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Title

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Permalink

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Journal

The Journal of experimental medicine, 200(4)

ISSN

0022-1007

Authors

Auerbuch, Victoria
Brockstedt, Dirk G
Meyer-Morse, Nicole
et al.

Publication Date

2004-08-01

DOI

10.1084/jem.20040976

Peer reviewed

Mice Lacking the Type I Interferon Receptor Are Resistant to *Listeria monocytogenes*

Victoria Auerbuch,¹ Dirk G. Brockstedt,³ Nicole Meyer-Morse,¹ Mary O’Riordan,¹ and Daniel A. Portnoy^{1,2}

¹Department of Molecular and Cell Biology and ²School of Public Health, University of California, Berkeley, Berkeley, CA 94720

³Cerus Corporation, Concord, CA 94520

Abstract

Listeria monocytogenes is a facultative intracellular pathogen that induces a cytosolic signaling cascade resulting in expression of interferon (IFN)- β . Although type I IFNs are critical in viral defense, their role in immunity to bacterial pathogens is much less clear. In this study, we addressed the role of type I IFNs by examining the infection of *L. monocytogenes* in BALB/c mice lacking the type I IFN receptor (IFN- α / β R^{-/-}). During the first 24 h of infection in vivo, IFN- α / β R^{-/-} and wild-type mice were similar in terms of *L. monocytogenes* survival. In addition, the intracellular fate of *L. monocytogenes* in macrophages cultured from IFN- α / β R^{-/-} and wild-type mice was indistinguishable. However, by 72 h after inoculation in vivo, IFN- α / β R^{-/-} mice were ~1,000-fold more resistant to a high dose *L. monocytogenes* infection. Resistance was correlated with elevated levels of interleukin 12p70 in the blood and increased numbers of CD11b⁺ macrophages producing tumor necrosis factor α in the spleen of IFN- α / β R^{-/-} mice. The results of this study suggest that *L. monocytogenes* might be exploiting an innate antiviral response to promote its pathogenesis.

Key words: TNF- α • CD11b antigen • macrophages • IL-12 • pathogen

Introduction

Type I IFNs (IFN- α / β) are expressed in response to microbial challenge and act as antiproliferative, immunomodulatory, and antiviral cytokines (1). The type I IFN family is comprised of multiple IFN- α 's and a single IFN- β , all using a common receptor (IFN- α / β R) that is expressed on a wide variety of cell types. In addition to being induced by viruses, type I IFNs are also produced in response to bacteria and their products (2, 3). Although IFN- α / β R^{-/-} and IFN- β ^{-/-} mice are highly sensitive to a number of viral infections (4, 5), a direct role of type I IFNs in resistance to bacterial pathogens is not yet appreciated.

Macrophages are mediators of inflammation and critical effector cells of the innate response. However, they also act as the primary host cell for many intracellular pathogens, including *Listeria monocytogenes*. *L. monocytogenes* is an intracytosolic human and animal pathogen that is readily phago-

cytosed by macrophages. Once in the host vacuole, *L. monocytogenes* produces an essential virulence factor encoded by the *hemolysin (hly)* gene, which enables the bacterium to permeabilize the vacuolar membrane and enter the cytosol (6). Entry into this cellular compartment is associated with induction of a cytosolic signaling cascade characterized by induction of IFN- β (3). In a recent study, microarray analysis of macrophage genes induced specifically by virulent *L. monocytogenes* revealed a response that was dominated by IFN-inducible genes (7).

In this paper, we addressed the role of type I IFNs in the immune response to *L. monocytogenes* infection. Surprisingly, mice lacking the IFN- α / β R were 400–1,500-fold more resistant to infection, suggesting that induction of IFN- β during *L. monocytogenes* intracytosolic growth leads to enhanced bacterial survival. Resistance to *L. monocytogenes* infection in IFN- α / β R^{-/-} mice was associated with an increase in the number of CD11b⁺ cells producing the inflammatory cytokine TNF- α . These data suggest that intracytosolic *L. monocytogenes* induces IFN- β expression, thereby suppressing the accumulation of TNF- α -producing phagocytic cells at sites of bacterial growth and promoting infection.

Address correspondence to Daniel A. Portnoy, Dept. of Molecular and Cell Biology, 508 Barker Hall, University of California, Berkeley, Berkeley, CA 94720. Phone: (510) 643-3925; Fax: (510) 643-6334; email: portnoy@berkeley.edu

Mary O’Riordan’s present address is Dept. of Microbiology and Immunology, University of Michigan, Ann Arbor, MI 48109.

