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Type I IFN Drives Experimental Systemic Lupus Erythematosus by Distinct Mechanisms in CD4 T Cells and B Cells

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ABSTRACT
Myriad studies have linked type I IFN to the pathogenesis of autoimmune diseases, including systemic lupus erythematosus (SLE). Although increased levels of type I IFN are found in patients with SLE, and IFN blockade ameliorates disease in many mouse models of lupus, its precise roles in driving SLE pathogenesis remain largely unknown. In this study, we dissected the effect of type I IFN sensing by CD4 T cells and B cells on the development of T follicular helper cells (TFH), germinal center (GC) B cells, plasmablasts, and antinuclear dsDNA IgG levels using the bm12 chronic graft-versus-host disease model of SLE-like disease. Type I IFN sensing by B cells decreased their threshold for BCR signaling and increased their expression of MHC class II, CD40, and Bcl-6, requirements for optimal GC B cell functions. In line with these data, ablation of type I IFN sensing in B cells significantly reduced the accumulation of GC B cells, plasmablasts, and autoantibodies. Ablation of type I IFN sensing in T cells significantly inhibited TFH expansion and subsequent B cell responses. In contrast to the effect in B cells, type I IFN did not promote proliferation in the T cells but protected them from NK cell–mediated killing. Consequently, ablation of either perforin or NK cells completely restored TFH expansion of IFNAR−/− Tfh and, subsequently, restored the B cell responses. Together, our data provide evidence for novel roles of type I IFN and immunoregulatory NK cells in the context of sterile inflammation and SLE-like disease. ImmunoHorizons, 2020, 4: 140–152.

INTRODUCTION
The overproduction of type I IFN is a prominent feature associated with the development of systemic lupus erythematosus (SLE) and has been implicated in the pathology of other autoimmune diseases including Sjögren syndrome, systemic sclerosis, adult-onset rheumatoid arthritis, and type I diabetes mellitus (1, 2). A role for type I IFN in driving disease pathology has been demonstrated in several genetic and inducible murine models of SLE (3–7). Moreover, the therapeutic use of IFN to treat patients with hepatitis C virus and cancer has been associated with the induction of SLE (8–10), and the administration of IFN to lupus-prone mice induced earlier onset, lethal disease (11). Furthermore, a type I IFN gene signature correlates with disease severity in SLE patients (12, 13) and approaches blocking type I IFN, or its receptor have shown some efficacy in clinical trials (14–17). Although these studies...
strongly implicate type I IFN in promoting SLE pathogenesis, relatively little is known about the precise functions of IFN in SLE.

Type I IFN comprises 13 IFN-α proteins, IFNβ, IFNκ, and IFNω, all of which signal through a common receptor known as the IFN α and β receptor, or IFNAR. IFNAR. IFNAR is expressed by essentially all nucleated cells and plays well-described, key roles in antimicrobial defenses (18). In SLE, one prevailing hypothesis is that by some combination of genetic and environmental factors, an accumulation of dying cells leads to increased exposure to self-RNA and self-DNA, which induces high levels of IFN production by plasmacytoid dendritic cells (DCs) (19–21). Relatively few studies, however, have sought to characterize the mechanism of action of IFN specifically in SLE.

Studies of SLE peripheral blood-derived DCs by Blanco et al. (22) indicated a likely role of IFN sensing by DCs in SLE pathogenesis. It is likely that type I IFN sensing by B cells and CD4 T cells also contributes to disease development, given that type I IFN is known to promote B cell activation, class switching, and support the generation of Ab-secreting plasma cells (23–25), and type I IFN sensing by CD4 T cells can dramatically enhance their priming in the context of certain viral infections and vaccines (25–27). Further dissection of the relative contribution of type I IFN sensing in different cell types in disease development and progression would provide valuable insights that could be exploited for therapeutic gain.

In this study, we investigated the potential role for type I IFN sensing by B cells and CD4 T cells in the bm12 chronic graft-versus-host (cGVHD) disease model of SLE, a well-established murine model of SLE with clinical signs that correspond to those of SLE patients, including the development of antinuclear Abs, lupus nephritis, and a recognized role for type I IFN (28, 29). Our data show that direct sensing of type I IFN by B cells is required for the maximal development of germinal center (GC) B cells, plasmablasts, and anti-dsDNA IgG through the upregulation of membrane-associated molecules that promote T:B interactions, induction of the GC-promoting transcription factor Bcl-6, and increasing the proliferative capacity to BCR stimuli. Direct sensing of type I IFN by CD4 T cells was critically important for T follicular helper cell (TFH) accumulation and subsequent disease development. However, in this scenario type I IFN sensing was required to protect the TFH from NK cell–mediated killing and elimination of NK cells or genetic ablation of perforin allowed for normal accumulation of IFNAR−/−TFH and disease-associated sequelae.

