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IFN- γ and self-absorbed CD4⁺ T cells: a regulatory double negative

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Abstract

Interferon- γ exerts many effects on the immune system. A new report shows that it induces both autophagy and Irgm1, a GTPase that protects activated CD4⁺ T cells from executing autophagy.

Autophagy, an internal cellular degradation process activated in response to nutrient or growth-factor starvation, begins with the formation of an 'autophagosome', an intracellular membrane that engulfs portions of cytoplasm. After fusion with late endosomes or lysosomes, the contents of the fused autophagolysosome are degraded and recycled for the anabolic needs of the cell. Autophagy can have prosurvival effects as a short-term nutrient recycler, but left unchecked, it can have pro-death effects as well. Studies have emphasized the importance of autophagy in the turnover of damaged or inefficient organelles¹, the control of pathogens^{2,3} and lymphocyte homeostasis⁴. Although much progress has been made in identifying the core machinery of autophagy, factors regulating the activation or suppression of autophagy remain unclear, particularly in the immune system.

In this issue of *Nature Immunology*, Feng *et al.* show that Irgm1 (also called LRG47) is an interferon-inducible GTPase that seems to suppress interferon- γ (IFN- γ)-induced autophagy in CD4⁺ T cells⁵. Building on published observations that Irgm1-deficient (*Irgm1*^{-/-}) mice develop severe CD4⁺ T cell lymphopenia during mycobacterial infection⁶, they now provide evidence that suggests this infection-induced CD4⁺ T cell attrition is due to autophagy and that superimposed IFN- γ deficiency negates such attrition. Hence, IFN- γ drives both a counter-regulatory mechanism (which would limit T helper type 1 (T_H1) responses) and an inhibitor of this negative regulatory mechanism—a regulatory 'double negative' (**Fig. 1**).

Optimal population expansion of CD4⁺ T cells and, in particular, T_H1 cells is critical for many aspects of immune responses. These include the promotion of antibody responses, priming of CD8⁺ T cells and induction of CD8⁺ T cell memory, activation of phagocytic cells and limiting of T_H2 and interleukin 17-producing T helper (T_H-17) responses. However, the clonal burst size of CD4⁺ T cell responses can vary by an order of magnitude

depending on the immunization protocol. Although various mechanisms can contribute to optimal CD4⁺ T cell population expansion, the survival of proliferating CD4⁺ T cells is probably critical.

In their latest paper, Feng *et al.* focus on the mechanisms underlying the infection-induced CD4⁺ T cell loss that occurs in *Irgm1*^{-/-} mice⁵. First, they find in standard proliferation assays with T cell receptor (TCR)-transgenic CD4⁺ T_H0 cells that incorporation of ³H is impaired in *Irgm1*^{-/-} T cells relative to that in wild-type cells after peptide or TCR stimulation. This apparent failure to proliferate is probably not due to defects in T cell activation, as they show that CD25 expression, incorporation of the thymidine analog 5-bromodeoxyuridine, activation of the kinase Erk, degradation of the inhibitor IκBα and phosphorylation of the kinase Akt are all normal after TCR stimulation of *Irgm1*^{-/-} CD4⁺ T cells. Instead, by counting cell bodies, they find that most *Irgm1*^{-/-} CD4⁺ T cells die roughly 2–3 days after stimulation. IFN-γ is the main culprit that limits cell survival because blocking antibodies to IFN-γ prevent the death of activated *Irgm1*^{-/-} CD4⁺ T cells. This impaired survival is also present after restimulation of *Irgm1*^{-/-} T_H1 (but not T_H2) cells *in vitro*. Further, superimposed loss of IFN-γ expression restores ‘proliferation’ to *Irgm1*^{-/-}*Ifng*^{-/-} CD4⁺ T cells *in vitro* and prevents attrition of CD4⁺ T cells after mycobacterial infection of *Irgm1*^{-/-}*Ifng*^{-/-} mice *in vivo*. Finally, the addition of IFN-γ to cell cultures enhances the death of *Irgm1*^{-/-}*Ifng*^{-/-} T cells but not *Ifng*^{-/-} T cells because IFN-γ upregulates cell death-protective *Irgm1* in *Ifng*^{-/-} T cells. These data collectively suggest that IFN-γ drives the death of activated CD4⁺ T cells but also induces *Irgm1*, which protects against such cell death.

Although a published report has identified a function for IFN-γ in controlling activation-induced cell death of CD4⁺ T cells through modulation of signaling by the death receptor Fas⁷, the studies here suggest that IFN-γ can control CD4⁺ T cell death in ways that do not seem to involve Fas or its ligand. Furthermore, Feng *et al.* show that caspase inhibition does not prevent IFN-γ-induced death of *Irgm1*^{-/-} CD4⁺ T cells⁵, again challenging the idea of involvement of death receptors in this cell death. Finally, they demonstrate that expression of several members of the family of the antiapoptosis protein Bcl-2 is not affected by either IFN-γ or the absence of *Irgm1*, which suggests a lack of involvement of the mitochondrial cell death pathway. However, this does not completely rule out the possibility of involvement of either death receptor or mitochondrial pathways; more definitive assessment of these pathways with knockout and/or transgenic mice is needed before such a conclusion can be drawn.

