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Type I IFN Protects Against Murine Lupus

Jonathan D. Hron* and Stanford L. Peng2•†

Both the type I (IFN-αβ) and type II (IFN-γ) IFNs have been heavily implicated in the pathogenesis of systemic lupus erythematosus (SLE). 1) Increased levels of IFNs are commonly observed in lupus patients, often correlating with disease activity (1–4), and IFN genetic signatures characterize the immune system in SLE (5, 6); 2) therapeutic administration of IFN-α often adversely induces autoimmunity, particularly autoantibody production by B cells (7); 3) both types I and II IFNs can promote class switch recombination to Th1-like IgG isotypes (IgG2a, IgG2b, IgG3), pathogenic, complement-fxing isotypes in lupus (8–11); 4) inappropriate expression of the IFNs appears to be capable of breaking tolerance and/or at least augmenting humoral autoimmunity, in part via the activation of dendritic cells (12); and 5) multiple murine lupus studies, involving genetically altered and/or therapeutically manipulated animals, have demonstrated the clear importance of the type II IFNs to autoantibody production and subsequent organ damage (13–19).

In addition, one recent study has demonstrated that deficiency in IFNAR1, the gene encoding the α subunit of the heterodimeric IFN-I receptor, protected from anti-erythrocyte autoantibodies, hemorrhagic anemia, anti-DNA Abs, kidney disease, and mortality in the New Zealand Black (NZB) model of autoimmune hemolytic anemia and immunocomplex glomerulonephritis (20). Similarly, IFNAR1 deficiency protected lpr mice of a mixed 129 × C57BL/6 background from lymphadenopathy and renal immune complex deposition, and sustained administration of the IFN-γ inducer polyinosinic-polycytidylic acid to C57BL/6/lpr mice aggravated autoantibodies, hypergammaglobulinemia, lymphocyte activation, and renal disease (21). Thus, the IFNs have emerged as a dominant target in the pathogenesis of SLE.

The type I and type II IFN systems significantly interact with each other during immune responses, sometimes bolstering and/or attenuating the effect of the other, such as the promotion (22) vs inhibition (23) of IFN-γ-producing Th1 and/or dendritic cells by type I IFNs (4, 24, 25). As such, type I IFNs may contribute to SLE by promoting and/or augmenting the production of IFN-γ, as opposed to playing a direct pathogenic role in the promotion of autoantibody production. Indeed, the only definitively demonstrated pathogenic target of the IFNs in humoral autoimmunity, the T-box transcription factor T-bet, is an IFN-γ- induced gene (11, 26), suggesting that perhaps the role of the type I IFNs is additive to IFN-γ. At the same time, several other type I and type II IFN-induced or -related genes, such as Ifi202, have been implicated in genetic and animal studies, at least in the NZB background (20, 27); therefore, the direct importance of the type I IFNs remains an open question.

Such considerations raise the possibility that the IFNs may play different roles in different SLE subtypes. Indeed, the previously published studies on murine lupus in the setting of IFN-RI deficiency have used autoimmune-prone backgrounds of relatively low-to-moderate severity, NZB (20) and a mixed 129 × C57BL/6-lpr (21). The former develops moderate autoimmune hemolytic anemia as well as chronic Ig deposition in the kidney related to anti-DNA Abs; however, only the F1 NZB × NZW hybrids develop severe, lethal crescentic glomerulonephritis related to high titer, high affinity anti-DNA Abs (28). Similarly, without the MRL genetic background, the lpr mutation induces only a mild anti-DNA response and renal Ig deposition (29, 28). Thus, it remains unclear whether or not type I IFNs are also pathogenic in severe lupus, as might be characterized by multisystem disease and/or high titer, high affinity autoantibodies of multiple specificities.

Of the known inbred lupus-prone mouse strains, MRL/lpr develops the most intense syndrome that resembles severe human disease, developing multisystem inflammation, including

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severe, crescentic glomerulonephritis, and a similar spectrum of autoantibodies including anti-dsDNA and anti-small nuclear RNP (28, 30). We therefore sought to determine directly the relative contributions of the type I and II IFNs in this model and generated congenic MRL/lpr animals deficient in IFN-RI and/or IFN-RII. Surprisingly, IFN-RI-deficient animals developed worsened serological, cellular, and histological parameters of autoimmunity, in contrast to IFN-RII-deficient animals, which were largely protected from disease. Thus, at least in this model of humoral autoimmune disease, the type I IFNs suppress autoimmunity. Further studies of the pathogenesis and therapy of murine lupus should exercise caution with respect to the divergent roles of the type I and II IFNs.

