7 and 9 from ER to endolysosomes.

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These findings have stimulated considerable interest in creating treatments based on blocking reagents against either the multiple IFN-αs and the single IFN-β, or their common receptor. The potential utility of these approaches would be considerably advanced by further defining the role of type I IFNs in lupus mice with diverse genetic abnormalities, the potential difference in pathogenicity between the IFN-α subtypes and IFN-β, and the clinical stage where blockade of signaling by these cytokines is effective. Here, we address some of these issues and demonstrate that the disease-promoting effect of type I IFNs in lupus is primarily mediated by the IFN-αs, type I IFN signaling significantly contributes to disease in BXSB mice but minimally in MRL-*Fas*^{lpr} mice, treatment with an anti-IFNAR antibody has therapeutic efficacy even with partial IFNAR blockade, and effectiveness is most evident when treatment is initiated at early disease stages. These findings provide support for the potential utility of IFNAR blockade for the treatment of human SLE, but suggest that the type of patient and timing of treatment may be crucial factors in determining the outcome.

Materials and Methods

Mice

BXSB.*Yaa*, MRL-*Fas*^{lpr}, and C57BL/6 (B6) mice were obtained from The Jackson Laboratory (Bar Harbor, ME) or The Scripps Research Institute Animal Facility. NZB mice deficient for IFNAR1 (*Ifnar1*$^{-/-}$) have been reported (8), and marker-assisted congenic NZB mice deficient for IFN-β (*Ifnb*$^{-/-}$) were generated as described (8). Mice were housed under specific pathogen-free conditions and all experimental protocols were performed according to the NIH Guide for the Care and Use of Laboratory Animals and approved by The Scripps Research Institute Animal Care and Use Committee.

Treatment with a monoclonal Anti-IFNAR Antibody

Male BXSB mice were treated with a monoclonal anti-IFNAR antibody of mouse origin (clone MAR1-5A3, Leinco Technologies) (11). Injections (i.p., 500 μg for 3 consecutive days, followed by 250 μg three times per week until experiment termination) were started either before (12 weeks of age) or after (17 weeks of age) appearance of disease manifestations, as suggested by detectable autoantibody titers and proteinuria. MRL-*Fas*^{lpr} mice were similarly treated, but starting at 7 wks of age due to the expedited disease course in this strain.

Cell Preparations

Single cell suspensions were prepared from bone marrow (BM), blood, peritoneal cavity, spleen and lymph nodes (LN, inguinal, axillary, brachial, cervical), as described (12). B cells were purified from spleen or peritoneal cavity using magnetic beads (MACS, Miltenyi Biotec), while conventional DCs (cDCs) and plasmacytoid DCs (pDCs) were prepared by stimulating BM cells with recombinant mouse GM-CSF or Flt3 ligand (R&D Systems), respectively (13).

Flow Cytometry

Monoclonal antibodies to mouse CD4, CD8, B220, CD11b, CD11c, PDCA-1, IFNAR1, CD69, CD86, CD25, CD21, CD23, AA4.1, CD138, I-A^b, H2-K^b, and GR-1 were obtained from BD Pharmingen, Biolegend or eBioscience. For surface staining, cells were sequentially incubated with various combinations of antibodies or streptavidin (BD Pharmingen). Cell events were acquired on four-color FACSCalibur™, and data analyzed using FlowJo software (Tree Star).

In Vitro Studies

Purified splenic B cells and BM-derived cDCs and pDCs were cultured in complete medium and stimulated or not with mouse IFN-α11 (1000 U/ml, Miltenyi Biotec), the TLR7 ligand R848 (30 ng/ml, InvivoGen), or both, in the presence or absence of the anti-IFNAR antibody (10 μg/ml). Splenic T cells were stimulated with plate-bound anti-CD3 and plate-bound or soluble anti-CD28 antibodies in the presence or absence of anti-IFNAR antibody (10 μg/ml). At the indicated time-points, cells were harvested, counted, and analyzed by flow cytometry, while supernatants were assayed for cytokines or IgM titers by ELISA.

ELISA

ELISA for polyclonal IgM and IgG was performed using 96-well plates coated with goat anti-mouse immunoglobulin (Jackson ImmunoResearch Laboratories), and for anti-chromatin and anti-ribonucleoprotein (RNP) autoantibodies using plates coated with chromatin or RNP (Inova Diagnostics), respectively. Bound antibodies were detected using alkaline phosphatase-conjugated goat antibodies specific for mouse IgM, IgG and IgG isotypes (Southern Biotech), and standard curves were generated using calibrated mouse serum (Nordic Immunology). Commercial ELISA kits were used to examine B cell and DC culture supernatants for the presence of IL-6, IL-10 (Biolegend), or IFN-α (PBL InterferonSource).

Anti-Nuclear and Anti-Erythrocyte Autoantibodies

Anti-nuclear autoantibodies (ANA) were detected using HEp-2 slides (Bion Enterprises), serial serum dilutions (1/10 to 1/3000), and Alexa-Fluor 488-conjugated goat anti-mouse IgG (Invitrogen), and the results were expressed as the inverse of the maximal serum dilution giving positive staining. Erythrocyte-bound autoantibodies were quantified by flow cytometry after sequential incubation of washed red blood cells (RBC) with biotinylated goat anti-mouse IgG (MP Biomedicals) and streptavidin-PE (Biolegend).

Kidney Pathology and Immunohistology

Proteinuria was determined using Albustix strips (Bayer Corporation) and graded semiquantitatively (0 = negative to traces, 1 = 30mg/ml; 2 = 100 mg/ml, 3 = 300 mg/ml, 4 = 2000 mg/ml). Zinc formalin-fixed and PAS/hematoxylin-stained tissue sections were scored blindly on a 0–4 scale for degree of glomerulonephritis (GN) (14). Kidney sections were examined by immunofluorescence for the presence of immune deposits and cellular infiltrates using antibodies to mouse IgG2a (Invitrogen), C3 (Nordic Immunology), CD11c, or CD11b (BD Bioscience), while spleen sections were stained with antibodies to CD19 (B cells), PDCA-1 (pDC) (BD Bioscience), or TLR7 (Imgenex). Glomerular IgG2a deposits and CD11b and CD11c staining were scored blindly on a 0–4 scale based on the intensity of the immunofluorescence signal.

Statistical Analysis

Group comparisons were analyzed by unpaired two-tailed Student's *t* test. Survival was analyzed by Kaplan-Meier plot and log rank test. $p < 0.05$ was considered significant.

