

Myeloid cell responsiveness to interferon-gamma is sufficient for initial resistance to *Listeria monocytogenes*



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ABSTRACT

The type II interferon (IFN γ) promotes resistance to intracellular pathogens. Most immune and somatic cells also express the IFN γ receptor (IFNGR) and respond to IFN γ . While myeloid cells have been implicated as important targets of IFN γ , it remains unknown if IFN γ signaling to myeloid cell types suffices for resistance to infection. Here, we addressed this question by generating mice in which IFNGR1 is selectively expressed by myeloid cells. These “MSGR1” (myeloid selective IFNGR1) mice express an epitope-tagged *Ifngr1* transgene (fGR1) from the myeloid-specific *c-fms* promoter in a background lacking endogenous *Ifngr1*. IFNGR staining was selectively observed on myeloid cells in the MSGR1 mice and correlated with responsiveness of these cells to IFN γ . During systemic infection by the bacterium *Listeria monocytogenes*, activation marker staining was comparable on monocytes from MSGR1 and control B6 mice. Bacterial burdens and survival were also equivalent in MSGR1 and wildtype B6 animals at a timepoint when B6.*Ifngr1*^{-/-} mice began to succumb. These data confirm that activation of inflammatory monocytes and neutrophils is a key mechanism by which IFN γ promotes innate anti-bacterial immunity and suggest that IFN γ targeting of myeloid cells is largely sufficient to mediate protection against systemic *L. monocytogenes*.

Introduction

At steady-state, tissue macrophages, blood monocytes, and granulocytic neutrophils promote tissue homeostasis and surveil for the presence of invasive microbes. The appearance of microbial and inflammatory stimuli induces rapid differentiation of these “resting” myeloid cells towards an anti-microbial and pro-inflammatory state of activation often referred to as M1 “activation” (Mills et al., 2014). The host-derived type II IFN, IFN γ , drives this activation and is required for optimal resistance to a variety of intracellular pathogens (Schroder et al., 2004; Kearney et al., 2013a; Bustamante et al., 2014). IFN γ stimulation induces myeloid cells to upregulate expression of numerous gene products, some of which have been shown to directly interfere with survival or replication of ingested microbes (Schneider et al., 2014; Coers et al., 2018). IFN γ also induces elevated myeloid cell expression of immune-stimulatory gene products such as class II major histocompatibility complex molecules (MHCII), CD54 (ICAM1), and CD64 (Schroder et al., 2004). IFN γ stimulation of non-myeloid cells can impact their differentiation and survival as well as the production of tissue protective

factors such as indoleamine 2,3-dioxygenase (IDO) (Desvignes and Ernst, 2009; Lee et al., 2017).

Listeria monocytogenes is an intracellular bacterial pathogen that can replicate within diverse myeloid and non-myeloid cell types to cause severe systemic infections. A systemic model of *L. monocytogenes* infection has been widely used in immunological studies. In this model, IFN γ plays a critical role in mediating host resistance. Specifically, deficiency in IFN γ or either subunit of the IFNGR significantly increases bacterial burdens and reduces host survival within the first few days of infection (Buchmeier and Schreiber, 1985; Dalton et al., 1993; Huang et al., 1993; Harty and Bevan, 1995; Lu et al., 1998). Further, mice deficient for the IFNGR-stimulated signaling component, STAT1 are unable to clear *L. monocytogenes* infection (Meraz et al., 1996; Kernbauer et al., 2012). Most immune and non-hematopoietic cell types express IFNGR and can respond to IFN γ (Schroder et al., 2004; Kearney et al., 2013a; Bach et al., 1997). Thus, the protective effects of IFN γ could be due to its activity in some or all of these cells. Studies using mice with transgenic expression of a dominant negative IFNGR1 suggested myeloid cells are an important target of IFN γ during the immune response to *L. monocytogenes* (Dighe

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et al., 1995; Lykens et al., 2010). More recent studies using mice with a floxed *Ifngr1* or *stat1* allele also confirmed that hematopoietic cells are vital targets of protective IFN γ early after infection (Kernbauer et al., 2012; Lee et al., 2013). However, the extent to which IFN γ signaling is protective due to its effects on myeloid cells versus other hematopoietic and non-hematopoietic cells has not been previously determined.

Here, we sought to investigate how myeloid cell-restricted responsiveness to IFN γ impacts host resistance to systemic *L. monocytogenes* infection. To this end, we generated a novel mouse strain in which only a myeloid cell-restricted flag epitope-tagged IFNGR1 is expressed. These mice utilized a previously reported transgenic strain that expresses flag-IFNGR1 from the myeloid-specific *c-fms* promoter (Eshleman et al., 2017). To eliminate expression of the endogenous *Ifngr1*, we crossed the fGR1 transgene to a B6.*Ifngr1*^{-/-} background. Our studies with these MSGR1 (myeloid selective IFNGR1) mice are reported here. We observed restricted IFNGR1 expression and responsiveness to endogenously produced IFN γ in myeloid cells of infected MSGR1 mice. Further, we determined that the myeloid cell-restricted fGR1 expression in these animals sufficed to mediate host resistance following systemic *L. monocytogenes* infection. Our results confirm that myeloid cells are a key target of IFN γ and demonstrate for the first time that IFN γ stimulation of myeloid cells is sufficient to promote host resistance to infection in the absence of other IFN γ -responsive cells.

Materials and methods

Mice

Adult male and female mice were used at 8–12 weeks of age. MSGR1 mice were generated by crossing fGR1 transgenic mice (Eshleman et al., 2017) to B6.129S7-*Ifngr1*^{tm1Agt/J} (B6.*Ifngr1*^{-/-}) animals (Huang et al., 1993). WT C57BL6/J control mice and B6.*Ifngr1*^{-/-} mice were purchased from Jackson Laboratories and maintained in our specific pathogen free (SPF) colony at the University of Colorado Medical Campus. All studies were conducted with approval by the Animal Care and Use Committees at the University of Colorado School of Medicine (protocol #00313). Animal studies follow standards enacted by the United States Public Health Service and Department of Agriculture.

