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BRIEF REPORT

# Type I Interferon is Pathogenic During Chronic *Mycobacterium africanum* Infection

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Type I interferons (IFNs, including IFN-αβ) contribute to the pathogenesis of *Mycobacterium tuberculosis* strains that induce high IFN-αβ levels. In the current study we examined the role of IFN-αβ during infection with a *Mycobacterium africanum* strain that induces low IFN-β levels. We infected wild-type and IFN-αβ receptor knockout mice with *M. africanum* and monitored bacterial growth, lung disease, and survival over 292 days. We found reduced lung bacterial burdens and less severe histopathological findings in the absence of IFN-αβ signaling. We conclude that IFN-αβ is pathogenic during chronic *M. africanum* infection and that the pathogenic effects may be mediated through poorer control of bacterial growth.

**Keywords.** *Mycobacterium africanum*; type I interferon; tuberculosis; IFN-αβ.

*Mycobacterium tuberculosis* is a highly successful pathogen, yet tuberculosis disease never develops in the majority of infected individuals [1]. Immune responses are sufficient to contain the bacteria in these individuals but insufficient to clear them. Although the reasons for this are largely unknown, it is clear that a balance of proinflammatory and anti-inflammatory cytokines is essential for bacterial containment. Type I interferons (IFNs, including IFN-αβ) are an important example of this. A certain level of IFN-αβ is required for protection, especially early in *M. tuberculosis* infection [2]. However, several studies have shown that higher levels of IFN-αβ are associated with poorer outcomes of infection with pathogenic mycobacteria. For example, lepromatous *Mycobacterium leprae* lesions are enriched in IFN-αβ–inducible genes [3], persons with active tuberculosis have an IFN-inducible gene signature that is more marked than in those with latent tuberculosis infection [4, 5], and interleukin-1 confers resistance to *M. tuberculosis* by limiting IFN-αβ production [6].

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It is unclear whether IFN-αβ signaling is pathogenic during infection with all *M. tuberculosis* complex (MTBC) strains. Hypervirulent *M. tuberculosis* strains are associated with enhanced IFN-αβ production in mice, and these strains are attenuated in mice lacking the IFN-αβ receptor (IFNAR$^{-/-}$) [7, 8]. This supports the evidence that IFN-αβ contributes to pathogenesis during human *M. tuberculosis* infection. However, it is unknown whether IFN-αβ is pathogenic during infection with other MTBC strains, especially strains that induce lower levels of IFN-αβ. Addressing this issue is important because drugs that limit IFN-αβ induction have been developed to improve the efficacy of tuberculosis treatment [6]. We need to know whether these drugs will be effective during infection with diverse MTBC strains or whether their efficacy will depend on the strain infecting a given patient.

The goal of the current study was to examine the effects of IFN-αβ signaling during infection with a *Mycobacterium africanum* strain characterized by lower induction of IFN-β than other strains in the MTBC [9] and by reduced virulence in humans and in mice [10, 11]. Thus, we infected C57BL/6 wild-type and IFNAR$^{-/-}$ mice on the same background with *M. africanum* and monitored bacterial burdens, lung disease, lung inflammation, and survival over 292 days. We find that IFN-αβ signaling is pathogenic during chronic *M. africanum* infection, and that the pathogenic response may be due to higher bacterial counts in the presence of IFN-αβ signaling.

## METHODS

### Bacterial Strain and Culture Conditions

*M. africanum* GM041182 was obtained from an human immunodeficiency virus–uninfected man with pulmonary tuberculosis in The Gambia (courtesy of Bouke de Jong, Institute of Tropical Medicine, Antwerp, Belgium). The strain was grown at 37°C in Middlebrook 7H9 liquid or 7H11 solid medium supplemented with 10% albumin, dextrose, and catalase. The strain was grown for approximately 7 days in liquid culture with shaking before infection. Infection was performed when the strain was in exponential growth phase (optical density at 580 nm, 0.4–0.7).

