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_J Immunol_ 2007; 178:3126-3133; doi: 10.4049/jimmunol.178.5.3126
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Type I IFN Signaling Is Crucial for Host Resistance against Different Species of Pathogenic Bacteria

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It is known that host cells can produce type I IFNs (IFN-αβ) after exposure to conserved bacterial products, but the functional consequences of such responses on the outcome of bacterial infections are incompletely understood. We show in this study that IFN-αβ signaling is crucial for host defenses against different bacteria, including group B streptococci (GBS), pneumococci, and Escherichia coli. In response to GBS challenge, most mice lacking either the IFN-αβR or IFN-β died from unrestrained bacteremia, whereas all wild-type controls survived. The effect of IFN-αβR deficiency was marked, with mortality surpassing that seen in IFN-γR-deficient mice. Animals lacking both IFN-αβR and IFN-γR displayed additive lethality, suggesting that the two IFN types have complementary and nonredundant roles in host defenses. Increased production of IFN-αβ was detected in macrophages after exposure to GBS. Moreover, in the absence of IFN-αβ signaling, a marked reduction in macrophage production of IFN-γ, NO, and TNF-α was observed after stimulation with live bacteria or with purified LPS. Collectively, our data document a novel, fundamental function of IFN-αβ in boosting macrophage responses and host resistance against bacterial pathogens. These data may be useful to devise alternative strategies to treat bacterial infections. The Journal of Immunology, 2007, 178: 3126–3133.

The IFNs were the first cytokines discovered, having been described nearly 50 years ago as products of virus-infected cells capable of inducing a virus-resistant state (1). IFNs are classified into type I and II based on receptor usage, molecular structure, and sequence homology (2, 3). Type I IFNs, which comprise multiple IFN-α and single IFN-β and IFN-ω subtypes, bind to a common, ubiquitously expressed heterodimeric receptor (IFN-αβ receptor or IFNAR),3 composed of IFNAR1 and IFNAR2 subunits. Type II IFN, or IFN-γ, signals through a different receptor composed of two IFN-GR1 and two IFN-GR2 chains. Together with few other mediators, including TNF-α and IL-12, type I IFNs are considered as primary cytokines that are produced directly in response to microbial products and are therefore likely to shape downstream events in innate and adaptive immune responses (2, 4).

The two IFN types have distinct, nonredundant activities and may have evolved to complement each other as innate host defense factors. Each IFN type regulates hundreds of partially overlapping secondary genes, the products of which orchestrate a comprehensive antimicrobial program (2–4). Although endowed with a more limited range of antiviral activities than type I IFNs, IFN-γ can potently activate macrophages to produce proinflammatory cytokines and to kill ingested nonviral organisms, including bacterial, fungal, and protozoan pathogens (3). Conversely, type I IFN has been traditionally assigned a minor role in antibacterial host defenses (5). Recently, however, conserved bacterial products, including LPS and prokaryotic DNA, were found to activate distinctive signal transduction pathways that merge, after pathogen recognition, with those activated by viruses and lead to high-level type I IFN production (6).

Whether type I IFN has a role in antibacterial defenses is presently unclear. Contrasting results have been obtained according to the pathogen species and experimental model (e.g., in vivo vs in vitro). In in vitro models, treatment of macrophages or fibroblasts with type I IFNs generally limited intracellular replication of bacteria, including Chlamydia, Legionella, and Shigella spp (7–10). However, the addition of IFN-α or IFN-β to human macrophages increased the intracellular replication of Mycobacterium bovis (11). In in vivo mouse models, administration of type I IFN exacerbated pulmonary tuberculosis (12), but had protective effects in infections by Salmonella typhimurium (13) or Listeria monocytogenes (14).

Very little is known of the physiologic role of endogenously produced, as opposed to exogenously added, type I IFN in the context of bacterial infections. The effects of type I IFN signaling have been studied recently in IFN-αβR-deficient mice infected with the facultative intracellular pathogens Mycobacterium tuberculosis or L. monocytogenes. There were only slight differences between IFN-αβR-deficient and wild-type (WT) mice in lung CFU after aerosol challenge with M. tuberculosis (15). Surprisingly, three recent studies indicate that type I IFN signaling may actually decrease host resistance against L. monocytogenes infection (16–18). The mechanisms underlying these detrimental effects are presently unknown, but may be related to the proapoptotic effects of Listeria-induced type I IFN (17–19).