Materials and Methods

**Mice**

BALB/cJ, MRL/Mpj (MRL+/+) and MRL/Mpj-CD95<sup>−/−</sup> (MRL/lpr) mice were obtained from The Jackson Laboratory (Bar Harbor, ME). IFN-RI (IFNAR1, CD118)<sup>−/−</sup> IFN-RII (IFNGR, CD119)<sup>−/−</sup> doubly deficient mice of the 129 background (AG129; Refs. 31 and 32) were graciously provided to us by Drs. H. Virgin and W. Yokoyama (Washington University School of Medicine, St. Louis, MO). To derive lupus-prone mice, CD4<sup>+</sup> CD8<sup>+</sup> T cells were cleared of RBC by osmotic lysis. Abs used included FITC-145-2C11 (anti-CD4; BD PharMingen, San Diego, CA). Proteinuria was determined on collected urine by alkaline phosphatase anti-mouse Ig using Luminex (Southern Biotechnology Associates, Birmingham, AL). IgG anti-DNA activity was determined by ELISA on sera at 1/100 dilution using calf thymus DNA (Sigma-Aldrich, St. Louis, MO). Total α-chain rheumatoid factor activity was determined by ELISA on sera at 1/100 dilution using pooled λ-chain IgG1, IgG2a, IgG2b, and IgG3 Abs for capture, followed by alkaline phosphatase anti-mouse Ig for detection (BD Pharmingen, San Diego, CA). Proteinuria was determined on collected urine by the DC protein assay kit (Bio-Rad Laboratories, Hercules, CA).

Histopathology was evaluated on paraffin-embedded, formalin-fixed tissue sections by routine H&E staining. IgG immune deposits were determined by direct immunofluorescence on 5 μm OCT-embedded frozen kidney sections, using FITC anti-mouse IgG (Pierce Biotechnology, Rockford, IL). Histopathological assessments were determined in blinded fashion by one of us (S.L.P.).

**Flow cytometry**

Flow cytometric analyses were performed on a FACS Calibur System (BD Biosciences, San Jose, CA) using lymph node cells and/or splenocytes cleared of RBC by osmotic lysis. Abs used included FITC-145-2C11 (anti-CD3), PE-53-6.7 (anti-CD8a), APC-RA3-6B2 (anti-CD45R/B220), and PE-Cy7-RM4-4 (anti-CD4; BD Pharmingen).

**In vitro B cell assays**

Naïve splenic B cells were purified by negative selection against CD43 and cultured in RPMI medium supplemented with LPS, 25 μg/ml (Sigma-Aldrich); anti-CD40, 2 μg/ml (BD Pharmingen); IL-12, 1 ng/ml; IL-18, 2.5 ng/ml, and/or IFN-γ, 100 ng/ml (PeptoTech, Rocky Hill, NJ), as described (40). Universal type I IFN (IFN-A/D), which activates the murine type I IFN receptor, was supplemented at 100–10,000 U/ml as indicated in the text (R&D Systems, Minneapolis, MN). At the times indicated, Ig and cytokine secretion was assayed in culture supernatants by ELISA, and cell proliferation was determined by BrdU incorporation, as described (40, 41).

**Results**

**Generation of IFN-RI-, IFN-RII-deficient MRL mice**

To determine the relative roles of the types I and II IFNs in MRL/lpr lupus, congenic MRL animals of the IFN-RI<sup>−/−</sup> IFN-RII<sup>−/−</sup> CD95<sup>+/+</sup> genotype were intercrossed to generate IFN-RI<sup>−/−</sup> or IFN-RI<sup>+/+</sup>, IFN-RII<sup>−/−</sup> or IFN-RII<sup>+/+</sup>, CD95<sup>++/−</sup> or lpr/lpr animals. For consistency with prior nomenclature (32, 42), the 8 genotypes produced are referred herein as MRL/+ (IFN-RI<sup>+/+</sup>IFN-RII<sup>++/−</sup>CD95<sup>++/−</sup>), AMRL/+ (IFN-RI<sup>−/−</sup>IFN-RII<sup>++/−</sup>CD95<sup>++/−</sup>), GMRL/+ (IFN-RI<sup>−/−</sup>IFN-RII<sup>−/−</sup>CD95<sup>++/−</sup>), MRL/lpr (IFN-RII<sup>−/−</sup>IFN-RII<sup>−/−</sup>CD95<sup>++/−</sup>lpr/lpr), AMRL/lpr (IFN-RI<sup>−/−</sup>IFN-RII<sup>−/−</sup>CD95<sup>++/−</sup>lpr/lpr), GMRL/lpr (IFN-RI<sup>−/−</sup>IFN-RII<sup>−/−</sup>CD95<sup>++/−</sup>lpr/lpr), and AGMRL/lpr (IFN-RI<sup>−/−</sup>IFN-RII<sup>−/−</sup>CD95<sup>++/−</sup>lpr/lpr). All genotypes were generated at expected Mendelian ratios.