### Aerosol Infection and Tissue Processing

C57BL/6 wild-type and IFNAR$^{-/-}$ mice on the C57BL/6 background were infected by aerosol with a target inoculum of 100 colony-forming units (CFUs) per mouse. All animal experiments were done in accordance with procedures approved by the New York University School of Medicine Institutional Animal Care and Use Committee (Laboratory Animal Care Protocol 150502-01), which conformed to the guidelines provided by the Guide for the care and Use of Laboratory Animals of the

National Institutes of Health. At the time of harvest, the left lung lobes were homogenized in 0.5% phosphate-buffered saline–Tween 80, and CFUs were quantified by serial dilution on 7H11 agar plates. Plates were incubated at 37°C for approximately 21 days before CFUs were counted. The remainder of the lung homogenate was filtered through Spin-X filters (Corning) to remove bacteria, and the following cytokines were quantified using enzyme-linked immunosorbent assay: tumor necrosis factor (TNF; eBioscience), interleukin-10 (IL-10; R&D Systems), interferon-γ (IFN-γ; BD Biosciences), and interleukin-1β (IL-1β; eBioscience). At harvest, the right lung lobes were sectioned and fixed in formalin. Fixed lung sections were stained with hematoxylin-eosin and visualized with a Leica SCN400F Whole Slide Scanner.

## RESULTS

### Inverse Association of IFN-αβ Signaling With Bacterial Counts During Chronic *M. africanum* Infection

To determine whether the absence of IFN-αβ signaling altered the course of chronic infection with *M. africanum*, we compared bacterial counts in the lungs of wild-type and IFNAR$^{-/-}$ mice. We euthanized infected mice 292 days after infection and counted CFUs. We found that wild-type mice had more bacteria in the lungs than IFNAR$^{-/-}$ mice at this time point (Figure 1*A*; $P < .001$ by Mann–Whitney *U* test), although there was no difference in bacterial growth during early stages of *M. africanum* infection (Supplementary Figure 1).

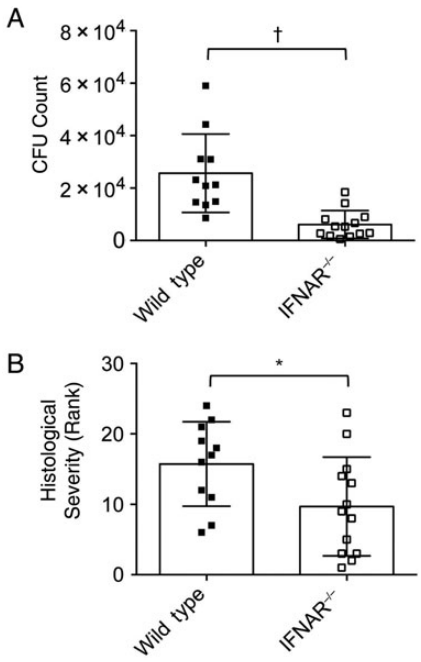


**Figure 1.** Interferon (IFN) αβ signaling is inversely associated with bacterial counts during chronic *Mycobacterum africanum* infection. C57BL/6 mice were infected with a target inoculum of 100 *M. africanum* colony-forming units (CFUs). *A*, CFU counts in the lungs of the surviving wild-type mice (n = 11) and mice lacking the IFN-αβ receptor (IFNAR$^{-/-}$; n = 13) were determined 292 days after infection. †$P < .001$ by Mann–Whitney *U* test; means are shown with standard deviations. *B*, Histological ranks of lungs of surviving wild-type (n = 11) and IFNAR$^{-/-}$ (n = 13) mice were determined 292 days after infection; increasing rank indicates increasing severity. *$P < .05$ by Mann–Whitney *U* test; means are shown with standard deviations.

### Association of IFN-αβ Signaling With Lung Disease and Inflammation During Chronic *M. africanum* Infection

To compare inflammation and lesions in the lungs 292 days after infection with *M. africanum*, we ranked lung sections in order of increasingly severe lung histopathological findings, in a blinded manner. Lungs with the most severe inflammation and lesions received the highest histopathology scores (Figure 2). Using this analysis, we found that wild-type mice overall had worse lung disease than IFNAR$^{-/-}$ mice (Figure 1*B*; $P < .05$ by Mann–Whitney *U* test), although there was notable heterogeneity among the mice (Figures 1*B* and 2). We quantified the levels of cytokines in lung homogenates as an additional measure of inflammation. We found that wild-type mice produced more TNF and IL-10 than IFNAR$^{-/-}$ mice (Supplementary Figure 2*A* and 2*B*). We also found that wild-type and IFNAR$^{-/-}$ mice produced similar amounts of IFN-γ and IL-1β, although the majority of the samples were below the limit of detection of the IL-1β secretion assay (Supplementary Figure 2*C* and 2*D*). There were weak but significant positive correlations between CFU counts and histopathological severity (Spearman $r = 0.47$; $P < .05$), TNF levels (Spearman $r = 0.52$; $P < .01$), and IL-10 levels (Spearman $r = 0.60$; $P < .01$). These results indicated that the overall effect of IFN-αβ signaling during chronic *M. africanum* infection was pathogenic. Furthermore, we propose that the pathogenic effects of IFN-αβ signaling were due to reduced bacterial growth in the lungs, with secondary effects on inflammatory histopathological findings.