Our data provide evidence for novel roles of type I IFN and immunoregulatory NK cells in SLE, providing rationale and potential new targets for the development of future combination immunotherapies.

**MATERIALS AND METHODS**

**Mice and cell lines**

All experiments involving mice were conducted following protocols approved by the Cincinnati Children’s Hospital Medical Center’s Institutional Animal Care and Use Committee. Mice were maintained under specific pathogen-free conditions in accordance with guidelines by the Association for Assessment and Accreditation of Laboratory Animal Care International. C57BL/6J, B6.5SJL-Ptprc−/− (CD45.1), B6.PL-Thy1α/CyJ (CD90.1), B6.129S2-Ighm−/−mCg/J (µMT), C57BL/6-Prf1−/−mIdz/J (Prf1−/−), B6-(H-2)-Abl1bm12/KhEgJ (bm12), and B6.129(Cg)-Foxp3−/−mDTR/GFP/Ayr/J (Foxp3-DTR) mice were originally purchased from The Jackson Laboratory (Bar Harbor, ME) and bred in-house. IFNAR1−/− mice were originally a gift from Dr. J. Sprent and have since been bred and backcrossed to the C57BL/6 background in our facility (30). Both male and female mice were used, but each experiment consisted of only one aged-matched gender. ISRE-L929 IFN reporter cells have been described previously (31). Mixed bone marrow (BM)–chimeric mice were generated using lethally irradiated mice of indicated strains on a C57BL/6 background. Mice received an initial dose of 700 rad followed by an additional 475 rad dose 3 h later using a 137Cs irradiator (JL Shepherd & Associates, San Fernando, CA). BM from indicated strains (on different congenic backgrounds) were mixed at indicated ratios prior to reconstitution, and 6 million total BM cells were transferred via tail vein injection. All BM-chimeric mice were given 12 wk for hematopoietic reconstitution.

**CD4 T cell isolation**

For most in vivo and in vitro assays, CD4 T cells were sorted to >97% purity by negative selection using magnetic bead sorting technologies from Miltenyi Biotec (Bergisch Gladbach, Germany) and BioLegend (San Diego, CA). Post sort purity was determined by flow staining for live cells, CD45, CD3, and CD4. In several experiments, cells were labeled with Cell Trace Violet (CTV) or CFSE as proliferation-tracking dyes. For isolation of CD4bm12 T cells for the TFH in vivo–killing assay, CD4bm12 T cells were repurified from spleens 2 wk after their initial transfer by negative selection for CD4, followed by positive selection for the congeneric marker using biotinylated Ab to CD45.1 (clone A20; BioLegend) and antibiotin labeled Miltenyi beads. Flow cytometric analysis showed >95% viability and >95% purity (CD45.1+CD4+), with >95% of the cells expressing TFH markers PD1 and CXCR5.

**Bm12 model of cGVHD**

The bm12 model experiments were performed as previously described (7, 28). Briefly, 7 × 105 naïve donor CD4bm12 T cells from indicated strains were injected i.p. into recipient mice of the specified strains. Fourteen days later, unless indicated otherwise, spleens were harvested, weighed, and processed into single-cell suspensions for counting and flow staining. Serum was harvested by retro-orbital bleeding prior to sacrificing mice, processed using serum separator tubes (BD Biosciences, San Jose, CA) and stored at −80°C for analysis of cytokine and anti-dsDNA IgG levels.

**In vivo depletion studies**

NK cells were depleted via an i.p. injection of 10 μL of anti–Asialo-GM1 rabbit antiserum (FUJIFILM Wako Chemicals, Richmond, VA) at −1, 1, 5, and 10 d after CD4bm12                        T cell transfer. Parallel
studies used NK1.1 (clone PK136, 25 μg/mouse) on day −1 and 1. CD8 T cells were depleted by i.p. injection of 25 μg of anti-CD8β (clone YTS156.7.7, 20 μg per mouse) 1 d prior to CD4<sub>bm12</sub>T cell transfer. Regulatory T cell (Treg) depletion in Foxp3-DTR mice was achieved by administration of DT (i.p. 20 mg/kg) −1, 3, and 10 d after CD4<sub>bm12</sub> T cell transfer. Depletion efficacy was assessed at the end of the experiments by flow cytometry.