Materials and Methods

Animals. IFN- α/β R^{-/-} mice in the 129Sv background were originally generated by Müller et al. (8). The 129Sv animals were backcrossed to the BALB/c background for seven generations. Heterozygotes were bred to obtain homozygote knockouts, which were intercrossed for one or two generations. Because IFN- α/β R^{-/-} mice are susceptible to viral infections, serum from resting animals was tested and found to be negative for antibodies to a panel of murine viruses. In addition, no inflammatory cytokines were detected in the blood of IFN- α/β R^{-/-} mice and the hematocrit differential showed no increased levels of circulating neutrophils, monocytes, or lymphocytes. Age- and sex-matched BALB/c control mice were obtained from The Jackson Laboratory and Charles River Laboratories.

Cell Culture and Bacteria. Bone marrow-derived macrophages (BMDMs) were isolated using media containing 30% L cell-conditioned media as a source of CSF-1 and resident peritoneal macrophages were isolated as described previously (9, 10). *L. monocytogenes* 10403S and Δ hly *L. monocytogenes* DP-L2161 were used in this work. Bacteria for in vitro (3) and in vivo infections (11) were prepared as described previously.

In Vivo *L. monocytogenes* Infections. 6–8-wk-old mice were injected intravenously with *L. monocytogenes* in 200 μ l PBS. Mice were killed 1–7 d after inoculation or were rechallenged 3–4 wk after primary inoculation. CFUs in the spleen and liver were determined as described previously (11). Nonterminal ocular and terminal heart puncture bleeds were performed under isoflurane anesthesia. Serum was obtained using serum separator tubes (Capiject; Terumo) or by removing blood clots after overnight incubation at 4°C, followed by centrifugation to remove excess RBCs.

Ex Vivo Stimulation of Splenocytes. Heat-killed *L. monocytogenes* (HKLM) were prepared as described previously (12). Single cell suspensions were prepared from collagenase D (Roche)-treated spleens. RBCs were lysed using NH₄Cl₂. For detection of secreted TNF- α , 5 \times 10⁵ splenocytes/ml of RPMI supplemented with 10% fetal bovine serum and penicillin/streptomycin (RPMI⁺) were plated in tissue culture (TC)-treated plates and either left untreated or stimulated with 4 \times 10⁶ HKLM/ml for 18 h. TNF- α -expressing cells in the spleen were determined by intracellular cytokine staining as described previously (13). In brief, 5 \times 10⁵ splenocytes/ml of RPMI⁺ were cultured in TC-treated plates \pm 4 \times 10⁶ HKLM/ml for 4 h in the presence of 1 μ g/ml brefeldin A (GolgiPlug; BD Biosciences). Cells were resuspended with gentle pipetting and the remaining adherent cells were treated with Cell Stripper (CellGro) and harvested. Cells were stained for cell surface markers using anti-CD11b-FITC (M1/70), anti-CD11c-PE, anti-GR-1-FITC (RB6-8C5), anti-CD4-FITC (GK1.5; all from eBioscience), and anti-CD8-PerCP (53-6.7) and anti-CD3e-APC (145-2C11BD; both from BD Biosciences). Cells were fixed in Cytotfix/Cytoperm, permeabilized with Perm/Wash buffer (BD Biosciences), and incubated with anti-TNF- α -PE (MP6-XT22) and Mac-3-FITC (M3/84; both from eBioscience). Samples were acquired on a FACSCalibur™ flow cytometer and data were analyzed using CELLQuest™ software (BD Immunocytometry System) and FlowJo (Tree Star, Inc.).

In Vitro *L. Monocytogenes* Infection. Growth curves in BMDMs and peritoneal macrophages were performed as described previously (10), except the BMDM experiments were performed in the presence of CSF-1. For mRNA analysis, BMDMs were seeded onto TC-treated dishes, incubated overnight, and infected with *L. monocytogenes* at a multiplicity of infection (MOI) of 10:1 for wild-type *L. monocytogenes* and 100:1 for the Δ hly strain for 30 min. These inoculation doses were used to achieve a similar

number of wild-type and Δ hly bacteria per cell at the end of the experiment. The infected cells were washed, gentamicin was added 30 min later, and RNA was isolated 6 h after inoculation using the RNeasy Mini kit (QIAGEN) according to the manufacturer's instructions. For detection of DNA fragmentation, BMDMs were seeded onto glass coverslips, incubated overnight, and infected with *L. monocytogenes* at an MOI of 16:1 for 30 min. The infected cells were treated as for the RNA isolation described above, except at 8 h after inoculation monolayers were fixed with 3.2% paraformaldehyde. The in situ cell death detection kit (Roche) was used according to the manufacturer's instructions. Stained coverslips were mounted using Vectashield with DAPI (Vector Laboratories). 10 frames per condition were captured using a 60 \times objective and the number of TdT-mediated dUTP nick-end labeling⁺ nuclei out of the total number of nuclei was quantified. The experiment was performed twice.

