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Type I Interferon Production Enhances Susceptibility to *Listeria monocytogenes* Infection

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Abstract

Numerous bacterial products such as lipopolysaccharide potently induce type I interferons (IFNs); however, the contribution of this innate response to host defense against bacterial infection remains unclear. Although mice deficient in either IFN regulatory factor (IRF)3 or the type I IFN receptor (IFNAR)1 are highly susceptible to viral infection, we show that these mice exhibit a profound resistance to infection caused by the Gram-positive intracellular bacterium *Listeria monocytogenes* compared with wild-type controls. Furthermore, this enhanced bacterial clearance is accompanied by a block in *L. monocytogenes*–induced splenic apoptosis in IRF3- and IFNAR1-deficient mice. Thus, our results highlight the disparate roles of type I IFNs during bacterial versus viral infections and stress the importance of proper IFN modulation in host defense.

Key words: IRF3 • IFNAR • apoptosis • intracellular bacteria • IFN target gene

Introduction

Type I IFNs (IFN-α/β) were the first cytokines to be discovered, being named for their potent effects on viral "interference" (1). These cytokines play a critical role in the innate antiviral response by inhibiting cellular proliferation, promoting apoptosis, activating the microbicidal function of macrophages and NK cells, and by linking innate and adaptive immune responses (2–4). Induction of type I IFN gene expression in response to viral infection is accomplished by a sophisticated interplay of transcription factors of the IFN regulatory factor (IRF) family (5). Upon detection of viral entry into the cytoplasm, IRF3 becomes phosphorylated and translocates to the nucleus to drive transcription of the *IFN*-β gene. Binding of IFN-β to the type I IFN receptor (IFNAR), composed of subunits IFNAR1 and IFNAR2, results in the phosphorylation of the transcription factor STAT1α/β, leading to induction of IFN target genes (2). Studies have also found that bacterial products, such as LPS, can induce IRF3-dependent type I IFN gene expression through interaction with Toll-like receptors (6–9). Although the molecular mechanisms by which bacterial products induce type I IFNs have been studied extensively (10), little is known of the functional significance of type I IFN induction during a bacterial infection.

Listeria monocytogenes is a Gram-positive intracellular bacterium that causes sepsis and meningitis in immunocompromised individuals and severe fetal infections in pregnant women (11–13). Furthermore, *L. monocytogenes* has been used widely as a laboratory model for intracellular bacterial infections (12). Recent studies have indicated that *L. monocytogenes* can induce high levels of IFN-β once inside the cytoplasm of infected macrophage cells (14–17). To better understand the functional consequences of type I IFN signaling during a bacterial infection, we have analyzed mice

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Abbreviations used in this paper: BHI, brain heart infusion; BMM, bone marrow–derived macrophage; IFNAR, type I IFN receptor; IRF, IFN regulatory factor; LLO, listeriolysin O; Q-PCR, quantitative real-time PCR; TUNEL, TdT-mediated dUTP nick-end labeling; USF, upstream stimulating factor.

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deficient in various components of the type I IFN system in the context of an *L. monocytogenes* infection.

Materials and Methods

Mice. 6–12-wk-old C57BL/6 (The Jackson Laboratory) mice were used as wild-type controls in all experiments, unless noted. *MyD88*$^{-/-}$ mouse lines were provided by S. Akira (Osaka University, Osaka, Japan). To obtain *IFNAR1*$^{-/-}$, *IFNAR1*$^{+/-}$, and matched littermate wild-type controls on a C57BL/6 genetic background, A129 mice (B&K Universal Limited) were backcrossed with C57BL/6 (The Jackson Laboratory) mice for five generations. All mice, including F9 C57BL/6 *IRF3*$^{-/-}$ mice, were maintained and bred under specific pathogen-free conditions in the UCLA-DLAM mouse facility and experiments were conducted within the parameters of our approved protocol.

Bacteria. *L. monocytogenes* strain 10403S was used for the in vitro portions of this study and all in vivo *L. monocytogenes* infection experiments were performed using *L. monocytogenes* expressing an immunodominant H-2D^{b}–restricted epitope from the lymphocytic choriomeningitis virus–NP protein. The epitope was embedded in an *hly* fusion protein that was integrated into the *L. monocytogenes* chromosome using a genetic system provided by P. Lauer and R. Calendar (University of California, Berkeley, Berkeley, CA). Although we found this particular strain of *L. monocytogenes* to be approximately five times less virulent than wild-type *L. monocytogenes* as determined by LD_{50}, IRF3$^{-/-}$ mice were also more resistant to infection by wild-type *L. monocytogenes* compared with control mice (unpublished data). *L. monocytogenes* was grown in brain heart infusion (BHI) broth with 200 μg/ml streptomycin. Bacteria were grown to midlogarithmic phase, pelleted, washed three times using PBS, and stored as glycerol stocks at −80°C in small aliquots until use. The concentration of *L. monocytogenes* was quantified by plating serial dilutions on BHI agar plates and counting colonies after growth at 37°C for 24–36 h.

Cell Culture. Murine bone marrow–derived macrophages (BMMs) were differentiated from adherent bone marrow cells from mice as described previously (18). In brief, bone marrow cells were isolated and maintained in macrophage differentiating media (1× DMEM [Mediatech Inc.], 10% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 30% L929 conditioned medium) for 7 d. Purity was assayed to be 94–99% CD11b^{+} and negative for several contaminating markers.