In vivo NK cell–killing assay
To assess the susceptibility of the T<sub>Flt</sub> to NK cell–mediated killing, naive wild type (WT), and IFNAR<sup>−/−</sup> CD45.1 CD4<sub>bm12</sub> T cells were injected into Prf<sup>−/−</sup> mice. After 14 d, T<sub>Flt</sub> were sorted and mixed in a 1:1 ratio with naive CD4 T cells from CD90.1 mice. A total of 4 × 10<sup>5</sup> cells were injected i.v into intact and NK-depleted (anti-Asialo-GM1–treated) WT recipients. After 24 h, spleens were collected and the ratio of CD45.1 CD4<sub>bm12</sub>T cells to CD90.1 control CD4 T cells was determined by flow cytometry.

Serum cytokine and anti-dsDNA IgG analyses
Circulating cytokines were analyzed in the serum of mice by Luminex Multiplex Technology (Austin, TX). Serum type I IFN was measured using the highly sensitive ISRE-L929 bioassay. Serum levels of anti-dsDNA were determined using ELISA essentially as described previously (7, 28). Anti-dsDNA IgG levels are plotted as relative units toward a reference standard that was used in all experiments.

In vitro T and B cell assays
Spleens and lymph nodes were harvested from indicated strains, and CD4 T cells or B cells were isolated by positive selection using magnetic bead-sorting technology (CD4<sup>+</sup> T cells and CD19<sup>+</sup> B cells Isolation Kits; Miltenyi). Cells were labeled with a proliferation tracking dye (CTV or CFSE), per the manufacturer’s protocol, and cultured in IMDM supplemented with 10% FBS, penicillin, streptomycin, and l-glutamine. CD4 T cells were stimulated with CD3 (1 μg/ml plate-bound, clone 17A2; BioLegend) and soluble CD28 (1 μg/ml, clone 37.51; BioLegend) in the absence or presence of 50–200 U/ml rIFN-α or IFN-β (PBL Assay Science, Piscataway, NJ). B cells were stimulated with goat anti-mouse IgM F(ab')<sub>2</sub> fragment (10 μg/ml, unless otherwise noted; Jackson Immuno-Research Laboratories, West Grove, PA) and anti-mouse CD40 (10 μg/ml, unless otherwise noted, clone FJK4.5; BioXCell). Analysis was performed by flow cytometry at indicated timepoints.

Flow cytometry
Unless otherwise specified, cells were collected and immediately stained directly ex vivo. For the bm12 model, cells were stained essentially as described previously (7, 28) using combinations of a Fixable Viability dye and Abs to CD3, CD4, PD1, CXCR5, ICOS, CD45.1/CD45.2, CD90.1/CD90.2, and CD25. Intracellular staining for Bcl-6, Foxp3, Bcl-2, Bcl-X, Bim, Bcl2A1, Ki67, and pH2AX was performed using the Foxp3 Staining Buffer Set (eBioscience, San Diego, CA). NK cells were defined as NK1.1<sup>+</sup>NKp46<sup>+</sup> and were evaluated further using combinations of Abs toward CD27, CD11b, NKG2A, NKG2D, Ly49A, C, D, F, G2, H, I, CD244, and CD122.

Serum cytokine and anti-dsDNA IgG analyses
Circulating cytokines were analyzed in the serum of mice by Luminex Multiplex Technology (Austin, TX). Serum type I IFN was measured using the highly sensitive ISRE-L929 bioassay. Serum levels of anti-dsDNA were determined using ELISA essentially as described previously (7, 28). Anti-dsDNA IgG levels are plotted as relative units toward a reference standard that was used in all experiments.

RESULTS

Type I IFN sensing by the hematopoietic compartment is required for disease development in the bm12 cGVHD model of SLE
Analysis of serum cytokines showed that type I IFN levels increased over time, concomitant with an increase in the serum levels of the type I IFNs and the IFN-inducible chemokines CXCL9 and CXCL10 (Fig. 1A). Additional inflammatory cytokines including TNF-α, IL-12, and IL-6 were also induced, although these were present in relatively low levels and at generally delayed kinetics compared with the type I IFNs and the IFN-inducible chemokines (Fig. 1A).

To assess the relative contribution of type IFN sensing in the hematopoietic and nonhematopoietic compartments to disease development, we generated BM-chimeric mice for which WT and IFNAR<sup>−/−</sup> mice were reconstituted with either WT or IFNAR<sup>−/−</sup> BM. After 12 wk, CD45.1 CD4<sub>bm12</sub>T cells were transferred and splenic mass, development of T<sub>Flt</sub> (PDL1<sup>CXCR5</sup><sup>+</sup> within live CD4<sup>+</sup>CD45.1<sup>+</sup> cells), GC B cells (live, CD19<sup>+</sup>Fas<sup>GL7</sup><sup>+</sup>), plasmablasts (live, B220<sup>med</sup><sup>low</sup>CD138<sup>+</sup>), and anti-dsDNA IgG levels were assessed 2 wk later (Fig. 1B, 1C) (28).

