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Dynamic Roles of Type I and Type II IFNs in Early Infection with *Mycobacterium tuberculosis*

Ludovic Desvignes,* Andrea J. Wolf,*1 and Joel D. Ernst*2,3,‡

Although the protective role of type II IFN, or IFN-γ, against *Mycobacterium tuberculosis* has been established, the effects of type I IFNs are still unclear. One potential confounding factor is the overlap of function between the two signaling pathways. We used mice carrying null mutations in the type I IFNR, type II IFNR, or both and compared their immune responses to those of wild-type mice following aerosol infection with *M. tuberculosis*. We discovered that, in the absence of a response to IFN-γ, type I IFNs play a nonredundant protective role against tuberculosis. Mice unable to respond to both types of IFNs had more severe lung histopathology for similar bacterial loads and died significantly earlier than did mice with impaired IFN-γ signaling alone. We excluded a role for type I IFN in T cell recruitment, which was IFN-γ dependent, whereas both types of IFNs were required for optimal NK cell recruitment to the lungs. Type I IFN had a time-dependent influence on the composition of lung myeloid cell populations, in particular by limiting the abundance of *M. tuberculosis*-infected recruited macrophages after the onset of adaptive immunity. We confirmed that response to IFN-γ was essential to control intracellular mycobacterial growth, without any additional effect of type I IFN. Together, our results imply a model in which type I IFN limit the number of target cells that *M. tuberculosis* can infect in the lungs, whereas IFN-γ enhances their ability to restrict bacterial growth. *The Journal of Immunology*, 2012, 188: 6205–6215.

Both innate and adaptive immune responses are necessary to control *Mycobacterium tuberculosis*, but they are not sufficient to achieve sterilizing immunity. This allows the bacteria to establish chronic infection and complicates efforts to develop effective tuberculosis (TB) vaccines. Understanding the protective mechanisms underlying immune responses to *M. tuberculosis* and their limitations are key to developing new strategies to control the global TB epidemic.

In the armamentarium deployed by the immune system against *M. tuberculosis*, cytokines, IFNs in particular, play an essential role (1). IFNs are a group of closely related cytokines that share transcription factors and signaling pathways; however, they are able to induce very diverse, even apparently opposing, immune responses. Type I IFNs, which include multiple forms of IFN-α and one IFN-β, signal through the same receptor (IFN-α/β receptor [IFNAR]) and have been commonly associated with innate immune responses to viruses. However, type I IFNs can also be induced by nonviral infections, during which they display diverse immunomodulatory properties (2). Type II IFN, or IFN-γ, which signals through a distinct receptor, IFN-γ receptor (IFNGR), is the canonical cytokine of adaptive Th1 immunity and is essential for immune responses to intracellular bacteria, including *M. tuberculosis*.

The absolute requirement for IFN-γ in the immune control of TB is well established in animal models (3) and humans (4). IFN-γ-dependent protection is commonly believed to act through increasing the mycobacterial activity of macrophages, but recent work revealed additional important regulatory roles for IFN-γ during *M. tuberculosis* infection (5, 6). The role of type I IFNs in TB is also incompletely understood; however, in contrast to IFN-γ, there is evidence that type I IFNs actually promote, rather than control, *M. tuberculosis* infection. Production of type I IFNs by restimulated peripheral blood monocytes from patients with active, but not latent, infection (7), as well as a type I IFN-inducible transcriptional signature in blood leukocytes from patients with active TB (8), suggests a positive correlation between type I IFNs and disease. However, reports show that *M. tuberculosis* can inhibit production of (9) and response to (10) type I IFNs, which indicate that these cytokines may also benefit the host. In fact, type I IFNs were found to have a beneficial effect against pulmonary TB in a clinical trial (11). Type I IFNs were also suggested to be a potential therapy for infections with multidrug-resistant strains (12) or in patients with IFNγR deficiencies (13). However, exacerbation of TB was reported in a patient receiving IFN-γ treatment for viral hepatitis (14). In vitro models also demonstrate both beneficial and deleterious effects of type I IFNs during mycobacterial infections. Although type I IFNs can impair the ability of human macrophages to control the growth of *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) (15), IFN-β was shown to improve BCG immunogenicity by increasing human dendritic cell (DC) maturation (16). A recent study revealed the inhibitory role of *M. tuberculosis*-induced type I IFN upon IL-1β production, considered essential for the control of mycobacterial infection, in human macrophages (17). These discrepancies could be attributed to the fact that type I IFNs act differently on distinct cell types (18), and therefore, in vitro systems cannot reflect the sum of their effects in vivo. Likewise,

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Abbreviations used in this article: BCG, bacillus Calmette-Guérin; DC, dendritic cell; dpi, days postinfection; IFNAR, IFN-α/β receptor; IFNGR, IFN-γ receptor; MFI, mean fluorescence intensity; NYULMC, New York University Langone Medical Center; TB, tuberculosis.

