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IRGM1 supports host defense against intracellular bacteria through suppression of type I interferon in mice

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IFN-γ enhances cell-autonomous host defense by inducing several families of antimicrobial target genes, including Immunity-Related GTPases (IRGs). Animals deficient in IRGM1, the best studied IRG, succumb to numerous bacterial and protozoal infections in a manner that nearly phenocopies IFN-γ-null mice (1). This infection susceptibility has been attributed to the cell-intrinsic role of IRGM1 in xenophagy and targeting of pathogen-containing vacuoles (1).

Recently, we reported that \( \text{Irgm1}^{-/-} \) mice spontaneously produce excess type I IFN (IFN-I) (2). Although IFN-I is protective against virus, it can compromise antibacterial host defense (3). We hypothesized that IFN-I, rather than defect cell-intrinsic defenses, drives the susceptibility of \( \text{Irgm1}^{-/-} \) mice to bacteria. Consistent with this, we found that \( \text{Irgm1}^{-/-} \) mice succumbed to \( \text{Mycobacterium tuberculosis} \) and \( \text{Listeria monocytogenes} \) (LM), as previously reported (1), but \( \text{Irgm1}^{+/+} \) mice lacking the IFN-I receptor, IFNAR1 (i.e., \( \text{Irgm1}^{-/-}\text{Ifnar}^{-/-} \)), were resistant (Figure 1A). Similarly, the increased pathogen burden in \( \text{Irgm1}^{-/-} \) mice following infection with \( \text{Salmonella typhimurium} \) was rescued in \( \text{Irgm1}^{-/-}\text{Ifnar}^{-/-} \) mice (Figure 1A). By contrast, during infection with \( \text{Toxoplasma gondii} \), a pathogen for which IFN-I is host-protective (4), \( \text{Irgm1}^{-/-} \) mice had reduced survival, and this was not rescued in \( \text{Irgm1}^{-/-}\text{Ifnar}^{-/-} \) animals (Figure S1A).

To investigate the mechanism of compromise of \( \text{Irgm1}^{-/-} \) mice by IFN-I, we pursued the LM infection model. After infection, \( \text{Irgm1}^{+/+} \) mice had elevated biomarkers of organ damage in serum (Figure S1B) and increased inflammation and necrosis in liver and spleen (Figures S1C-E), phenotypes that were rescued in \( \text{Irgm1}^{-/-}\text{Ifnar}^{-/-} \) mice. Increased cell death in \( \text{Irgm1}^{+/+} \) liver and spleen was dependent on IFN-I signaling (Figures 1B-C, S1F-G). Compared to WT and \( \text{Irgm1}^{-/-}\text{Ifnar}^{-/-} \), there was also increased LM growth in \( \text{Irgm1}^{-/-} \) organs (Figures S1H-J). Increased growth was seen by 4h post-infection in the peritoneum (Figure 1D), the site of LM inoculation in our model, indicating that IFN-I suppresses clearance of LM upon initial encounter. Indeed, \( \text{Irgm1}^{-/-} \) F4/80\( ^{hi} \) peritoneal macrophages internalized LM normally in vitro (Figure S2A) but had reduced killing capacity (Figure 1E). This was associated with decreased lysosomal delivery of LM (Figures 1F, S2B), despite normal lysosomal mass (Figure S2C) and pH (not depicted) in \( \text{Irgm1}^{+/+} \) macrophages. LM-challenged \( \text{Irgm1}^{-/-} \) F4/80\( ^{hi} \) peritoneal macrophages also had higher expression of STAT1, STAT2, (Y701-)PO\(_{4}\)-STAT1, and (Y689-)PO\(_{4}\)-STAT2 than WT and \( \text{Irgm1}^{-/-}\text{Ifnar}^{-/-} \) counterparts (not depicted). In vivo, 4h post-infection by GFP-expressing LM, only \( \text{Irgm1}^{-/-} \) F4/80\( ^{hi} \) macrophages showed increased bacterial load (Figure 1G, S2D).