ELISA. Serum and supernatant samples were assayed simultaneously for levels of IFN- γ , IL-12p70, TNF- α , MCP-1, IL-6, and IL-10 using the mouse inflammation cytometric bead array kit (BD Biosciences) according to the manufacturer's instructions, except 20 μ l of sample, 4 μ l of each antibody-conjugated bead, and 20 μ l of PE-conjugated detection reagent were used per reaction. Data was acquired on a Beckman Coulter XL flow cytometer and analyzed using BD Biosciences analysis software. For detection of serum TNF- α , a TNF- α ELISA kit (eBioscience) was used according to the manufacturer's instructions. Results were obtained using a plate reader (SpectraMax 340; Molecular Devices).

Quantitative PCR. To synthesize cDNA, 1.5 μ g of total RNA, M-MLV reverse transcriptase, Random Primers, and RNaseOUT ribonuclease inhibitor (Invitrogen) were used. SYBR[®] Green-based quantitative PCR amplification was performed in 96-well plates using SYBR[®] Green PCR core reagents (Applied Biosystems), the Stratagene Mx3000P Real-Time PCR System, and a 60°C annealing temperature. Results were analyzed with Stratagene Mx3000P software. The following mouse primer sequences were designed using Applied Biosystems Primer Express software: *ifnb*-F: 5'-ctggagcagctgaatggaaag; *ifnb*-R: 5'-cttgaagtc-gccctgtaggt; β actin-F: 5'-agggtgtgatggtggaatgg; β actin-R: 5'-gcctctgccaccacatagga; *tnfa*-F: 5'-gcaccacatcaaggactcaa; and *tnfa*-R: 5'-tcgaggctcaggaattcg.

Results and Discussion

IFN- α/β R^{-/-} Mice Are 400–1,500-fold More Resistant to *L. monocytogenes* Infection. To evaluate the role of type I IFNs during infection with *L. monocytogenes*, we infected BALB/c and IFN- α/β R^{-/-} mice with 2 \times 10⁴ *L. monocytogenes* (equivalent to 1 LD₅₀ in BALB/c mice; reference 10), and CFUs in the spleen and liver were monitored (Fig. 1, A and B). After 1 d of infection, bacterial numbers were indistinguishable between the wild-type and IFN- α/β R^{-/-} mice in the liver and similar in the spleen. As expected, the bacterial load in both organs continued increasing logarithmically in BALB/c mice. However, the number of CFUs only decreased over the time course in IFN- α/β R^{-/-} mice. At 3 d after inoculation, there were three logs more bacteria in the spleens of the wild-type mice compared with the IFN- α/β R^{-/-} mice. During low dose *L. monocytogenes* infection (equivalent to 0.1 LD₅₀ in BALB/c mice), the results were not as dramatic, yet differences in bacterial numbers at

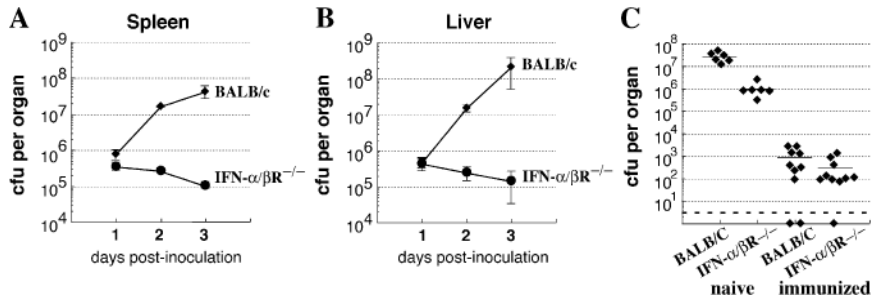


Figure 1. *L. monocytogenes* infection of BALB/c and IFN- α / β R $^{-/-}$ mice. Naive BALB/c and IFN- α / β R $^{-/-}$ mice (three per group) were infected with 2×10^4 wild-type *L. monocytogenes* and bacterial growth in the spleen (A) and liver (B) was followed over 3 d. (C) BALB/c and IFN- α / β R $^{-/-}$ mice were immunized with 2×10^3 *L. monocytogenes* 3–4 wk before challenge with 4×10^4 bacteria. Naive animals were infected with 4×10^4 bacteria. Dashed line denotes limit of detection. CFUs from the spleen 48 h after inoculation are shown. Data represents two separate experiments.