A suppressive role for the IFN-I receptor in the autoimmune hypergammaglobulinemia of MRL/lpr mice

Disease parameters were first assessed in 12-wk-old animals, when the penetrance of both humoral autoimmunity and end organ disease in MRL/lpr mice has reached 90–100% (28). Total serum Ig analysis (Fig. 1) demonstrated that MRL/lpr animals developed an expected hypergammaglobulinemia of all isotypes examined, as compared with their MRL/+ counterparts (p < 0.0001 for all isotypes). GMRL/lpr animals developed substantially lower serum titers of the pathogenic isotypes IgG2a, IgG2b, and IgG3, as well
as IgM and IgA, but higher titers of IgG1 (p < 0.0001 for all indicated comparisons, MRL/lpr vs GMRL/lpr). Interestingly, pathogenic IgG titers in MRL/lpr mice were not always reduced to normal levels: although their titers of IgG2a and IgG3 were comparable with those of MRL/+ animals (p not significant, comparing MRL/+ with GMRL/lpr), GMRL/lpr mice developed clearly elevated IgG2b titers (p < 0.0001, comparing MRL/+ with GMRL/lpr); nonetheless, these titers were substantially elevated compared with nonautoimmune C57BL/6 or BALB/c mice, which spontaneously produce serum IgG1, IgG2a, IgG2b, and IgG3 in titers <100–200 μg/ml (p < 0.0001 for all isotypes, compared with GMRL/lpr; Ref. 43 and data not shown). These findings persisted in older animals examined at 24 wk of age (Fig. 2; p < 0.0001 for all aforementioned comparisons). Thus, reminiscent of prior studies (13–15), type II IFN was critically required for the full blown development of hypergammaglobulinemia of the Th1-like isotypes IgG2a, IgG2b, and IgG3 in MRL/lpr mice, although some degree of elevated Ig, presumably autoimmune-related, persisted.

Surprisingly, absence of IFN-RI often worsened hypergammaglobulinemia. AMRL/lpr animals developed higher titers of IgM, and IgA by 12 wk of age (Fig. 1; p < 0.001) and by 24 wk of age possessed higher titers of IgG2b and IgG3 than their MRL/lpr counterparts (Fig. 2; p < 0.001). At the same time, AGMRL/lpr animals interestingly developed modestly lower titers of all IgG isotypes, compared with their GMRL/lpr counterparts (Figs. 1 and 2; p < 0.10). Thus, type I IFNs here play divergent roles depending on the presence or absence of IFN-γ activity; they suppress autoimmune hypergammaglobulinemia in the presence of IFN-RI but instead promote hypergammaglobulinemia in the absence of IFN-RII. Still, for all pathogenic isotypes (IgG2a, IgG2b, and IgG3), AGMRL/lpr animals developed significantly lower titers than their AMRL/lpr counterparts (p < 0.0001), indicating that whatever the specific effect of type I IFNs, IFN-γ contributed a substantial pathogenic role to autoimmune hypergammaglobulinemia.

Expression of a type I IFN receptor suppresses humoral autoimmunity in MRL/lpr mice, independently of IFN-II