### Marginally Enhanced Survival of Mice Infected With *M. africanum* in the Absence of IFN-αβ Signaling

We found that the majority of mice survived 292 days after infection with *M. africanum* (Supplementary Figure 3), which was consistent with previous findings that *M. africanum* is characterized by lower virulence than *M. tuberculosis* strains in humans and in mice [10, 11]. We also found that all of the mice that died were wild type; all of the IFNAR$^{-/-}$ mice survived (Supplementary Figure 3). The difference in survival between wild-type and IFNAR$^{-/-}$ mice was small, which was consistent with our previous findings that *M. africanum* induces low levels of IFN-β [9]. These data were consistent with the CFU counts and lung pathological results and supported the conclusion that IFN-αβ signaling was pathogenic during *M. africanum* infection.

## DISCUSSION

We found that IFN-αβ signaling is pathogenic during infection with *M. africanum*, an MTBC strain that is characterized by low

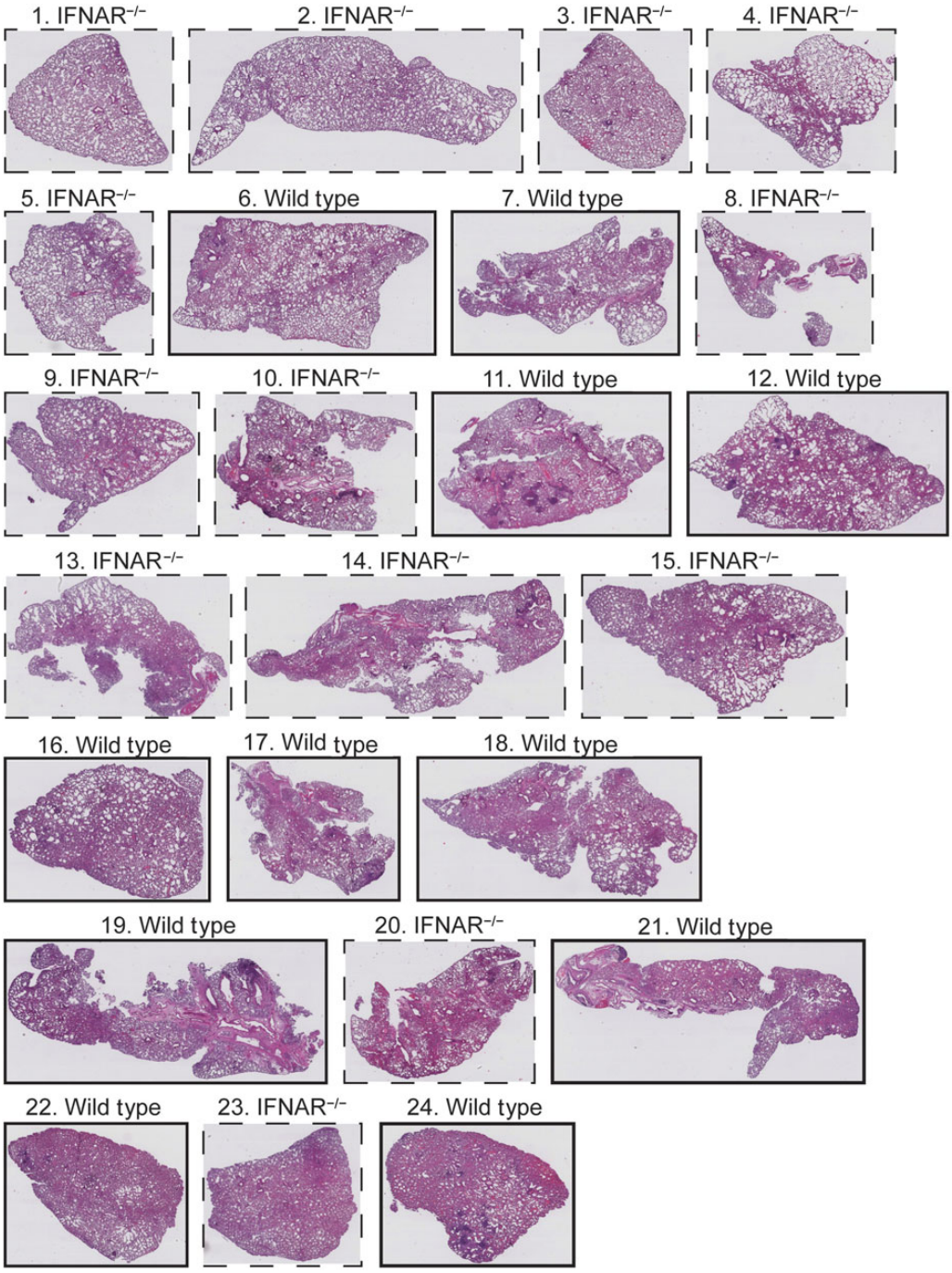


**Figure 2.** Histological severity ranking of lung sections from wild-type and IFNAR$^{-/-}$ mice during chronic *Mycobacterum africanum* infection. Lungs of *M. africanum*–infected wild-type mice (n = 11) and mice lacking the IFN-αβ receptor (IFNAR$^{-/-}$; n = 13) mice at 292 days after infection were fixed in formalin, cut in 5-μm sections, stained with hematoxylin-eosin, and imaged. Lung samples were ranked in order of increasing histopathological severity, in a blinded manner. Lung sections are shown in order of ranking, with the genotype of the mouse indicated. Lung sections from IFNAR$^{-/-}$ mice are bordered with dashed lines; sections from wild-type mice are bordered with solid lines.

virulence and induces low levels of IFN-β [9–11]. We propose that IFN-αβ contributes to *M. africanum* pathogenesis by supporting bacterial growth in the lungs. Our results fit the model that IFN-αβ signaling is pathogenic during bacterial infections and chronic viral infections [12]. Likewise, our data are consistent with previous findings showing that MTBC strains are attenuated in the absence or reduction of IFN-αβ signaling [7, 8]. The differences in survival between wild-type and IFNAR$^{-/-}$ mice infected with *M. africanum* were less striking than seen in studies with *M. tuberculosis*. In addition, the differences in lung bacterial burden between wild-type and IFNAR$^{-/-}$ mice were detected at later stages of infection than in previous

*M. tuberculosis* studies. These differences are most likely due to the reduced virulence of *M. africanum* and its reduced capacity to induce IFN-β [9–11].