Bacterial infections

Male and female sex and aged-matched mice were infected with WT mouse-passaged *L. monocytogenes* (Lm), strain 10403s. Frozen aliquots of mouse-passaged bacteria were thawed and grown to log phase (Optical Density (OD) of 0.1) in Tryptic Soy Broth (TSB) supplemented with 50 μ g/mL of streptomycin at 37 °C with shaking. Lm was diluted in PBS prior to inject via the lateral tail vein (I.V.) at a sublethal dose of 1–1.5 \times 10⁴ CFUs/mouse. Livers were harvested into 0.02% NP-40 and homogenized for 1 min with a tissue homogenizer (IKA Work, Inc.). Spleens were processed into single-cell suspensions as previously described (Eshleman et al., 2017) and 2 \times 10⁶ splenocytes were lysed in 0.02% NP-40. Serial dilutions were plated on TSB agar plates containing 50 μ g/mL of streptomycin. Agar plates were incubated overnight at 37 °C and CFUs were counted. To estimate bacterial burdens in the whole spleen, the number of counted CFUs was multiplied by a ratio of the total spleen cell number divided by the plated cell number (2 \times 10⁶).

Cell culture and cytokine stimulations

To culture bone marrow derived macrophages (BMDM), bone marrow was flushed from the femurs and tibias of mice with DM10 media (DMEM (Gibco) supplemented with 10% FBS, 1% sodium pyruvate, 1% L-glutamine, 1% penicillin/streptomycin). The bone marrow cells were cultured for 6 days in BM macrophage media containing 1% P/S (DMEM media supplemented with 10% Bovine Growth Serum, 1% sodium pyruvate, 1% L-glutamine, 50 μ M 2-mercaptoethanol, 10% L-cell

conditioned media). Cells were provided fresh media on Day 3 and on Day 6 BMDMs were harvested and plated without P/S for experiments to be conducted on Day 7. Peritoneal cells were isolated from naïve mice by injecting 10-mL of ice-cold PBS into the peritoneal cavity and were cultured in DM10 media. For flow cytometric analysis, peritoneal cells were cultured in non-tissue culture treated suspension plates (Gibco) to minimize cell adherence. Spleens were digested and disrupted into single cell suspension as previously described (Eshleman et al., 2017). To determine MHCII expression, cells were stimulated with 100 U/mL murine IFN γ (Life Technology, #PMC-4031) for 18–24 h. All cell cultures were maintained at 37 °C with 7.5% CO₂.

Flow cytometry

Murine Fc receptors were blocked before staining using supernatant from hybridoma 2.4G2 (rat anti-CD16/32). Monoclonal antibodies (mAbs) were diluted in FACS Buffer (1% BSA, 0.01% NaN₃, PBS) to final concentrations that were empirically determined by pilot staining experiments. The following mAbs clones were used: CD11b (M1/70, eBioscience, 1:400), CD11c (N418, Biolegend, 1:200), Ly6C (Hk1.4, eBioscience, 1:200), Ly6G (1A8, BioLegend, 1:200), CD90.2 (53-2.1, eBioscience, 1:200), IgM (11/41, eBioscience, 1:200), CD64 (X54/5/7.1, BioLegend, 1:200), CD80 (16-10A1, eBioscience, 1:200), MHCII (I-A/I-E) (M5/114.15.2, eBioscience, 1:300), F480 (CL-A3-1, BioRad, 1:200), IFNGR1/CD119 (2E2, BD Bioscience, 1:100), ICAM-1 (CD54) (YN1/1.7.4, eBioscience, 1:100). IFNGR1 (CD119) was stained using a secondary Streptavidin-APC (eBioscience). Cells were analyzed on a BD LSR Fortessa (BD Biosciences) and data were processed with FlowJo software (Treestar).

Statistical analysis

All experiments were repeated at least three times unless otherwise noted. For statistical analysis of flow cytometry data, the geometric mean fluorescent intensity (MFI) was measured for individual samples. For experiments where cultured cells were stimulated *in vitro*, MFI values were normalized across experiments prior to statistical analysis, as previously described (Kearney et al., 2013b). For studies using cells from infected animals, raw MFIs were pooled from at least 2 experiments using 2–4 mice per group prior to analysis, except where otherwise noted. Error bars represent the mean \pm SEM. Statistical calculations were performed using GraphPad Prism software. Significance was determined by one-way ANOVA with Tukey's Multiple Comparisons Test, paired two-tailed t-tests, or log-rank Mantel-Cox test as indicated in figure legends. A p-value of <0.05 was considered significant. Approximate p values are indicated as follows: *p = 0.05-0.01; **p = 0.01-0.005; ***p = 0.005-0.0001, ****p < 0.0001.

Results

IFNGR1 staining is selectively restored on monocytes and macrophages from MSGR1 mice

Upon generating fGR1 x B6.*ifngr1*^{-/-} (MSGR1) mice, we evaluated splenocyte subsets (see Fig. 1A for gating strategy) including inflammatory monocytes (CD11b^{hi}, Ly6C^{hi}, Ly6G^{lo}), neutrophils (CD11b^{hi}, Ly6C^{hi}, Ly6G^{hi}), dendritic cells (DCs) (CD11c^{hi}, MHCII^{hi}), T cells (Thy1/CD90.2⁺), and B cells (CD90.2⁻ MHCII^{hi} IgM⁺) in naïve mice for IFNGR1 (CD119) expression by flow cytometry. Each of these cell populations stained positive for IFNGR1 in samples from control B6 mice while all cell populations from B6.*Ifngr1*^{-/-} mice showed negligible IFNGR1 staining (Fig. 1B–D). In the MSGR1 mice, specific IFNGR1 staining was observed on monocytes and neutrophils (Fig. 1B, D), but not on DCs, T cells or B cells (Fig. 1B–D). A comparison of geometric mean fluorescence intensity (MFI) confirmed that the comparable cell surface IFNGR1 staining on monocytes and neutrophils from MSGR1 and B6 mice was significantly