As expected, MRL/lpr mice developed significantly elevated serum activities of anti-DNA and κ-chain rheumatoid factor (RF) autoantibodies, compared with their MRL/+ counterparts (Fig. 3; p < 0.0001). As also expected, GMRL/lpr animals developed significantly lower serum activities of both anti-DNA (p < 0.0001) and RF (p < 0.001) Abs than MRL/lpr mice, although the effect on the latter was less pronounced. Indeed, the ELISA-positive anti-DNA Abs of GMRL/lpr animals were likely anti-ssDNA, rather than anti-dsDNA, in specificity: whereas 7 of 10 and 5 of 5 MRL/lpr sera at 12 and 24 wk of age, respectively, reacted with the dsDNA-containing kinetoplast of Crithidia luciliae substrates, 0 of 12 and 0 of 5 GMRL/lpr sera at 12 and 24 wk of age, respectively, did (Fig. 2; p < 0.00001). Analogous findings were seen in MRL/+ vs GMRL/+ sera, with 2 of 8 MRL/+ sera developing Crithidia-positive anti-dsDNA activity by 24 wk of age, in contrast to 0 of 10 GMRL/+ (p < 0.01). GMRL/+ also produced significantly, albeit modestly, lower titers of RF compared with their MRL/+ counterparts (p < 0.01 for 12 wk, p < 0.10 for 24 wk). Thus, type II IFN was critically required for the development of humoral autoimmunity, as evidenced by effects of IFN-RII deficiency on both anti-DNA and RF autoantibodies.

Surprisingly, however, IFN-RI clearly played a suppressive role in the development of pathogenic autoantibodies in both MRL/+
and MRL/lpr mice, independent of IFN-γ. At 12 wk of age, 7 of 7 (100%) of AMRL/lpr sera were Crithidia positive, in contrast to the 7 of 10 (70%) MRL/lpr (p < 0.05); and by 24 wk, 7 of 13 (54%) of AGMRL/lpr sera were Crithidia positive, in contrast to 0 of 5 GMRL/lpr sera (p < 0.0001). Similar findings were seen with MRL/+ sera, where by 24 wk 2 of 8 (25%) were Crithidia positive, in contrast to 5 of 8 (63%) AMRL/+ , 0 of 10 (0%) GMRL/+ , and 4 of 12 (33%) AGMRL/+ sera (p < 0.001 comparing MRL/+ with AMRL/+ or GMRL/+ with AGMRL/+). Interestingly, IFN-RI deficiency had a variable effect on RF activity, with higher titers in AMRL/+ animals than their MRL/+ counterparts, and higher titers in AMRL/lpr animals than their MRL/lpr counterparts at 24 wk (p < 0.01), but seemingly lower titers in AGMRL/lpr animals compared with their GMRL/lpr counterparts at 24 wk (p < 0.10). Nonetheless, these results strongly suggest that type I IFNs play a critical role in antagonizing the development of pathogenic humoral autoimmunity in MRL mice.

### Table 1. Lymphoaccumulation in MRL/lpr mice in the absence of IFN-RI and/or IFN-RII

<table>
<thead>
<tr>
<th>IFN-RI</th>
<th>IFN-RII</th>
<th>wt (g)</th>
<th>CD4⁺B220⁻</th>
<th>CD8⁺B220⁻</th>
<th>CD4⁺B220⁺</th>
<th>CD8⁺B220⁺</th>
<th>DN B220⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/+</td>
<td>+/+</td>
<td>1.4 ± 0.2</td>
<td>1.7 ± 0.8</td>
<td>0.7 ± 0.4</td>
<td>3.0 ± 0.1</td>
<td>0.4 ± 0.3</td>
<td>58.2 ± 0.1</td>
</tr>
<tr>
<td>+/-</td>
<td>+/+</td>
<td>1.9 ± 0.6</td>
<td>1.6 ± 0.0</td>
<td>1.2 ± 0.1</td>
<td>2.9 ± 0.7</td>
<td>0.3 ± 0.0</td>
<td>57.3 ± 0.6</td>
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<tr>
<td>+/+</td>
<td>-/-</td>
<td>0.2 ± 0.1</td>
<td>11.5 ± 0.7</td>
<td>11.8 ± 0.2</td>
<td>3.0 ± 0.3</td>
<td>0.6 ± 0.2</td>
<td>16.5 ± 0.7</td>
</tr>
<tr>
<td>+/-</td>
<td>-/-</td>
<td>0.4 ± 0.1</td>
<td>5.9 ± 1.4</td>
<td>12.4 ± 2.0</td>
<td>3.7 ± 0.5</td>
<td>0.5 ± 0.2</td>
<td>29.3 ± 6.9</td>
</tr>
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</table>

* Animals of the indicated genotypes were assessed for peripheral lymph node weights (cervical, inguinal, axillary, brachial), as well as indicated cell populations (percentages) by flow cytometry at 12 wk of age. Splenic populations were similar (not shown). Shown are means ± SD of 5–7 animals assessed per genotype.