Immunoblotting. Cell fractionation and nuclear and cytoplasmic immunoblotting were performed as described previously (6). Anti-IRF3 was obtained from Zymed Laboratories. Anti–upstream stimulating factor (USF)-2 and anti-STAT1 were obtained from Santa Cruz Biotechnology, Inc. Antibodies specific to the phosphorylated forms of STAT1 were obtained from Cell Signaling Technologies. Cell lysates were quantified and 40 μg of nuclear extract or 25 μg of cytoplasmic extract was loaded in each lane of an acrylamide gel and separated by SDS-PAGE. Gels were transferred to Immobilon-P membranes (Millipore) and immunoblotted according to the antibody manufacturer's recommended instructions.

ELISA. To assay cytokine levels, serum was isolated from infected and uninfected mice as indicated. IFN-γ and IL-6 levels were assessed using the mouse IFN-γ (AN-18) and IL-6 ELISA sets from BD Biosciences according to the manufacturer's protocol.

Quantitative Real-Time PCR (Q-PCR). RNA was isolated using TRIzol Reagent (Invitrogen) according to the manufacturer's protocol. RNA was quantitated and 2 μg RNA was used to make cDNA templates using Superscript II (Invitrogen) according to the manufacturer's instructions and oligo-dT primers. Q-PCR analyses were performed using the iCycler thermocycler (Bio-Rad Laboratories). Q-PCR was conducted in a final volume of 20 μl containing the following: Taq polymerase, 1× Taq buffer (Stratagene), 125 μM dNTP, SYBR™ Green I (Molecular Probes), and Fluorescein (Bio-Rad Laboratories), using oligo-dT cDNA as the PCR template. Amplification conditions were as follows: 95°C for 3 min, 40 cycles of 95°C for 20 s, 55°C for 30 s, and 72°C for 20 s. Primers were used to amplify a specific 100–120-bp fragment corresponding to these respective genes. Primer sequences for *L32*, *IFN-β*, *Mx1*, *IL-6*, and *IP10* are the same as those published previously (6, 7). Primers for *TRAIL*, *TGTP*, *PML*, *Daxx*, *PKR*, and *IRF1* are as follows: *TRAIL*: forward, ACCTCAGCTTCAGTCAGCACTTC, reverse, TGTAAGTCACAGCCACAGACACAG; *TGTP*: forward, CGATTTCCCCATTTGTTTGC, reverse, TGGCCCACCAGTAACTGAAGA; *PML*: forward, CAGGCTCTTAGCACCCACATG, reverse, AGGCATCCCTTACTTTCAGCTTT; *Daxx*: forward, TTCGGGAAAAT CGAACCTTG, reverse, CCTCAGTCTTGTCTTGCATCATTG; *PKR*: forward, GGAGCACGAAGTACAAGCGC, reverse, GCACCGGGTTTTGTATCGA; and *IRF1*: forward, TCCAAGTCCAGCCGAGACA, reverse, TGCTGAGTCCATCAGAGAAAGTGT. *L32* expression measurements were conducted in tandem with the gene of interest. All data is presented as relative expression units after normalization to the average *L32* value to control for loading of total RNA. Measurements were conducted in triplicate.

Infections. For in vitro infections, bacteria were diluted from glycerol stocks into media (1× DMEM, 7% fetal bovine serum, no antibiotics), added onto cells, and incubated at 37°C, 10% CO_2, until harvest. Concentration of bacteria in the infectious stimulus was verified by plating serial dilutions on the appropriate agar plates. For in vivo infections, *L. monocytogenes* was grown to midlogarithmic phase, pelleted, washed, resuspended in PBS three times, and quantified by visible spectrometry readings at 600 nm. For intravenous injection, bacteria were diluted in pyrogen-free saline and 200 μl was injected into the lateral tail vein. Concentration of bacteria in the infectious dose was controlled by plating serial dilutions on BHI/streptomycin agar plates. For determination of bacterial recovery, mice were killed and the livers and spleens were homogenized in 1% Triton-X solution. Serial dilutions of homogenates were plated on BHI/streptomycin agar plates and colonies were counted after growth at 37°C for 24–36 h.

Histology. Wild-type, *IRF3*$^{-/-}$, and *IFNAR*$^{-/-}$ mice were inoculated with 5×10^5 CFUs of *L. monocytogenes* intravenously. Livers were harvested 3 d later, fixed in formalin, and embedded in paraffin. Sections were cut at 4 μm and mounted onto poly-L-lysine–coated slides (Fisher Scientific). Serial sections were then processed for hematoxylin and eosin staining according to standard protocols.

TdT-mediated dUTP Nick-End Labeling (TUNEL) Assays. Spleens were removed from infected mice and snap-frozen in OCT (Sakura Finetek). 4-μm sections were cut and mounted onto poly-L-lysine–coated slides. Splenic tissue was fixed using 4% paraformaldehyde, permeabilized using 0.1% Triton and 0.1% sodium citrate, and then double stranded DNA breaks were labeled using an in situ Cell Death Kit (Roche) according to the manufacturer's protocol. Sections were then counterstained using DAPI and analyzed using a Leica DM IRBE fluorescent microscope at a magnification of 10. Openlab software (Improvision) was used for image acquisition.

Results

Induction of IFN-β by L. monocytogenes Requires IRF3. The transcription factor IRF3 has been shown to mediate the induction of the *IFN*-β gene in response to both viruses and Toll-like receptor signaling (6, 19). To determine if induction of type I IFNs by *L. monocytogenes* also involves IRF3, primary murine BMMs were challenged with *L. monocytogenes* and IRF3 activation was assayed by nuclear translocation. As shown in Fig. 1 A, *L. monocytogenes* can induce strong IRF3 nuclear translocation by 4 h after infection. We also found that *L. monocytogenes* induced nuclear translocation of the NF-κB subunit p65 by 1 h, whereas USF-2 was monitored as a loading control (Fig. 1 A). Next, we sought to assess whether IRF3 is required for the induction of the type I IFN system. *L. monocytogenes* induction of IFN-β occurred normally in BMMs deficient in the Toll-like receptor and IL-1R adaptor molecule MyD88, which has been shown to play an important protective role during an *L. monocytogenes* infection in vivo (Fig. 1 B; references 20 and 21). However, *IRF3*$^{-/-}$ BMMs infected with *L. monocytogenes* were unable to induce expression of IFN-β. As a control, we found that IFN-β production in BMMs deficient in the IFNAR subunit IFNAR1 was similar to wild-type.