Given that IFN-I may induce cell death (3), we examined peritoneal myeloid cells for viability (Figure S2E). Lytic death was increased in the \( \text{Irgm1}^{-/-} \) peritoneum at day 1 and 3 post-infection and was IFN-I-dependent (Figure S3A). Fewer neutrophils were recruited by 4h post-LM to \( \text{Irgm1}^{-/-} \) peritoneum, but neutrophil accumulation increased dramatically after 24h in an IFN-I-dependent manner (Figure 1H) and increased citrullinated histones, a marker of lytic neutrophil death by NETosis, was detected at day 3 (Figure S3B). Increased IFN-I-dependent lytic death was also observed among F4/80\( ^{hi} \) macrophages (Figure S3A), perhaps explaining their depletion 24h post-infection (Figures 1I-J). Notably, increased staining of phosphorylated-Mixed Lineage Kinase Domain-Like Pseudokinase, a necroptosis effector, was observed only in \( \text{Irgm1}^{+/+} \) macrophages (Figures S3C-D). Thus, IFN-I promotes multiple modes of pro-inflammatory lytic cell death in \( \text{Irgm1}^{-/-} \) mice. Accordingly, \( \text{Irgm1}^{-/-} \) peritoneal fluid exhibited an
IFN-I-dependent increase in lactate dehydrogenase activity and pro-inflammatory cytokines (Figures S3E-F).

During peritonitis, death of resident macrophages leads to recruitment and reprogramming of Ly6CHiF4/80− monocytes into Ly6C− F4/80Hi macrophages, often through an MHCII+F4/80Lo/Int intermediate (5). We observed emergence of a small F4/80+ population at day 3 post-LM (Figure S2E). Unlike WT, Ifnar−/−, and Irgm1−/−Ifnar−/− counterparts, all CD11b+F4/80Hi macrophages in the Irgm1−/− peritoneum at day 3 post-LM retained high Ly6C and did not express TIM4 (Figures S4A-B), a maturity marker of peritoneal macrophages (5). The CD11b+F4/80Lo population in Irgm1−/− remained Ly6CHi at day 3 and lacked a MHCII+ subpopulation (Figure S4C). The receptor for Colony-Stimulating Factor-1 (CD115), which is critical for survival and differentiation of monocytes, was repressed in Irgm1−/−Ly6CHi cells in an IFN-I-dependent manner (Figure S4C). Ly6CHi monocytes were also elevated in Irgm1−/− blood and showed reduced CD115 and MHCII (Figure S4E). These results suggest that excess IFN-I in Irgm1−/− mice impairs maturation of inflammatory Ly6CHi monocytes into macrophages, possibly by repressing CD115.

To specifically examine myeloid IFN-I signaling, we infected Irgm1−/− mice lacking IFNAR1 solely in myeloid cells (Irgm1−/−LysM:Cre+IfnarFx/Fx). These mice showed decreased necrotic death of peritoneal myeloid cells, partial rescue of CD115 in CD11b+F4/80LoLy6C− cells (Figures S4F-G), and reduced bacterial burden (Figure 1K) compared to controls, indicating that myeloid IFN-I signaling compromises myeloid cell fate and host defense in Irgm1−/− mice.

Our findings challenge the long-prevailing paradigm that IRGM1 serves as an IFNγ-induced cell-autonomous host defense effector (1), and suggest instead that IRGM1 supports host defense by preventing excess autocrine and/or paracrine IFN-I from compromising myeloid cell fate and function. Future studies will be required to distinguish autocrine vs. paracrine mechanisms.
References


Figure 1. IFN-I induces susceptibility to bacterial infection in Irgm1−/− mice. (A) Survival curves after infection with M. tuberculosis (N=7-10) and L. monocytogenes (N=4-5). Spleen CFU at day 21 after S. typhimurium infection (N=4-9). (B) Liver at day 3 post-Listeria stained for TUNEL and DAPI. (C) Quantification of TUNEL+ foci (N=3). (D) Peritoneal lavage CFU at 4h, day 1 and 3 post-Listeria (N=4-8). (E) Isolated F4/80Hi peritoneal macrophages exposed to Listeria were permeabilized for CFU count after 24h. (F) Macrophages were stained for Listeria and lysosome (LAMP1) at 6h and quantified for volumetric pixels of Listeria that were LAMP1-positive (N=32 images). (G) Percent GFP-positive after 4h infection by GFP-tagged Listeria (N=9-10). (H-I) Neutrophil (H) and F4/80Hi tissue macrophage (I) numbers in infected peritoneal lavage (N=7-11). (J) Representative plot showing depletion of CD11b+ F4/80Hi macrophages at 24h. (K) CFU in peritoneal cavity (PerC), liver, and spleen at day 3 post-Listeria (N=5-8). Data are mean ± s.e.m. #P<0.08, *P<0.05, **P<0.01, ***P<0.001, and ****P<0.0001 (one-way ANOVA with Tukey’s adjustment or Student’s t-test [panel K]).