Suppressive vs pathogenic roles for IFN-I and IFN-II receptors in lpr-related lymphoproliferation

Similarly, examination of lpr-related lymphoproliferation revealed divergent roles for the type I and II IFNs (Table 1). MRL/lpr mice developed massive lymphadenopathy, with peripheral lymph node weights of typically ∼1.2–1.6 g, composed largely of CD3⁺CD4⁻CD8⁻ B220⁺ T cells at the expense of normal CD4⁺B220⁻ and CD8⁺B220⁻ T cells (p < 0.00001 comparing MRL/lpr to MRL/+, which typically yielded lymph node weights of 20–40 mg with CD4⁺ B220⁺ and CD8⁺ B220⁻ percentages of 20–25%). This lymphoaccumulation was greatly reduced in GMRL/lpr mice, which developed significantly lower lymph node weights (0.1–0.3 g) in association with lower percentages of CD3⁺CD4⁻CD8⁻ B220⁺ T cells (15–20%) and more normal percentages of CD4⁺B220⁻ and CD8⁺B220⁻ cells (10–12%; p < 0.001 comparing weights and indicated percentages between

**FIGURE 4.** End organ disease in MRL/lpr mice reflects suppressive roles for IFN-RI vs pathogenic roles for IFN-RII. Indicated organs of animals of the indicated MRL/lpr genotypes were assessed by routine H&E staining of paraffin-embedded tissues. Note the development of moderate inflammatory infiltrates in the salivary glands of MRL/lpr animals, which were substantially worsened in AMRL/lpr animals, and largely abrogated in GMRL/lpr counterparts. Five of ten AGMRL/lpr animals developed mild inflammatory salivary gland infiltrates (shown), which nonetheless were substantially less severe than MRL/lpr animals. Similar findings were encountered in the lung and liver. Note also in MRL/lpr animals the development of proliferative glomerulonephritis accompanied by moderate perivascular inflammation, which was worsened in AMRL/lpr animals. Renal disease was largely abrogated in GMRL/lpr animals, although mild perivascular inflammation generally persisted. AGMRL/lpr kidneys were uniformly protected from glomerular disease but developed mild-moderate perivascular infiltrates. Except as noted, data are from individual animals of each genotype, representative of 10 animals examined. Original magnification, ×20.
## Table II. Renal pathology of AGMRL mice*  

<table>
<thead>
<tr>
<th>IFN-R1</th>
<th>IFN-R2</th>
<th>CD95</th>
<th>Age (wk)</th>
<th>Glomerular Disease</th>
<th>Vascular Inflammation</th>
<th>Glomerular IgG Deposition</th>
<th>Nuclear IgG Deposition</th>
<th>Proteinuria</th>
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<tr>
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* Renal disease was assessed by light microscopic assessment of glomerular disease and perivascular inflammation; Ig deposition was assessed by direct immunofluorescence of IgG at the glomeruli and tubular nuclei, each on a – to ++++ scale as previously described (30). Proteinuria was rated as: –, < 30 mg/ml; +, 30–100 mg/ml; or ++, >100 mg/ml.
MRL/lpr and GMRL/lpr mice). Thus, IFN-II plays a critical role in the development of lpr-related lymphoaclumulation.

Conversely, AMRL/lpr mice displayed modestly elevated lympho-adenopathy as evidenced by organ weights (p < 0.05 compared with MRL/lpr counterparts), although percentages of T cell subpopulations were comparable between MRL/lpr and AMRL/lpr animals. More strikingly, though, AGMRL/lpr mice developed increased lymphoaclumulation compared with GMRL/lpr counterparts, as evidenced by total lymph node weights (0.4 ± 0.1 g vs 0.2 ± 0.1 g; p < 0.001), increased percentages of CD3^+CD4^+CD8^+ B220^+ (29.3 ± 6.9 vs 16.5 ± 0.7; p < 0.0001), and diminished percentages of normal CD4^+B220^- T cells (5.9 ± 1.4 vs 11.5 ± 0.7; p < 0.0001). Thus, whether in the presence or absence of IFN-RII, type I IFNs suppressed lpr-related lymphoaclumulation.