Results

Differential Roles of IFN-α and IFN-β in systemic autoimmunity

We have reported that lupus-predisposed NZB mice lacking the IFNAR1 subunit of the common receptor for type I IFNs showed significant reductions of disease manifestations (8). We generated *Ifnb*$^{-/-}$ NZB mice to determine whether the disease-inhibiting effect of *Ifnar1* gene deletion results from absence of signaling by the multiple IFN-α or the single

IFN-β. We found that, unlike *Ifnar1*$^{-/-}$ NZB mice, disease progression and severity were unaltered in *Ifnb*$^{-/-}$ NZB mice compared to wild-type (WT) controls, including autoantibody production, hemolytic anemia, kidney disease and mortality (Fig 1). The results indicated that the autoimmunity-promoting effects of type I IFNs in this model are primarily mediated by IFN-αs. Therefore, because of the multiplicity of IFN-αs, therapeutic inhibition of signaling by these cytokines may be best accomplished using an anti-IFNAR antibody.

In vitro Effects of IFNAR blockade

Using the anti-IFNAR monoclonal antibody MAR1-5A3 (11), we assessed surface IFNAR expression in immunocytes from male BXSB mice, which carry the *Yaa*-associated *Tlr7* gene duplication (15, 16). IFNAR levels were comparable in CD4 and CD8 T cells, B cells, and monocytes (CD11b^{+}CD11c^{-}), but lower in conventional DCs (CD11b^{+}CD11c^{+} and CD11b^{-}CD11c^{+}) and higher in pDCs (PDCA-1^{+}CD11c^{low}) (Fig 2A), and similar findings were obtained with cells from control C57BL/6 mice (not shown). IFNAR expression remained unchanged in B cells cultured for 24 hr in the absence of stimuli, modestly decreased following incubation with IFN-α, and increased ~2-fold upon TLR7 stimulation, whereas the combination of IFN-α and a TLR7 ligand had a null effect (Fig 2B). Thus, ligand-induced IFNAR down-modulation, a mechanism critical for signal termination (17–19), is compensated by TLR7 engagement, likely to sustain an efficient response.

We next examined the effect of anti-IFNAR antibody on in vitro responses by B cells, T cells and DCs. Complete IFNAR blockade (Fig 3A) significantly inhibited the synergistic effect of IFN-α and TLR7 stimulation on B cells, including upregulation of CD69 and CD86, and production of IL-6, IL-10 and IgM (Fig 3B–C). The antibody treatment also partially inhibited TLR7 ligand-induced production of IL-6 by cDCs and pDCs, and IFN-α by pDCs (Fig 3D), but T cell activation through CD3 and CD28 ligation was unaffected, as suggested by equal upregulation of CD25 and CD69 (not shown). These results indicate that type I IFNs and TLR7 act synergistically, and IFNAR blockade partially inhibits the effects of TLR7 engagement in B cells and DC subsets.

Early Anti-IFNAR Treatment of BXSB Mice

To examine the effect of IFNAR blockade on systemic autoimmunity, male BXSB mice were treated with anti-IFNAR antibody starting at the preclinical stage (12 wk of age). Treatment consisted of three consecutive daily injections of 500 μg of antibody, followed by 250 μg three times weekly until termination. Assessment of IFNAR expression 4 weeks after treatment commencement showed significant, although incomplete, receptor blockade (Fig 4A). Nonetheless, considerable reductions in most disease parameters were observed. Thus, at 20 wks of age, anti-IFNAR antibody-treated mice showed decreases in polyclonal IgG isotypes (IgG2a, 1.5 ± 0.9 vs. 2.7 ± 1.0 mg/ml; IgG2b, 1.2 ± 0.7 vs. 2.1 ± 1.0 mg/ml, $p<0.05$) and IgG2a anti-chromatin autoantibodies (Fig 4B). Moreover, while ANA patterns of non-treated mice were diverse (homogeneous, nucleolar, and in some mice speckled and peripheral), those of treated mice were primarily homogeneous and with reduced titers (Fig 4B and not shown). In addition, at the termination of the experiment (38 wks of age), GN scores, kidney deposits (IgG2a, C3) and cellular infiltrates (monocytes, DCs) were significantly reduced and survival was extended compared to controls (Fig 4C–E). Because glomerular CD11b staining (Fig 4C) in untreated BXSB mice could be a reflection of the monocytosis in this strain, we performed control staining comparing perfused and unperfused kidneys and found minimal differences (Supplemental Fig 1). Notably, the glomerular CD11b staining of perfused kidneys remained markedly more intense than that of uperfused kidneys from anti-IFNAR-treated mice. Thus, the reduction of renal mononuclear cell infiltration following IFNAR blockade could not be accounted for by the

degree of reduction of these cells in the circulation, but rather suggests that type I IFN signaling mediates attraction of inflammatory cells in the afflicted organs, either directly by inducing specific sets of chemokines or indirectly by promoting autoantibody production and immune complex deposition.

Lymphoid organ examinations of 38 wk-old anti-IFNAR antibody-treated mice showed reduced splenomegaly and lymphadenopathy (Fig 5A), mostly resulting from numerical decreases in B cells (Fig 5B), which are typically expanded in this strain (20). These decreases primarily affected $CD21^{-}CD23^{-}$ B cells, which include the $AA4.1^{low}$ population phenotypically similar to a subset that accumulates in aged normal mice (21, 22) and found in this study to expand in untreated male BXSB mice (Fig 5C). T2-follicular (T2-FO) B cells ($CD21^{low}CD23^{+}$) were also reduced in several of the treated mice, whereas the low number of marginal zone B cells ($CD21^{+}CD23^{-}$) characteristic of male BXSB and other *Yaa*$^{+}$ mice (23–25) was retained (Fig 5B). The percent of $CD4^{+}$ and $CD8^{+}$ T cells expressing the CD69 activation marker was also decreased (19 ± 5% vs. 32 ± 0.3% for $CD4^{+}$ cells, and 2 ± 1% vs. 12 ± 3% for $CD8^{+}$ cells, $p<0.05$), as were the number of monocytes ($CD11b^{+}CD11c^{-}$) and the level of MHC class I expression by cDCs (GMFI 68.3 ± 0.2 vs. 142.5 ± 37.5, $p<0.05$). Other changes in the spleen included reductions in follicle sizes, number of pDCs clustering at the marginal sinus, and TLR7 expression within follicles (Fig 5E). Overall, the results indicate that early anti-IFNAR antibody treatment significantly reduces disease in male BXSB mice likely by inhibiting activation and expansion of B cells, monocytes and DC subsets.