3 d after inoculation were still >40-fold in the spleen and 20-fold in the liver (Table I). These data suggest that type I IFNs favor the growth of *L. monocytogenes* in vivo.

Adaptive Immunity to L. monocytogenes Is Unchanged in the Absence of Type I IFN Signaling. Recently, there has been a heightened interest in the function that type I IFNs might play in bridging innate and adaptive immunity. Type I IFNs are known to induce the proliferation of memory CD8 $^+$ T cells through the induction of IL-15 and have been implicated in the persistence of both CD4 $^+$ and CD8 $^+$ memory T cells by preventing apoptosis (14, 15). However, at least during some viral infections, IFN- α / β R $^{-/-}$ mice are able to mount normal T cell responses (8, 16). To determine whether IFN- α / β R $^{-/-}$ mice are able to mount an adaptive immune response to *L. monocytogenes*, we immunized IFN- α / β R $^{-/-}$ and BALB/c mice with *L. monocytogenes* and challenged them 3–4 wk later (Fig. 1 C). In contrast to the results seen in naive animals, both wild-type and IFN- α / β R $^{-/-}$ mice displayed equivalent numbers of bacteria in the

spleen 48 h after challenge inoculation. Although the adaptive immune response against *L. monocytogenes* is known to be dependent on CD8 $^+$ T cells (17), our data suggest that IFN- α / β R $^{-/-}$ mice mount a fully productive adaptive immune response to *L. monocytogenes*.

Interaction of L. monocytogenes with Macrophages In Vitro. Macrophages are the primary host cell for *L. monocytogenes* in vivo. To identify any potential differences in the interaction between *L. monocytogenes* and macrophages in the absence of type I IFN signaling, we characterized *L. monocytogenes* infection of primary cultured macrophages. Intracellular bacterial growth/survival was indistinguishable in both wild-type and IFN- α / β R $^{-/-}$ BMDMs (Fig. 2 A), and in resident peritoneal macrophages (not depicted). Because type I IFNs promote apoptosis under some conditions (1, 18), we analyzed apoptosis of BALB/c and IFN- α / β R $^{-/-}$ BMDMs infected with *L. monocytogenes* for 8 h by the TdT-mediated dUTP nick-end labeling assay. At this time point, infected cells contained 20–100 bacteria

Table I. Serum Cytokine Levels During *L. monocytogenes* Infection of BALB/c and IFN- α / β R $^{-/-}$ Mice

	Days after inoculation				
	1	2	3	4	
Spleen CFU ^a	$1.2 \times 10^5 \pm 2.3 \times 10^4$	$1.2 \times 10^5 \pm 1.4 \times 10^4$	$4.9 \times 10^5 \pm 2.4 \times 10^5$	$2.5 \times 10^4 \pm 6.2 \times 10^3$	BALB/c
	$6.5 \times 10^4 \pm 1.7 \times 10^4$	$3.3 \times 10^4 \pm 5.9 \times 10^3$	$1.1 \times 10^4 \pm 4.6 \times 10^3$	$1.3 \times 10^3 \pm 7.9 \times 10^1$	IFN- α / β R $^{-/-}$
Liver CFU	$7.8 \times 10^4 \pm 1.7 \times 10^4$	$3.0 \times 10^4 \pm 1.3 \times 10^4$	$7.4 \times 10^4 \pm 3.2 \times 10^4$	$3.4 \times 10^4 \pm 9.6 \times 10^3$	BALB/c
	$4.2 \times 10^4 \pm 1.7 \times 10^4$	$2.2 \times 10^4 \pm 7.2 \times 10^3$	$3.3 \times 10^3 \pm 1.6 \times 10^3$	$8.8 \times 10^3 \pm 5.0 \times 10^3$	IFN- α / β R $^{-/-}$
IL-12p70 ^b	24 ± 9	10 ± 4	25 ± 6	9 ± 5	BALB/c
	89 ± 26	103 ± 20	35 ± 13	0	IFN- α / β R $^{-/-}$
IFN- γ	351 ± 61	735 ± 111	372 ± 125	48 ± 23	BALB/c
	299 ± 57	237 ± 28	26 ± 5	7 ± 2	IFN- α / β R $^{-/-}$
MCP-1	522 ± 71	395 ± 54	182 ± 8	38 ± 16	BALB/c
	515 ± 63	180 ± 27	84 ± 10	58 ± 16	IFN- α / β R $^{-/-}$
IL-6	126 ± 31	101 ± 15	80 ± 26	18 ± 6	BALB/c
	166 ± 49	81 ± 14	23 ± 4	12 ± 3	IFN- α / β R $^{-/-}$

^aMice were infected with 1.2×10^3 *L. monocytogenes*. Average CFU per organ from four mice per group \pm SEM is shown. Values in bold are significantly different between BALB/C and IFN- α / β R $^{-/-}$ mice ($P = 0.03$) according to the Mann-Whitney nonparametric test.