WT→WT and WT→IFNAR<sup>−/−</sup> recipients showed comparable increases in splenic mass, frequencies, and absolute numbers of T<sub>Flt</sub>, GC B cells, plasmablasts, and anti-dsDNA IgG levels, indicating that type I IFN sensing by the nonhematopoietic compartment was not required for disease development (Fig. 1D). In contrast, IFNAR<sup>−/−</sup>→WT mice showed significantly diminished disease compared with mice reconstituted with WT BM. There was no added effect of loss of IFNAR on the nonhematopoietic compartment (IFNAR<sup>−/−</sup>→IFNAR<sup>−/−</sup> compared with IFNAR<sup>−/−</sup>→WT) (Fig. 1D). Together, these data identify type I IFN sensing by the hematopoietic compartment as the critical component in disease development.
Type I IFN sensing by B cells is required for optimal GC and plasmablast formation

As it is likely that multiple components in the hematopoietic compartment require type I IFN sensing to confer disease development, we next assessed the contribution of type I IFN sensing on the development of GC B cells, plasmablasts, and anti-dsDNA IgG levels.

To generate mice that lacked IFNAR only on the B cell compartment, WT mice were reconstituted with a mix of BM from B cell–deficient muMT mice together with either WT or IFNAR−/−
BM in a 10:1 ratio, effectively generating mice in which the B cells are either WT or IFNAR\(^{-/-}\) (Fig. 2Ai). Transfer of CD45.1 CD4\(_{bm12}\) T cells into the WT/\(\mu\)MT mice resulted in the expansion of T\(_{FH}\) and induction of GC B cells, plasmablasts, and anti-dsDNA IgG. However, in the IFNAR\(^{-/-}/\mu\)MT recipients, GC B cells, plasmablasts, and anti-dsDNA IgG levels were significantly reduced, suggesting a direct effect of type I IFN sensing on B cells (Fig. 2B). However, IFNAR\(^{-/-}/\mu\)MT recipient mice also had lower levels of T\(_{FH}\) than WT/\(\mu\)MT recipient mice. T\(_{FH}\) and GC B cells are reciprocally supportive of each other: the frequency of T\(_{FH}\) is associated with the magnitude of the B cell response, and GC B cells have been shown to promote T\(_{FH}\) expansion (32–34). Therefore, the observed reduction in B cell responses in the IFNAR\(^{-/-}/\mu\)MT mice could result from changes in reciprocal T\(_{FH}\)-B cell interactions, rather than a sole effect on B cells.

**FIGURE 2.** B cell IFN sensing augments plasmablast and GC B cell development. (A) Schematics used for the mixed BM chimeras in (B) and (C), respectively. (B) BM from WT or IFNAR\(^{-/-}\) CD45.1 mice was combined with BM of B cell–deficient CD45.2 \(\mu\)MT mice at a 1:10 ratio and transplanted into lethally irradiated C57BL/6 mice. After 12 wk, mice received CD45.1/2 WT\(_{bm12}\) CD4 T cells. B cell and donor T\(_{FH}\) response 2 wk after T cell transfer presented as absolute number of cells per spleen. (C) BM from WT (CD45.2) and IFNAR\(^{-/-}\) (CD45.1) mice was combined at a 1:1 ratio and transplanted into lethally irradiated C57BL/6 mice. Twelve weeks later, mice received CD45.1/2 WT\(_{bm12}\) CD4 T cells and composition of the B cell compartments in each part of the graft was assessed. Depicted are the percentages of WT versus IFNAR\(^{-/-}\) cells within GC or plasmablast B cell subsets normalized to the percentage of each genotype within the total B cell graft. For (B) and (C), data from one representative experiment (out of two experiments with \(n = 4–6\) per group) are shown as mean \(\pm\) SEM. (D) CTV-labeled purified B cells were cultured with 1 or 10 \(\mu\)g/ml anti-IgM and anti-CD40 with or without 100 U/ml IFN-\(\alpha\), and proliferation was assessed 96 h later. (E and F) Purified B cells were cultured under the indicated conditions with or without 50 U/ml IFN-\(\alpha\). Expression of CD40 and MHCII was assessed over time, and Bcl-6 expression was assessed after 3 d by flow cytometry. Data are shown as mean \(\pm\) SEM (\(n = 3–4\) per group for each timepoint). One of two experiments is shown. *\(p \leq 0.05\), ***\(p < 0.001\).