Suppressive vs pathogenic roles for IFN-I and IFN-II receptors in MRL end organ disease

MRL/lpr animals developed severe end organ inflammation of the salivary gland, liver, lung, and kidney, all of which were largely absent in MRL/+, AMRL/+, GMRL/+, and AGMRL/+ animals (p < 0.0001; Fig. 4 and Table II). Renal disease was characterized by membranoproliferative glomerulonephritis, as well as glomerular and often tubular and intratubular immune deposits (Figs. 4–6 and Table II). GMRL/lpr animals were largely protected from all end organ manifestations: at 12 wk of age, none of the animals developed detectable infiltrates of the salivary glands, liver or lung; and their renal disease was reduced in terms of inflammatory infiltrates, glomerular proliferation, and immune deposits, although mild renal disease was seen in older animals (Table II). Therefore, IFN-RII played a critical role in the development of autoimmune end organ disease in MRL/lpr animals.

In contrast, AMRL/lpr animals developed significantly worsened end organ disease, particularly more severe infiltrates of the salivary gland, liver, lung, and kidney (Fig. 4 and Table II). Interestingly, the latter was further accompanied by more pronounced glomerular hypercellularity and mesangial proliferation (Fig. 4), as well as more intense immune deposits (Figs. 5 and 6 and Table II). Similarly, at 12 wk of age, AMRL/+ animals developed significantly detectable renal immune deposits, in contrast to their MRL/+ counterparts, although overt glomerulonephritis was not seen until 24 wk (Figs. 5 and 6 and Table II). As such, in the presence of IFN-RII, IFN-RI plays a protective role in the development of end organ disease.

Similarly, AGMRL/lpr animals in general developed worsened end organ disease compared with their GMRL/lpr counterparts (Fig. 4), developing mild-to-moderate infiltrates of the salivary gland, liver, and lung, all of which were largely absent in GMRL/lpr animals (p < 0.0001). Interestingly, AGMRL/lpr animals developed worsened renal disease in comparison with GMRL/lpr counterparts, accumulating significant immune deposits as well as overt glomerular hypercellularity (Figs. 4–6 and Table II; p < 0.0001). In addition, whereas only minimal, if any, perivascular lesions were seen in GMRL/lpr kidneys, AGMRL/lpr kidneys consistently developed mild-to-moderate perivascular lesions (Fig. 4 and Table II) that were clearly distinguishable from GMRL/lpr specimens, although they did not reach the intensity of MRL/lpr or AMRL/lpr animals.

In accordance with all these findings, IFN-RII deficiency protected MRL animals from proteinuria (100% of MRL/lpr vs 13% of GMRL/lpr animals at 24 wk developed proteinuria >30 mg/ml; p < 0.0001; Table II, which shows only animals examined histopathologically, and data not shown), but IFN-RI deficiency significantly worsened proteinuria (at 24 wk, 58% of MRL/lpr vs 100% of AMRL/lpr animals developed proteinuria >100 mg/ml; 0% of GMRL/lpr vs 44% of AGMRL/lpr animals developed proteinuria >30 mg/ml; p < 0.0001 for both comparisons; Table II and data not shown; n = 10, 10, 12, and 12 for MRL/lpr, AMRL/lpr, GMRL/lpr, and AGMRL/lpr, respectively). Furthermore, whereas MRL/lpr animals demonstrated ~40% mortality by 24 wk of age, AMRL/lpr animals demonstrated 80%, GMRL/lpr animals demonstrated 0%, and AGMRL/lpr animals demonstrated 25% (n = 10, 10, 12, and 12; p < 0.001 for any comparison between the groups). Thus, type I IFNs play a critical protective role in the development of pathogenic humoral autoimmunity and end organ inflammatory disease.

Role of type I IFN in MRL B cell activation

As such, type I IFNs likely play a suppressive role in B cell activation, at least in MRL mice. However, although autoantibody
activities were strongly affected by IFN-RI deficiency (Figs. 3, 5, and 6), total serum Igs were in comparison only modestly affected (Figs. 1 and 2), suggesting a context-specific role and/or importance for type I IFNs in B cell suppression. Because autoantibody activity is critically dependent on T cell help, whereas hypergammaglobulinemia is not, at least in MRL mice (30, 39), type I IFNs might differentially regulate MRL B cell activation during T-dependent vs T-independent Ab responses, as has been found in some prior studies in nonautoimmune strains (12, 25, 44), explaining such observations.