Once produced, type I IFNs can signal in an autocrine and paracrine manner leading to the phosphorylation-dependent activation of STAT1 through IFNAR (2). Consistent with our observations that IRF3 is required for *L. monocytogenes*–induced IFN-β production, STAT1 phosphorylation also required IRF3 (Fig. 1 C). In contrast, *L. monocytogenes*–challenged *MyD88*$^{-/-}$ BMMs, which were capable of IFN-β production after *L. monocytogenes* infection, induced STAT1 phosphorylation similar to wild-type controls (Fig. 1 C). STAT1 phosphorylation was dependent upon type I IFNs, as BMMs deficient in *IFNAR1* did not activate STAT1 (Fig. 1 C). Collectively, these findings indicate that like some viruses, *L. monocytogenes* induction of IFN-β and subsequent STAT1 activation also occur through an IRF3-dependent, MyD88-independent pathway.

Mice Deficient in IRF3 Have an Enhanced Ability to Clear Infection by L. monocytogenes. IRF3 plays a protective role during viral infection (19). To assess the functional role of IRF3 activation during a bacterial infection, we inoculated both wild-type and *IRF3*-deficient mice with a strain of *L. monocytogenes* expressing an epitope from the NP protein (see Materials and Methods). Surprisingly, we found that 56% of *IRF3*-deficient mice survived a dose of *L. monocytogenes* that was lethal for all mice in the wild-type control group (Fig. 2 A). Although bacterial titers were roughly equivalent on day 1 (not depicted), *IRF3*-deficient mice had ~100-fold fewer bacteria compared with wild-type controls in both the spleen and liver by day 3 after a sublethal *L. monocytogenes* infection (Fig. 2 B). A similar pattern was noted when using a dose of *L. monocytogenes* that was ap-

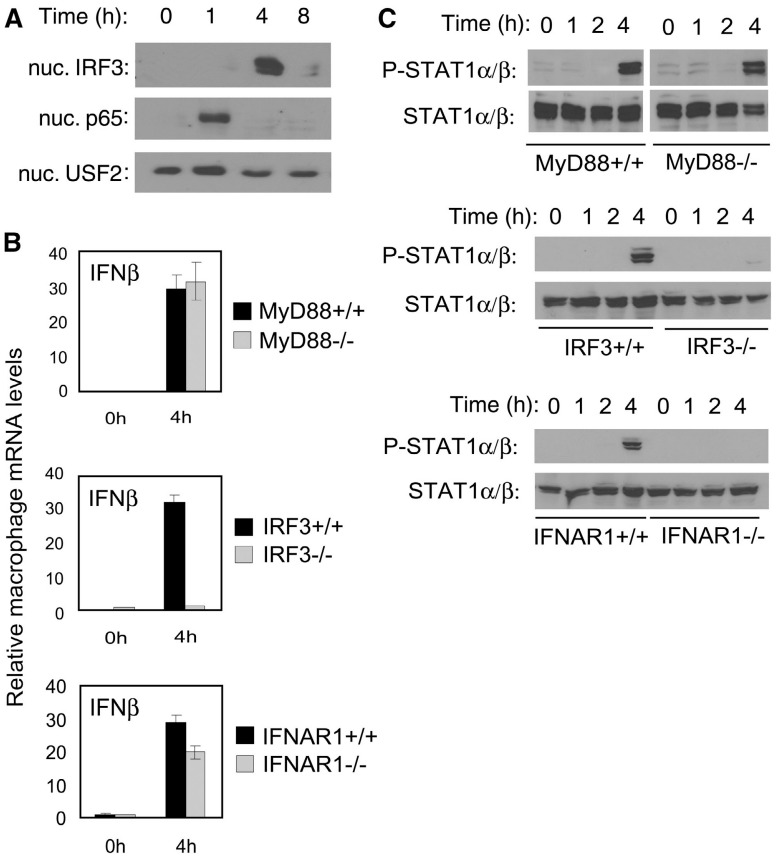

Figure 1. Induction of IFN-β by *L. monocytogenes* requires IRF3. (A) BMMs from C57BL/6 wild-type mice were infected with *L. monocytogenes* at a multiplicity of infection of 1 and IRF3 and p65 nuclear translocation were assayed at the indicated times by immunoblotting using nuclear extract. USF-2 was also assayed as a loading control. (B) *MyD88*$^{-/-}$, *IRF3*$^{-/-}$, *IFNAR1*$^{-/-}$, and wild-type BMMs were infected with *L. monocytogenes* as described above and assayed at the indicated time points for *IFN*-β gene expression by Q-PCR and (C) phospho-STAT1α/β, or total STAT1α/β by immunoblotting. Results shown are representative of at least two independent experiments, and Q-PCR results are expressed in relative expression units and have been normalized to L32 mRNA levels.