^bMice were bled immediately before death. Average cytokine pg/ml serum \pm SEM is shown. Values in bold are significantly different between BALB/C and IFN- α / β R $^{-/-}$ mice ($P < 0.05$).

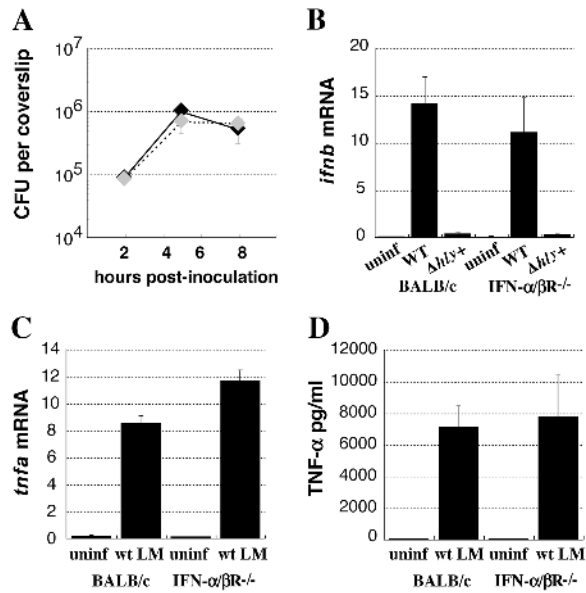


Figure 2. IFN- α / β R $^{-/-}$ BMDMs support normal *L. monocytogenes* growth. BALB/c and IFN- α / β R $^{-/-}$ BMDMs were (A) infected with wild-type *L. monocytogenes* and bacterial growth was monitored in the presence of 50 μ g/ml gentamicin. Black diamonds, BALB/c; gray diamonds, IFN- α / β R $^{-/-}$. BMDMs were infected with an MOI of 10:1 wild-type or 100:1 Δ hly *L. monocytogenes*, which does not enter the host cytosol and does not grow intracellularly, and *ifnb* (B) or *tnfa* (C) mRNA levels (normalized to β actin) and TNF- α supernatant protein levels (D) were quantified 6 h after inoculation. All experiments were performed at least twice.

each. The percentage of apoptotic cells was similar between wild-type and IFN- α / β R $^{-/-}$ BMDMs (9 and 7%, respectively). We concluded that type I IFN signaling under the in vitro conditions described here did not sensitize macrophages to *L. monocytogenes*-induced apoptosis.

Macrophages are an important source of cytokines during listeriosis (19). To ensure that signaling originating from primary infected cells is intact in the absence of the IFN- α / β R, we characterized the expression of cytokines downstream of two distinct pathways of microbial detection. Cytosolic but not vacuolar *L. monocytogenes* induces IFN- β expression (3), which is MyD88 and TRIF independent (unpublished data and reference 7), but IRF-3 dependent (20). In contrast, surface or vacuolar detection of bacterial

products leads to induction of TNF- α via the Toll-like receptor pathway (17). Expression of both IFN- β and TNF- α was similar in *L. monocytogenes*-infected BALB/c and IFN- α / β R $^{-/-}$ BMDMs (Fig. 2, B–D). We conclude that *L. monocytogenes* interaction with its primary host cell proceeds normally in the absence of type I IFN signaling.

Increased IL-12p70 and TNF- α , But Not IFN- γ , in IFN- α / β R $^{-/-}$ Mice. To gain insight into the resistance of IFN- α / β R $^{-/-}$ mice to *L. monocytogenes*, we quantified the levels of several inflammatory cytokines in the serum of BALB/c and IFN- α / β R $^{-/-}$ mice infected with a low dose of *L. monocytogenes* (Table I). Consistent with the greater number of CFUs per organ, levels of IFN- γ , IL-6, and MCP-1 were higher in wild-type mice than in IFN- α / β R $^{-/-}$ mice. In contrast, there was a 10-fold increase in IL-12p70 serum levels in the IFN- α / β R $^{-/-}$ mice 48 h after inoculation, even though at this time point IFN- α / β R $^{-/-}$ spleens harbored fourfold fewer CFUs than wild-type. These data were confirmed by analyzing IL-12p70 and IFN- γ serum levels in BALB/c and IFN- α / β R $^{-/-}$ mice infected with a higher dose of *L. monocytogenes* (Fig. 3 A).