It is reasonable to assume that at least a portion of the antiviral program triggered by IFN-αβ is ineffective against bacteria, owing
to fundamental differences between viruses and prokaryotic organisms (20–22). In contrast, it is difficult to understand how the host has evolved specific mechanisms to produce IFN-α in response to bacteria, if such responses are useless or detrimental. To gain further insights, it may be useful to assess the role of type I IFN in defenses against pathogens differing in their relationship with host cells. Because only intracellular pathogens have been studied to date, the present study investigated the consequences of defective type I IFN signaling during infection with extracellular pathogens, including group B streptococci (GBS), Escherichia coli and encapsulated Salmonella Typhimurium, and encapsulated Escherichia coli. These encapsulated bacteria impose an enormous burden on human health. S. pneumoniae is estimated to cause 10 million deaths annually in the world (23). GBS and encapsulated E. coli are major causes of sepsis and meningitis in the newborn and are becoming increasingly associated with invasive disease in the adult population (24). Moreover, several signal transduction pathways contributing to innate resistance against these bacteria have been elucidated recently (25–28).

We found in this study that type I IFN is a requirement for resistance against GBS, pneumococci, and E. coli. Increased susceptibility to infection could be related to defective production of IFN-γ, NO, and TNF-α by macrophages in the absence of IFN-α signaling.

Materials and Methods

Mice

IFN-αR−/−, IFN-γR−/−, IFN-αβ, and IFN-γR−/− double knockout (KO) and 129Sv/Ev control WT mice were purchased from B&T Universal. IFN-β−/− mice were developed, as described (29). C57BL/6 mice (Charles River Laboratories) were used as controls for the IFN-β-deficient mice. Mice used in the present study were housed under specific pathogen-free conditions in enclosed filter top cages of the Department of Pathology and Experimental Microbiology of the University of Messina. The mice were fed clean food and water ad libitum. All of the procedures described in the present study were in agreement with the European Union guidelines of animal care and were approved by the relevant committees.

Reagents and bacterial strains

Chemicals were purchased from Sigma-Aldrich, unless indicated otherwise. E. coli O111:B4 ultrapure LPS was obtained from InvivoGen (distributed by Labogen). The following bacterial strains were used in the present study: COH-1, a highly virulent type III GBS strain, provided by C. Rubens (University of Washington, Seattle, WA); GBS β-hemolysin−/− (30), a hemolysin-defective mutant of COH-1, provided by V. Nizet (University of California, San Diego, La Jolla, CA); strain 2603 V/R, a type V GBS (31); L. monocytogenes 265, a previously described clinical isolate (32). S. pneumoniae strain D39 and E. coli strain K1E-R8 were provided, respectively, by M. Oggoni (University of Siena, Siena, Italy) and A. Caprioli (Istituto Superiore di Sanità, Rome, Italy). All bacterial strains were grown to the mid-log phase in Todd-Hewitt broth, washed three times in PBS (0.01 M phosphate and 0.15 M NaCl (pH 7.2)), and diluted to the appropriate concentration. The number of viable bacteria used in each experiment was carefully determined by plate counting. A Limulus amebocyte lysate test (Pyrotest assay; Associates of Cape Cod, conducted according to the instruction of the manufacturers, revealed no detectable amounts of LPS (i.e., ≤0.01 EU/ml) in any of the Gram-positive bacterial preparations.

Bacterial infection models

In GBS sepsis models, neonatal or adult mice of either sex were injected locally (s.c. or i.p.) with viable bacteria. Under these conditions, GBS are contained at the inoculation site or spread systemically, depending on the bacterial dose and the intrinsic ability of the host to control microbial growth (26, 33). Infection has a rapid course in these models, and the animals either die of septic shock or clear the infection within 1–4 days. Mouse pups (24–48 h old) were infected s.c. with the type III strain COH-1 in 30 μl of PBS. Adult (6-wk-old) mice were challenged i.p. with strain 2603 V/R, belonging to a serotype (type V) that accounts for most of adult human infections. Mice were observed daily for 14 days after infection, but deaths were never recorded after 4 days. In additional experiments, mice were killed at 18 or 36 h after challenge for the determination of bacterial burdens.
of blood and kidney CFU using blood agar. Because kidney weight did not differ in different groups of mice, results were expressed as CFU/kidney.