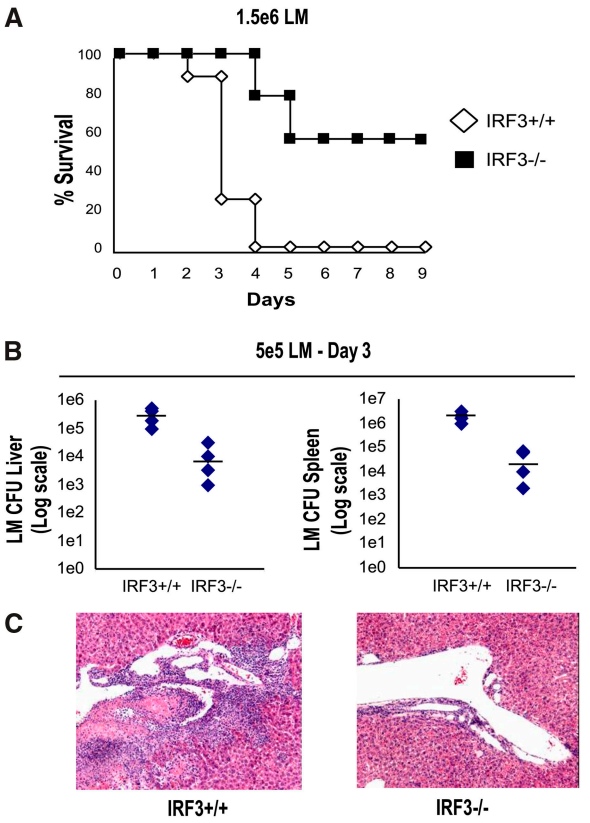

Figure 2. Mice deficient in *IRF3* have an enhanced ability to clear infection by *L. monocytogenes*. (A) $IRF3^{-/-}$ ($n = 8$) and wild-type control mice ($n = 8$) were infected intravenously with 1.5×10^6 *L. monocytogenes* and viability was assayed daily for 9 d. (B) $IRF3^{-/-}$ ($n = 4$) and wild-type mice ($n = 4$) were infected intravenously with 5×10^5 *L. monocytogenes* for 3 d, at which time the *L. monocytogenes* titer in the spleen and liver was assayed and presented as CFUs. (C) A portion of the $IRF3^{-/-}$ and wild-type livers from B were fixed, sectioned, and subjected to a histological analysis after hematoxylin and eosin staining. All data are representative of at least two independent experiments.

proximately one tenth the LD_{50} (not depicted). Histological analysis showed that the *L. monocytogenes*–infected wild-type livers displayed more severe tissue pathology relative to $IRF3^{-/-}$ livers at 3 d after infection (Fig. 2 C). Livers from wild-type mice demonstrated extensive perivascular infiltrate and multiple intravascular thrombi, whereas $IRF3^{-/-}$ livers were relatively clear. Collectively, these data demonstrate that IRF3 activation inhibits the host's ability to control *L. monocytogenes* growth during the early stages of infection.

Type I IFNs Sensitize Mice to L. monocytogenes Infection. It has been clearly shown that mice lacking the IFNAR are extremely susceptible to many viruses (22); however, initial studies using mice on a 129SV/EV genetic background did not find a significant difference in survival between *L. monocytogenes*–infected wild-type and $IFNAR1^{-/-}$ mice (23). To determine if the *L. monocytogenes*–resistant phenotype observed in the $IRF3^{-/-}$ mice, which are on a C57BL/6 background, was due to the absence of type I IFN signaling or by means of a type I IFN–independent pathway, we used $IFNAR1^{-/-}$ mice that were crossed onto the C57BL/6 genetic background for five generations. These mice, along with their littermate wild-type and heterozygous controls, were challenged with *L. monocytogenes*. Only 7% of $IFNAR1^{+/+}$ and 33% of $IFNAR1^{+/-}$ mice remained viable, whereas >77% of $IFNAR1^{-/-}$ mice survived the infection (Fig. 3 A). These data strongly indicate that IRF3-mediated induction of type I IFNs results in enhanced severity of *L. monocytogenes* infection. Furthermore, functional analysis of type I IFNs during *L. monocytogenes* infection in various genetic backgrounds may improve our understanding of both antibacterial and antiviral immune responses in heterogenous human populations.

To further challenge the hypothesis that type I IFNs interfere with the host's ability to control an *L. monocytogenes* infection, we chose to investigate whether elevated type I IFN levels could enhance susceptibility to an *L. monocytogenes* infection. We and others have found that intravenous injection of poly I:C results in strong induction of type I IFNs, including both IFN-β and IFN-α4 (24 and unpublished data). Therefore, wild-type mice were inoculated with a combination of poly I:C and *L. monocytogenes*, or either agent alone. Mice given a sublethal dose of *L. monocytogenes* remained healthy and viable (Fig. 3 B). Likewise, the administration of poly I:C alone did not appear to influence the health status of the mice by day 9 (Fig. 3 B). However, when mice were given the combination of a sublethal dose of *L. monocytogenes* along with poly I:C, all of the mice died by day 4 (Fig. 3 B).

To test whether the increased *L. monocytogenes* resistance observed in the $IFNAR1^{-/-}$ versus wild-type mice was due to improved *L. monocytogenes* clearance, we infected both wild-type and $IFNAR1^{-/-}$ mice with a sublethal dose of *L. monocytogenes*. After 3 d of infection, both the liver and spleen of the $IFNAR1^{-/-}$ mice had a substantially lower *L. monocytogenes* titer compared with wild-type controls (Fig. 3 C). Furthermore, wild-type livers suffered from significant *L. monocytogenes*–induced tissue pathology, whereas $IFNAR1^{-/-}$ livers appeared relatively clear from insult (Fig. 3 D). To determine if the decreased survival observed in mice receiving poly I:C in addition to *L. monocytogenes* was mediated by type I IFNs, we gave both wild-type and $IFNAR1^{-/-}$ mice the same poly I:C + *L. monocytogenes* treatment and monitored *L. monocytogenes* clearance from the spleen and liver by day 3. As shown in Fig. 3 C, wild-type mice receiving *L. monocytogenes* plus poly I:C exhibited drastically higher bacterial titers in both the liver and spleen compared with wild-type mice receiving *L. monocytogenes* alone. When these same studies were performed in $IFNAR1^{-/-}$ mice, poly I:C had almost no effect on the levels of bacteria in the liver or spleen (Fig. 3 C). Histological analysis of wild-type livers from poly I:C and *L. monocytogenes* coadministered mice revealed multiple parenchymal abscesses compared with the less severe lesions found in the those mice injected with *L. monocytogenes* alone (Fig. 3 D). Consistent with the CFU analysis, livers from $IFNAR1^{-/-}$ mice with or without coadministration of poly I:C were relatively clear of tissue abscesses (Fig. 3 D). These findings indicate that the adverse effects elicited by

