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## Interferon-inducible Immunity-Related GTPase Irgm1 regulates IFN- $\gamma$ -dependent host defense, lymphocyte survival and autophagy

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### SUMMARY

IFN- $\gamma$  is a pleiotropic cytokine that plays a key role in host resistance yet when not properly regulated can become host-detrimental. The interferon-inducible Immunity Related GTPase family M member 1 (Irgm1), previously characterized as an effector molecule required for macrophage microbicidal activity, has been shown recently to play a previously unrecognized role in regulating IFN- $\gamma$ -dependent cell survival and host resistance. Irgm1 regulates the expansion / survival of mature effector CD4<sup>+</sup> T lymphocytes by protecting them from IFN- $\gamma$  induced autophagic cell death. Importantly, mice deficient in both IFN- $\gamma$  and Irgm1 were rescued from the lymphocyte depletion and increased mortality that occurs in single *Irgm1*<sup>-/-</sup> animals following pathogen exposure. We propose that Irgm1 plays a major role in maintaining T lymphocyte homeostasis during host IFN- $\gamma$  responses by protecting these cells from autophagy-dependent cell death.

### Keywords

autophagy; cell death; IFN- $\gamma$ ; Th1; lymphocyte homeostasis; GTPase

While IFN- $\gamma$  is required for host control of a wide range of intracellular pathogens by inducing NOS2 and other effector molecules in infected macrophages, unregulated production of or signaling by the cytokine can be host detrimental. Interestingly, the regulatory mechanisms that determine the beneficial versus detrimental outcomes of the IFN- $\gamma$  response are poorly understood. A set of these genes strongly induced by interferons encodes the p47 kDa Immunity-Related GTPase (IRG) family <sup>1, 2</sup>. We and others have shown that mice deficient in one member of the family, Irgm1 (also known as LRG47) <sup>3</sup> are highly susceptible to a wide variety of different intracellular bacterial and protozoan infections <sup>2, 4</sup>. Because Irgm1 has been shown to enhance intracellular pathogen killing by promoting phagolysosomal maturation and autophagy in vitro <sup>5, 6</sup>, it is characterized as an effector molecule required for microbicidal

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Addendum to:

The immunity-related GTPase Irgm1 promotes the expansion of activated CD4<sup>+</sup> T cell populations by preventing IFN- $\gamma$ -induced cell death

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activity in IFN- $\gamma$ -activated macrophages. Unexpectedly, we observed that *Mycobacterium avium* 7 and *Trypanosoma cruzi* 8 infected *Irgm1*<sup>-/-</sup> mice develop a profound pancytopenia, a defect that we have recently shown to be associated with impaired expansion of *Irgm1*<sup>-/-</sup> hematopoietic stem and progenitor cell populations<sup>9</sup>. These observations suggest that *Irgm1* plays a broader and more fundamental regulatory role in host responses to infections beyond its proposed function in pathogen killing.

Focusing on mature CD4<sup>+</sup> T lymphocytes we recently demonstrated that IFN- $\gamma$  induced *Irgm1* is critical for the survival of activated mature lymphocytes since in the absence of *Irgm1*, IFN- $\gamma$  triggered the death of these cells. Importantly, the lymphopenia and mortality previously observed in *M. avium*-infected *Irgm1*<sup>-/-</sup> animals was completely abrogated in *Irgm1*<sup>-/-</sup>*Ifng*<sup>-/-</sup> double-deficient mice<sup>10</sup>. These findings identified a previous unknown regulatory function for *Irgm1* in IFN- $\gamma$ -dependent host defense and lymphocyte survival in vivo. They are consistent with the conclusions from a recent in vitro study demonstrating that *Irgm* proteins negatively regulate the activation status of other GTPases within the same family and such that in the absence of this pathway some IRG members form aggregates and fail to reach pathogen-containing vacuoles<sup>11</sup>. Together, these two studies argue that in the absence of *Irgm1*, dysregulated cellular responses, rather than defective effector cell function, account for the impaired resistance of *Irgm1*<sup>-/-</sup> mice to certain infections. This is explained for *Mycobacterium avium* infection in Figure 1.