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studies in animal models characterized the role of type I IFNs during mycobacterial infections and found distinct effects, depending on the bacterial species or strain. For example, infusion of IFN-β enhanced the resistance of mice to *Mycobacterium avium* infection (19). In contrast, the apparently hypervirulent strain of *M. tuberculosis* HN878 was reported to induce high levels of type I IFNs, and pathology in HN878-infected wild-type mice was worsened by intranasal treatment with IFN-α/β (20). Infection of *Ifnar*−/− mice revealed that type I IFNs were necessary for the early control of *M. bovis* BCG infection (21), whereas *Ifnar*−/− mice showed improved control of late infection with the HN878 strain (22). This time-dependent relationship between type I IFNs and control of TB could also contribute to the lack of consensus on the role of these cytokines.

A crucial element to understanding the role of type I IFNs in TB is their ability to influence recruitment and/or differentiation of myeloid cells (23), which are primary cellular targets of mycobacteria. In vivo administration of the potent type I IFN inducer polynosinic-polycytidylic acid, stabilized with poly-L-lysine, induced type I IFN-dependent recruitment of a CD11b+F4/80+Gr1intCD11cnegCD11b+Gr-1lo/neg macrophages. These observations do not preclude the presence of high levels of IFN-γ,

### Materials and Methods

#### Mice

Wild-type and IFN-γR−/−null (*Ifngr*−/−) mice on the C57BL/6 background were originally purchased from The Jackson Laboratory (Bar Harbor, ME). IFN-α/βR−/−null (*Ifnar*−/−) and STAT1-null (Stat1−/−) mice, both backcrossed to C57BL/6 mice for ≥10 generations, were generously provided by Professors Jonathan Sprent (The Scripps Research Institute, La Jolla, CA) and David Levy (New York University Langone Medical Center [NYULMC]), respectively. *Ifnar*−/− and *Ifngr*−/− mice were crossed to obtain double-null mutant mice (*Ifnar*−/−*Ifngr*−/−). All mice were bred and maintained under specific pathogen-free conditions at the NYULMC.

For infections with *M. tuberculosis*, animals were housed under barrier conditions in the Animal Biosafety Level 3 facility at NYULMC. For tissue harvest, mice were euthanized by CO2 asphyxiation, followed by cervical dislocation. All mice were between 8 and 12 wk of age at the beginning of the experiment. All experiments were performed with the prior approval of the NYULMC Institutional Animal Care and Use Committee.

#### Bacterial infection

*M. tuberculosis* H37Rv was grown as previously described (25). In some experiments, a strain of *M. tuberculosis* H37Rv expressing GFP (26) was used. Mice were infected via the aerosol route, using an inhalation exposure system (Glas-Col) (26), calibrated to deliver ~100 CFU/animal. The infectious dose was confirmed on day 1 by plating whole-lung homogenates from five mice on Middlebrook 7H11 agar. CFU were counted after incubation at 37°C for 2–3 wk. To determine the bacterial load throughout the infection, lungs and mediastinal lymph node were harvested and homogenized, and serial dilutions were plated on Middlebrook 7H11 agar.

### Histopathology

The left lung was harvested and processed, as previously described (5), for histopathological examination.

#### Flow cytometry and microscopy

Lungs were removed and processed into single-cell suspensions for flow cytometry and enumeration of GFP-expressing *M. tuberculosis* per cell in specific subsets, as previously described (26). Abs conjugated to various fluorophores and directed against the following surface markers were used: CD49b (DX5; BD Biosciences), NK1.1 (PK136; BD Biosciences), CD4 (RM-4-5; BioLegend), CD8 (53-6-7; BD Biosciences), CD26L (MEL-14; BioLegend), CD107a (1D4B; BD Biosciences), H2Kb (AF6-88.5; BioLegend), CD11c (HL3; BD Biosciences), CD11b (M1/70; BD Biosciences), Gr-1 (RB6-8C5; BD Biosciences), CD40 (HM40-3; BD Biosciences), CD80 (B7-1; BD Biosciences), CD86 (B7-2; BD Biosciences), and 1-AET (2G9; BD Biosciences). A minimum of 200,000 events per sample, gated on single live cells using forward and side scatter parameters, was acquired using a FACSCalibur and CellQuest software or an LSRII and FACSDiva software (BD Biosciences). Data were analyzed using FlowJo software (TreeStar).

#### ELISA measurement of cytokine production

 Supernatants from whole-lung homogenates harvested at various time points following *M. tuberculosis* infection were analyzed by ELISA, using commercial kits to quantitate IFN-β (R&D Systems) and IFN-γ (BD Biosciences).

#### Statistics

Experiments were routinely performed two or three times or more, and one representative set of results is presented. Results are expressed as mean and SE. Unless otherwise stated, the Student two-tailed t test was used to compare experimental groups, with p < 0.05 considered significant.

### Results

#### Type I IFNs play a nonredundant protective role during TB

To characterize the role of type I IFNs during TB without the potential interference of type II IFN signaling, we used mice incapable of responding to both types of IFNs by lack of specific receptors (*Ifnar*−/−*Ifngr*−/−) or their common transcription factor (Stat1−/−). We first studied the consequences of a low-dose aerosol infection with *M. tuberculosis* on survival and lung bacterial load in Stat1−/− and *Ifnar*−/−*Ifngr*−/− mice and compared them with their single-null mutant counterparts and with wild-type C57BL/6 mice.