In additional sepsis models, E. coli E-R8 and S. pneumoniae D39 were injected i.v. in 6-wk-old mice of either sex. We also used a pneumococcal meningitis model in which adult mice were inoculated with S. pneumoniae D39 via an intracranial, subarachnoidal route, as previously described (34). In this model, which mimics several features of naturally occurring bacterial meningitis, pneumococci replicate in the subarachnoidal space and cause little or no encephalitis (34).

Culture and stimulation of peritoneal macrophages

Resident or thioglycolate-elicited mouse macrophages were obtained, as described (35), and incubated with live bacteria at different multiplicities of infection for 2 h. Subsequently, the plates were washed with warm medium and cells were further cultured for various times in the presence of gentamicin (100 µg/ml) before harvesting cells and/or supernatants. In control experiments, cells were cultured continuously in the presence of E. coli LPS. In reconstitution experiments, rIFN-β (with a sp. act. of 2.42 × 10^7 U/mg; PBL Biomedical Laboratories, distributed by R&D Systems) was added to macrophages from IFN-β−/− mice at the same time of addition of the bacterial stimuli. After 2 h, the monolayers were washed and further cultured in the presence of medium containing the recombinant cytokine and gentamicin. Macrophage viability was checked at the end of the experiments and was always higher than 90%.

Cytokine and NO measurements

TNF-α, IFN-γ, IFN-β, and IL-6 concentrations were measured by ELISA in culture supernatants or in the plasma of infected mice using the mouse TNF-α module set (Bender MedSystems), the murine IFN-γ reagent set (Euroclone), the mouse IFN-β ELISA (PBL Biomedical Laboratories), and the murine IL-6 reagent set (Euroclone). The lower detection limits of these assays were 16, 25, 15.6, and 15.6 pg/ml, respectively. NO concentration was determined by measuring nitrite formation, as described (36), using the Griess reagent (1% sulfanilamide in 2.5% phosphoric acid-0.1% n-1-naphthylethylenediamine dichlorique). After a 30-min incubation at room temperature with agitation, absorbance was measured at 540 nm. NO_2^- was quantified using NaNO_2 as a standard.

IFN-αβ mRNA analysis by quantitative real-time PCR

IFN-α4 and IFN-β mRNA were measured in peritoneal macrophages or in the spleens of infected mice. Peritoneal macrophages (5 × 10^6) were collected after culture by scraping and vigorous pipetting in cold PBS, sedimented by centrifugation, and mixed with the RNAeasy kit lysis buffer (Qiagen). Spleens, obtained immediately after sacrifice, were homogenized in the same lysis buffer using the Tissue Lyser MM301 (Retsch; distributed by Incotec Italia). mRNA was extracted with the RNAeasy kit, per the manufacturer’s instructions.

For the quantification of IFN-α4 and IFN-β mRNA, real-time quantitative RT-PCR assays were conducted, in duplicate, with an Applied Biosystems 7500 (Applied Biosystems), as described (37). PCR conditions were as follows: 50°C, 30 min; 95°C, 10 min; (95°C, 15 s; 60°C, 1 min) × 40 cycles. Real-time PCR data were normalized in each individual sample by the level of β-actin expression. Gene expression was measured by the comparative cycle threshold method (ΔΔC_T) and was reported as the n-fold difference relative to the normalized expression of unstimulated samples (37). Primers and TaqMan MGB probes for β-actin, IFN-α4, and IFN-β have been previously described (38, 39) and were purchased from Applied Biosystems.

Statistical analysis

Kaplan-Meier survival curves were compared using the log rank test (JMP Software; SAS Institute) on an Apple Macintosh computer. The evaluation of differences in cytokine levels was performed with one-way ANOVA and Student-Newman-Keuls test. The evaluation of differences in organ CFU...
Results

IFN-αβR-deficient neonatal mice are hypersusceptible to GBS infection

To study the role of type I IFN in host responses against GBS, we infected IFN-αβR-deficient neonatal mice and WT 129Ev/Sv controls with a low GBS dose (20 CFU of the COH-1 strain). IFN-γR−/− and double KO IFN-αβ and IFN-γR−/− mice were also studied for comparison, because IFN-γ is known to play a central role in defenses against intracellular (3, 40) as well as extracellular (41, 42) bacteria, including GBS (43–45). Fig. 1A shows that all WT neonates survived the low-dose inoculum, whereas 65% of the IFN-αβR−/− and 32% of the IFN-γR−/− mice died. Differences in lethality between IFN-αβR−/− and IFN-γR−/− mice were statistically significant. Double IFN receptor KO mice were extremely susceptible to GBS, and all died within 72 h.