A complex link between type I IFNs, IL-12, and IFN- γ induction has been established (21). Type I IFNs can inhibit production of IL-12 (22) and our results concur with this finding. In addition, type I IFNs have been shown to inhibit IL-12-induced IFN- γ production (21). However, the increased IL-12 levels in the absence of type I IFNs we report here did not lead to increased IFN- γ expression. However, another cytokine, IL-18, has been shown to synergize with type I IFNs to induce NK and T cell IFN- γ expression (23). Because IFN- γ is known to contribute to protection against *L. monocytogenes* (19), the findings of elevated IL-12 yet decreased IFN- γ in the IFN- α / β R $^{-/-}$ mice do not explain their resistance to this pathogen.

TNF- α is essential for controlling *L. monocytogenes* infections and acts synergistically with IFN- γ to enhance macrophage killing of *L. monocytogenes* (9, 19). However, TNF- α is rapidly cleared from the serum (24). Consistent with this, we detected low levels of TNF- α in the serum from both BALB/c and IFN- α / β R $^{-/-}$ mice during high dose *L. monocytogenes* infection (2×10^4 ; not depicted). Therefore, we isolated splenocytes from BALB/c and IFN- α / β R $^{-/-}$ mice infected 48 h previously with *L. monocytogenes* and directly measured TNF- α secretion in the presence

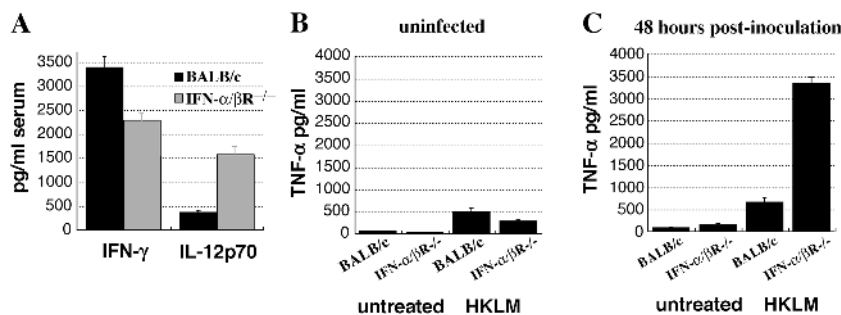


Figure 3. Elevated IL-12p70 and TNF- α , but not IFN- γ , in the absence of type I IFN signaling. (A) BALB/c and IFN- α / β R $^{-/-}$ mice were infected with 2.5×10^4 wild-type *L. monocytogenes*, bled 24 h after inoculation, and serum was assayed for cytokine levels. Data represents the average from seven mice per group over two separate experiments \pm SEM. Splenocytes from uninfected BALB/c and IFN- α / β R $^{-/-}$ mice (B) or from mice infected 48 h previously with 2×10^4 *L. monocytogenes* (C) were cultured $\pm 4 \times 10^6$ HKLM/ml for 18 h. Culture supernatants were assayed for TNF- α . Data represent the average from six mice per group over two separate experiments \pm SEM.