Late Anti-IFNAR Treatment of BXSB Mice

A group of male BXSB mice was treated starting at 17 wks of age, when anti-chromatin autoantibodies and proteinuria had begun to emerge. This treatment had no significant effects on autoantibody titers, lymphadenopathy, splenomegaly or mortality (not shown). However, at 26 to 33 wks of age, there were significant reductions of blood $Gr\text{-}1^{-}CD11b^{+}$ "resident" monocytes (Fig 6A), the expansion of which is a characteristic of this lupus strain (26), and splenic $CD21^{-}CD23^{-}$ B cells (Fig 6B). Although GN scores were not modified by this late treatment (2.83 ± 1.15 vs. 2.95 ± 0.87 in controls) and proteinuria was only marginally affected (Fig 6C), there were significant reductions in IgG2a deposits and mononuclear cells infiltrates (Fig 6D). Interestingly, in contrast to the untreated mice in which the $CD11b^{+}$ and $CD11c^{+}$ cells were within the glomeruli, in treated animals these cells were confined to the interstitium, suggesting that signaling by type I IFNs contributes to the entry of inflammatory cells in the glomerulus. Thus, therapeutic anti-IFNAR treatment of male BXSB mice initiated after appearance of disease manifestations has measurable effects on monocytosis, B cell expansion and kidney disease, but is significantly less efficient than treatment initiated at an earlier disease phase.

Anti-IFNAR treatment of *Fas*^{lpr} mice

Prophylactic anti-IFNAR antibody treatment was also initiated in 7 wk-old MRL-*Fas*^{lpr} mice. At 12 wks of age, both anti-RNP autoantibody titers and proteinuria were significantly reduced. However, with advancing age, these parameters and mortality converged with those of control mice (Fig 7). Yet, there was no disease enhancement in the treated mice, in contrast to a previous report with *Ifnar1*$^{-/-}$ MRL-*Fas*^{lpr} mice (27).

Discussion

In this study, we further examined the pathogenic role of type I IFNs in mouse models of lupus. We demonstrated that IFN-β does not contribute to disease, pointing to the multiple IFN-αs as the most critical type I IFNs in lupus. Moreover, we showed that antibody-

mediated IFNAR blockade significantly reduced disease in male BXSB mice as well as, transiently, in MRL-*Fas*^{lpr} mice. Of clinical relevance, this treatment was considerably less effective in BXSB mice when initiated late in the disease process.

The contribution of type I IFNs in lupus pathogenesis is supported by several findings, including the predominant representation of IFN-inducible genes correlating with disease flares in peripheral blood mononuclear cells of SLE patients (2, 28–33). Moreover, in lupus-predisposed mice, administration of recombinant IFN-α enhanced autoimmunity (34–37), whereas *Ifnar1* gene deletion reduced disease manifestations (8, 9). These findings have raised the possibility of blocking type I IFN signaling as a means to treat lupus and other syndromes mediated by these cytokines (1). Classically, interference with pathogenic cytokines entails neutralization with antibodies or recombinant receptors or direct blockade of the receptors with antibodies. The first approach is limited by the multiplicity of IFN-α subtypes, demonstrated here to be the main pathogenic mediators of mouse lupus. Therefore, soluble recombinant receptors or direct receptor blockade with antibodies appear to be the methods of choice.

The availability of an anti-mouse IFNAR antibody of mouse origin allowed us to examine the effects of long-term treatment without interference by a host immune response. This anti-IFNAR antibody has been shown to potently inhibit type I IFN-mediated Stat1 phosphorylation, MHC class I upregulation, and iNOS induction in cell lines (11). Here, we showed that this antibody also significantly inhibited in vitro TLR7- and type I IFN-mediated B cell, DC and pDC activation and production of proinflammatory cytokines.

Through *Ifnar1* gene deletion, we previously established the central role of type I IFNs in NZB mice (8). Here, we extend these observations to BXSB male mice with the *Tlr7* gene duplication (15, 16) and MRL-*Fas*^{lpr} mice with a mutation in the major apoptosis-controlling *Fas* gene (38). It has been demonstrated that the *Tlr7* gene duplication is responsible for the accelerated disease phenotype in male BXSB mice, and that transgenic overexpression of TLR7 can cause systemic autoimmunity even in normal background mice (24, 25, 39). In the present study, we found that IFNAR blockade significantly reduced most autoimmune manifestations in male BXSB mice, including autoantibody production, splenomegaly, lymphadenopathy, kidney disease and mortality. These results clearly indicate that the disease-enhancing effect of the *Tlr7* gene duplication is primarily mediated by hyperproduction of type I IFNs.

IFNAR blockade in BXSB mice was optimally effective when initiated at an early stage of disease. This is consistent with our proposed two-phase model of lupus pathogenesis (5), in which the initiation phase mediated by the innate immune system is required for the subsequent amplification phase mediated by the adaptive immune system. Nonetheless, even when treatment was initiated at a relatively advanced disease stage, some reductions in glomerular IgG2a deposits and mononuclear cell infiltrates were noted, although most disease parameters were unaffected. These findings are compatible with previous studies showing that local engagement of TLR7 and type I IFN signaling are involved in renal pathology of humans and mice with lupus (40–42). The limited effect of late anti-IFNAR antibody treatment suggests that IFNAR blockade is less efficient once autoantibody production has exceeded a certain threshold, or inflammatory molecules produced by the adaptive immune system may sustain end organ damage in a type I IFN-independent manner. These molecules may include IFN-γ, which has also been shown to promote disease in lupus-predisposed models (43). Overall, if the results can be translated to human SLE, they suggest that IFNAR blockade should be applied at relatively early disease stages and, as disease progresses, this treatment may require supplementation with additional

interventions aimed at inhibiting downstream injurious adaptive responses and associated inflammatory factors.

It is notable that while the dose and schedule of anti-IFNAR antibody injections led to incomplete occupation of the receptor, the disease reducing effects were still remarkable. This is consistent with our previous study, in which not only homozygous but also heterozygous *Ifnar1* gene-deleted NZB mice showed significantly reduced disease and increased survival (8). Together, these findings strongly suggest that effectiveness of this treatment does not require complete receptor blockade, but rather reduction of receptor availability below a threshold level. Therefore, it could be surmised that translation of this treatment to humans may, upon proper titration, reduce autoimmunity and yet permit relatively normal responses to microbial pathogens.

Considerable evidence indicates that, in most cell types, production of IFN-α requires a preceding induction of IFN-β that, upon binding to the common receptor, induces the transcription factor IRF7 necessary for IFN-α expression (44–46). In contrast, evidence has been presented that this IFN-β feedback loop is not required for IFN-α production by pDCs, presumably due to constitutive expression of IRF7 by these cells (46–48). Our finding that IFN-β is not involved in lupus progression provides indirect evidence that pDCs, likely acting by hyperproduction of type I IFNs, are intimately involved in disease pathogenesis. Interestingly, we also found that IFNAR blockade inhibited in vitro production of type I IFNs by pDCs, consistent with reports in both humans and mice showing that signaling by these cytokines promotes their own expression via autocrine stimulation (49–51). Accordingly, in vivo anti-IFNAR treatment of BXSB mice reduced both follicle size and number of pDCs clustering at the marginal sinus. The latter finding may also relate to the observation that nucleic acid-sensing TLRs and type I IFNs affect expression of chemokine receptors and thus the migration patterns of pDCs (49).