To ascertain whether increased lethality was associated with a defective ability of the host to control in vivo GBS growth, we determined bacterial burden at 18 and 36 h after GBS challenge (Fig. 1, B and C). No bacteria were detected in the blood and kidney in any of the WT mice, indicating that these animals were capable of preventing systemic spreading of GBS from the inoculation site. In contrast, 50, 70, and 100% of, respectively, the IFN-γR−/−, IFN-αβR−/−, and double KO mice were bacteremic at 18 h after infection. Similar data were obtained after measuring CFU in kidneys (Fig. 1C).

Next, we determined circulating cytokine levels in infected mice. Plasma elevations in TNF-α and IL-6 were found in the receptor-deficient, but not in the WT, mice in association with bacteremia (Fig. 1, D and E). It is likely that elevated cytokine levels merely reflected the presence of systemic infection, which was frequent in the immunodeficient mice and absent in the controls. Therefore, these experiments could not clarify whether type I IFN has a regulatory role on cytokine production in the course of GBS infection.

This first set of data indicated the following: 1) IFN-αβR KO animals were more susceptible to GBS-induced lethality than WT mice, secondary to a relative inability to control systemic bacterial spreading from a local focus; 2) this defect was marked, with lethality surpassing that seen in IFN-γR-deficient mice; and 3) mice lacking both type I and II IFN receptors displayed an additive phenotype and were extremely susceptible to low-dose infection.

Effects of IFN-αβR deficiency in GBS-infected adult mice

Because early age is associated with a number of defects in host defenses, it could not be excluded that the IFN-αβR deficiency phenotype observed in the neonatal mouse model of GBS disease was at least in part age related. Moreover, GBS infections are frequent in both neonates and adults (24, 46, 47). For these reasons, we tested IFN-αβR-defective adult mice for susceptibility to GBS. As in previous studies (26), much higher GBS doses (i.e., 2 × 107 CFU of the type V strain 2603) were used in adults, relative to the neonates, reflecting the higher resistance of the former to infection. Under these conditions, 60, 80, and 100% of
FIGURE 5. IFN-β signaling amplifies macrophage responses to GBS. Upper panels. Responses in resident peritoneal macrophages from 129SV/Ev (WT), IFN-αβR−/−, and IFN-γR−/− mice. Middle panels. Responses in resident peritoneal macrophages from C57BL/6 (WT) and IFN-β-defective adult mice. Monolayers (1 × 10^6 cells) were infected with the indicated CFU of GBS strain 2603 and cultured for 24 h. Culture supernatants were assayed for TNF-α (A and D), IFN-γ (B and E), or NO (C and F) production. The levels of these mediators were always below the limit of detection of the assays in supernatants from unstimulated macrophages. Lower panels. Effect of the addition of various doses of rIFN-β, simultaneously to GBS (1 × 10^6 CFU), on the production of TNF-α (G) or IFN-γ (H) in macrophages from IFN-β-defective mice. Data represent means ± SDs of five separate and duplicate determinations. * and #, p < 0.05 relative to WT and IFN-αβR−/− peritoneal macrophages, respectively, by oneway ANOVA and Student-Newman-Keuls test. N = nondetectable.

the IFN-γR−/−, IFN-αβR−/−, and double KO mice, respectively, succumbed to infection, whereas all WT mice survived (Fig. 2A). As in the neonates, increased lethality was associated with systemic spreading of GBS (Fig. 2, B and C). Therefore, very similar effects of IFN I receptor deficiency were observed in adult and neonatal models of infection.

Role of IFN-β in GBS infection

The above data indicated that type I IFN is of crucial importance in restricting GBS replication in vivo. However, these results did not differentiate between the roles of IFN-α and IFN-β. We therefore investigated the effects of deletion of the IFN-β gene on the progression of GBS infection. Adult IFN-β−/− mice were challenged i.p. with a GBS dose (1 × 10^7 CFU), found in previous studies to be sublethal for control WT C57BL/6 mice (26). Under these conditions, IFN-β gene-deleted mice were significantly more susceptible to infection in terms of lethality (Fig. 2D). Moreover, GBS were detected in the blood and kidneys of IFN-β−/− mice (with log CFU values of 3.33 ± 2.79/ml and 3.08 ± 2.63/kidney, respectively, means ± SD of eight determinations each conducted on a different animal), but not in WT mice at 18 h after GBS challenge. These data indicated that the inability of type I receptor-deficient mice to control GBS infection was at least partially accounted for by defective IFN-β signaling.