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To circumvent this problem, we generated chimeric mice with WT (CD45.2) and IFNAR−/− (CD45.1) BM mixed in a 1:1 ratio (Fig. 2Aii). In this setting, the WT B cells facilitate the development of Tfh, allowing the assessment of the direct effect of type I IFN sensing on B cell responses during disease by the comparison of WT and IFNAR−/− B cells in the same animal. Upon transfer of CD45.1/2 CD4bm12 T cells, all mice developed high frequencies of Tfh and anti-dsDNA IgG levels (data not shown). However, compared with the WT B cell compartment, the development of both GC B cells and plasmablasts was significantly reduced in the IFNAR−/− B cell compartment (Fig. 2C). Together, these data demonstrate a critical role for type I IFN signaling in the B cell response that cannot be overcome by other mediators involved in the disease development.

Type I IFN sensing by B cells has been shown to promote B cell survival and lower the threshold for BCR signaling, enhancing the proliferation of naive B cells when stimulated with anti-IgM ([35], Fig. 2D). Indeed, B cells exposed to IgM and type I IFN also increase expression of MHCII and CD40, molecules involved with cognate T:B interaction and requirements for optimal GC formation (Fig. 2E). Moreover, type I IFN sensing in IgM- and CD40-stimulated B cells increased the expression of Bcl-6, the transcription factor that is associated with GC B cell formation (Fig. 2F).

Together, these data indicate that type I IFN has a direct effect on B cells and promotes B cell responses on the transcriptional level as well as stimulatory capacity toward T cells.

**Tfh require type I IFN for their accumulation**

Genetic ablation of IFNAR can completely prevent disease development in various experimental models of SLE (4–6). Although our data show that a lack of IFN sensing by the hematopoietic compartment was critically important for disease development, the transfer of IFNAR-sufficient CD45.1 CD4bm12 T cells into IFNAR recipients still resulted in partial disease (Fig. 1D and (7)). As these transferred cells are the drivers of disease, we next tested the contribution of IFN sensing by the donor CD4bm12 T cells on disease development.

Compared with the transfer of WT CD4bm12 T cells, the transfer of IFNAR−/− CD4bm12 T cells (into WT recipients) resulted in a significantly reduced disease, illustrated by reduced Tfh, GC B cells, plasmablasts, and anti-dsDNA IgG levels (Fig. 3A, 3C). Importantly, transfer of IFNAR−/− CD45.1 CD4bm12 T cells into IFNAR−/− recipients completely abrogated disease development. Because Tfh and GC B cell responses are reciprocally supportive of one another, we considered the possibility that type I IFN may have acted in trans to promote Tfh expansion. We reasoned that type I IFN sensing by CD4bm12 T cells may increase their expression of costimulatory molecules, such as IL-21, that support a more robust GC B cell response. An augmented GC B cell response may subsequently drive greater Tfh expansion. To test this hypothesis, we cotransferred congenically marked WT CD4bm12 T cells (CD45.2) and IFNAR−/−CD4bm12 T cells (CD45.1) into CD45.1/2 WT recipients (Fig. 3B). In this model the WT CD4bm12 T cells drive comparable GC B cell and plasmablast responses to those found in mice that received WT CD4bm12 T cells only. Direct comparison between the WT and IFNAR−/−CD4bm12 T cells in the same animals still showed a significant reduction in Tfh development in the IFNAR−/−CD4bm12 T cells (Fig. 3C), indicating a critical role of type I IFN sensing by the T cells that could not be overcome by other inflammatory mediators.

**Type I IFN is not required for proliferation or Tfh development**

To identify the mechanisms that drive the reduced IFNAR−/− Tfh accumulation, we first assessed when Tfh accumulation of WT and IFNAR−/− CD45.1 CD4bm12 T started to diverge. A time course indicated that small differences were apparent within 3 d of transfer and became significant within 5 d after transfer (Fig. 4A). Proliferation dye indicated that only a proportion of transferred WT and IFNAR−/− CD4bm12 T cells proliferated and that the WT and IFNAR−/− CD4bm12 T cells that did proliferate showed a similar loss of the dye, indicating similar proliferation rates (Fig. 4B). The absolute number of nonproliferating cells remained similar between WT and IFNAR−/− CD4bm12 T cells, whereas the absolute number of proliferating cells was significantly reduced for the IFNAR−/− CD4bm12 T cells (Fig. 4B). Analysis of Ki67 expression was low/absent in undivided cells and high in divided cells, resulting in a trend toward reduced Ki67 levels in the bulk IFNAR−/− CD4bm12 T cell population (Fig. 4C). A comparable percentage of WT and IFNAR CD4bm12 T cells also expressed phospho–H2AX (pH2AX), a well-characterized marker for the DNA damage response, shown to be highly elevated in vivo activated and proliferating T cells (36).