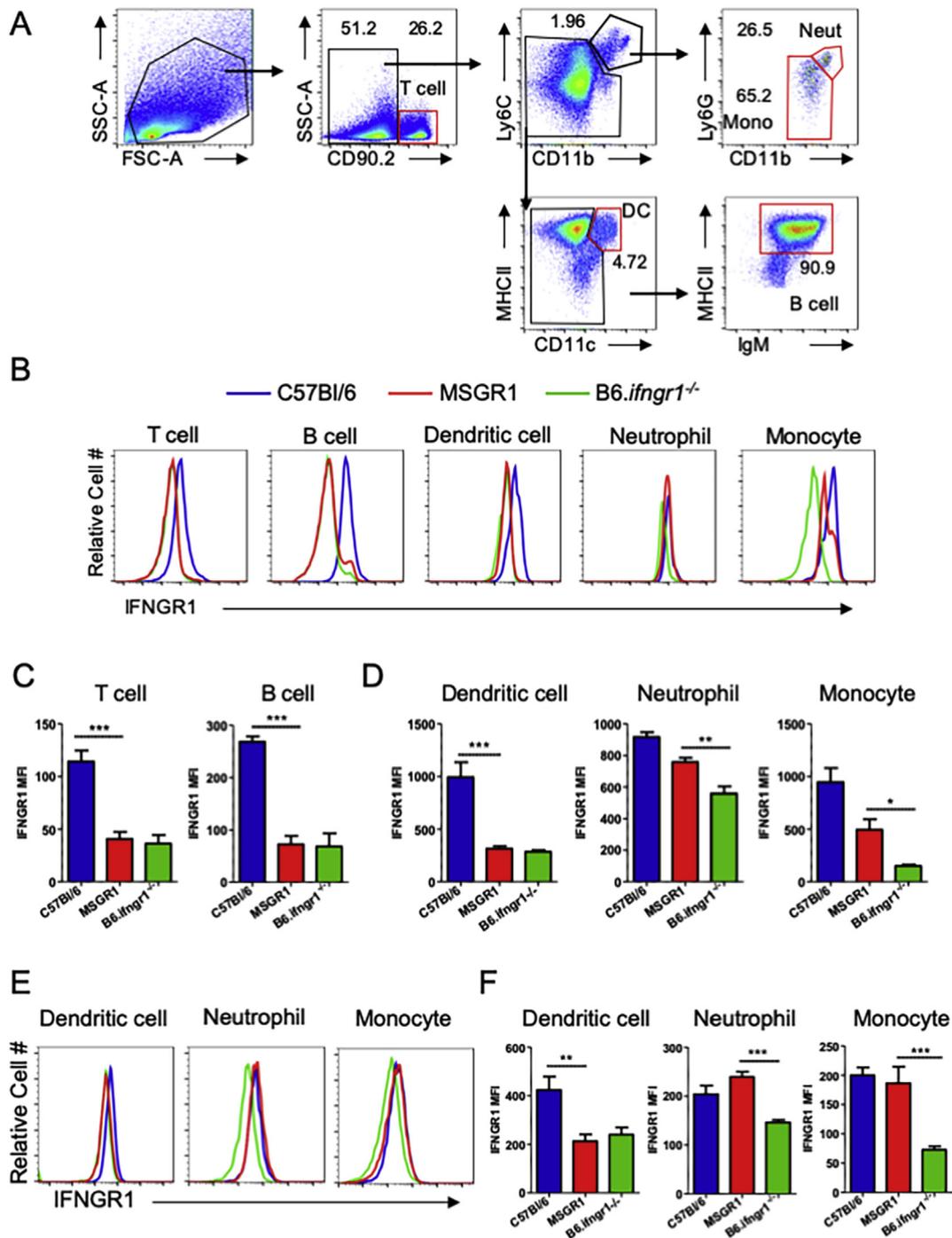


Fig. 1. IFNGR1 is selectively expressed on splenic monocytes and neutrophils from MSGR1 mice. A) Gating strategy for different immune cell populations, red gates indicate the final gate for each population. B) Histogram overlays for IFNGR1 expression for each of the cell populations gated in panel A. C) IFNGR1 MFI in splenic lymphocytes and D) indicated myeloid cell populations. Naïve mice were used for staining in A-D. E) Histogram overlays and F) plots of IFNGR1 MFIs for indicated splenic myeloid cell populations at 72 hpi with *L. monocytogenes*. For all panels, C57Bl/6-blue, MSGR1-red, B6.*Ifngr1*^{-/-}-green. Geometric MFIs were pooled from at least 2-independent experiments with 3–4 mice per group per experiment. (*two-tailed t-test).

higher than that seen on B6.*Ifngr1*^{-/-} cells (Fig. 1D). Likewise, the lack of staining for IFNGR1 was comparable on DCs, T cells and B cells from MSGR1 and B6.*Ifngr1*^{-/-} mice and significantly lower than that observed on B6 cells (Fig. 1C and D). After *L. monocytogenes* infection, splenic neutrophils and inflammatory monocytes, but not DCs, from MSGR1 mice retained IFNGR1 staining that was comparable to cells from infected B6 mice and significantly greater than that observed on B6.*Ifngr1*^{-/-} cells (Fig. 1E and F).

We next determined the expression of IFNGR1 in myeloid cell populations present in other organs including the liver (Fig. 2A), lung (Fig. 2B), and mesenteric lymph nodes (Fig. 2C). Consistent with the spleen, IFNGR1 was only detected in inflammatory monocyte (CD11b^{hi}, Ly6C^{hi}, Ly6G^{lo}) and macrophage (CD11b^{hi}, F480^{hi}) populations isolated from these organs, including alveolar macrophages (gated as CD11c⁺, F480^{hi}) in the lung (Fig. 2B). DC (CD11c^{hi}, MHCII^{hi}) populations evaluated in these organs also failed to express IFNGR1 in MSGR1 mice.