We also investigated the mechanism of IFN- $\gamma$ -induced death of *Irgm1*<sup>-/-</sup> T cells and found that the *Irgm1*-dependent death is distinct from previously described T cell receptor triggered T cell death mechanisms and is independent of Fas ligand-Fas interactions. Moreover, both morphological analysis as well as studies with pharmacological inhibitors failed to reveal a significant role for caspase-dependent apoptosis in the IFN- $\gamma$ -induced death of *Irgm1*<sup>-/-</sup> cells. Instead, we observed that following IFN- $\gamma$  stimulation many living *Irgm1*<sup>-/-</sup> cells appeared to have significantly increased numbers of membrane-bound vacuoles closely resembling autophagosomes and autolysosomes in their morphology. Importantly, IFN- $\gamma$ -induced death of *Irgm1*<sup>-/-</sup> T cells could be reduced by treatment with inhibitors of phosphoinositide 3-kinase or by silencing the expression of beclin-1 thus directly implicating the involvement of an autophagic pathway. Based on these observations, we suggested that *Irgm1* controls a non-apoptotic cell death program that depends on autophagy.

The *Irgm1*-dependent autophagic death program in lymphocytes is clearly distinct from the autophagic cell death mechanism previously described in macrophages which requires caspase inhibition and receptor interacting protein (RIP) kinase induction<sup>12, 13</sup>. Interestingly, the proposed functions for *Irgm1* in autophagy also appear to be cell type-specific. Thus, in IFN- $\gamma$ -stimulated macrophages *Irgm1* has been shown to promote<sup>6, 14</sup> rather than inhibit autophagy as observed in T cells. Although the mechanisms underlying this distinction are presently unclear, it is possible that *Irgm1* plays a role in fine-tuning the extent of autophagy depending on the specific functions of the cell type. Macrophages are terminally differentiated non-dividing cells in which high levels of autophagy have been proposed to contribute both to their anti-microbial effector activity and enhanced capacity to degrade house keeping proteins. In contrast, effector T lymphocyte function requires extensive cycling activity. While basal autophagy is beneficial for normal cellular homeostasis and survival, excessive catabolic activity during mitosis would inhibit generation of essential organelles / proteins and lead to cell death. Indeed, we found that IFN- $\gamma$  triggers death of proliferating but not resting *Irgm1*<sup>-/-</sup> CD4 T cells, suggesting that molecular events associated with increased nutrient uptake and protein turnover in cycling *Irgm1*<sup>-/-</sup> lymphocytes may contribute to their death. This observation also raises the question of whether in addition to directly regulating autophagy pathway, *Irgm1* may be critical in balancing IFN- $\gamma$ -dependent cellular events upstream of *Atg* (autophagy related genes) that, if not properly regulated, could trigger autophagy related

protein degradation cascades. Our current works focus on the mechanisms by which Irgm1 regulates the autophagic process in lymphocytes. Autophagy has now been shown to play a generalized role in maintaining cellular homeostasis through its involvement in both cell survival as well as cell death<sup>15–18</sup>. Interestingly, the autophagic cell death pathway utilizes almost the same machinery that promotes cell survival. In this regard, our findings emphasize the pivotal role played by non-Atg specific factors, such as extracellular signal, level of autophagy, cell type and rate of metabolism, in determining the threshold of autophagy-dependent death.

In conclusion, our findings demonstrate that a single GTPase, Irgm1, can dictate the beneficial outcome of the IFN- $\gamma$  response by providing a feedback mechanism essential for protecting activated CD4<sup>+</sup> lymphocytes from IFN- $\gamma$ -mediated death while enhancing the anti-microbial functions of the cytokine (Figure 2). We propose that this activity of Irgm1 is of special importance in the establishment and maintenance of Th1 responses to persistent intracellular infections. It is however also likely that the GTPase serves a similar role in the maintenance of cellular homeostasis in other non- T lymphocyte IFN- $\gamma$  responsive lineages.