Upon transfer, both WT and IFNAR−/− CD4bm12 T cells rapidly adopted a Tfh phenotype and both genotypes expressed comparable levels of PD1, CXC5, ICOS, and Bcl-6 (Fig. 4D). Together, these data suggest that differences in proliferation rate, either directly driven by type I IFN or as a consequence of different phenotype development, were not responsible for the differences in Tfh accumulation.

In vivo proliferation data can be skewed by silent clearance of dying cells or migration away from target organs. Analysis of a wide variety of lymphoid and nonlymphoid tissues eliminated the differences in migratory capacity as a critical contributor to the phenotype, as all lymphoid tissues and the peritoneal cavity showed similar reduction in IFNAR−/− CD4bm12 T cells and the transferred cells (WT and IFNAR−/−) were not detectable in nonlymphoid tissues at this timepoint (data not shown).

To assess the effect of IFN sensing on CD4 T cell proliferation and survival in vitro, purified CD4bm12 T cells from WT (CD45.2) and IFNAR−/− (CD45.1) donors were combined at a 1:1 ratio, labeled with a proliferation-tracking dye, and stimulated with anti-CD3/CD28 in the absence or presence of type I IFN. Type I IFN inhibited the proliferation of activated WT but not IFNAR−/− CD4 T cells, demonstrating that the antiproliferative effect of IFN-involved direct signaling in the CD4bm12 T cells (Fig. 4E). As these data were seemingly at odds with the phenomenon observed in vivo, in which IFN sensing allowed for the increased accumulation of WT CD4bm12 T cells, we therefore shifted our focus toward the
potential effect of type I IFN on the survival of these cells. However, analysis of pro- and antiapoptotic molecules did not show a difference between WT and IFNAR$^{-/-}$CD4$^{bm12}$ T cells at 5 d after transfer, and IFNAR$^{-/-}$CD4$^{bm12}$ T cells had comparable levels of pyH2AX$^+$ cells (Fig. 4C, 4F). In line with this finding, both WT and IFNAR$^{-/-}$CD4$^{bm12}$ T cells showed similar rates of cell death when cultured ex vivo in medium or in the presence of type I IFN (data not shown).

**Type I IFN sensing protects T$_{FH}$ from perforin-dependent cell death**

Because type I IFN imparted no obvious cell-intrinsic survival advantage, we hypothesized that IFN may be protecting T$_{FH}$ from cell-extrinsic pressure, either control by Tregs or killing by NK cells or CD8 T cells.

The frequency and total number of CD4$^+$Foxp3$^+$CD25$^+$ cells in the spleens did increase slightly between day 3 and day 7 after transfer, but no difference between WT and IFNAR$^{-/-}$ recipient groups was seen. Similarly, 7 d after transfer, WT and IFNAR$^{-/-}$CD4$^{bm12}$ T cells contained comparable proportions of Tregs, and elimination of recipient Tregs before and during the T cell transfer did not restore the IFNAR$^{-/-}$CD4$^{bm12}$ T$_{FH}$ accumulation (Supplemental Fig. 1). These observations argue against a specific role for Treg-mediated suppression of the IFNAR$^{-/-}$ T$_{FH}$.

To assess susceptibility to killing, WT and IFNAR$^{-/-}$CD4$^{bm12}$ T cells were transferred into WT or perforin-deficient (Prf1$^{-/-}$)
FIGURE 4. Normal development but lack of accumulation of IFNAR\(^{−/−}\) TFH.

(A) CTV-labeled WT and IFNAR\(^{−/−}\) CD4\(^{+}\) CD45.1 CD4\(_{bmi12}\) T cells were transferred into CD45.2 WT recipients, and the absolute number of splenic donor cells was assessed over time. Data are shown as mean ± SEM (with \(n = 5–6\) per group) and exhibit significant interaction by a two-way ANOVA.