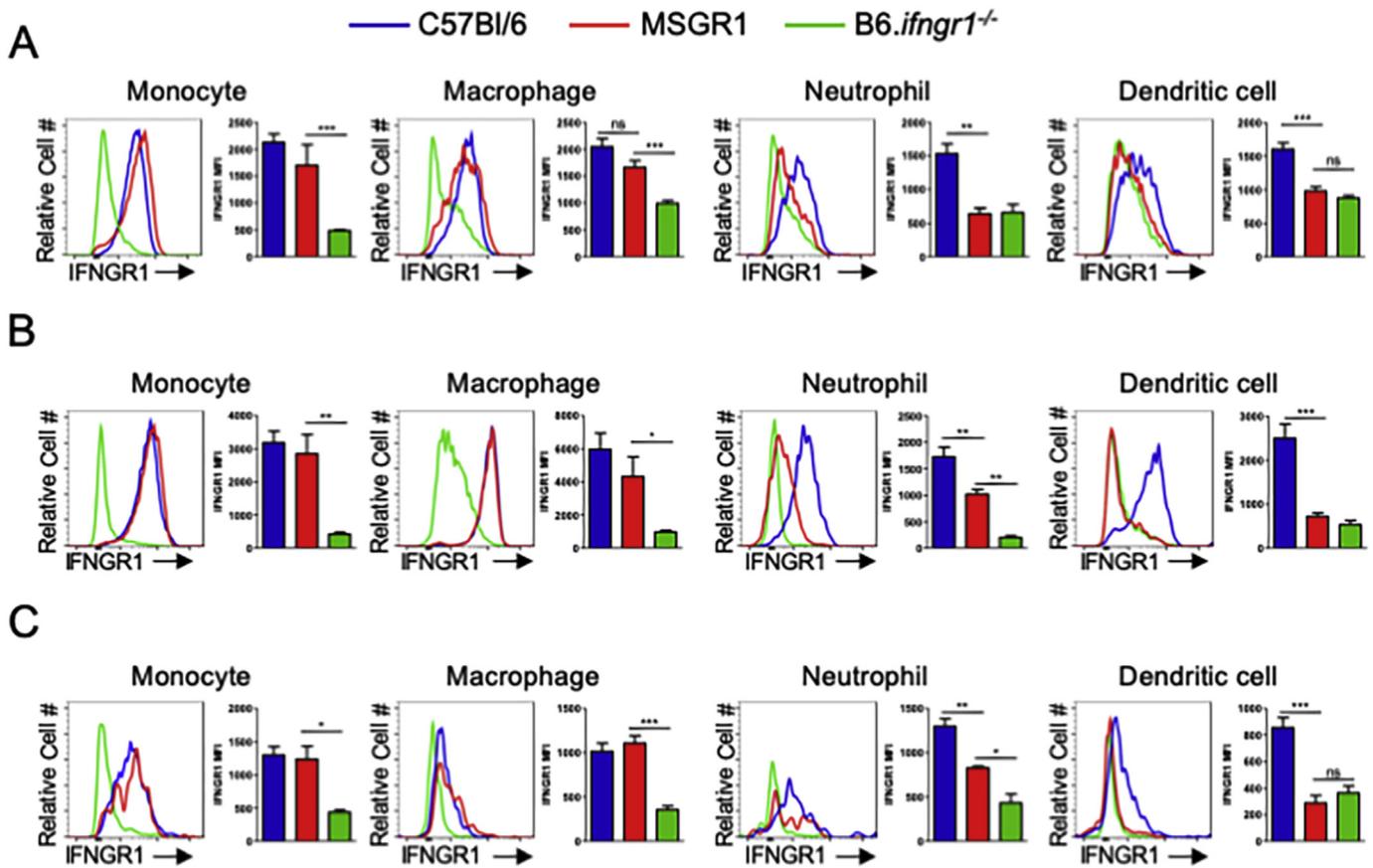


Fig. 2. IFNGR1 expression is restricted to macrophage and monocytes. Myeloid cell populations were assessed 72 hpi in the A) liver, B) lung, and C) mesenteric lymph nodes. C57Bl/6-blue, MSGR1-red, B6.*Ifngr1*^{-/-}-green. Geometric MFIs are from two experiments 3 mice per group per experiment. (*one-way ANOVA).

Interestingly, MSGR1 neutrophils (CD11b^{hi}, Ly6C^{hi}, Ly6G^{hi}) present in the lung (Fig. 2B) and mesenteric lymph nodes (Fig. 2C) have higher IFNGR1 than B6.*ifngr1*^{-/-} cells, but lower than B6 mice, while MSGR1 neutrophils from the liver are devoid of IFNGR1 (Fig. 2A). Together, these data support the conclusion that fGR1 expression sufficed to selectively restore cell surface IFNGR1 on monocyte/macrophage cell populations in MSGR1 mice.

MSGR1 and WT myeloid cells respond comparably to IFN γ stimulation

To evaluate the responsiveness of MSGR1 myeloid cells to IFN γ , splenocytes, peritoneal cells, and BMDMs were isolated and cultured with IFN γ (100 U/ml). After 24 h cells were stained and analyzed by flow cytometry. When evaluated for cell surface MHC class II (MHCII) we observed that IFN γ treatment significantly increased staining for MHCII on gated splenic monocytes (Fig. 3A), peritoneal macrophages (Fig. 3B), and BMDMs (Fig. 3C) from both B6 and MSGR1 mice. By comparison and as expected, staining for MHCII was not increased in the IFN γ -treated B6.*Ifngr1*^{-/-} cells. These results demonstrate that expression of fGR1 in the MSGR1 animals restores responsiveness of macrophages and monocytes to IFN γ . Moreover, there was no significant difference in the responses of myeloid cells from B6 and MSGR1 mice.

Activation of inflammatory monocytes is comparable following infection of WT B6 and MSGR1 mice

Given evidence that IFN γ responsiveness was restored in cultured myeloid cells from MSGR1 mice, we evaluated upregulation of several cell surface markers indicative of IFN γ responsiveness and “M1” activation on myeloid cells during *L. monocytogenes* infection. Spleens were harvested from control mice and at 72 hpi, stained, and analyzed by flow

cytometry. Gating on inflammatory monocytes, we observed that in B6 and MSGR1 mice the infection significantly increased staining for MHCII (Fig. 4A), CD64 (Fig. 4B) and ICAM-1 (Fig. 4C). In contrast, there was no significant increase in staining for these IFN γ -responsive activation markers on inflammatory monocytes from the infected B6.*ifngr1*^{-/-} mice. Together with the results from cultured cells in Fig. 3 these findings indicate that myeloid cells from MSGR1 mice are as responsive as B6 cells to both exogenous IFN γ and endogenous IFN γ produced in response to systemic *L. monocytogenes* infection.

Survival/retention of T cells and inflammatory monocytes is increased in spleens of MSGR1 mice

Adhesion molecules such as ICAM-1 on immune cells interact with LFA-1 on endothelial cells to increase tethering and extravasation of immune cells to sites of infection (Kolaczowska and Kubek, 2013). Hence we evaluated spleen immune cell numbers in control infected B6, B6.*ifngr1*^{-/-}, and MSGR1 mice. Systemic *L. monocytogenes* infection was previously shown to trigger apoptosis of splenic lymphocytes in a manner that is independent of IFN γ (Merrick et al., 1997). Consistent with this the total number of splenocytes was significantly reduced by the infection in all three groups of mice (Fig. 5A). In particular, T cell numbers were reduced by ~60–75% (Fig. 5B). The reduction in overall splenocyte and T cell numbers were comparable in B6 and MSGR1 mice (Fig. 5A and B). Interestingly, the numbers of total splenocytes and T lymphocytes were reduced further in infected B6.*Ifngr1*^{-/-} animals. These data suggest myeloid cell responsiveness to IFNGR1 improved the survival of IFN γ -non-responsive T cells in the infected spleens.