We therefore examined the effect of type I IFN on naive MRL B cells stimulated with LPS, modeling T-independent stimulation, vs anti-CD40, modeling T-dependent stimulation (40), using IFN-A/D, a known murine IFN-RI agonist (11). Remarkably, IFN-A/D significantly inhibited Ig secretion by anti-CD40-stimulated MRL B cells, generally 5- to 10-fold (p < 0.0001; Fig. 7A and our unpublished data). In contrast, it had little, if any, effect on Ig secretion by LPS-stimulated cells. Converse results were seen with LPS- vs anti-CD40-stimulated B cells from MRL vs AMRL mice; the latter consistently secreted 3- to 7-fold higher Ig amounts in response to anti-CD40 stimulation (p < 0.001; Fig. 7B and our unpublished data). These results seemed unlikely to represent simply the isolation of more B cells preactivated in vivo from AMRL mice, because our initial B cell isolates were devoid of all IgG class-switch transcripts and unstimulated B cells failed to secrete significant amounts of Ig (Refs. 11 and 40 and data not shown), suggesting that they were indeed naive, non-class-switched cells; nonetheless, we cannot entirely rule out the possibility. Still, these results suggest that type I IFNs can suppress T-dependent humoral immune responses in MRL B cells and furthermore suggest that MRL B cells intrinsically express and/or use type I IFNs to contain and/or limit their overall activity during such responses. As a result, IFN-RI deficiency leads to the exaggerated production of T-dependent autoantibodies, like anti-dsDNA, by autoreactive B cells (Fig. 3 and Refs. 30 and 39).

Discussion

These results are somewhat surprising given the accumulating evidence for the importance of type I IFNs in the pathogenesis of human SLE (1–4), and particularly given the results of recent studies suggesting the importance of type I IFN activity in the NZB (20) and (129 × C57BL/6)-lpr (21) models. In the past, most findings with regard to disease pathogenesis have remained robustly consistent across all murine lupus models, such as the importance of αβ T cells (30, 39, 45), and IFN-γ (13–16, 18, 19). The present study emphasizes a critical difference between the pathogeneses of at least the NZB, (129 × C57BL/6)-lpr, and MRL models of SLE; the former both apparently rely on both the type I and II IFNs, although a direct genetic study of both IFNs in those models has not to our knowledge been performed, as done here for MRL. In contrast, in MRL lupus type I IFNs play a critical but protective role, likely by antagonizing the activity of IFN-γ in the propagation of autoimmune inflammation. Thus, it is likely that different subtypes of human SLE, as they become better codified (46, 47), will reflect different roles for the types I and II IFNs. Similarly, it will be of interest to compare the roles of the type I IFNs in the activation of autoreactive B cells in the various lupus-prone mouse strains.

Notably, these conclusions presume that IFNAR1 (CD118) deficiency abrogates all biological activity of type I IFNs in vivo, because it is thought to comprise a critical subunit of the single known type I IFN receptor, IFNAR (48). However, because IFNAR also consists of a second receptor subunit, IFNAR2, and possibly a third as yet undiscovered subunit (48), we cannot rule out the possibility that the remaining IFNAR gene(s) retain some...
functional ability to recognize type I IFNs with biological consequences below the limit of detection of currently available assays. As a result, some type I IFNs, via the residual IFNAR activity, may in fact exert a pathogenic role in MRL lupus; nonetheless, because by all known in vivo and in vitro assays IFNAR1 deficiency eliminates all biological responses to all type I IFNs tested (e.g., Refs. 32 and 44 and our unpublished data), the present results argue strongly that the predominant role of the type I IFNs is the protection against humoral autoimmunity, at least in the MRL model.

Because type I IFNs can inhibit IFN-γ expression via STAT1, at least under certain conditions (23), a more plausible mechanism to explain the phenotypes of AMRL and AGMRL animals involves the overexpression of IFN-γ and/or unopposed STAT1 activity in the absence of IFN-RI. However, cultured lymphocytes from AMRL animals failed to overproduce IFN-γ in the absence of IFN-RI. Nonetheless, because type I IFNs, at least compared with type II IFNs, their STAT1 dependency in lupus-prone mice: relationship of the Fas apoptosis gene to disease manifestations and/or unopposed STAT1 activity in AMRL animals (Figs. 4–6 and Table II). Therefore, the IFN pathways responsible for disease pathogenesis could actually be IFN-γ-dependent yet STAT1-independent (51, 52), and a further understanding of the relationship between the types I and II IFNs and their STAT1-dependent and-independent functions will likely shed substantial light on the molecular pathogenesis of SLE.

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References