IFNAR blockade also inhibited accumulation of monocytes, cDCs and B cells. Monocytosis has been described as a unique characteristic of male BXSB mice (26), but what drives this expansion is incompletely defined. It appears that the *Tlr7* gene duplication, although in itself insufficient, is required for severe monocytosis (25). Our results showing reduction of monocytosis by IFNAR blockade strongly suggest that type I IFNs are key mediators of this feature. The role of monocytosis in lupus pathogenesis remains unresolved, but possible contributions may include production of proinflammatory cytokines or conversion to mature antigen-presenting DCs (52).

B cell responses in vitro were potentiated by the combined IFN-α and TLR7 stimulation, an effect abolished by IFNAR blockade. Accordingly, in vivo treatment with anti-IFNAR antibody reduced B cell expansion, particularly $CD21^{-}CD23^{-}AA4.1^{low}$ B cells, a subset recently shown to derive from overstimulated follicular B cells, to expand with aging and autoimmunity, and to be enriched in autoreactive clonotypes (21, 22). Expansion of these age-associated B cells (ABCs) in normal mice was reported to depend on signaling by TLR7, but not IFNAR (22). In contrast, our results clearly showed that, in male BXSB mice, expansion of these cells is partly dependent on type I IFNs, perhaps due to engagement of the overexpressed TLR7.

At variance with the strong evidence in both humans and mice that type I IFNs are critical effectors of lupus pathogenesis, one study reported disease acceleration in $Ifnar1^{-/-}$ MRL-Fas^{lpr} mice, suggesting that these cytokines may exert a protective effect in this model (27). We observed that both anti-RNP autoantibody levels and proteinuria were reduced when assessed 5 wks after initiation of anti-IFNAR antibody treatment, but rebounded 4 wks later to levels similar to those in untreated controls. Other parameters, such as anti-chromatin

autoantibodies, lymphadenopathy and mortality, were not modified by this treatment. However, no disease acceleration or enhanced severity was observed in anti-IFNAR antibody-treated mice compared to controls. These results suggest that, early autoimmune events in this model are also influenced by type I IFNs, particularly anti-RNP autoantibody responses known to be dependent on TLR7 engagement (7, 10, 15, 16, 25) and to correlate with type I IFN signaling in SLE (29–31) and mouse models of lupus (36, 53–55). However, the beneficial effect of IFNAR blockade in MRL-*Fas*^{lpr} mice was eventually overcome, likely due to apoptosis defects that may favor the engagement of type I IFN-independent pathogenic pathways. In fact, genome-wide mRNA expression analysis in this strain indicated a predominant IFN-γ-induced gene expression signature, whereas type I IFN genes were minimally modulated (42). The differential disease expression in anti-IFNAR antibody-treated vs. *Ifnar1* gene-deleted MRL-*Fas*^{lpr} mice might be explained by partial vs. complete inhibition of IFNAR signaling, respectively. Thus, it may be hypothesized that initiation and/or continuance of the pathogenic process in lupus requires high IFNAR availability and signaling, whereas a lower degree of type I IFN signaling is sufficient for the beneficial anti-proliferative effects of these cytokines (56).

To summarize, the results clearly establish the pathogenic role of IFN-α in several models of lupus, and strongly support the notion that anti-IFNAR antibodies should be contemplated as a potential treatment for human SLE. In addition, because pDCs, TLRs and type I IFNs have been implicated in the pathogenesis of several other autoimmune diseases, including rheumatoid arthritis, Sjogren's syndrome, and type I diabetes (1, 5), this approach may also be applicable to these diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations used in this paper

SLE	systemic lupus erythematosus
IFNAR	type I IFN receptor
BM	bone marrow
LN	lymph node
cDC	conventional dendritic cell
pDC	plasmacytoid dendritic cell
RNP	ribonucleoprotein
ANA	anti-nuclear autoantibody
GN	glomerulonephritis
WT	wild-type