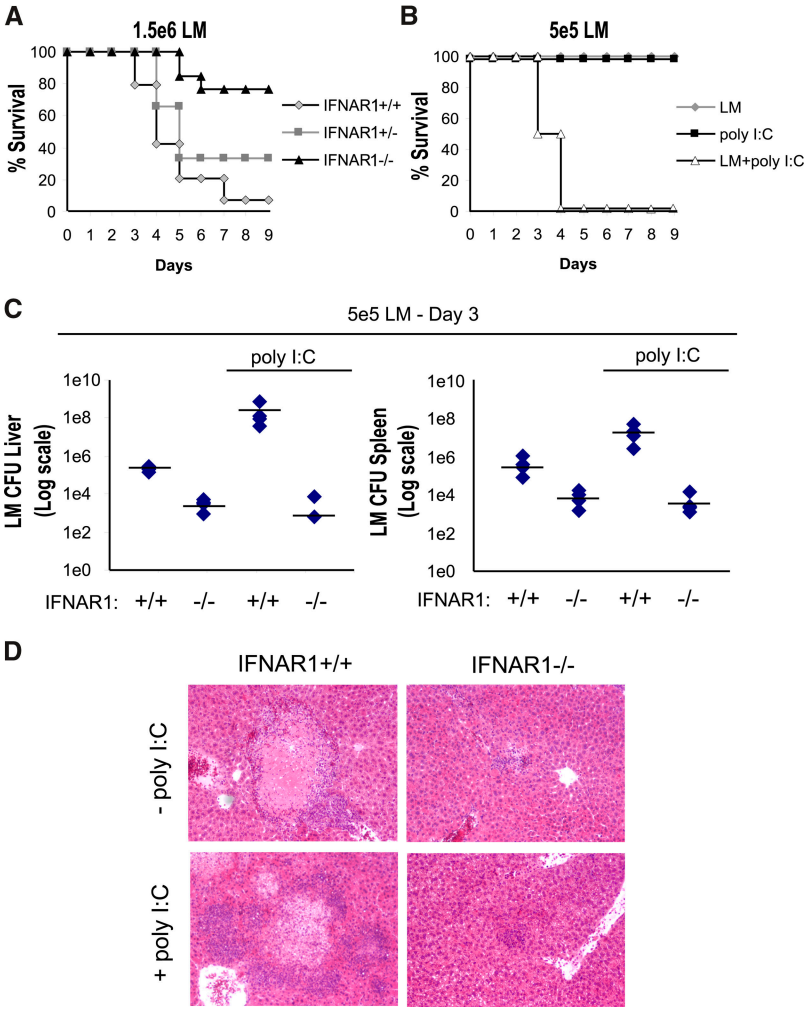

Figure 3. Type I IFNs sensitize mice to *L. monocytogenes* infection. (A) *IFNAR1*$^{-/-}$ (n = 13), *IFNAR1*$^{+/-}$ (n = 8), and *IFNAR1*$^{+/+}$ (n = 14) mice were infected intravenously with 1.5 × 10^6 *L. monocytogenes* and viability was assayed daily for 9 d. (B) Wild-type mice were challenged with 5 × 10^5 *L. monocytogenes* (n = 6), 200 μg poly I:C (n = 6), or the combination of the two (n = 6), and viability was assayed daily for 9 d. (C) *IFNAR1*$^{-/-}$ and wild-type control mice were infected intravenously with 5 × 10^5 *L. monocytogenes* either in the absence or presence of 200 μg poly I:C. After 3 d, the mice were killed and the *L. monocytogenes* titer was determined in the liver (n = 4) and spleen (n = 4), and presented as CFUs. (D) A portion of the livers from C were fixed, sectioned, and subjected to a histological analysis after hematoxylin and eosin staining. All data are representative of at least two independent experiments.

poly I:C during the *L. monocytogenes* infection are largely mediated by type I IFNs, and confirm the ability of type I IFNs to potently enhance *L. monocytogenes* pathogenicity.