*Ifnar*−/−*Ifngr*−/− mice died significantly earlier than did *Ifngr*−/− mice (median survival = 33 d versus 40 d, p < 0.0001; Fig. 1A), whereas none of the *Ifnar*−/− or wild-type mice succumbed during the first 70 d following *M. tuberculosis* infection. Stat1−/− mice infected simultaneously died even earlier than did *Ifnar*−/−*Ifngr*−/− mice (median survival = 30 d versus 33 d, p < 0.0001; Fig. 1A). In a parallel experiment, quantitation of lung bacterial loads revealed that Stat1−/− mice harbored >2 log10 more bacteria in their lungs than did wild-type mice at day 28 postinfection, whereas the bacterial loads in the two groups were indistinguishable a week earlier (Fig. 1B). Similarly, there was no difference in lung bacterial loads in wild-type, *Ifnar*−/−,* Ifngr*−/−, or *Ifnar*−/−*Ifngr*−/− mice before 18 d postinfection (dpi) (Fig. 1C). On day 18, all groups of receptor-null mice had ~40% fewer bacteria in their lungs than did wild-type mice. By day 25, *Ifnar*−/− mice still had fewer *M. tuberculosis* in their lungs than did any other groups. However, bacterial loads in the lungs of *Ifngr*−/− and *Ifnar*−/−*Ifngr*−/− mice increased exponentially after day 18; by day 25, they were 3- and 6-fold greater, respectively, than in wild-type mice. In the mediastinal lymph node, there was no difference in bacterial loads in any of the groups of mice until 25 d postinfection. At that time, the lymph node GFP mirrored that in the lungs, with a significantly lower bacterial burden in *Ifnar*−/−;
FIGURE 1. Type I IFNs play an early, nonredundant role in protection against *M. tuberculosis*. (A) Survival of wild-type (*n* = 5), *Ifngr*<sup>−/−</sup> (*n* = 20), *Ifnar*<sup>−/−</sup> (*n* = 5), *Ifnar*<sup>−/−</sup>*Ifngr*<sup>−/−</sup> (*n* = 20), and *Stat1*<sup>−/−</sup> (*n* = 20) mice following aerosol infection with *M. tuberculosis*. ***p* < 0.0001; log-rank test. (B) Bacterial load in the lungs of wild-type and *Stat1*<sup>−/−</sup> mice (*n* = 5) 21 and 28 dpi with *M. tuberculosis*. ***p* < 0.0001. Lung (C) and mediastinal lymph node (MdLN) (D) bacterial load in wild-type, *Ifngr*<sup>−/−</sup>, *Ifnar*<sup>−/−</sup>, and *Ifnar*<sup>−/−</sup>*Ifngr*<sup>−/−</sup> mice after *M. tuberculosis* infection; (Figure legend continues)
mice and a greater number of *M. tuberculosis* in the lymph nodes of *Ifngr*−/− and *Ifnar*−/− *Ifngr*−/− mice (Fig. 1D).

Histopathological examination of the lungs revealed that, on day 25, wild-type mice presented a classic granulomatous response, with few limited infiltrates of inflammatory cells and with alveolar spaces retaining most of their structural integrity (Fig. 1E). In *Ifngr*−/− mice, the extent of the inflammatory infiltrates was noticeably greater than in wild-type mice and was more neutrophilic in nature (Fig. 1E). However, those infiltrates remained regionally localized, and a substantial proportion of healthy tissue remained.

In contrast, in *Ifnar*−/− *Ifngr*−/− mice, infiltration of the tissue by inflammatory cells, neutrophils in particular, severely compromised the architecture of the entire lung (Fig. 1E). In addition, tissue liquefaction was observed, and centers of granulomas were characterized by tissue necrosis (Fig. 1E). When we enumerated total lung cells, we observed that, in the first 18 d of infection, *Ifnar*−/−, *Ifngr*−/−, and *Ifnar*−/− *Ifngr*−/− mice had lower cell counts (between 3.3 ± 0.3 and 3.8 ± 0.2 × 10⁵ cells) than did wild-type mice (5.8 ± 1.4 × 10⁵ cells) (Fig. 1F). At day 25, total cell yields from the lungs were more scattered among the groups; *Ifnar*−/− *Ifngr*−/− mice exhibited the greatest cellularity, with 9.6 ± 1.4 × 10⁶ cells, consistent with the histological observations (Fig. 1E).

Finally, considering that single IFNR-null mice are able to respond to other types of IFN, we assessed the effects of IFNR deletion on the levels of IFNs in the lungs during TB (Fig. 1G). Early in infection, IFN-β and IFN-γ were produced in similar quantities in wild-type mice: 62 ± 10 and 107 ± 34 pg/ml, respectively, on day 18. Deletion of type I IFNR had divergent effects on type I and type II IFN in the lungs, with a significant reduction in IFN-β (12.1 ± 0.8 pg/ml; *p* = 0.038) and a significant increase in IFN-γ (408 ± 55 pg/ml; *p* = 0.0152). Interestingly, increased levels of IFN-γ were also observed in *Ifnar*−/− *Ifngr*−/− mice (304 ± 48 pg/ml), suggesting IFN-γ-independent mechanisms. In contrast, deletion of type II IFNR had little or no effect on the respective levels of IFN-β or IFN-γ at that time of infection. By day 25, the levels of IFN-γ in the lungs of *M. tuberculosis*-infected wild-type mice increased to 950 ± 620 pg/ml, a 12-fold level of IFN-β, which remained similar to the levels measured a week earlier (75 ± 12 pg/ml). Deletion of IFNRRs—type I, type II, or both—had the same effect on decreasing the levels of IFN-β at day 25 as at day 18. Levels of IFN-γ in the lungs of *M. tuberculosis*-infected mice at day 25 were only marginally affected by the deletion of either IFNFR.