GBS induce production of type I IFN in the spleen and in peritoneal macrophages

Because the above data demonstrated a critical role of type I IFN signaling in host defenses to GBS, it was of interest to determine whether GBS stimulate type I IFN responses. To determine whether IFN-α or IFN-β mRNA is expressed during GBS infection, spleens of WT mice (129Ev/Sv) were removed for RT-PCR analysis at different times after i.p. challenge. In these experiments, the animals were injected with 1 × 10^8 CFU, a dose resulting in systemic infection. Under these conditions, we detected significant increases over baseline values in the expression of both IFN-α and IFN-β mRNA at 18–24 h after challenge (Fig. 3, upper panel), in association with the presence of GBS in the spleen (Fig. 3, lower panel).

Because macrophages have a central role in innate immunity and are abundant in the spleen, we next examined whether they express type I IFNs in vitro in response to GBS. After adherent peritoneal cells from 129Ev/Sv mice were exposed to live GBS, IFN-α4 and IFN-β mRNA expression was measured by RT-PCR. LPS and L. monocytogenes, which are known to induce type I IFN in macrophages, were included for comparison. IFN-α4 mRNA moderately increased within 4 h after GBS stimulation, reached the highest levels at 6 h, and returned to normal at 12 h (Fig. 4A). Moreover, GBS could induce even higher elevations in IFN-β mRNA levels (Fig. 4B). The kinetics of IFN-αβ induction by GBS or Listeria was delayed relative to LPS. The degree and kinetics of IFN-α4 and IFN-β mRNA elevations after GBS stimulation were similar in macrophages from IFN-αβR−/− and WT mice (data not shown). In additional experiments, we investigated whether increased IFN expression occurred not only at the mRNA, but also at the protein level. To this end, IFN-α and IFN-β concentrations were determined by ELISA in macrophage supernatants collected at 24 h after stimulation with GBS, using the same conditions of the experiments depicted in Fig. 4. We measured 102 ± 18 pg/ml IFN-β in the supernatants of GBS-stimulated macrophages (means ± SD of five separate experiments), whereas cytokine levels were below the limit of detection of the assay in unstimulated macrophages. In contrast, in both GBS-stimulated and unstimulated macrophages, IFN-α was below the limit of detection of the assay (data not shown). This was not surprising, because only moderate IFN-α4 mRNA elevations were previously detected by RT-PCR, which is generally considered a more sensitive assay than ELISA (Fig. 4A). Collectively, these findings indicated that GBS are capable of inducing type I IFN expression both during in vivo infection and in vitro cultured macrophages.
Pneumoniae and Student-Newman-Keuls test. ND, Nondetectable. To WT macrophages, respectively, by one-way ANOVA (100 ng/ml). After culture, supernatants were assayed for TNF-α and IFN-γ, which are known to be essential for host defenses against GBS (26, 43–45). Moreover, macrophages produce NO, an important antibacterial factor, after GBS stimulation. Therefore, we tested the hypothesis that the hypersusceptibility to GBS of IFN-αβR-deficient mice is associated with decreased macrophage TNF-α, IFN-γ, and/or NO production. Resident peritoneal macrophages from IFN-αβR−/− or WT 129Ev/Sv mice were stimulated with different GBS doses, and supernatants were collected after 24 h for cytokine assays (Fig. 5, upper panels). Because macrophage production of IFN-γ is known to amplify, in an autocrine fashion, proinflammatory cytokine responses (including the IFN-γ response), IFN-γR−/− macrophages were also included for comparison. Moreover, similar experiments were conducted on macrophages from IFN-β−/− mice and WT C57BL/6 controls (Fig. 5, lower panels).