In contrast to the reductions in T cell numbers, the proportion and numbers of splenic inflammatory monocytes and neutrophils increase 2–4 d after systemic *L. monocytogenes* infection (Eshleman et al., 2017;

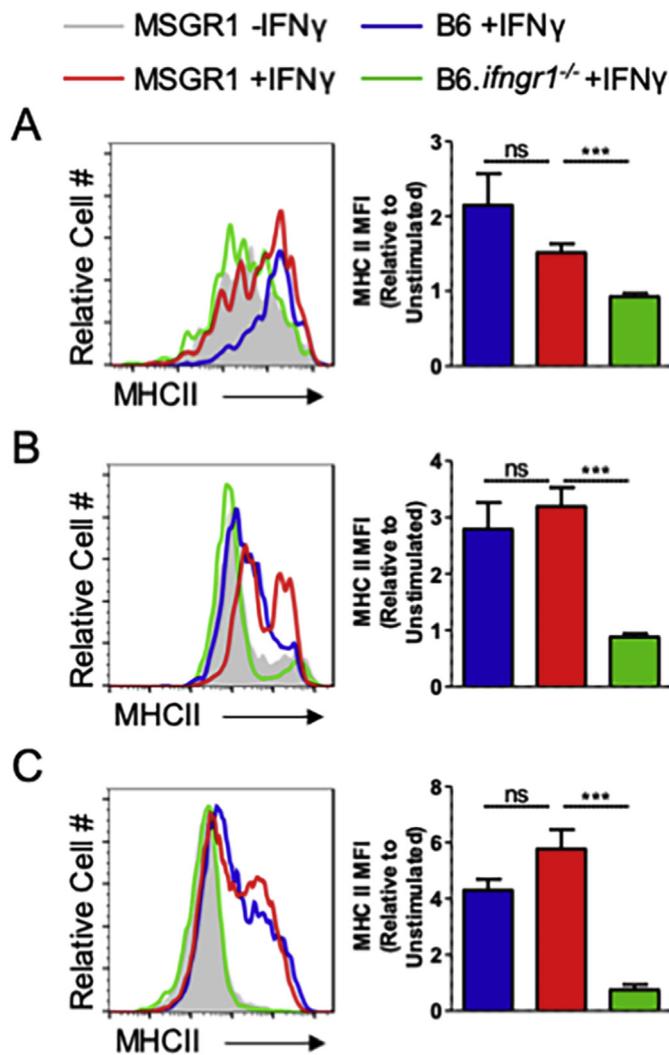


Fig. 3. MSGR1 macrophages respond to IFN γ similar to WT cells. Naïve A) splenic monocytes (CD11b^{hi}, Ly6C^{hi}, Ly6G^{lo}), B) peritoneal macrophages (CD11b^{hi}, F480^{hi}), and C) BMDM were stimulated with 100 U/mL of IFN γ for 24 h then analyzed by flow cytometry for MHCII expression. Geometric MFIs were normalized to unstimulated controls and pooled from 2-independent experiments with 1–3 mice per group per experiment. (*two-tailed t-test).

Serbina et al., 2003). We thus observed a large population of CD11b⁺Ly6C⁺ inflammatory myeloid cells in spleens of the infected mice (Fig. 5C). The increase in inflammatory myeloid cells was more pronounced in the B6 and MSGR1 spleens when compared to spleens of infected B6.*ifngr1*^{-/-} animals. Similar numbers of Ly6G⁻ inflammatory monocytes and Ly6G⁺ neutrophils were recovered from spleens of the infected MSGR1 and B6 mice (Fig. 5D and E). Fewer monocytes were recovered from the B6.*ifngr1*^{-/-} mice, but there were comparable numbers of neutrophils in the B6 and B6.*ifngr1*^{-/-} spleens. These data suggest that expression of IFNGR1 on myeloid cells improves survival and/or accumulation of inflammatory monocytes and T cells in infected tissues.

Myeloid IFNGR1 suffices for resistance to systemic *L. monocytogenes* infection

To determine if the restoration of myeloid cell IFN γ responsiveness in MSGR1 mice sufficed to enhance host resistance, we evaluated bacterial burdens from livers and spleens of B6, B6.*ifngr1*^{-/-} and MSGR1 mice at 72 h after systemic *L. monocytogenes* infection. A dose of 10⁴ bacteria (~1/2 LD₅₀) was used in these experiments. Quantification of bacterial

burdens confirmed that universal IFN γ responsiveness of cells significantly reduced bacterial burdens in spleens and livers of B6 mice when compared to B6.*ifngr1*^{-/-} mice (Fig. 6A and B). Bacterial burdens in these organs were also lower in tissues from MSGR1 mice versus those from B6.*ifngr1*^{-/-} mice. Most strikingly, the burdens recovered from both organs were nearly identical in the MSGR1 and WT B6 mice (Fig. 6A and B). These results indicate that at least at this early timepoint after infection the selective restoration of IFN γ responsiveness in myeloid cells enabled mice to limit bacterial replication equivalently as mice in which all cell types respond to IFN γ . Interestingly, serum IFN γ concentrations were similarly elevated at 72 hpi in MSGR1 and B6.*ifngr1*^{-/-} mice. This suggests that IFNGR expression by non-myeloid cells (presumably T or NK cells) regulates early serum concentrations of this cytokine (Fig. S1). Data in this figure further show that serum IL-6 was similarly elevated in the infected MSGR1 and B6.*ifngr1*^{-/-} mice, whereas IL-12p70 was not affected by IFNGR1 deficiency and TNF α was selectively elevated in the mice that lack IFNGR1 on myeloid cells. These data demonstrate distinct effects of myeloid and non-myeloid cell IFNGR1 on the overall serum cytokine abundance and resistance to the systemic infection.