These data show that type I IFN plays a nonredundant role, which is most readily demonstrated in the absence of IFN-γ signaling, for the control of lung bacterial burdens and histopathology, as well as survival, following *M. tuberculosis* infection in mice. In addition, the relationship among type I IFN, type II IFN, bacterial control, and recruitment of cells to the lungs during TB changes over time, with a transition at 3 wk of infection during the onset of adaptive immunity that is characterized by a marked increase in IFN-γ production.

**Type I IFN only marginally affects T cells in lungs of *M. tuberculosis*-infected mice**

To evaluate whether the early protective effect of type I IFN responsiveness during TB is mediated through enhanced priming or trafficking of T lymphocytes, we examined populations of CD4+ and CD8+ T cells in the lungs of infected wild-type and IFNR-null mice.

At day 18 postinfection, CD4+ T cell populations were equivalent in wild-type mice and those lacking type I and/or type II IFNRs (Fig. 2A). In contrast, there were fewer CD8+ T cells in the lungs of *Ifnar*−/− *Ifngr*−/− mice compared with wild-type mice, whereas mice lacking individual IFNRs had intermediate numbers of CD8+ T cells in their lungs.

By day 25, both CD4+ and CD8+ T cell populations had increased similarly in the lungs of wild-type and *Ifnar*−/− mice: 2- to 3.5-fold compared with day 18. In contrast, the number of CD4+ T cells increased minimally in *Ifngr*−/− mice, with 60% fewer CD4+ T cells than in the lungs of wild-type mice at that time. The increase in CD4+ T cells was also limited in *Ifnar*−/− *Ifngr*−/− on day 25, with ~40% fewer CD4+ T cells than in the lungs of wild-type mice. In comparison with wild-type and *Ifnar*−/− mice, the increase in CD8+ T cell populations in the lungs of *Ifngr*−/− mice was also limited, independently of their capacity to respond to type I IFN. Overall, responsiveness to IFN-γ was the strongest determinant of the recruitment of CD4+ and CD8+ T cells to the lungs early postinfection with *M. tuberculosis*; the ability of mice to respond to type I IFN had no apparent effect on T cell recruitment in the first month of infection.

Consistent with other studies using BCG (27) or *M. tuberculosis* (28), we found that the frequency of effector, CD4+CD62Llowneg T cells in the lungs increased sharply during the third week of infection, from days 17 to 21 (Fig. 2B, 2C); this was not affected by the capacity to respond to IFN-α/β or IFN-γ. However, *Ifnar*−/− *Ifngr*−/− mice displayed a significantly higher frequency of effector CD4+CD62Llowneg T cells (79.4 ± 0.8%; *p* = 0.0049) in their lungs than did wild-type mice (72.5 ± 1.6%) at day 21. Nine days later, the frequency of effector CD4+CD62Llowneg T cells in the lungs had increased only slightly in all groups, but it was still higher in *Ifnar*−/− *Ifngr*−/− mice (81.1 ± 2.4%) and significantly higher in *Ifngr*−/− mice (82.8 ± 1.3%; *p* = 0.0164) compared with that in wild-type mice (76.4 ± 2.4%). Wild-type mice also retained a larger population of naive CD4+CD62L+ T cells in their lungs than did *Ifnar*−/− or *Ifnar*−/− *Ifngr*−/− mice (Fig. 2B), possibly reflecting the higher bacterial burdens in these IFNR-null mice at that time.

Cytolytic CD8+ T cell activity in the lungs, as assessed by surface expression of the degranulation marker CD107a/LAMP-1 (Fig. 2D), was significantly higher in wild-type mice (3.3 ± 0.4%; *p* = 0.0478) and *Ifnar*−/− *Ifngr*−/− mice (4.3 ± 0.6%; *p* = 0.0115) than in *Ifngr*−/− mice (2.0 ± 0.3%) at day 25, indicating that responsiveness to type I IFNs is modulating the effects of type II IFN on development and/or degranulation of cytolytic CD8+ T cells in this context (Fig. 2E). However, because the higher frequency of CD8+ T cell degranulation did not translate to improved control of bacterial growth in the lungs, this effect of type I IFNs is clearly insufficient to influence control of *M. tuberculosis* at this stage of infection.

Overall, these results indicate that, in addition to their marginal effect on T cell recruitment to the lungs, type I IFNs play no more than a transient or secondary role in CD4+ and CD8+ T cell ac...
tivation during early infection with *M. tuberculosis*, particularly in the context of IFN-γ responsiveness.

**NK cell responses during *M. tuberculosis* infection require both type I and type II IFN responsiveness**

Type I IFNs have been implicated in NK cell homeostasis and activation against tumors (29) or viral infections (30), indicating that differences in NK cells might provide at least a partial explanation for the poorer control of *M. tuberculosis* that we observed in *Ifnar−/−*/*Ifngr−/−* mice versus *Ifngr−/−* mice. To test this hypothesis, we first determined whether recruitment of NK cells to the lungs during TB was dependent on responsiveness to type I and/or type II IFNs.