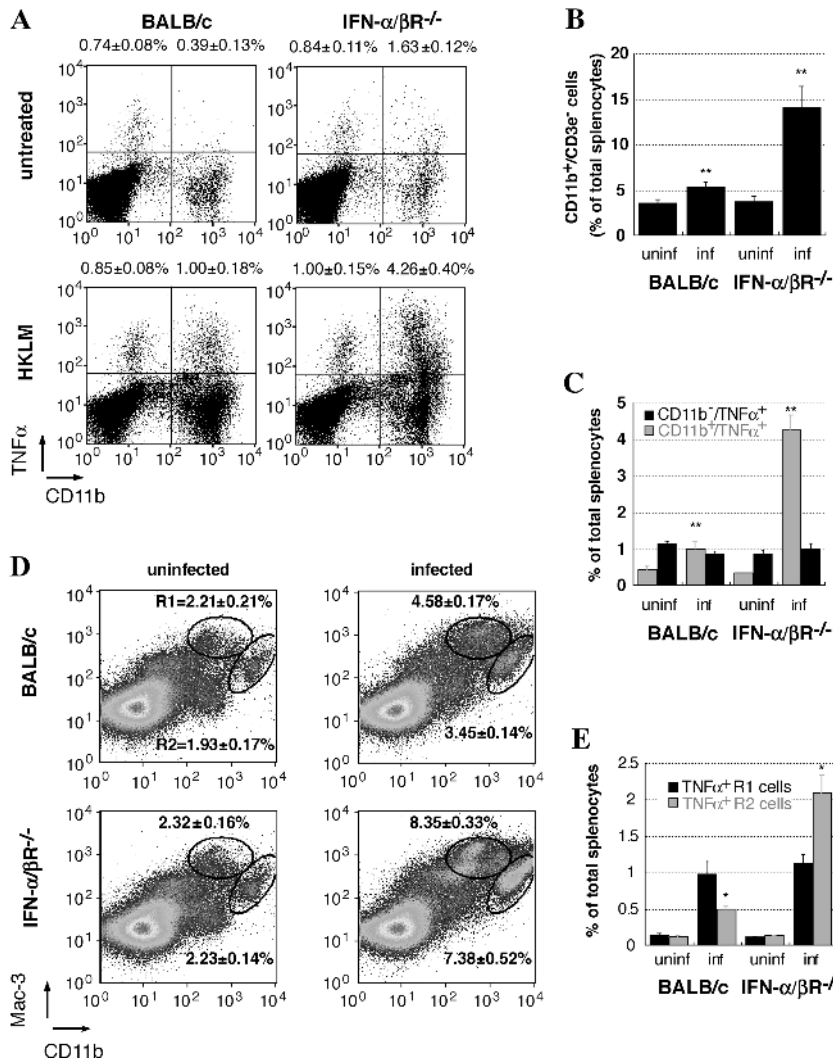


Figure 4. Increased number of CD11b⁺ TNF-α-producing cells in IFN-α/βR^{-/-} mice. Splenocytes from uninfected (uninf) or *L. monocytogenes*-infected (inf) BALB/c and IFN-α/βR^{-/-} mice were cultured ± 4 × 10⁶ HKLM/ml for 4 h. The absolute number of splenocytes was similar between BALB/c and IFN-α/βR^{-/-} mice. Cells were stained for cell surface markers and intracellular TNF-α and Mac-3. (A–C) Cells were stained for CD11b, CD3e, and TNF-α, gated on CD3e⁻ events, and expression of CD11b and TNF-α was analyzed. Data represents results from six mice per group over two separate experiments. (A) Representative dot plots from infected mouse splenocytes. Average percentage of total splenocytes ± SEM that are TNF-α-producing CD11b⁻ (top left) and CD11b⁺ (top right) cells are shown. (B) Average percentage of total splenocytes that are CD11b⁺/CD3e⁻. (C) Average percentage of total splenocytes stimulated with 4 × 10⁶ HKLM/ml that are CD11b^{int}/TNF-α⁺ or CD11b^{high}/TNF-α⁺. (D and E) Cells stimulated with 4 × 10⁶ HKLM/ml were stained for CD11b, Mac-3, and TNF-α. (D) Representative dot plots from one of three mice per group. Values shown are average percentage of total splenocytes ± SEM residing within the R1 and R2 populations. (E) Cells within the R1 and R2 gates were analyzed for intracellular TNF-α staining. Average percentage of total splenocytes ± SEM that are CD11b^{int}/Mac-3^{high}/TNF-α⁺ (R1) or CD11b^{high}/Mac-3^{int/high}/CD11c⁻ (R2). Statistical significance was determined by using the Mann-Whitney nonparametric test. *, P = 0.081; **, P = 0.005.

or absence of HKLM stimulation ex vivo (Fig. 3 C). IFN-α/βR^{-/-} splenocytes produced fivefold greater TNF-α than BALB/c splenocytes. In contrast, BALB/c and IFN-α/βR^{-/-} splenocytes from uninfected animals produced equivalent low levels of TNF-α upon HKLM stimulation (Fig. 3 B).

IFN-α/βR^{-/-} Mice Contain More TNF-α-producing CD11b⁺ Cells in the Spleen During *L. monocytogenes* Infection. To identify the cell type(s) responsible for producing the increased amount of TNF-α in the IFN-α/βR^{-/-} mice, we performed intracellular cytokine staining of splenocytes from BALB/c and IFN-α/βR^{-/-} mice infected for 2 d with *L. monocytogenes*. Very few CD4⁺ or CD8⁺ T cells produced TNF-α in either BALB/c or IFN-α/βR^{-/-} mice (not depicted). However, fourfold more TNF-α-producing CD11b⁺ cells were detected in the infected IFN-α/βR^{-/-} spleens as compared with wild-type (Fig. 4, A and C). Accounting for this increase, both the total number of CD11b⁺ cells (Fig. 4 B) and the percentage producing TNF-α (35% of IFN-α/βR^{-/-} CD11b⁺ cells, 22% of BALB/c upon HKLM stimulation; P = 0.045, Mann-

Whitney nonparametric test) were greater in the absence of type I IFN signaling.