To further explore the longer-term consequences of myeloid cell-selective responsiveness to IFN γ , we evaluated survival of the B6, B6.*ifngr1*^{-/-}, and MSGR1 over the course of one week after initiating systemic *L. monocytogenes* infection. Consistent with the known importance of IFN γ , the infection dose used (1.5 \times 10⁴ CFU) caused severe morbidity in all B6.*ifngr1*^{-/-} mice by 4 dpi (Fig. 6C). However, most MSGR1 mice remained healthy at 4–5 dpi and over 35% survived until termination of the experiment on day 7. There was nonetheless a difference in survival of the B6 and MSGR1 strains, suggesting an important protective role for IFN γ signaling in one or more cell type that does not express fGR1. Data from animals of both sexes are included in Fig. 6C. When broken down by sex, we found that male MSGR1 mice were much more resistant than females and in fact showed equivalent survival as B6 males (Fig. S2A). By contrast, female B6 and MSGR1 mice showed increased morbidity and reduced survival (Fig. S2B). The difference in survival of B6 and MSGR1 mice was not significant by the test used (p~0.052) but may suggest non-myeloid cell IFN γ signaling has increased importance in female mice. Nevertheless, both sexes of MSGR1 mice showed significantly improved survival when compared to sex-matched B6.*ifngr1*^{-/-} animals completely lacking IFNGR1. Hence, myeloid cell activation by IFN γ is largely responsible for the protective effects of this cytokine at early stages of the bacterial infection.

Discussion

The goal of these studies was to improve understanding of the respective roles for IFN γ stimulation in myeloid versus non-myeloid cells during innate resistance to microbial infection. We employed a well-established systemic infection model with the intracellular bacterial pathogen *Listeria monocytogenes* to build on results from several prior studies that used this model to demonstrate IFN γ -dependent control of infection (Buchmeier and Schreiber, 1985; Kernbauer et al., 2012; Dighe et al., 1995; Lykens et al., 2010; Eshleman et al., 2017; Portnoy et al., 1989). By developing and utilizing a murine model (MSGR1) in which only myeloid cells could respond to endogenous IFN γ , we were for the first time able to directly test if such activation suffices to limit bacterial replication and increase host survival in the context of a systemic *L. monocytogenes* infection. Our results confirmed that bacterial burdens were significantly lower in MSGR1 mice than in B6.*ifngr1*^{-/-} animals. Further, the MSGR1 mice showed improved survival following a high-dose challenge infection. The finding that bacterial burdens in MSGR1 mice were similar to those in infected wildtype B6 mice is suggestive that IFN γ activation of myeloid cells is the major mechanism by which IFN γ promotes early bacterial containment in wildtype animals. Thus, our findings confirm that myeloid cell activation by IFN γ is important for resistance to infection and further argue that the targeting of these cells is the key mechanism by which IFN γ promotes innate

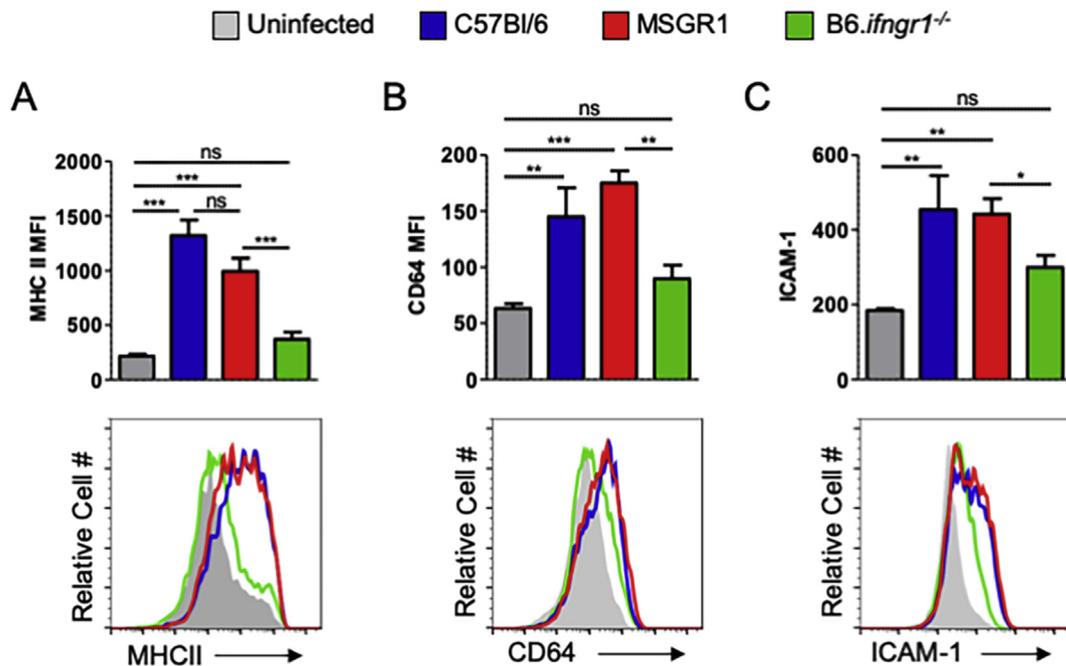


Fig. 4. *L. monocytogenes* infection similarly induces IFN γ -dependent activation markers in splenic MSGR1 and WT myeloid cells. Inflammatory monocytes (as gated in Fig. 1A) were analyzed from spleens 72 hpi and assessed for expression of A) MHCII, B) CD64, and C) ICAM-1. Uninfected controls-grey, C57Bl/6-blue, MSGR1-red, B6.*Ifng1*^{-/-}-green. Geometric MFI values were pooled from 3-independent experiments with 3–4 mice per group per experiment, (*one-way ANOVA).

resistance.