GMFI	geometric mean fluorescence intensity
ABCs	age-associated B cells

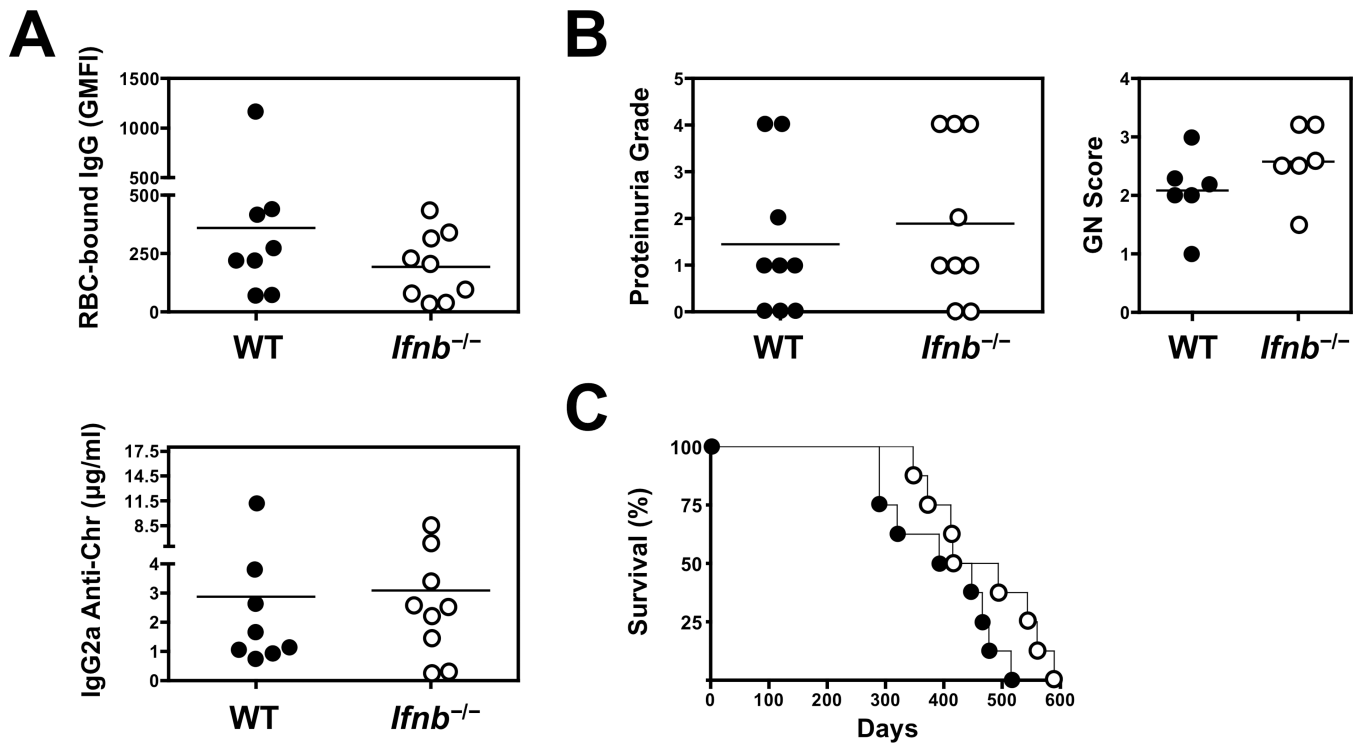

Figure 1. IFN-β Is Not Required for Lupus-Like Disease in NZB Mice

Groups of *Ifnb*$^{-/-}$ and WT NZB mice (n = 8–9/group) were followed for disease manifestations. (A) Anti-red blood cells (RBC) and anti-chromatin autoantibody levels at 9 mo of age. (B) Proteinuria and glomerulonephritis scores at 10 mo of age. (C) Survival. p>0.05 for A–C.

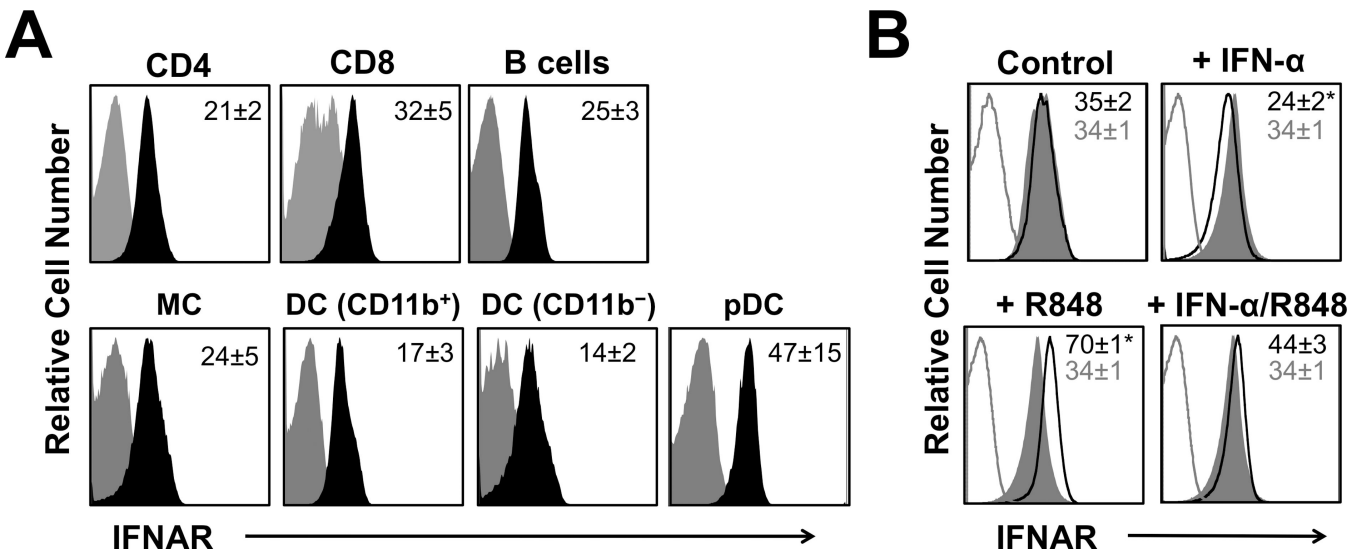

Figure 2. IFNAR Expression in BXSB Mice

(A) Ex vivo analysis. Spleen cells from BXSB mice (16 wks old) were examined by flow cytometry to assess cell surface IFNAR expression after gating on T cells ($CD4^+$ and $CD8^+$), B cells ($B220^+$), monocytes ($CD11b^+CD11c^-$), conventional DCs ($CD11c^+CD11b^+$ and $CD11c^+CD11b^-$), and pDCs ($PDCA1^+CD11c^{low}$). Gray-filled histograms indicate background staining. (B) IFNAR modulation. Splenic B cells from BXSB mice were cultured in vitro for 24 hrs with medium alone (control), or medium containing IFN-α, the TLR7 ligand R848, or both. IFNAR expression on gated $B220^+$ cells (black-lined histograms) was examined as in panel A. Background staining (gray-lined histograms) and IFNAR levels in non-cultured ex vivo B cells (gray-filled histograms) are also depicted. Numbers in histograms represent geometric mean fluorescence intensity (GMFI) ± SD. Asterisks indicate statistical significance ($p<0.05$).