In an initial experiment, we observed that CD3−Ddx5+ NK cells were recruited to the lungs of mice following *M. tuberculosis* infection, with a peak frequency at day 21 (13.3 ± 0.7% of total lung cells), followed by a rapid decline (Fig. 3A). This recruitment peak was significantly blunted in *Ifngr−/−* mice (4.5 ± 0.2%; *p < 0.0001), as well as in *Ifnar−/−*/*Ifngr−/−* mice (6.4 ± 0.7%; *p = 0.0001). In an additional experiment, we also determined that the number of NK cells in the lungs of *Ifnar−/−*/*Ifngr−/−* mice was reduced by 60% in comparison with wild-type mice 18 d post-infection (Fig. 3B). This effect was due to combined unresponsiveness to both types of IFNs, because *Ifnar−/−* and *Ifngr−/−* mice only displayed a reduction of 20 and 30% of NK cell populations, respectively, in comparison with wild-type mice.

Although NK cells were less abundant in the lungs of *Ifnar−/−*, *Ifngr−/−*, and *Ifnar−/−*/*Ifngr−/−* mice, their cytolytic activity, as assayed by surface expression of CD107a/LAMP-1 in CD3− NK1.1+ cells (Fig. 3C), was significantly higher in *Ifngr−/−* and *Ifnar−/−*/*Ifngr−/−* mice compared with wild-type mice after 25 d of *M. tuberculosis* infection (Fig. 3D). Because NK cell degranulation can be triggered by low levels of MHC class I on target cells (31), we measured this parameter on diverse myeloid cell subsets in the lungs by flow cytometry (Fig. 3E). Myeloid subsets were defined based on the expression of specific surface markers (Fig. 4). We observed that the level of expression of MHC class I on CD11b+CD11c− myeloid DCs was significantly lower in *Ifngr−/−* mice (mean fluorescence intensity [MFI]: 351 ± 28; *p < 0.0001) and *Ifnar−/−*/*Ifngr−/−* mice (MFI: 262 ± 13; *p < 0.0001) compared with wild-type mice (MFI: 1506 ± 87) (Fig. 3F). Similarly, low levels of MHC class I were observed on other myeloid cells from *Ifnar−/−*, *Ifngr−/−*, and *Ifnar−/−*/*Ifngr−/−* mice, although these differences did not reach statistical significance in comparison with wild-type mice (data not shown).

These observations indicate the importance of responsiveness to types I and II IFNs for optimal development and/or recruitment of NK cells to the lungs following *M. tuberculosis* infection, as well as their roles in MHC class I expression and subsequent NK cell activation.

**Types I and II IFN signaling orchestrates the accumulation and activation of myeloid cells in the lung during *M. tuberculosis* infection**

Types I and II IFNs were reported to individually influence recruitment, differentiation, and activation of myeloid cells under inflammatory conditions or during infections (23, 32). Our approach, based on single- and double-null–mutant mice for their respective receptors, allowed us to examine how their interaction affects lung myeloid cell populations during *M. tuberculosis* infection.

As shown by flow cytometry (Fig. 4A), lung myeloid cell populations were dominated by CD11c+CD11b−Gr-1− macrophages and CD11c+CD11b+Gr-1+ neutrophils in all groups of mice until day 18 (Fig. 4B). At that time, responses to either types I or II IFN were necessary for optimal recruitment and/or differentiation of all cell types (i.e., monocytes, recruited...
macrophages, myeloid DCs, and neutrophils). On day 25 postinfection, a further increase in the myeloid DC population occurred in the lungs of wild-type mice. This increase depended upon the ability of mice to respond to IFN-γ. Interestingly, the deficit of myeloid DCs in the lungs of Ifngr−/− mice was dependent on type I IFN signaling, because Ifnar−/−Ifngr−/− mice had a normal population of myeloid DC in their lungs, even though type I IFNs did not show any independent effect on myeloid DC recruitment and/or differentiation at this time of infection. Also on day 25 postinfection, mice unable to respond to type I IFN possessed, on average, twice as many recruited macrophages (MFI: 61,6; *p < 0.05, **p < 0.0001) as MFI = 5). (E) Representative flow cytometry graph of MHC class I expression (H-2Kb) among NK cells in the lungs of wild-type, Ifngr−/−, Ifnar−/−, and Ifnar−/−Ifngr−/− mice at day 25 dpi; data are expressed as the percentage of CD107a+ cells among NK cells in the lungs of wild-type, Ifngr−/−, and Ifnar−/−Ifngr−/− mice 21 dpi. (F) Level of expression of MHC class I (H-2Kb) in M. tuberculosis-infected mice 21 dpi. (*p < 0.05, **p < 0.0001) 18 d postinfection (Fig. 6C). That enhancement was entirely IFN-γ dependent, because recruited macrophages and myeloid DCs in Ifnar−/−Ifngr−/− mice showed similar levels of MHC class II compared with wild-type mice. In contrast, IFN-γ did not have any impact of its own at this early stage of M. tuberculosis infection. However, on day 25, IFN-γ responsiveness was clearly responsible for a sharp increase in MHC class II levels on recruited macrophages and for a more limited one on myeloid DCs. At that time of infection, the effects of type I IFN signaling on MHC class II expression waned in both cell types. Similarly, levels of costimulatory molecules (i.e., CD40, CD80, and CD86) on myeloid DCs were also completely dependent on the capacity of cells to respond to IFN-γ, whereas type I IFN had no noticeable effect (Fig. 4D).