A similar, marked decrease in TNF-α and NO production was found in macrophage supernatants from either IFN-αβR−/− or IFN-γR-deficient macrophages after GBS stimulation, compared with WT cells (Fig. 5, A and C). Strikingly, no or very little IFN-γ was detected in the supernatants of IFN-αβR−/− cells, whereas robust production was observed in WT macrophages (Fig. 5B). The defect in IFN-γ production observed in IFN-αβR−/− macrophages was significantly more pronounced than that observed in IFN-γR-deficient cells. The results observed in macrophages from IFN-β-deficient mice were similar to those observed with IFN-αβR−/− cells and showed significantly impaired TNF-α, IFN-γ, and NO responses to GBS (Fig. 5, middle panels). Moreover, the addition of rIFN-β at the same time of bacterial stimulation restored the ability of macrophages to produce TNF-α or IFN-γ in response to GBS (Fig. 5, lower panels). These data indicate that type I IFN, and specifically IFN-β, plays an important role in GBS-induced production of optimal levels of TNF-α, IFN-γ, and NO.

**Type I IFN is required for host resistance against pneumococci and E. coli**

The above data showing the importance of type I IFN in GBS infection were in sharp contrast with the detrimental role of this *Listeria* is dependent on the production of listeriolysin, a pore-forming toxin, which mediates bacterial escape in the cytosol. Because GBS possess hemolysin, which is also a pore-forming toxin, in additional experiments we examined whether hemolysin had a role in IFN-β induction. This was not the case, because a GBS mutant defective in hemolysin was equally potent, relative to the parental WT strain, in its ability to induce IFN-β mRNA expression in macrophages (Fig. 4C).

**FIGURE 7.** Type I IFN signaling amplifies macrophage responses to *S. pneumoniae* and *E. coli*. Thioglycolate-elicited peritoneal macrophages (1 × 10^6 cells) from 129Sv/Ev (WT) or IFN-αβR−/− mice were infected with the indicated CFU of *S. pneumoniae* or *E. coli* or stimulated with *E. coli* LPS (10 and 100 ng/ml). After culture, supernatants were assayed for TNF-α (A–C) and IFN-γ (D–F). The levels of these mediators were always below the limits of detection of the assays in supernatants from unstimulated macrophages. Columns and bars represent means ± SDs of five separate and duplicate determinations. *p < 0.05 relative to WT macrophages, respectively, by one-way ANOVA and Student-Newman-Keuls test. ND, Nondetectable.
mediator previously demonstrated in experimental listeriosis (16–18). Therefore, it was of interest to explore the effects of defective IFN-αβ signaling on the outcome of other bacterial infections. To this end, IFN-αβR-deficient adult mice and WT 129Ev/Sv controls were infected with S. pneumoniae via an i.v. or intracranial (subarachnoidal) route to mimic pneumococcal sepsis and meningitis, respectively (Fig. 6, A and B). Using either inoculation route, approximately one-half of the IFN-αβR−/− mice succumbed to infection, whereas all WT mice survived. Moreover, increased lethality was associated with severe bacteremia in the defective mice (Fig. 6, C and D). Similar effects of IFN-αβR deficiency were observed in a model of E. coli K1 sepsis (Fig. 6, E and F). Thus, type I IFN signaling played a protective role not only against GBS, but also against pneumococci and E. coli.

Next, it was of interest to determine whether increased susceptibility to pneumococcal and E. coli infection in the absence of IFN-αβ signaling was associated with decreased macrophage production of TNF-α or IFN-γ, as previously observed with GBS. To this end, macrophages from IFN-αβR-deficient mice were stimulated with live bacteria and E. coli LPS. Initially, we found that, contrary to our previous findings with GBS (Fig. 5), there was little production of TNF-α or IFN-γ in resident WT macrophages upon exposure to live pneumococci (data not shown). However, these cytokines were readily measurable in supernatants from thioglycolate-elicited macrophages, which were thus used thereafter. There was a marked reduction of TNF-α and IFN-γ responses to S. pneumoniae and E. coli in IFN-αβR-deficient macrophages relative to WT ones (Fig. 7). The production of these cytokines was reduced also after stimulation with LPS. However, the defect could be rescued using high LPS doses (Fig. 7, C and F). These data demonstrated that IFN-αβ signaling is essential for optimal production of TNF-α and IFN-γ upon exposure to pneumococci and E. coli, and for in vivo resistance against these pathogens.