A remaining question is how IFN-αβ signaling results in sustained bacterial growth during *M. africanum* infection. IFN-αβ induces anti-inflammatory cytokines such as IL-10, inhibits proinflammatory cytokines, such as IFN-γ and IL-1β, and inhibits production of antimicrobial peptides and major histocompatibility complex class II molecules, all of which could enhance *M. africanum* growth [12]. We found that during *M. africanum* infection, lung disease was overall worse in the presence of IFN-αβ signaling and that pathological severity and survival correlated with bacterial burden. Furthermore, TNF production was reduced in the absence of IFN-αβ signaling and was correlated with bacterial burden, despite a simultaneous reduction in IL-10. These results suggested that the anti-inflammatory effects of IFN-αβ did not result in increased *M. africanum* growth during chronic infection and, instead, that the increased disease was a result of increased bacterial burden in the presence of IFN-αβ signaling. Another way in which IFN-αβ could promote mycobacterial growth is by inducing CCR2 expression or production of its ligands, which would recruit macrophages to the site of infection [2]. Although *M. africanum* is attenuated for growth in vivo, we found that *M. africanum* is fully capable of infecting macrophages, gaining access to the cytosol, and replicating in macrophages in vitro [9]. Therefore recruitment of macrophages to the site of infection could enhance *M. africanum* growth by increasing the number of cells in which it can replicate. It is also possible that IFN-αβ promotes *M. africanum* growth by a still-undetermined mechanism. IFN-αβ signaling modulates macrophage cholesterol metabolism [13], and probably also modulates other basic cellular processes, which could regulate mycobacterial growth.

Our results suggest that the ability to induce IFN-αβ at levels that are pathogenic for the host is a widespread virulence mechanism in MTBC strains and probably arose early in mycobacterial evolution. This indicates that therapies that limit IFN-αβ induction could be effective in treating infections with diverse MTBC strains. However, our results also show that treatments that limit IFN-αβ will not result in lung sterilization, even for attenuated MTBC strains such as *M. africanum* strains. Therefore, there are probably additional signaling pathways that act in concert with IFN-αβ signaling to promote mycobacterial growth. Identifying such pathways would provide additional targets for drugs that, in combination with those that inhibit IFN-αβ, might effectively prevent mycobacterial growth and tuberculosis disease progression.

## Supplementary Data

Supplementary materials are available at http://jid.oxfordjournals.org. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

## Notes

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