Despite the evidence here indicating myeloid cells are a vital and sufficient target for IFN γ -driven protective immunity, the receptor for this cytokine (IFNGR) is expressed ubiquitously and IFN γ -stimulation induces diverse gene expression changes in non-myeloid cell types. What are the benefits of targeting non-myeloid cells? Certainly IFN γ triggers antiviral gene expression in non-myeloid populations and can also mediate direct anti-tumor effects through the induction of apoptosis. In T cells, IFN γ -stimulation is also instrumental for enforcing differentiation of type 1 responses. Our focus here on early events after an acute infection would not be expected to reveal evidence for these non-myeloid cell effects of IFN γ . However, our data here do lend support for another beneficial role associated with IFN γ targeting of non-myeloid cells. Namely, we observed that despite harboring bacterial burdens equivalent to those seen in B6 mice, the overall survival of MSGR1 mice was significantly lower than that seen in B6 animals. Thus, IFN γ targeting of myeloid cells permits killing of bacteria but overall host survival appears to require targeting of a non-myeloid cell type(s) – particularly in female mice. While our experiments did not specifically discern whether IFN γ -responsiveness alters cell death or accumulation, the differences in survival of B6 and MSGR1 mice correlated with a (non-significant) trend towards higher numbers of neutrophils and monocytes in spleens of the infected MSGR1 mice. Hence, IFN γ effects on non-myeloid cells may improve survival by reducing inflammatory cell infiltration that damages host tissues. These data are consistent with previous work showing that IFNGR1 expression by stromal cells is required for production of indoleamine-2,3-dioxygenase (IDO) that reduces neutrophil influx and lung damage during *Mycobacterium tuberculosis* infection (Desvignes and Ernst, 2009). We did not observe any significant difference in overall T cell numbers between infected MSGR1 and wildtype animals, but another model worth investigating further in future studies is the possibility that the lack of IFN γ signaling to T cells favors over-active T cell responses in the MSGR1 mice. IFN γ signaling to T cells has been shown to promote death of these cells in chronic disease settings (Dalton et al., 2000; Berner et al., 2007). CD4⁺ T cells thus down regulate IFNGR2 to avoid this IFN γ -driven cell death (Bach et al., 1995; Pernis et al., 1995).

The regulation of cellular IFN γ responsiveness impacts host resistance

to a number of infectious agents (Crisler and Lenz, 2018). Many questions remain unanswered regarding the regulation of myeloid cell IFNGR expression and activity. Our lab and others previously demonstrated that responsiveness of myeloid cells to IFN γ can also be attenuated in response to type I IFNs and infections that elicit production of these cytokines (Rayamajhi et al., 2010; Yoshida et al., 1988; Ling et al., 1985). These effects are associated with transcriptional silencing of *Ifng1* (Kearney et al., 2013b). This downregulation of myeloid cell IFNGR1 is conserved in mice and humans, despite evolutionary divergence of the IFN receptors and ligands, and thus likely plays an important role in controlling myeloid cell activity. The fGR1 transgenic mice were developed to overcome the effects of *Ifng1* silencing (Eshleman et al., 2017). Hence, fGR1 expression in myeloid cells from MSGR1 mice might be expected to result in elevated cell surface IFNGR1 (vs. that in B6 cells) in the presence of type I IFNs or other stimuli that normally drive *Ifng1* silencing. However, we failed to observe obvious differences in the intensity of myeloid cell surface IFNGR1 staining in *L. monocytogenes* infected B6 and MSGR1 mice. Moreover, IFN γ stimulation induced similar expression levels of MHCII on monocytes and macrophages from naïve B6 and MSGR1 mice, and activation marker expression was comparable on monocytes from these mice after *L. monocytogenes* infection. The similar IFNGR1 staining and IFN γ responsiveness in myeloid cells from infected B6 and MSGR1 mice may be a fortuitous consequence of the low surface expression of the transgenic FLAG-tagged IFNGR1. Regardless, the available data argue that the amounts of IFNGR1 available at the myeloid cell surface and the responsiveness of myeloid cells to IFN γ is comparable in the infected B6 and MSGR1 myeloid cells.

Which myeloid cell populations must respond to IFN γ to support resistance? Results of prior studies using *Lyz2* or *Cd68* promoters to drive expression of dominant negative IFNGR1 support the conclusion that macrophages, monocytes, and/or neutrophils are key targets of IFN γ during *L. monocytogenes* and *Toxoplasma gondii* infection (Dighe et al., 1995; Lykens et al., 2010). A complementary study using *Vav-cre* or *Itgax-cre* to delete a floxed *Ifng1* allele implicated hematopoietic cells and suggested an important role for IFN γ signaling to CD8 α ⁺ DC (Lee et al., 2013). In our studies, we failed to observe a significant increase in IFNGR1 staining on DCs (Figs. 1 and 2). However, IFNGR1 staining and