(B–D) CTV-labeled WT, IFNAR\(^{−/−}\) CD45.1\(^{+}\) CD4\(^{+}\) bm12 T cells, or control CTV-labeled CD45.1\(^{+}\) CD4\(^{+}\) H-2K\(^{b}\) T cells were transferred into B6 mice. Spleens were harvested 5 d later. (B) Representative CTV-dilution plots (gated on live CD4\(^{+}\) CD45.1\(^{+}\)) and absolute numbers of divided and undivided donor CD4\(_{bmi12}\) T cells 5 d after transfer. Data are shown as mean ± SEM (with \(n = 4–5\) per group) and exhibit significant interaction by a two-way ANOVA. (C) Proportion of Ki67\(^{+}\) and p\(\gamma\)H2AX\(^{+}\) cells within the proliferating cells 5 d after transfer. (D) Comparable expression of TFH-associated molecules in proliferating donor WT and IFNAR\(^{−/−}\) CD4\(_{bmi12}\) T cells 5 d after transfer. Data are gated on live CD45.1\(^{+}\) CD4\(^{+}\) bm12 CTV-diluted cells or CD45.1\(^{+}\) CD4\(^{+}\) undiluted control cells. (E) Inhibition of proliferation by type I IFN. Purified naive WT (CD45.2) and IFNAR\(^{−/−}\) (CD45.1) CD4\(_{bmi12}\) T cells were mixed (1:1 ratio), CTV labeled, and cocultured with CD3/CD28 in the absence or presence of type I IFN. Proliferation was assessed 24, 48, and 72 h later. Representative CTV dilution plots are shown from one of three independent experiments. Day 3 proliferation data for varying concentrations of IFN-\(\alpha\) and IFN-\(\beta\) are also plotted using the Divisional Index calculated by the FlowJo proliferation modeling tool. (F) Expression of pro- and antiapoptotic markers in proliferating WT (black bar) and IFNAR\(^{−/−}\) (white bar) CD4\(_{bmi12}\) T cells 3 d after transfer (gating on live CD45.1\(^{+}\) CD4\(^{+}\) CTV-diluted cells). Data are shown as mean ± SEM (with \(n = 3–4\) per group). **\(p < 0.01\), ***\(p < 0.001\).
**levels in the serum were determined by ELISA. A representative experiment (of four) is shown as mean dsDNA IgG levels (Fig. 5).**

after transfer, splenic weight and the total numbers of splenocytes, donor TFH, GC B cells, and plasmablasts were determined. Total anti-dsDNA IgG

significantly. Transfer of IFNAR−/−CD4bm12 T cells into Prf1−/− recipients completely restored accumulation of IFNAR−/−CD4bm12 Tfh, concomitant with increases in plasmablasts, GC, and anti-dsDNA IgG levels (Fig. 5).

**Type I IFN protects Tfh from NK cell–mediated killing**

Based on the kinetic accumulation data, both CD8 T cells and NK cells could be involved in the elimination of the IFNAR−/− Tfh. Depletion of CD8 T cells before IFNAR−/−CD4bm12 T cell transfer did not restore Tfh accumulation and subsequent GC B cell, plasmablast and anti-dsDNA IgG level development (Fig. 6A). In contrast, NK cell depletion by either NK1.1 or anti-Asialo-GM1 prior to IFNAR−/−CD4bm12 T cell transfer restored Tfh accumulation and the number of GC B cells, plasmablasts and anti-dsDNA IgG levels (Fig. 5A).

To further confirm that IFNAR−/−CD4bm12 Tfh are target cells for NK cells, Prf−/− mice were injected with WT or IFNAR−/−CD4bm12 T cells and Tfh were harvested 2 wk later. Cells were added in a 1:1 ratio with CD90.1 CD4 control T cells and transferred into intact or NK-depleted WT mice. The ratio of CD4bm12 T cells/CD90.1 control cells was assessed 24 h later (Fig. 6E, 6F). As expected, the ratio of WT CD4bm12 T cells/control T cells in intact and NK-depleted recipients did not change significantly. Transfer of IFNAR−/−CD4bm12 Tfh into intact WT recipients resulted in a significant distortion of the Tfh/control T cell ratio. In support of our in vivo studies, the IFNAR−/−CD4bm12 Tfh/control CD4 T cell ratio remained unaltered and comparable to the WT Tfh/control T cell ratio when cells were transferred into NK-depleted WT mice (Fig. 6G, 6H).

Importantly, the protective effect of type I IFN sensing by the Tfh was not shared by the B cells. In mixed BM-chimeric mice from Fig. 2 (WT/IFNAR 1:1), NK-depletion did not restore GC B cell and plasmablast numbers in the IFNAR−/− B cell compartment (Supplemental Fig. 2). These data indicate that although both the T cell and the B cell response required type I IFN sensing for their accumulation, the underlying mechanisms are distinct.

**DISCUSSION**

A connection between the overproduction of type I IFN and the development of SLE has been well established, yet the mechanisms by which IFN promotes disease pathology have not been fully elucidated. In this study, we showed that type I IFN enhances development of pathogenic T and B cell responses in the bm12 cGVHD model of SLE-like disease through two different mechanisms: directly promoting B cell proliferation and GC differentiation and protecting Tfh from NK cell–mediated killing.