Type I, But Not Type II, IFN Signaling Is Impaired in L. monocytogenes–infected IFNAR1$^{-/-}$ *Mice.* To examine the biological basis by which type I IFNs increase sensitivity to *L. monocytogenes* infection, we examined potential targets for type I IFNs that are known to play important roles in *L. monocytogenes* clearance. Both type I and II IFNs are critical in combating certain viral infections through the up-regulation of overlapping and nonoverlapping gene subsets (25). However, only type II IFN, or IFN-γ, has been shown to contribute to antibacterial immunity, as mice deficient in the IFN-γ receptor quickly succumb to infection by *L. monocytogenes* (26). Furthermore, mice deficient in various proteins required for IFN-γ signaling, including IRF2 and IRF8, have been shown to be highly susceptible to infection by *L. monocytogenes* (27). To determine if the absence of type I IFN signaling can modulate the inducible levels of IFN-γ, we measured IFN-γ serum levels in mice that had been infected with *L. monocytogenes*. We found that IFN-γ serum levels in *IFNAR1*$^{-/-}$ and wild-type mice were induced to similar levels 24 h after *L. monocytogenes* infection (Fig. 4 A). IFN target genes such as *IP10*, *TGTP*, and *IRF1* were also induced to similar levels in both wild-type and *IFNAR1*$^{-/-}$ mice, suggesting that these genes are targets of IFN-γ signaling during *L. monocytogenes* infection (Fig. 4 B). The inflammatory cytokine IL-6 was also induced to similar levels in *IFNAR1*$^{-/-}$ and wild-type mice (Fig. 4 A). In contrast, induction of *Mx1*, *PKR*, *TRAIL*, and *Daxx* was completely ablated in the *IFNAR1*$^{-/-}$ spleen, indicating that this set of genes is specifically activated by type I IFNs (Fig. 4 B). These findings argue that the resistance to *L. monocytogenes* infection observed in *IRF3*- and *IFNAR1*-deficient mice compared with wild-type controls was not a result of altered IFN-γ production and subsequent signaling, or altered production of the proinflammatory cytokine IL-6.

L. monocytogenes–infected Macrophages Up-regulate a Type I IFN–dependent Apoptotic Gene Subset. Fig. 4 B demonstrates that among the type I IFN–specific genes up-regulated in the spleen during infection are genes with known roles in promoting apoptosis such as *TRAIL*, *PKR*, and *Daxx* (Fig. 4 B and reference 28). Macrophage cells have been shown to undergo type I IFN–dependent apoptosis in response to *L. monocytogenes* (16). Because macrophages make up a fraction of the splenocyte population, we wanted to

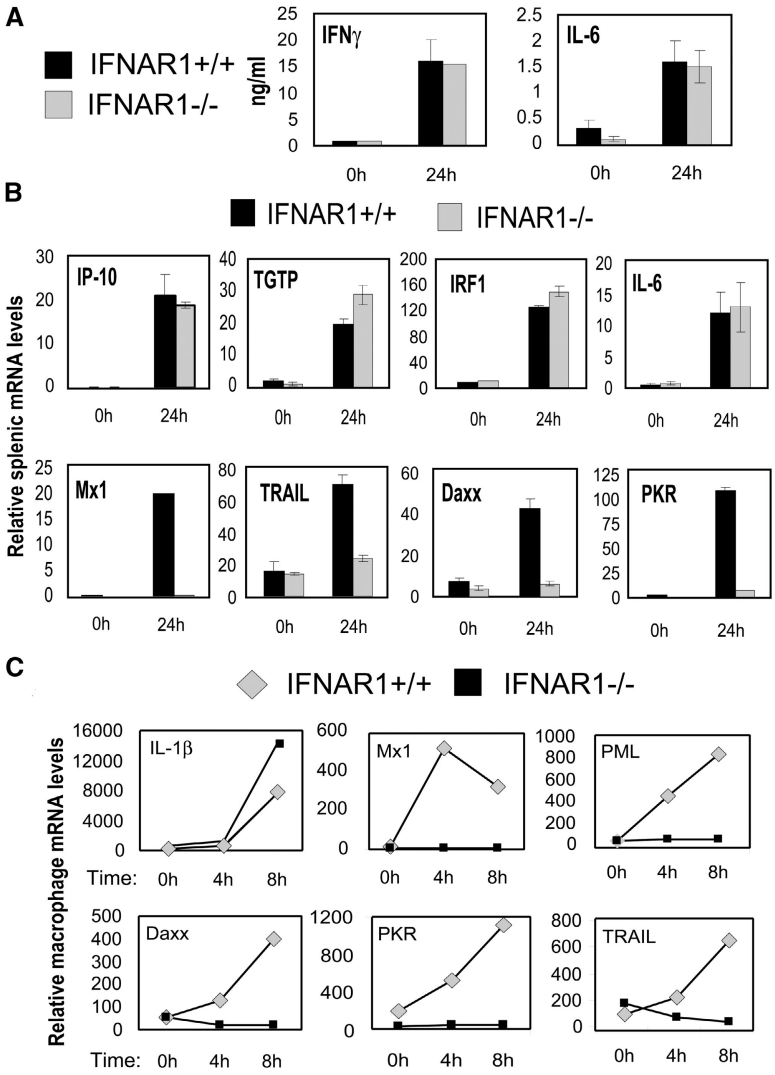

Figure 4. Type I, but not Type II, IFN signaling is inhibited in *L. monocytogenes*–infected *IFNAR1*$^{-/-}$ mice. (A) The spleens and serum from *IFNAR1*$^{-/-}$ and wild-type mice infected with 1.5×10^6 *L. monocytogenes* were removed 24 h after infection and serum IFN-γ and IL-6 levels were assayed by ELISA. (B) The splenocyte mRNA was subjected to a Q-PCR analysis to assay *IP10*, *IRF1*, *TGTP*, *IL-6*, *Mx1*, *TRAIL*, *PKR*, and *Daxx* expression. (C) *IFNAR1*$^{-/-}$ and wild-type BMMs were infected with *L. monocytogenes* at a multiplicity of infection of 1 and assayed at the indicated time points for *IL-1*β, *Mx1*, *PKR*, *Daxx*, *TRAIL*, and *PML* gene expression by Q-PCR. Results shown are representative of at least two independent experiments. Q-PCR data are presented in relative expression units and have been normalized to L32 mRNA levels.

determine whether they might be contributing to the type I IFN–dependent expression of apoptotic genes during the *L. monocytogenes* infection. Wild-type and IFNAR$^{-/-}$ BMMs were challenged with *L. monocytogenes* for up to 8 h. Gene expression was analyzed at the indicated time points by Q-PCR (Fig. 4 C). We found that macrophage cells, similar to the heterogenous splenocyte population, respond to *L. monocytogenes* by up-regulating numerous proapoptotic genes, such as *PKR*, *Daxx*, *TRAIL*, and *PML*, in a type I IFN–dependent manner. As a control, type I IFN was not required for the up-regulation of the proinflammatory cytokine IL-1β (Fig. 4 C). These data indicate that *L. monocytogenes* induction of type I IFN–dependent proapoptotic genes observed in the spleen likely involves splenic macrophages.