Recently, a population of CD11b^{int}/Mac-3^{high}/CD11c^{int} cells capable of producing TNF-α (TipDCs) was shown to be associated with enhanced *L. monocytogenes* clearance (25). To determine whether TipDCs were responsible for the enhanced TNF-α production in IFN-α/βR^{-/-} mice, we performed further flow cytometric analysis. Interestingly, the total numbers of both TipDCs (R1, CD11b^{int}/Mac-3^{high}/CD11c⁺) and a distinct CD11b⁺ cell population (R2, CD11b^{high}/Mac-3^{int/high}/CD11c⁻) were approximately twofold greater in the infected IFN-α/βR^{-/-} mice compared with wild-type (Fig. 4 D and not depicted). However, the total number of TipDCs producing TNF-α was similar in both BALB/c and IFN-α/βR^{-/-} mice (Fig. 4 E). In contrast, there were fourfold more CD11b^{high}/Mac-3^{int/high}/CD11c⁻ cells producing TNF-α in the IFN-α/βR^{-/-} mice compared with wild-type. These data suggest that a CD11b⁺ cell population distinct from TipDCs is responsible for the majority of the additional TNF-α produced by IFN-α/βR^{-/-} splenocytes.

The increased number of CD11b⁺ cells during *L. monocytogenes* infection in the absence of type I IFN signaling suggests that either (a) these cells are normally eliminated during listeriosis, (b) IFN- α/β normally prevent their expansion or differentiation from a precursor cell, or (c) an increased number traffic to sites of *L. monocytogenes* infection in the absence of type I IFN signaling. Type I IFNs have a role in determining cell fate by a number of mechanisms: induction of apoptosis, proliferation, activation, or maturation, depending on the environmental context (1, 18). Although we cannot at this time eliminate the latter possibilities, we favor the hypothesis that the proapoptotic properties of IFN- α/β suppress an innate immune response mediated by CD11b⁺ cells. Apoptosis of splenocytes has been observed during *L. monocytogenes* infection (26). Recently, a link between type I IFNs and apoptosis of naive T cells during infection with *L. monocytogenes* has been suggested (27). This apoptosis may result from induction of T cell CD69, an early activation marker, in the absence of other activation signals (27, 28). Similarly, we observed fewer CD69⁺ splenocytes from IFN- α/β R^{-/-} mice (not depicted). Indeed, O'Connell et al. (29) find extensive apoptosis in the spleens of *L. monocytogenes*-infected C57BL/6 mice only in the presence of type I IFN signaling. Extensive analysis will be required to determine the identity of the apoptotic cells seen in wild-type but not IFN- α/β R^{-/-} mouse spleens during *L. monocytogenes* infection. Because we observed a larger population of CD11b⁺ cells in the infected IFN- α/β R^{-/-} mice, we suggest that these cells are normally eliminated in the wild-type animal via induction of apoptosis.

Macrophages act as both primary host cells and effector cells during listeriosis. The results of this paper show that type I IFNs do not affect the interaction of *L. monocytogenes* with its primary host cell in terms of intracellular growth, induction of apoptosis, and expression of at least two different cytokines representing distinct signaling pathways. However, we show that a population of CD11b⁺ cells distinct from TipDCs are enhanced in both numbers and effector function (TNF- α expression) during *L. monocytogenes* infection of mice in the absence of type I IFN signaling. Additional studies will reveal whether other effector functions besides TNF- α contribute to CD11b⁺ cell-mediated resistance to *L. monocytogenes*.

We thank Christine Biron for the IFN- α/β R^{-/-} 129Sv animals. We gratefully acknowledge Caroline Moon for assistance with maintenance of the IFN- α/β R^{-/-} mouse colony and Lindsey Jennings for performing the ocular bleeds.

This research was supported by National Institutes of Health grants RO1 AI27655 and AI29619 (to D.A. Portnoy) and the Irvington Institute for Immunological Research (to M. O'Riordan).

The authors have no conflicting financial interests.

Submitted: 17 May 2004

Accepted: 8 July 2004

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