Our observations that type I IFN enhances B cell responses fit well with existing data on the direct and indirect roles of type I IFN and induction of humoral responses (23–25, 35, 37). Type I IFNs have been shown to promote B cell survival and isotype switching through the induction of BLyS and APRIL in DC and macrophages (24, 38). However, our mixed BM chimera models indicate that the lack of type I IFN sensing by B cells cannot be overcome by other inflammatory mediators, implicating a more-important contribution of direct type I IFN sensing in the B cell response. Intrinsic direct effects of type I IFN sensing encompass reducing BCR signaling threshold, increasing expression of molecules involved in cognate and reciprocal T–B cell interactions, cytokine production, migration, survival, as well as plasmablast development (25, 35, 37, 39–41). Although our BM chimera experiment would indicate a limited role for increased cytokine production, it is likely that combinations of several above-mentioned mechanisms are involved in the type I IFN–associated boosting of the B cell response in SLE. IFNAR−/− B cells were still able to mount a partial response, pointing to the involvement of other stimulatory mechanisms in the autoantibody response. Further dissection of the relative contribution of these mechanisms might point to
FIGURE 6. Type I IFN protects T_{FH} from NK cell-mediated killing.

(A–C) WT (black bar) or IFNAR^{−/−} (white bar) CD45.1+CD4_{bm12} T cells were transferred into intact or Ab-depleted CD45.2 mice. Two weeks after transfer, mice were assessed for the absolute splenic number of donor T_{FH}, GC B cells, and plasmablasts in each graft. Mice were kept intact or

(Continued)
Our studies show that type I IFN sensing by TFH protects them from NK cell–mediated killing, which is crucial for their accumulation and promotion of the B cell response. The potential for immunoregulation of T cell activity by NK cells has been uncovered in recent years. Mounting evidence has established a role for NK cells in limiting T cell responses toward lymphocytic choriomeningitis virus (LCMV) by direct killing of virus-specific T cells (42–45). Recently, NK cytotoxicity toward LCMV-specific T cells was shown to be inhibited by type I IFN (44, 46). These reports were consistent with data published over four decades earlier, in which Hansson, Welsh, and colleagues (47, 48) showed that either LCMV infection, the induction of type I IFN during certain conditions, or a combination of these. However, the remaining NK cells were still able to kill IFNAR–/– TFH, indicating that at least part of their cytolytic machinery was functional. As this remaining cytolytic capacity was not necessarily reinvigorated by NK cell–mediated killing through the differential expression of activating and inhibitory NK cell ligands. Comparing expression of various known NK cell–activating and –inhibiting ligands on WT and IFNAR–/– TFH in intact mice showed notable shifts in expression of activating (Rael, Mult-1, CD48, ICAM-1/2) and inhibitory (H-2D/β/Kβ) NK ligands. We consistently observed increases in the expression of several NK receptor–activating ligands in CD4bm12 T cells compared with CD4 T cells from naive mice; however, these changes were not unique to IFNAR–/– TFH from NK-depleted mice, suggesting the primary mechanism of protection from NK killing mediated by type I IFN was not through decreasing these particular signals (Supplemental Fig. 4). Class I MHC (H2-Dβ and H2-Kβ) was increased in WT TFH compared with IFNAR–/– TFH and naive CD4 T cells, indicating one possible mechanism for type I IFN–induced protection from NK cell killing, although these shifts were relatively small and were not completely restored in the IFNAR–/– TFH upon NK cell depletion. One of the recent studies on NK immunoregulation in the context of LCMV showed that type I IFN protected SMARTA and P14 T cells by upregulating an, as yet, unidentified Ncr1 ligand or ligands (46). However, we were unable to detect differences in Ncr1 ligands on our polyclonal endogenous WT and IFNAR–/– TFH upon staining with an Ncr1-HigG fusion protein. These data suggest that the lack of WT TFH sensitivity to NK cells could involve a complex combination of signals, a critical effector ligand or ligands that is/are still unknown, a TFH–intrinsic intracellular compensatory mechanism for the cytolytic assaults, or a combination of these.

With the rise of therapeutics that target the type I IFN pathway, it will be important to evaluate the relative effect of the treatment on the different immune cells. Although one can envision that simply eliminating or reducing type I IFN would render the TFH susceptible to NK cell–mediated killing, it does not necessarily reinvigorate the disease-affected NK cells. The capacity of the host to properly kill the TFH is critically important as our in vitro and in vivo data indicates that type I IFN has an antiproliferative effect. In vitro stimulation of human and mouse T cells in the presence of type I IFN significantly inhibits cell division [Fig. 4, (56, 57)]. Moreover, in the absence of perforin,
with type I IFN might be an appropriate therapeutic approach when combined with type I IFN–interfering drugs. Although the current data set justify targeting type I IFN in SLE-like disease, additional studies are needed to more precisely appreciate the many functions of type I IFN to enable the rational design of more targeted therapies for SLE and other autoimmune diseases with pathological type I IFN production.

**DISCLOSURES**

The authors have no financial conflicts of interest.

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