Our observations reveal that the interplay between types I and II IFNs for recruitment and/or differentiation of myeloid cells and their activation as APCs in the lungs during early M. tuberculosis infection is biphasic. During the initial 3 wk of infection, both IFNs act together to populate the lungs with most cell types, but they have divergent effects on the activation of APCs. Beyond 3 wk, types I and II IFNs also diverge in their recruitment/differentiation potential of myeloid cells as IFN-γ becomes the predominant regulator of APC activation.

Type I IFNs determine the number of M. tuberculosis-infected macrophages in the lungs

To better understand the impact of types I and II IFNs on myeloid cell populations and the increased bacterial burdens in the lungs of Ifnar−/−Ifngr−/− mice, we investigated the distribution of M. tuberculosis within specific cell subtypes, as well as bacterial burdens at the single cell level.

We used a GFP-expressing strain of M. tuberculosis (26), together with flow cytometry, to identify individual myeloid cells
FIGURE 4. Types I and II IFNs orchestrate optimal recruitment and/or differentiation and activation of lung myeloid cells during early TB. (A) Flow cytometry gating strategy for quantification of lung myeloid cell populations following aerosol infection with *M. tuberculosis*. R1 (CD11b<sup>−</sup>CD11c<sup>+</sup>): alveolar macrophages; R2 (CD11b<sup>−</sup>CD11c<sup>+</sup>): myeloid DCs; R4 (CD11c<sup>−</sup>CD11b<sup>−</sup>Gr-1<sup>lo</sup>/neg): recruited macrophages; R5 (CD11c<sup>−</sup>CD11b<sup>−</sup>Gr-1<sup>int</sup>): monocytes; and R6 (CD11c<sup>−</sup>CD11b<sup>hi</sup>Gr-1<sup>hi</sup>): neutrophils. (B) Abundance of lung myeloid cell populations in the lungs of wild-type, *Ifnar<sup>−/−</sup>*; *Ifngr<sup>−/−</sup>*; and *Ifnar<sup>−/−</sup>*Ifngr<sup>−/−</sup> mice 14, 18, and 25 dpi; data are expressed as mean ± SE (*n* = 3). (C) Expression of MHC class II (I-A/I-E) on recruited macrophages and myeloid DCs in the lungs of wild-type, *Ifnar<sup>−/−</sup>*; *Ifngr<sup>−/−</sup>*; and *Ifnar<sup>−/−</sup>*Ifngr<sup>−/−</sup> mice 18 and 25 dpi; data are expressed as MFI ± SE (*n* = 3). (D) Expression of costimulatory molecules (CD40, CD80, and CD86) on myeloid DCs in the lungs of wild-type, *Ifnar<sup>−/−</sup>*; *Ifngr<sup>−/−</sup>*; and *Ifnar<sup>−/−</sup>*Ifngr<sup>−/−</sup> mice 25 dpi with *M. tuberculosis*; data are expressed as MFI ± SE (*n* = 3). *p < 0.05, **p < 0.01, ***p < 0.0001.
that contain bacteria (Fig. 5A). Consistent with our prior observation with wild-type mice (26), on day 25, DCs were the most frequently infected cells (12–15% of the population) (Fig. 5B), and there was no apparent influence of responsiveness from these cells to IFNs. Alveolar macrophages were more susceptible to M. tuberculosis infection in the absence of IFN-γ signaling, without any additional effect of type I IFNs, but these differences were not translated into absolute numbers of infected cells (Fig. 5C). In contrast, monocytes and neutrophils were infected significantly less frequently (Fig. 5B) and in smaller numbers (Fig. 5C) in Ifngr−/− mice than in wild-type mice. With regard to frequency and absolute numbers, Ifnar−/−Ifngr−/− mice had significantly more infected monocytes and neutrophils than did Ifngr−/− mice. Finally, although the frequency of infected recruited macrophages was not influenced by the ability of mice to respond to IFNs, there was a clear role for type I IFNs on the absolute number of infected recruited macrophages. Significantly more infected recruited macrophages were present in the lungs of M. tuberculosis-infected Ifnar−/−Ifngr−/− mice (7.9 ± 2.1 × 104 cells; p = 0.049) than in Ifngr−/− mice (3.3 ± 0.4 × 104 cells). Interestingly, wild-type mice had a similar number of infected recruited macrophages (8.8 ± 0.5 × 104 cells) compared with Ifnar−/−Ifngr−/− mice. Because we observed a significantly higher lung bacterial load in Ifnar−/−Ifngr−/− mice than in wild-type mice (Fig. 1C), we hypothesized that, for an equal number of infected cells, the number of bacteria/cell was higher in mice unable to respond to both types of IFN. Indeed, enumeration of GFP-expressing M. tuberculosis in infected cells by fluorescence microscopy revealed that mice unable to respond to IFN-γ harbored significantly more bacteria/cell than did wild-type mice (Fig. 5D). It is noteworthy that this effect was not altered by the additional absence of type I IFN responsiveness.