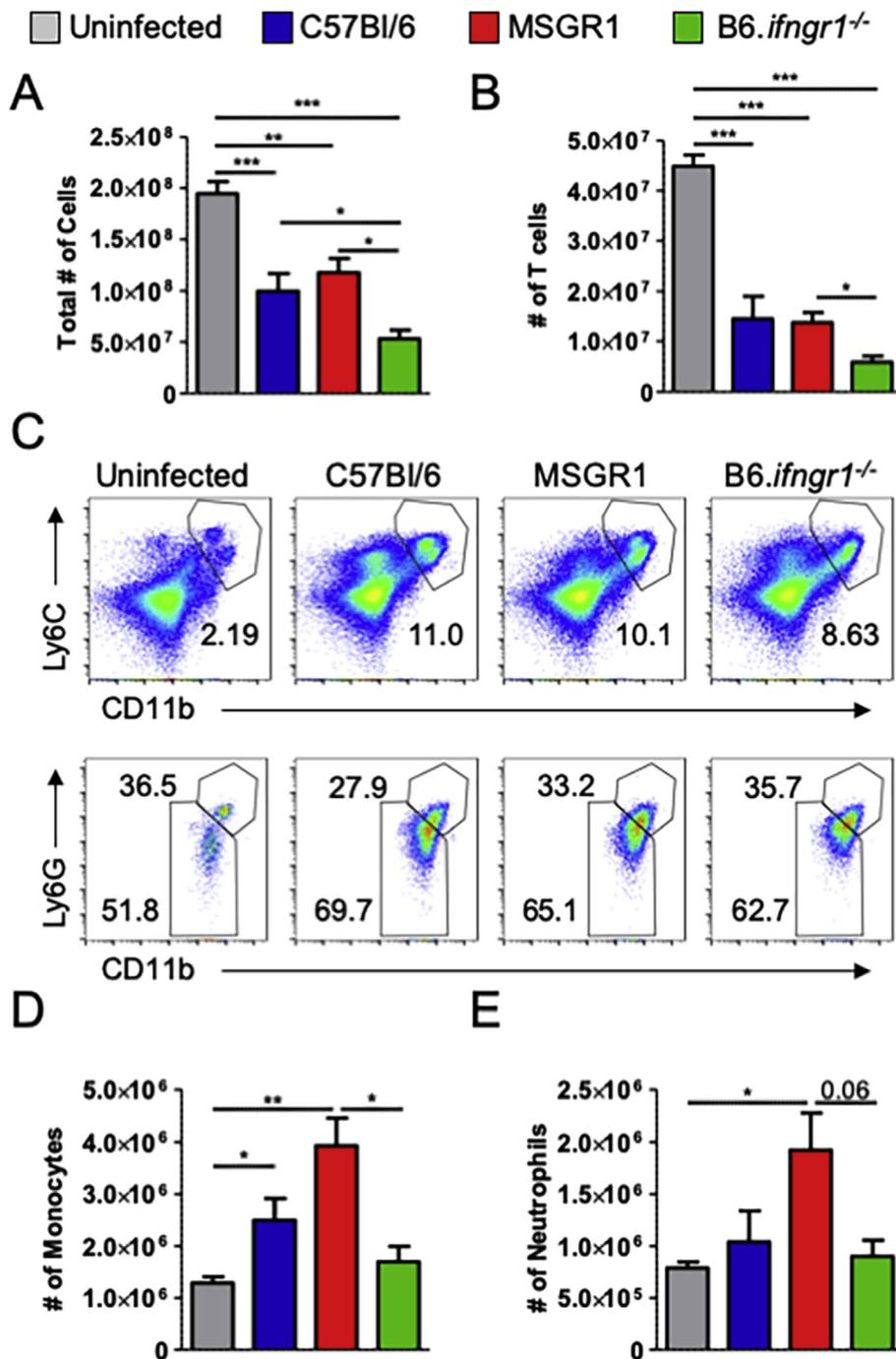


Fig. 5. Myeloid cell-restricted IFNGR1 expression restores inflammatory cell accumulation to *L. monocytogenes*-infected spleens. Mice were infected with *L. monocytogenes* and splenocytes harvested 72 hpi. A) Total number of splenocytes and B) T cells (CD90.2⁺). C) The frequency of inflammatory monocytes (CD11b^{hi}, Ly6C^{hi}, Ly6G^{lo}) and neutrophils (CD11b^{hi}, Ly6C^{hi}, Ly6G^{hi}). D) Total numbers of monocytes and E) neutrophils. Data were pooled from 3 independent experiments with 3–4 mice per group per experiment. (*one-way ANOVA).

IFN γ responsiveness was observed in macrophages, neutrophils, and inflammatory monocytes from infected MSGR1 animals. Inflammatory monocytes and neutrophils are recruited rapidly to sites of microbial infection and within 2–3 days are the most abundant splenic myeloid cell populations (Serbina et al., 2003; Clark et al., 2016; Clark et al., 2018). Further, we recently showed that these cells are heavily associated with *L. monocytogenes* and that IFN γ enhances their ability to inactivate or resist productive infection by this bacterium (Eshleman et al., 2017). Together with the findings from our studies here, these data suggest innate IFN γ -dependent resistance to systemic *L. monocytogenes* largely results from the activation of these accumulating inflammatory monocytes and neutrophils.

Over 35 years have passed since IFN γ was identified as a factor that stimulates M1-type antimicrobial macrophage activation (Nathan et al., 1983). In the interim, this cytokine has been firmly-established as a vital mediator of host resistance to pathogenic intracellular microbes. The efforts here establish for the first time that IFN γ responsiveness in myeloid cells suffices to mediate early host protection in the absence of responsiveness in other hematopoietic and non-hematopoietic cell populations. This information furthers our understanding of how IFN γ mediates protection and underscores the importance of further defining how IFN γ responsiveness is regulated in myeloid cells. Future efforts to further dissect the mechanisms by which IFN γ promotes resistance of MSGR1 inflammatory monocytes and neutrophils to *L. monocytogenes* and other

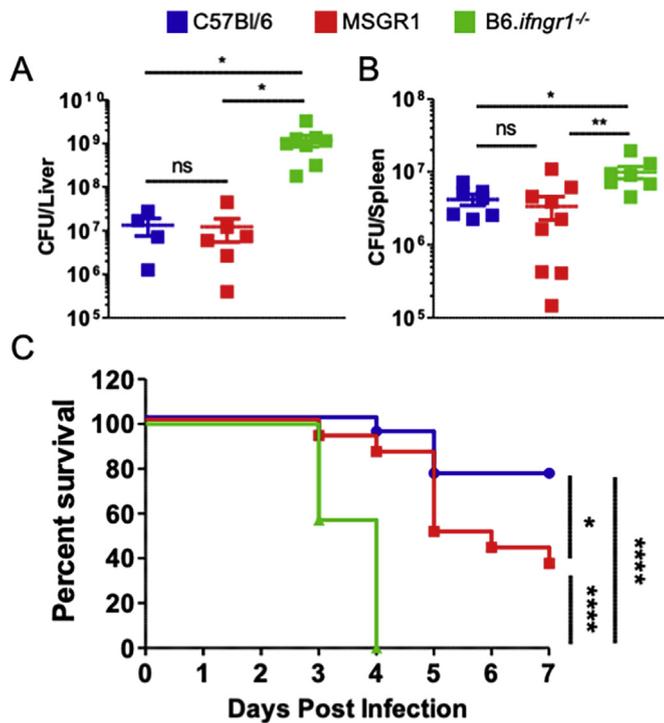


Fig. 6. Myeloid IFNGR1 suffices for early resistance to *L. monocytogenes*. Mice were infected with *L. monocytogenes* and bacterial burdens were determined in the A) liver and B) spleen 72 hpi. CFUs were pooled from 3-independent experiments with 3–4 mice per group per experiment. (*one-way ANOVA). C) Survival of mice infected with *L. monocytogenes*. Data pooled from 2-independent experiments with 8–9 mice per group per experiment. (*log-rank Mantel-Cox test).

pathogens could reveal new approaches to exploit or mimic the protective effects of IFN γ targeting in these myeloid cell populations for host-directed antimicrobial therapies.

Author contributions

EME and LLL conceived, wrote, and edited the manuscript. EME analyzed the data and prepared figures. NB, DSM, and WJC performed specific experiments and contributed to the curation of data. LLL was responsible for funding acquisition and project administration.

Conflict of interest

The authors declare that there are no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.crimmu.2020.01.001>.

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