L. monocytogenes–induced Splenic Apoptosis Is Dependent on Type I IFNs. One of the hallmark effects mediated by type I IFNs is the induction of apoptosis in infected cells, which has been shown to drastically inhibit the viral life cycle (28). To assess whether *L. monocytogenes* can also induce apoptosis, mice were infected with *L. monocytogenes* and splenocyte apoptosis was assessed 48 h later by TUNEL assay. We observed massive apoptosis, as indicated by TUNEL$^+$ cells, in the splenic tissue from infected mice compared with uninfected controls (Fig. 5 A). TUNEL$^+$ cells were widely distributed and highly prevalent throughout the spleens in a follicular pattern. To determine whether the type I IFN pathway contributes to splenic apoptosis after infection by *L. monocytogenes*, wild-type, *IFNAR1*$^{-/-}$, and *IRF3*$^{-/-}$ mice were infected with *L. monocytogenes* and TUNEL assays were performed 48 h later. Despite roughly equivalent bacterial loads at this time point (Fig. 5 B), *L. monocytogenes*–infected *IFNAR1*$^{-/-}$ and *IRF3*$^{-/-}$ spleens exhibited a drastic reduction in splenic apoptosis compared with wild-type controls (Fig. 5 A). These results demonstrate that *L. monocytogenes*–induced apoptosis in vivo is largely dependent on type I IFN production and signaling.

Discussion

Although type I IFN up-regulation plays an important role in fighting viral infection (23), our results demonstrate that

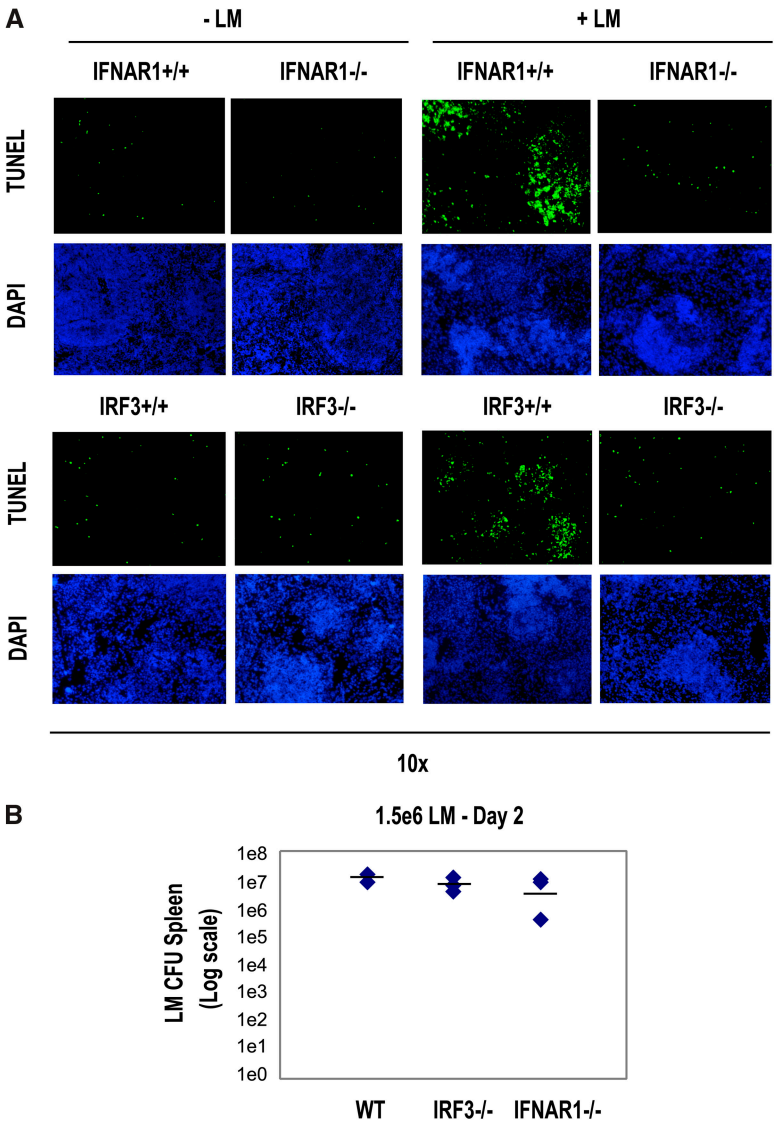

Figure 5. *L. monocytogenes*–induced splenic apoptosis is dependent on type I IFNs. (A) Spleens from *IFNAR1*$^{-/-}$, *IRF3*$^{-/-}$, and wild-type mice that had been infected with 1.5×10^6 *L. monocytogenes* 48 h previously were removed and subjected to a TUNEL assay followed by DAPI staining of the nuclei. Pictures were taken using a 10× lens. (B) Portions of the spleens from wild-type ($n = 3$), *IRF3*$^{-/-}$ ($n = 3$), and *IFNAR1*$^{-/-}$ ($n = 4$) mice used for TUNEL assays in A were assayed and presented as CFUs 2 d after infection with 1.5×10^6 *L. monocytogenes*. All data represent three independent experiments.

these cytokines actually inhibit host defense against *L. monocytogenes* infection. Compared with wild-type mice, *IRF3*- and *IFNAR1*-deficient animals have a greatly improved ability to control the bacterial challenge. Furthermore, exogenous delivery of the type I IFN–inducing agent poly I:C dramatically sensitized mice to *L. monocytogenes* inoculation in an IFNAR1-dependent manner. These studies indicate that *L. monocytogenes* benefits from the cellular programming mediated by type I IFNs. Although the complete mechanism by which type I IFNs inhibit host immunity to *L. monocytogenes* is likely complex due to the pleiotropic cellular effects mediated by these cytokines (2), our findings suggest that *IRF3*- and *IFNAR1*-deficient mice might be more resistant to *L. monocytogenes* infection because their splenocytes do not succumb to type I IFN–mediated apoptosis.