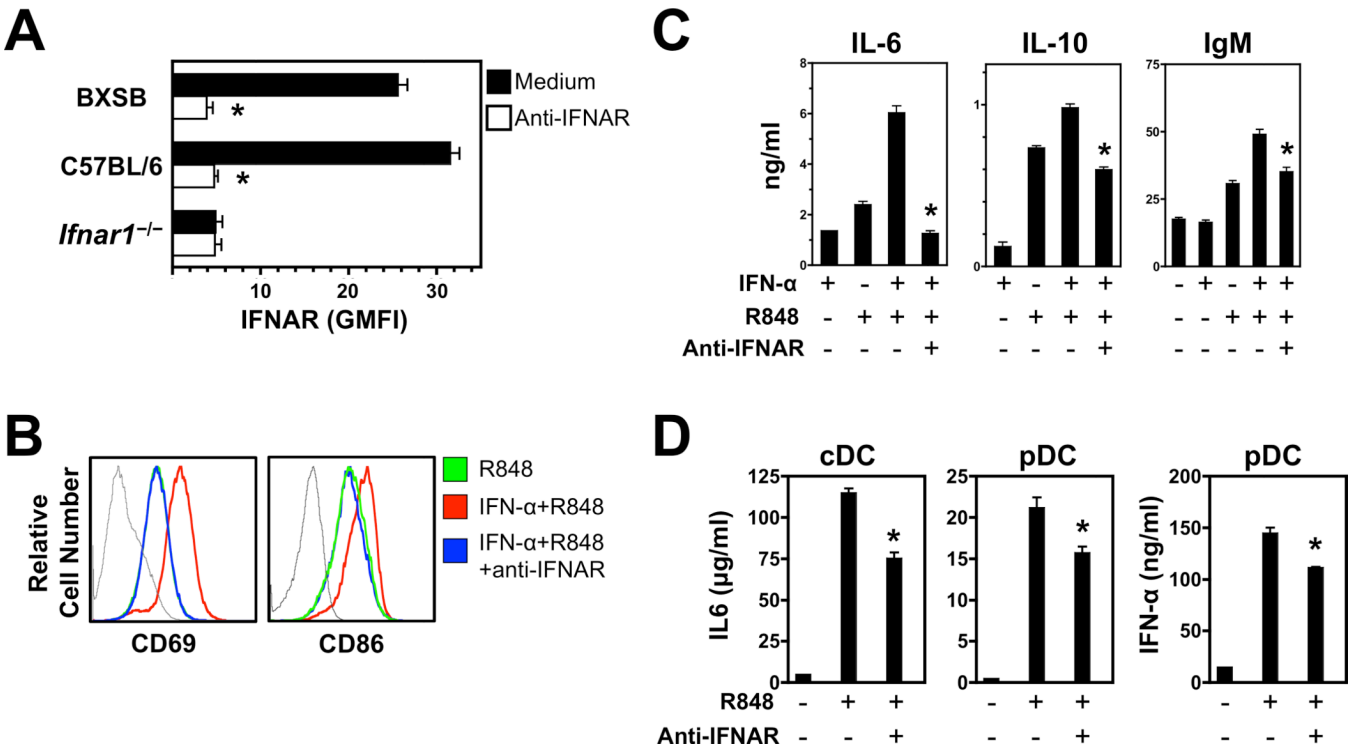

Figure 3. Anti-IFNAR Antibody Inhibits In Vitro Activation of B cells, DCs, and pDCs from BXSB Mice

(A–C) Effects of IFNAR blockade in B cells. (A) B cells from BXSB mice were incubated for 120 hrs in medium supplemented or not with anti-IFNAR antibody. Efficiency of IFNAR blockade on BXSB B cells (compared to similarly cultured B cells from WT and *Ifnr1*$^{-/-}$ C57BL/6 mice) was assessed by flow cytometry and expressed as GMFI ± SD. (B–C) B cells from BXSB mice were stimulated for 120 hrs with medium alone (control) or medium containing IFN-α, the TLR7 ligand R848, or both, in the presence or absence of anti-IFNAR antibody. The effect of IFNAR blockade on B cell activation was evaluated by measuring expression of CD69 and CD86 by flow cytometry (B) and production of IL-6, IL-10 and IgM by ELISA (C). (D) Effects of IFNAR blockade in DC subsets. Bone marrow-derived cDC and pDCs from BXSB mice were stimulated for 24 hrs with the TLR7 ligand R848 in the presence or absence of anti-IFNAR antibody. Production of IL-6 and IFN-α was determined by ELISA. Bars represent average (± SD) of individual mice. Asterisks indicate statistical significance ($p<0.05$).