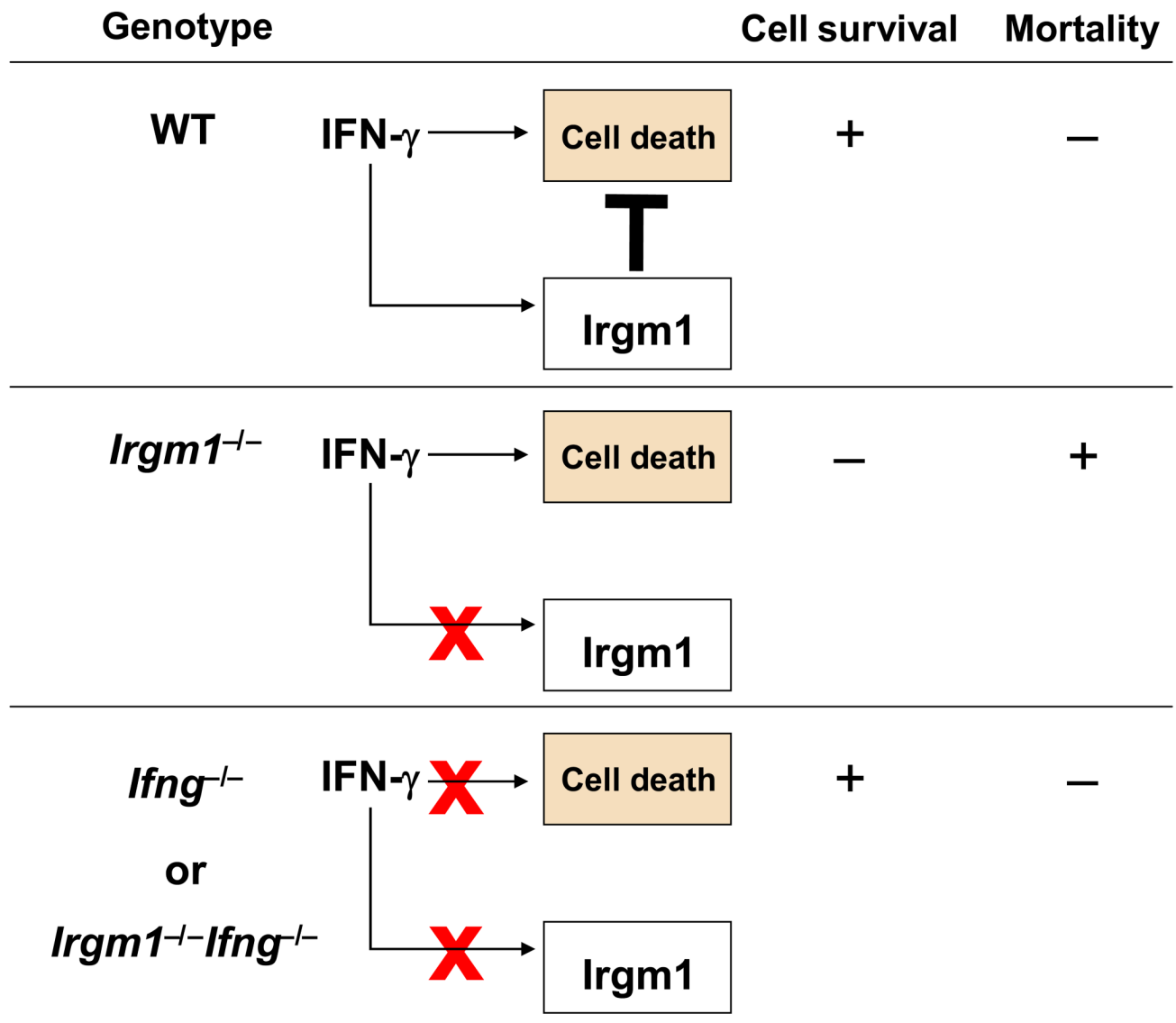
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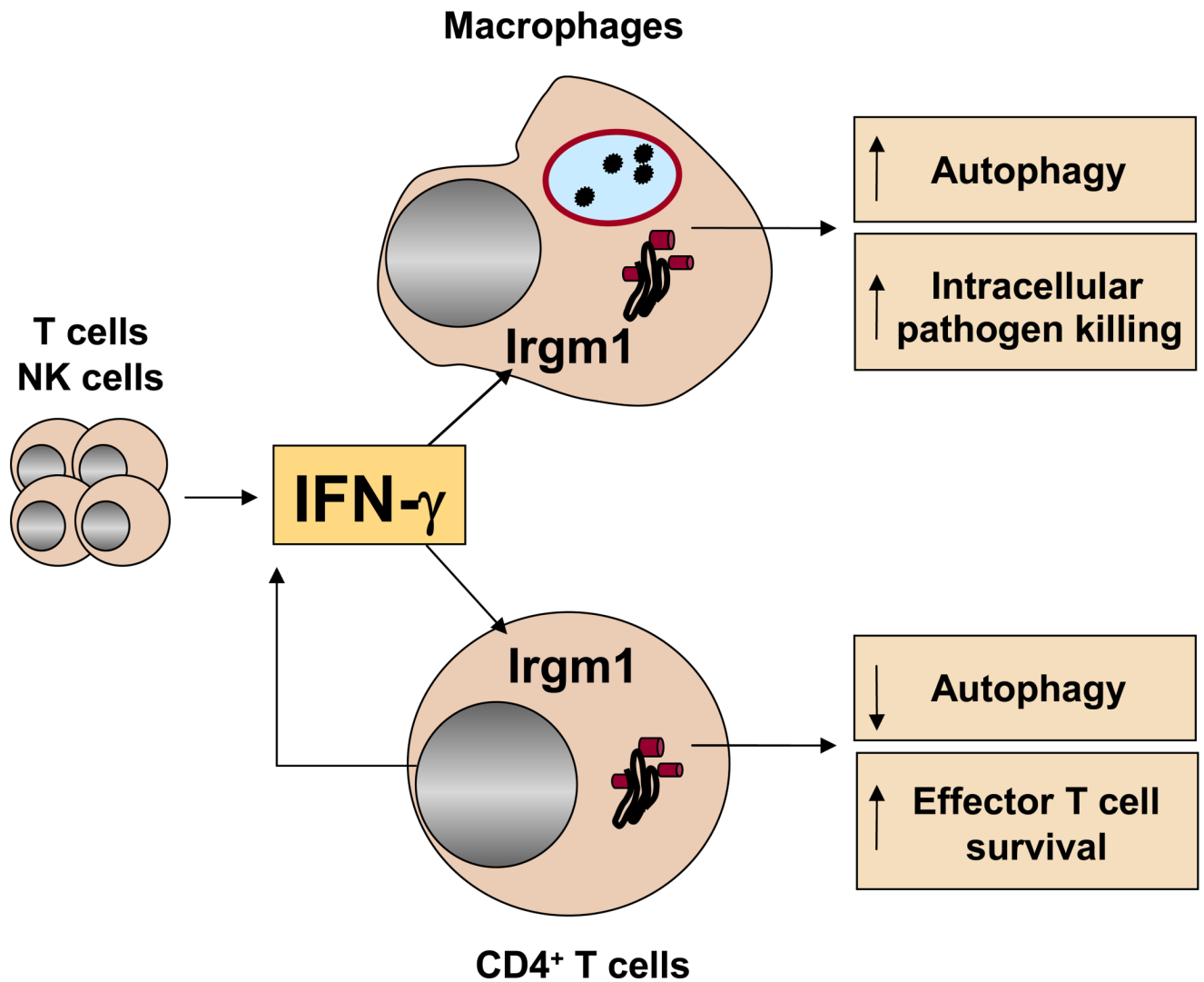
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**Figure 1.**

*Irgm1* regulates the outcome of IFN- $\gamma$  response to persistent intracellular pathogens. In this model, interferon-inducible *Irgm1* negatively regulates IFN- $\gamma$ -dependent death program ensuring expansion of effector lymphocyte populations and survival of infected hosts. In the absence of such protective mechanism as observed in *Irgm1*<sup>-/-</sup> mice, exposure of IFN- $\gamma$  leads to the death of activated CD4<sup>+</sup> T cells and increased mortality to mycobacterial infection. This regulatory mechanism is revealed only in the presence of IFN- $\gamma$ , as single IFN- $\gamma$  or double *Irgm1* and IFN- $\gamma$  deficiency does not result in cell death or increased mortality to the infection.



**Figure 2.** Distinct role for Irgm1 in the regulation of autophagy in macrophages and CD4<sup>+</sup> T cells. During Th1 response, IFN- $\gamma$  produced by activated NK and T cells induces Irgm1 expression to maximize host control of intracellular pathogens. While promoting IFN- $\gamma$ -dependent autophagy in macrophages to enhance clearance of intracellular pathogens, Irgm1 inhibits autophagic death of activated CD4<sup>+</sup> T cells to amplify Th1 effector populations.