Because IFN-γ can promote autophagy², the authors next investigate the function of autophagy in the death of activated *Irgm1*^{-/-} CD4⁺ T cells by assessing several criteria that are consistent with autophagy. First, they find that treatment of *Irgm1*^{-/-}*Ifng*^{-/-} CD4⁺ T cells with IFN-γ results in more autophagic vacuole-containing cells, as assessed by electron microscopy. Second, they use an overexpression system to assess aggregation of LC3 (also called ATG8), which is a distinguishing feature of autophagy. LC3 is conjugated to phosphatidylethanolamine and becomes incorporated and aggregated into the growing autophagosomal membrane⁸. They find that IFN-γ promotes LC3 aggregation in *Irgm1*^{-/-}*Ifng*^{-/-} CD4⁺ T cells—not a trivial experiment, given the sparse cytoplasm of

activated CD4⁺ T cells. Third, using small interfering RNA, they show that the death of activated *Irgm1*^{-/-}/*Ifng*^{-/-} but not *Ifng*^{-/-} CD4⁺ T cells is mostly prevented by knockdown of beclin-1, a molecule critical for autophagy initiation. These data collectively suggest that IFN- γ is able to induce autophagic death in CD4⁺ T cells but only when *Irgm1* is missing.

Notably, these data are in direct contrast to the purported function of *Irgm1* in other cells. For example, in mouse macrophages, *Irgm1* seems to be critical for IFN- γ -induced autophagy, which is itself a process critical for the intracellular elimination of mycobacteria^{2,9}. One possible explanation for this conundrum might be that *Irgm1* has cell context-dependent effects on autophagy induction. In this scenario, differential expression of genes encoding modifiers may control the cell type-specific autophagic function of *Irgm1*. Genetic screens comparing activated macrophages versus activated CD4⁺ T cells may be a first step in identifying differentially expressed gene products that control autophagic processes operative in the two disparate cell types. The identification of such target genes might not only aid the understanding of autophagy but also provide therapeutic targets for a new class of antimicrobial agents to induce autophagy selectively in macrophages.

The work of Feng *et al.*⁵ is also in contrast to other studies examining the function of autophagy in T cells. The autophagic protein ATG5 has been shown to be essential for normal lymphocyte homeostasis⁴, consistent with a prosurvival function for autophagy in T cells. One explanation might be that because of the low metabolic demands of resting lymphocytes, autophagy may provide supplementary nutrients to intermittently sustain the cells when environmental nutrients become limiting. However, in activated lymphocytes, metabolic demands are high and may supersede the ability of autophagy to safely supply them. Instead, activated CD4⁺ T cells increase glycolysis to meet energy demands^{10,11}. Thus, the induction of *Irgm1* may control the amount of autophagy in activated T cells to allow them to expand their populations without devouring themselves. Indeed, this would prevent a 'futile cycle' in which glycolysis would be supplying the building blocks necessary for cell division while autophagy would be breaking them down. Such a scenario would lead to metabolic catastrophe. Thus, *Irgm1* induction in activated CD4⁺ T cells would be essential to allow the survival of effector CD4⁺ T cells and to promote a sustained T_H1 response. Obviously, more work is needed to identify specifically how *Irgm1* interferes with autophagy in T cells. A good place to start would be to determine if *Irgm1* inhibits autophagy by mechanisms similar to or distinct from those of mTOR, another chief negative regulator of autophagy. Nonetheless, *Irgm1* could be envisioned as an 'Achilles' heel' of activated T_H1 cells, in that therapeutics targeting *Irgm1* (similar to the targeting of mTOR by rapamycin) would allow the induction of autophagy specifically in activated T cells.

Thus, it is possible that this autophagic process occurs in activated T cells only when *Irgm1* is missing and acts as a 'backup' mechanism. For example, both the proapoptotic Bim–antiapoptotic Bcl-2 and Fas–Fas ligand pathways are critical for the apoptosis of activated CD4⁺ T cells^{12–15}. It is unclear whether autophagy contributes the population expansion and/or contraction of antigen-specific CD4⁺ T cell responses *in vivo*. More definitive experiments with mice deficient in ATG5 or beclin-1 should address this point. Furthermore, if autophagy is important in determining the magnitude of the T_H1 response,

what might the effects be on differentiation to the T_H2 and T_H-17 lineages? Finally, given the importance of Irgm1 in promoting the formation of autophagic vacuoles in macrophages, why would it have the opposite function in CD4⁺ T cells? In this context, it would serve a 'double-positive' regulatory function, promoting more robust T_H1 responses that in turn would induce more Irgm1 in macrophages to enhance antimicrobial activity. In the end, the answer may be in the 'genes that fit' CD4⁺ T cells particularly well and prevent them from gorging on themselves while allowing them to become 'LRG'.

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Figure 1. IFN- γ drives a regulatory double negative. **(a)** Stimulation of wild-type CD4⁺ T cells in T_H1 conditions drives the production of IFN- γ . IFN- γ induces *Irgm1* expression and robust proliferation of T_H1 cells occurs. In the absence of *Irgm1*, stimulated CD4⁺ T cells can still produce IFN- γ but instead undergo autophagy, and the activated cells begin proliferating but do not survive. Additional loss of IFN- γ prevents autophagy and loss of activated CD4⁺ T cells, thereby restoring the proliferative response. **(b)** IFN- γ drives both autophagy (a counter-regulatory mechanism to limit T_H1 responses) and *Irgm1*, an inhibitor of this negative regulatory mechanism.