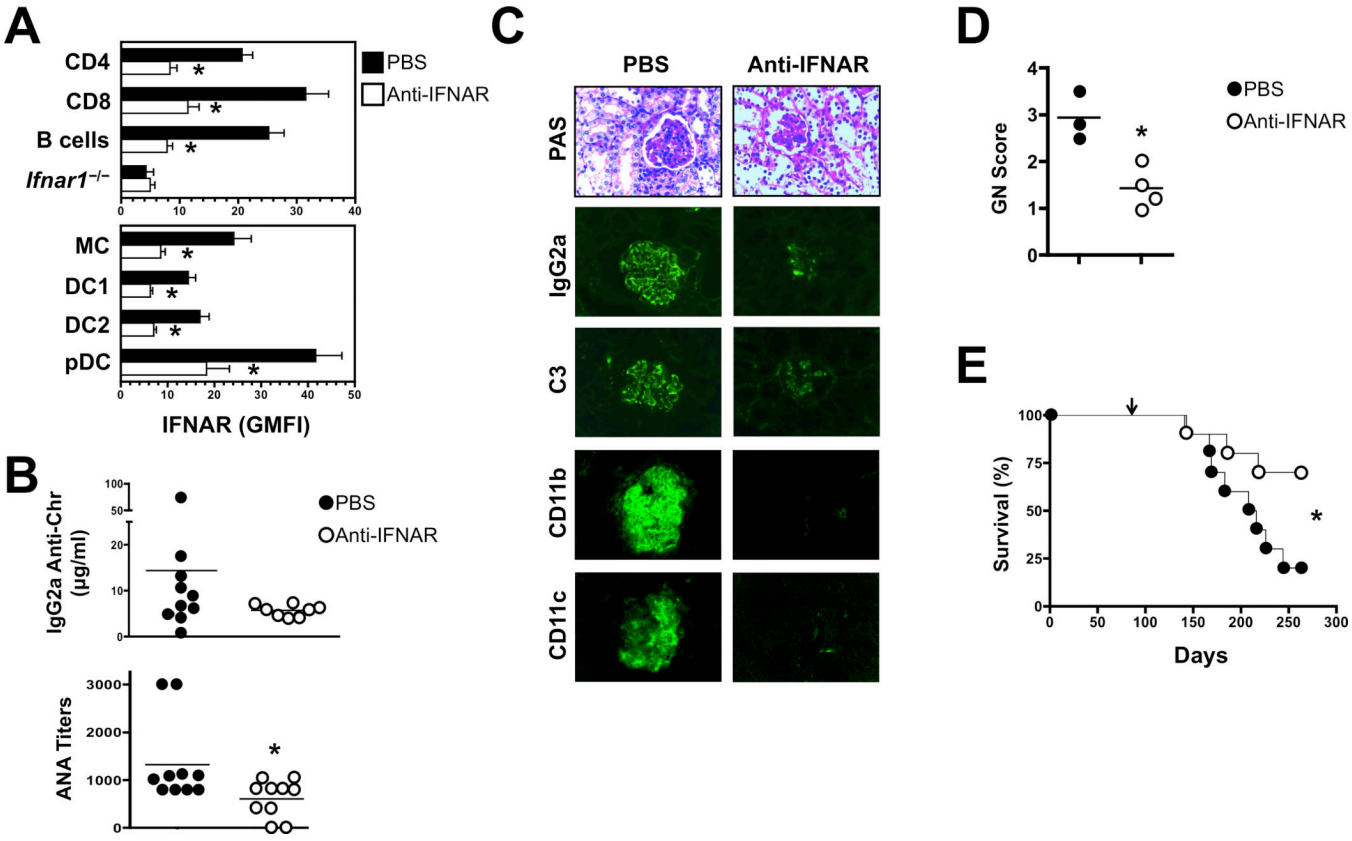

Figure 4. Early anti-IFNAR Antibody Treatment of BXSB Mice

Mice (n = 8–10/group) were treated with anti-IFNAR antibody (or PBS) starting at 12 wks of age. (A) Efficiency of IFNAR blockade as defined 4 wks post-treatment initiation by flow cytometry (average GMFI ± SD) in T cells ($CD4^+$ and $CD8^+$), B cells ($B220^+$), monocytes (MC, $CD11b^+CD11c^-$), DCs (DC1, $CD11c^+CD11^-$; DC2, $CD11c^+CD11b^+$), and pDCs ($PDCA1^+$, $CD11c^{low}$). *Ifnar1*$^{-/-}$ mice were used as negative staining controls (shown for B cells). (B) Serum autoantibody titers (anti-chromatin and ANA) at 20 wks of age. (C) Kidney histology showing glomerular size (PAS), immune deposits (IgG2a and complement C3), and immunocyte infiltration ($CD11b^+$ and $CD11c^+$ cells) at 38 wks of age (n = 3–4/group). (D) Glomerulonephritis scores at 38 wks of age (n = 3–4/group). (E) Survival. Arrow indicates start of antibody treatment. At the termination of the experiment (38 wks of age), three PBS-treated and five anti-IFNAR-treated mice were still alive. Asterisks indicate statistical significance ($p<0.05$).

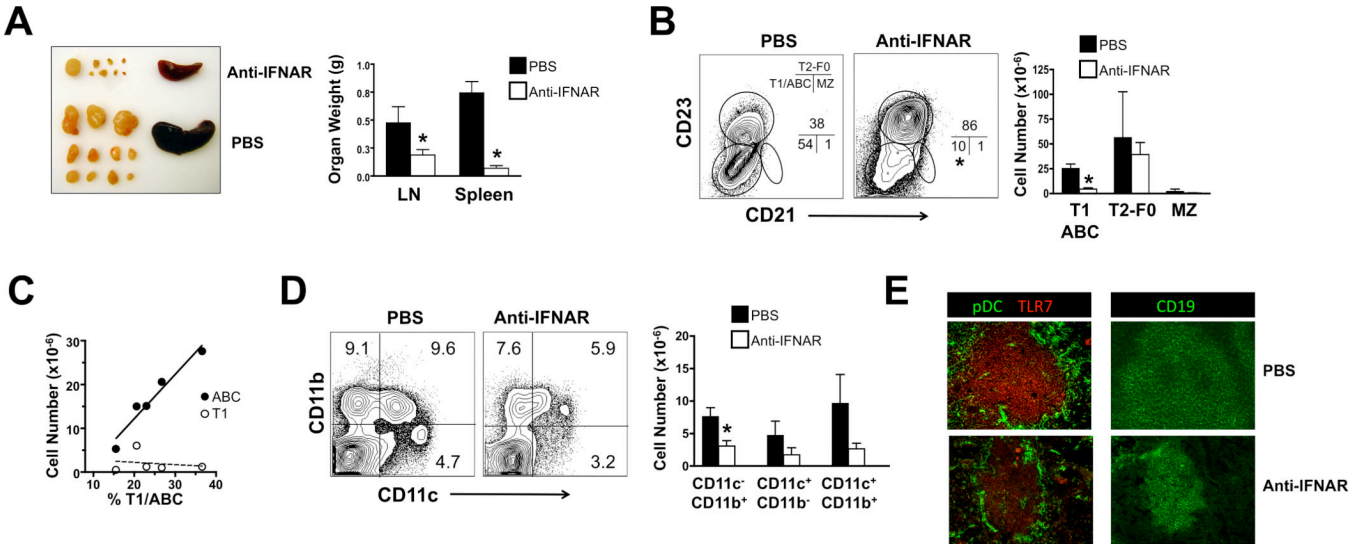

Figure 5. Anti-IFNAR Antibody Treatment Inhibits Expansion of B cells, Monocytes, and Dendritic Cells in BXSB Mice

Mice (n = 8–10/group) were treated with anti-IFNAR antibody (or PBS) from 12 wks to 38 wks of age. (A) Weights (± SD) of spleen and lymph nodes (LN, inguinal, axillary and cervical). (B) B cell subsets. Spleen cells were examined for CD21 and CD23 expression after gating on $B220^+IgM^+$ B cells to identify $CD21^-CD23^+$ T2/follicular (T2-FO), $CD21^+CD23^-$ marginal zone (MZ), and $CD21^-CD23^-$ T1 immature and age-associated B cells (ABCs). Numbers within plots correspond to average frequency of the indicated subsets. Bar graph indicates cell numbers ± SD. (C) Expansion of $CD21^-CD23^-AA4.1^{low}$ B cells in untreated BXSB mice. Spleen cells were obtained from male BXSB mice (n = 5, age = 21 to 26 wks) with varying degrees of splenomegaly. The numbers of $CD21^-CD23^-AA4.1^{low}$ (ABCs) and $CD21^-CD23^-AA4.1^+$ (T1) B cells were determined by flow cytometry and plotted as a function of the frequency of $CD21^-CD23^-$ B cells for each individual mouse. Linear regression was calculated using Prism 4 software. (D) Spleen monocytes ($CD11b^+CD11c^-$) and DC subsets ($CD11c^+CD11b^-$ and $CD11c^+CD11b^+$). Frequencies and cell numbers (± SD) were defined by flow cytometry. (E) pDCs and TLR7 expression in spleen B cell follicles. Spleen sections were stained with fluorescent anti-PDCA1 (pDC, green) and anti-TLR7 (red), or with anti-CD19 (B cells, green) antibodies. Asterisks indicate statistical significance ($p<0.05$).