Discussion
The role of IFN-αβ responses in resistance to bacterial pathogens has been studied previously in vivo using two intracellular pathogens, namely M. tuberculosis and L. monocytogenes. Type I IFN signaling had only minor effects on the outcome of experimental lung tuberculosis (15), whereas it was detrimental in listeriosis (16–19). We show in this study that defective type I IFN signaling results in impaired host resistance to different extracellular pathogens, including GBS, S. pneumoniae, and E. coli. The effect of IFN-αβR deficiency was marked, with mortality surpassing that seen in the absence of IFN-γ signaling, a function considered crucial for host defenses against intracellular (3), as well as extracellular (41, 42, 44) bacteria. Interestingly, IFN-αβR−/− and IFN-γR−/− double KO mice showed an extremely severe, additive phenotype and quickly succumbed to challenge with few CFU of GBS. Collectively, our data suggest that IFN-αβ is a fundamental factor for host resistance against highly frequent bacterial pathogens. Moreover, in this function, the role of IFN-αβ is nonredundant and complementary with that of IFN-γ.

In this study, the inability of IFN-αβR-deficient mice to control GBS infection was at least partially accounted for by defective IFN-β signaling, as shown by increased lethality and bacterial burden in IFN-β gene-deleted mice. However, a role for other members of the type I IFN family cannot be formally excluded from our data, because GBS induced not only IFN-β, but also IFN-α4 mRNA both in vivo and in vitro.

The protective effects of type I IFN could be related in this study to its ability to boost the antibacterial responses of macrophages. In the absence of IFN-αβ signaling, the production of TNF-α and IFN-γ was strikingly impaired after exposure to each of the pathogens tested. Because both TNF-α and IFN-γ are fundamental mediators of antibacterial host defenses (26, 41, 44), the increased susceptibility to infection of IFN-αβR−/− mice can be at least partly explained by defective production of these cytokines. Moreover, the production of NO, which has direct antimicrobial effects, was severely defective in the absence of IFN-αβR. It was found in this study that IFN-αβ signaling was required for optimal macrophage responses to purified LPS, although such requirement was no longer apparent using high LPS doses (Fig. 7). This dose dependency may explain the different results of two previous studies showing reduced and unchanged TNF-α responses in IFN-β-deficient mice using, respectively, LPS-stimulated macrophages (48) and a high-dose endotoxin shock model (38).

To our knowledge, this is the first study showing in macrophages that IFN-αβ is required for the production of high levels of IFN-γ. In this activity, IFN-αβ was significantly more potent than IFN-γ itself, which is known to act autocrinely on APCs in a positive feedback loop (3). Thus, our data extend to macrophages previous observations that IFN-αβ responses up-regulate IFN-γ production in dendritic cells (49) and T cells (50–52). However, it cannot be excluded from our data that, in addition to macrophages, small quantities of contaminating cells participated to IFN-αβ-dependent IFN-γ responses. Moreover, given the role of constitutive IFN-αβ signals for efficient IFN-αβ induction by viruses (2), further studies are required to ascertain the relative importance of constitutive vs induced type I IFN production in boosting macrophage responses to bacteria.

We show in this study that type I IFN expression can be induced in murine macrophages by bacteria, such as GBS, which do not have an intracytosolic lifestyle resembling that of L. monocytogenes. The latter, by virtue of lysteriolysin, can lyse endosomal membranes and escape into the cytosol, where its presence is sensed by as yet undefined cytoplasmic receptors, leading to IFN-β induction. However, no endosomal escape mechanism has been described for GBS, although leakage of bacterial products to the cytosol cannot be discounted a priori. Moreover, although L. monocytogenes-induced IFN-β responses are lysteriolysin dependent, a GBS mutant defective in hemolysin, a lysteriolysin homologue, was fully capable in this study of inducing IFN-β. Traditionally, bacterial induction of IFN-β has been considered solely a function of the LPS component of Gram-negative bacteria, based on observations that high doses (100 μg/ml) of heat-killed Gram-positive bacteria were unable to induce IFN-αβ expression in macrophages (53). We found, however, that, in addition to live bacteria, low (0.1 μg/ml), but not high (≥10 μg/ml), doses of heat-killed bacteria can also induce IFN-αβ expression in macrophages (our unpublished observations). Heat-killed GBS were previously found to induce TNF-α via a pathway involving MyD88, an adaptor protein that is involved in signaling by most TLRs (54). Studies are underway to determine whether MyD88 or TLRs are involved in GBS-induced IFN-αβ expression.

In conclusion, our data indicate that type I IFN is a crucial host defense factor against common extracellular bacterial pathogens. A major function of this mediator was to prime macrophages for increased proinflammatory responses. Our data support the possibility that type I IFN plays a fundamental role in host defenses against bacterial pathogens.

Disclosures
The authors have no financial conflict of interest.

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