Together, these results explain the higher bacterial burden in the lungs, and most likely the impaired survival, of Ifnar−/−Ifngr−/− mice compared with Ifngr−/− mice. This difference appears to be due to the role of type I IFNs in the modulation of myeloid cell populations, in particular recruited macrophages. However, the decisive factor determining susceptibility to M. tuberculosis at the individual cell level in vivo is the ability to respond to IFN-γ (Fig. 6).

Discussion

The results that we present in this article demonstrate that type I IFNs play a nonredundant protective role during the early stages of TB in vivo. Our data also contribute to explain the conflicting results surrounding type I IFNs in mycobacterial infections. In particular, we reveal both pro- and anti-inflammatory properties of type I IFNs in TB, a duality already observed in autoimmune diseases (33–35). The transition from pro- to anti-inflammatory effects is time dependent, and type I IFNs ultimately balance out proinflammatory IFN-γ by limiting the abundance of potential...
target cells in the lungs (Fig. 6). However, we also demonstrate that these effects of type I IFNs are intricately connected to, and ultimately masked by, the influence of IFN-γ, especially after the onset of adaptive immunity.

During the innate immune phase following *M. tuberculosis* infection, both types of IFN are produced in similar quantities. Types I and II IFNs activate parallel signaling pathways, resulting in phosphorylation, nuclear translocation, and binding of STAT molecules to IFN-γ-activated sequence enhancer elements located in the promoter regions of proinflammatory genes (2), such as MCP-1/CCL2 or iNOS. This common proinflammatory pathway could explain how both types of IFN act synergistically to mount an optimal immune response to TB, in particular the recruitment, differentiation, and/or survival of myeloid cells in the lungs. Paradoxically, this initial increase in myeloid cell populations (e.g., DCs and recruited macrophages) promotes infection by providing target cells for intracellular growth of *M. tuberculosis*. This phenomenon, well characterized in the zebrafish embryo model of mycobacterial infection (36), explains the lower bacterial loads in the lungs of mice unable to respond to one or both types of IFNs early in infection. These effects on myeloid cells also explain the higher susceptibility of mice treated with polyinosinic-polycytidylic acid stabilized with poly-L-lysine, which induces type I IFN-dependent recruitment of a subset of *M. tuberculosis*-permissive macrophages (24). Additionally, we observed that, when types I and II IFNs are present in similar concentrations in the lungs, type I IFN limits the expression of IFN-γ-induced MHC class II on APCs. This concentration-dependent regulatory role was described previously in vitro (37) and was shown to be due to downregulation of IFNγR, at least in the context of *Listeria monocytogenes* infection (38). Greater expression of MHC class II on APCs in *Ifnar−/−* mice at 3 wk postinfection was accompanied by a higher production of IFN-γ in the lungs but without evidence of a direct correlation, particularly because IFN-α/β was also shown to inhibit IFN-γ production (39). In any case, it did not improve bacterial control significantly compared with *Ifnar−/− Ifngr−/−* mice. Taken together, our results suggest that the availability of *M. tuberculosis*-susceptible myeloid cells in the lungs is a more decisive correlate of disease control than is response to IFN-γ before the onset of adaptive immune responses.

After the onset of adaptive immune responses to TB, CD4+ and CD8+ effector T cells traffic to the lungs where they produce IFN-γ, whose concentration then exceeds, by a factor of 10, that of type I IFN. Under these conditions, IFN-γ becomes the predominant regulator of T cell recruitment, activation of APCs, and control of *M. tuberculosis* growth, as demonstrated by our results in terms of bacterial load/cell. Although type I IFNs do not play any detectable role in the recruitment of T cells during TB, they still contribute to the control of lung myeloid cell populations, but this time by limiting the abundance of recruited macrophages and myeloid DCs.

The molecular mechanisms behind type I IFN fate decision as pro- or anti-inflammatory effectors are still largely unknown. In addition to IFN-γ-activated sites, type I IFN-induced transcription factors can bind to other activating elements, such as IFN-stimulated response elements (2). Differential usage of these activating elements can be modulated by the nature, the duration of phosphorylation, and/or the abundance of STAT molecules, all mechanisms regulated by IFNs themselves (40). Under these circumstances, it is possible that, in the presence of high concentrations of IFN-γ during TB, type I IFNs switch to an anti-inflammatory regulatory role. In particular, type I IFNs can inhibit inflammasome-mediated production of IL-1β by an IFN-stimulated response element-dependent mechanism (41). Additionally, type I IFNs can modulate STAT1 activity in myeloid cells by inducing a STAT3-dependent pathway, thus sequestering transcription factors in STAT1:STAT3 heterodimers (42). Activated STAT3 pathway can also increase IL-10 production and amplify anti-inflammatory responses by inducing BCL3 or IL-1R antagonist (43). Finally, the ubiquitous distribution of IFNAR and IFNγR implies that many cell types can respond to these cytokines but with distinct outcomes, with some cellular subsets more prone to playing an anti-inflammatory role. Recently, pulmonary