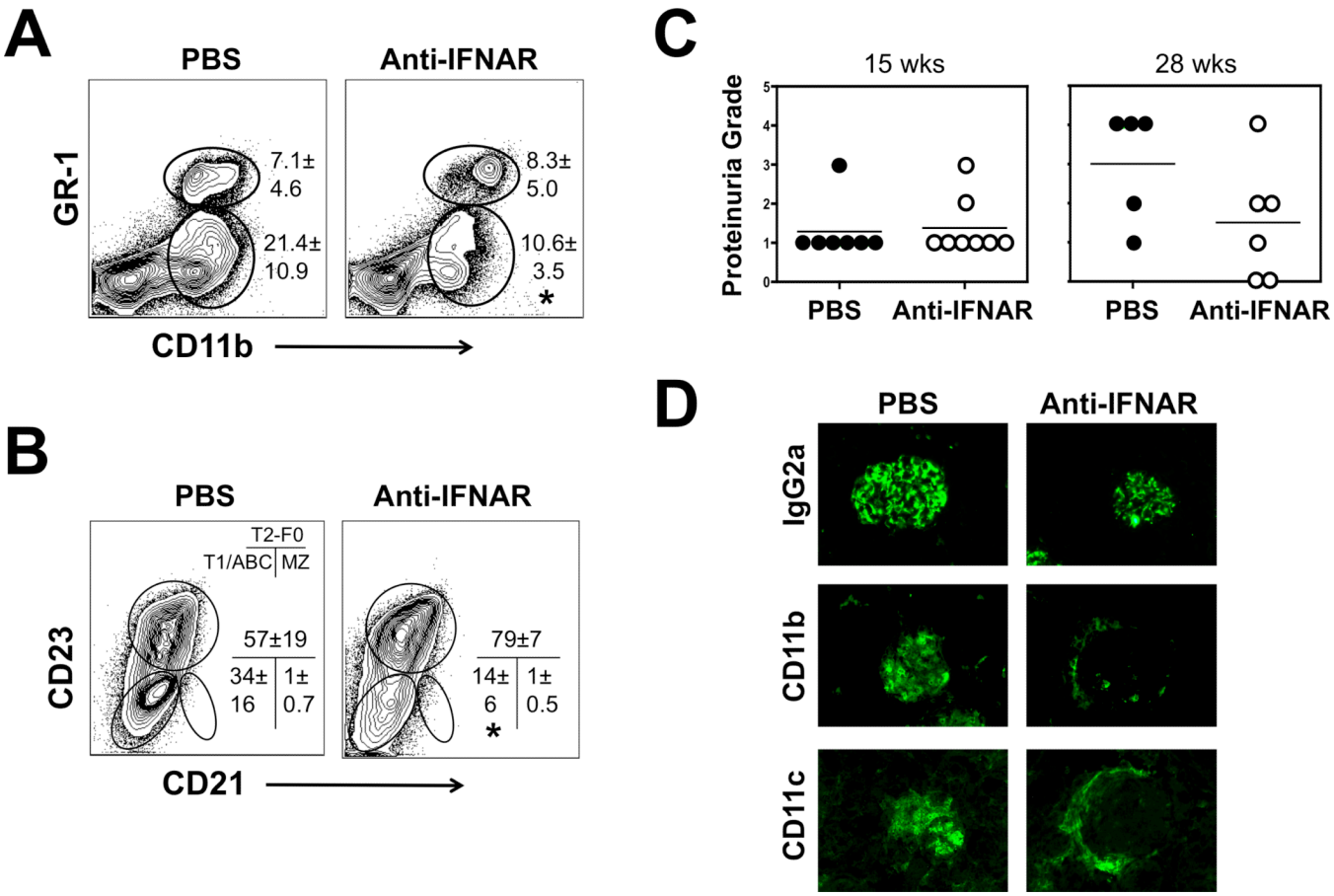

Figure 6. Late anti-IFNAR Antibody Treatment of BXSB Mice

Mice (*n* = 7–8/group) were treated with anti-IFNAR antibody starting at 17 wks of age. (A) Blood monocyte subsets ($CD11b^+Gr\text{-}1^-$ "resident", $CD11b^+Gr\text{-}1^+$ "inflammatory") at 26 wks of age. Numbers indicate average frequencies (± SD) of the gated cell subsets (* = $p < 0.05$). (B) Spleen B cell subsets at 33 wks of age. Numbers indicate average frequencies (± SD) of the gated cell subsets (* = $p < 0.05$). (C) Proteinuria at 15 and 28 wks of age. (D) Kidney glomerular IgG2a deposits and immunocyte infiltration ($CD11b^+$ and $CD11c^+$) at 33 wks. Shown are representative immunofluorescence images of kidneys from treated and control mice (n=4/group). Scoring of individual mice (0–4, based on fluorescence intensity) indicated significant treatment-associated reductions in IgG2a deposits (2.25 ± 1.26 vs. 3.75 ± 0.5, $p < 0.05$), CD11b staining (1.13 ± 0.63 vs. 3.13 ± 1.75, $p < 0.05$) and CD11c staining (1.00 ± 0.82 vs. 2.5 ± 1.29, $p < 0.05$).

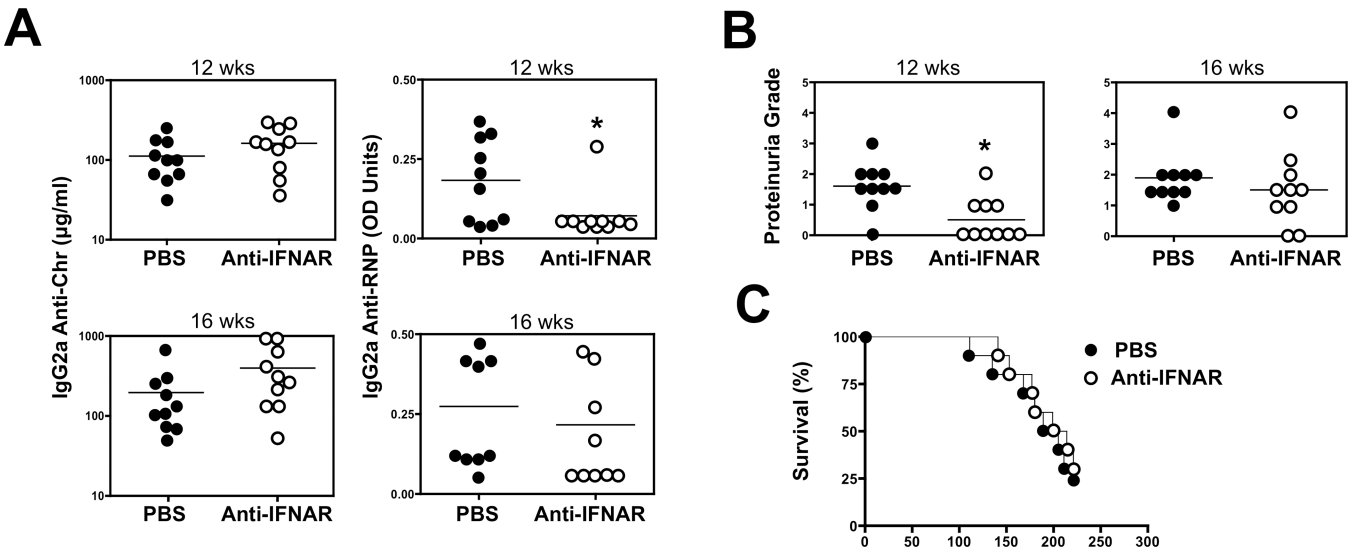

Figure 7. Prophylactic anti-IFNAR Antibody Treatment of MRL-*Fas*^{lpr} Mice

Mice (n = 10/group) were treated with anti-IFNAR antibody starting at 7 wks of age. (A) Serum anti-chromatin and anti-ribonucleoprotein (RNP) autoantibody titers. (B) Proteinuria. (C) Survival. Asterisks indicate statistical significance ($p<0.05$).