We observed massive apoptosis in lymphocyte-rich follicles in the spleens of *L. monocytogenes*–infected wild-type mice by 2 d after infection. Previous reports have found similar patterns of apoptosis clearly involving uninfected T and B lymphocytes, and potentially other cell types, within close proximity to *L. monocytogenes*–laden macrophages (29). These findings suggest that one or more autocrine/paracrine factors produced by the infected macrophages induce apoptosis in neighboring cells and possibly macrophages themselves. However, blocking such known mediators of apoptosis as TNF, IL-1, IFN-γ, and nitric oxide had no effect on the apoptotic lesion (29). In our study, we found greatly reduced numbers of TUNEL$^+$ cells in the spleens of mice deficient in either *IRF3* or *IFNAR1* compared with wild-type spleens despite the presence of similar bacterial loads by 2 d after infection. Thus, type I IFNs are likely the paracrine factors responsible for the splenic apoptosis observed in *L. monocytogenes*–infected mice.

Although splenic T and B lymphocytes are clearly being killed by *L. monocytogenes*, Jiang et al. (30) have suggested that nonspecific, but not antigen-specific, T cells are targeted for deletion in this situation. However, experiments using SCID mice have indicated that B and T cells do not

contribute to the early response against *L. monocytogenes* (12). Thus, it is highly plausible that other important cell types are also being targeted by *L. monocytogenes*–induced type I IFNs, resulting in apoptosis. Macrophage cells have been shown to undergo type I IFN–dependent apoptosis when incubated with *L. monocytogenes* in vitro (16). Consistent with these observations, we found type I IFN–dependent up-regulation of a subset of proapoptotic genes in *L. monocytogenes*–infected macrophage cells, similar to the expression profile observed in the *L. monocytogenes*–infected spleen. Furthermore, there were approximately twice as many macrophages and neutrophils in *IRF3*- and *IFNAR1*-deficient spleens 2 d after *L. monocytogenes* inoculation (unpublished data). Previous reports have shown that depletion of macrophages or neutrophils results in enhanced *L. monocytogenes* infection during the early stages of the infection (12). Hence, the reduction of these cell types in wild-type mice relative to *IRF3*- and *IFNAR1*-deficient animals may explain why wild-type mice exhibit decreased immunity against *L. monocytogenes*.

Both type I and II IFNs play important roles in combating viral infections through the up-regulation of overlapping and nonoverlapping gene subsets (25). In response to bacterial infections, only type II IFN has been shown to play a protective role through the induction of a Th1 cell immune response (25). As type II IFN production and subsequent signaling are not disrupted in *IFNAR1*$^{-/-}$ mice (Fig. 4), this particular cytokine appears to be sufficient for induction of IFN target genes necessary to mount an effective response to *L. monocytogenes*. Conversely, type I IFN–specific target genes such as *Mx1*, *PKR*, *Daxx*, and *TRAIL* have been shown to inhibit viral replication and regulate cellular apoptosis (25). Thus, although type I IFN target genes play an imperative role in antiviral defense, they might be detrimental when mounting an immune response to *L. monocytogenes*.

This study raises an open question as to why phagocytic cells would respond to a pathogen by secreting factors that potently inhibit host immunity. One attractive possibility is that *L. monocytogenes* has evolved the ability to activate type I IFNs to overcome host defenses. The *L. monocytogenes* protein listeriolysin O (LLO) has recently been shown to be required for *L. monocytogenes* induction of IFN-β in macrophage cells (15). Furthermore, *L. monocytogenes* deficient in LLO exhibit a drastic reduction in both virulence and splenic apoptosis compared with wild-type *L. monocytogenes* in vivo (29), whereas the LLO protein is alone sufficient to induce apoptosis in lymph nodes (31). Further studies are needed to determine if the LLO protein, or some other *L. monocytogenes*–produced ligand, is sufficient to induce expression of type I IFNs.

Type I IFNs may also play a different biological role in other genetic backgrounds or during later stages of the infection. For example, type I IFNs might contribute to the adaptive response to *L. monocytogenes* by regulating the expression of MHC and costimulatory molecules required for developing antigen-specific CTLs, as it has been shown to do during viral infections (4). However, Auerbuch et al. (32) have not found a significant defect in immunological memory during a secondary challenge in *L. monocytogenes*–immunized IFNAR$^{-/-}$ mice compared with wild-type controls. Further investigation is also required to characterize whether type I IFNs play harmful or protective roles against other bacteria or nonviral pathogens in general. A recent study has found that the administration of type I IFNs results in increased loads of *Mycobacterium tuberculosis* in the lungs (33), lending further evidence that other infectious pathogens may also benefit from these cytokines. Consequently, patients suffering from viral infections might be more susceptible to certain bacteria due to the effects of virally induced type I IFNs. Thus, our results highlight the importance of proper regulation of the IFN pathway in response to specific pathogens due to the disparate roles these cytokines play during bacterial versus viral infection.

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