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**FIGURE 6.** Interplay of IFNs controls early TB. Schematic model of myeloid cell recruitment, evolution of granulomatous lesions, and influence of types I and II IFNs in the lungs during early TB. In wild-type mice, IFN-α/β and IFN-γ are produced in similar amounts during the preadaptive immune phase (<3 wk), and they both contribute to the optimal recruitment, differentiation, and/or survival of myeloid cells to the lungs. In *Ifnar−/−* mice and *Ifngr−/−* mice and, even more markedly, in *Ifnar−/− Ifngr−/−* mice, this influx of potential cellular targets of *M. tuberculosis* is limited, leading to lower lung bacterial loads. After the onset of the adaptive immune response (>3 wk), IFN-γ is produced in greater amounts than type I IFNs and, in wild-type mice, it controls bacterial growth, including via activation of individual cells. At that time, type I IFNs limit the inflammatory effects of IFN-γ. For this reason, *Ifnar−/−* mice have an increased population of myeloid cells in the lungs, but they retain the ability to control intracellular bacteria in response to IFN-γ. In *Ifngr−/−* mice, cells cannot control the growth of *M. tuberculosis*, leading to severe granulomatous lesions and neutrophilic infiltrates. In *Ifnar−/− Ifngr−/−* mice, the additional defect in type I IFN signaling increases recruitment, differentiation, and/or survival of potential target cells of *M. tuberculosis*, which translates into a widespread granulomatous response that affects the whole lung and further impairs mouse survival.
CD11c+ cells were shown to suppress excessive IFN-γ response and prevent excessive lung damage following Pneumocystis-induced chronic inflammation via type I IFN-mediated signaling (44).

At the cell population level, the anti-inflammatory role of type I IFNs during TB can be explained by a reduction in recruitment, differentiation, and/or survival of these myeloid cell subsets. Type I IFN-induced apoptosis in macrophages infected with L. monocytogenes (45) was suggested as a mechanism to explain the excess of spleen macrophages in Ifnar−/− mice compared with wild-type mice (46). In the case of myeloid DCs, we showed previously that their abundance in the lungs of M. tuberculosis-infected mice depends on the expression of IFNγR on nonhematopoietic cells (5), which can explain the defect observed in Ifng−−/− mice in the current study. Surprisingly, we observed a restoration of myeloid DC populations in Ifnar−/−Ifng−−/− mice. This could represent an extension of in vitro data showing that human monocyte-derived DCs generated in the presence of type I IFN are more susceptible to apoptosis when exposed to activating stimuli, such as IFN-γ plus bacterial products (47); therefore, the reduced population of lung myeloid DCs in the absence of IFNγR would be compensated for by an increased survival in the absence of IFNAR.

Alternatives to the proapoptotic role of type I IFN were proposed to explain its influence on myeloid cells during infection with M. tuberculosis. In particular, in vitro studies indicate that M. tuberculosis can divert human monocyte differentiation from DCs into macrophage-like host cells in an IFN-α-dependent fashion (48). However, our experimental data do not necessarily corroborate or refute this mechanism, which has also been described for IFN-γ (49). Other hypotheses include antiproliferative properties displayed by type I IFN, in general (50), and on murine macrophages, in particular (51). Finally, the increased lung neutrophilia, correlated with the inability to respond to IFN-γ, is a known consequence of IL-17-driven inflammation during TB (6), on which we show in this study that type I IFNs have no additional effect.

In the current study, we also demonstrated the importance of both types of IFNs for the optimal recruitment of NK cells to the lungs during the early phase of TB. Previous work showed that IFN-α/β production in vitro by M. tuberculosis-infected human DCs leads to chemokine-mediated recruitment of NK cells (52). Although an earlier study questioned the role of NK cells in the control of M. tuberculosis (53), these cells have been characterized as an early, T cell-independent source of IFN-γ following infection (54) and could prove to be a valuable asset for protection in HIV/AIDS patients.

In our model, combined effects of IFN-γ on mycobacterial cell activity in target cells (e.g., recruited macrophages) and of type I IFN on their abundance in the lungs contribute to the overall immune control of TB. We detected no evidence of a detrimental role of type I IFN on the ability of individual cells to control intracellular growth of M. tuberculosis in vivo. Nevertheless, our results are compatible with previous studies showing that type I IFN can negatively influence the outcome of infection when its results are compatible with previous studies showing that type I IFN on their abundance in the lungs contribute to the overall activity in target cells (e.g., recruited macrophages) and of type I IFN on its ability to control intracellular growth of M. tuberculosis

The difference observed between the clear detrimental role of type I IFNs in other bacterial infections, such as L. monocytogenes, and the more complex situation following M. tuberculosis infection might be explained by the fact that the latter has proven to be a relatively weak inducer of type I IFNs (data not shown), as well as by the selective tropism of M. tuberculosis for professional phagocytic cells. Further studies will be required to define the conditions (i.e., type I IFN subtypes, cellular source, concentrations, tissue distribution, and timing) under which the effects induced by type I IFN transition from protective to deleterious. As we have shown in this article, these studies will also need to take into account the influence of IFN-γ.

The recent discovery of a relationship between IFNs and IL-1 production during M. tuberculosis infection in vivo highlights the partial overlap of function between types I and II IFNs (18). Finally, our results provide additional information, which may help to determine whether the type I IFN signature associated with active TB in patients is the cause or the consequence of a poorly controlled infection. This distinction is crucial for future treatments targeting type I IFN signaling in TB patients, as well as for patients with combined viral infections, like hepatitis C or HIV.

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Disclosures

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References


