The Mouse Resistance Protein Irgm1 (LRG-47): A Regulator or an Effector of Pathogen Defense?

Julia P. Hunn, Jonathan C. Howard*
Institute for Genetics, University of Cologne, Cologne, Germany

The interferon-γ (IFN-γ)–inducible IRG proteins are a distinctive cytoplasmic GTPase family encoded by about 20 genes in the C57BL/6 mouse [1]. All four IRG genes that have been knocked out (Irgm1, Irgm3, Irgd, Irga6) have caused more or less striking susceptibility phenotypes to Toxoplasma gondii [2,3] and O. Liesenfeld, I. Parvanova, J. Zerrahn, S-J. Han, F. Heinrich, et al., unpublished data). However, one single member of the IRG family, Irgm1 (formerly called LRG-47), stands out because it has additionally been implicated in a remarkable range of resistances in the mouse: resistance to Trypanosoma cruzi [4], Leishmania major [5], Listeria monocytogenes [2], Mycobacterium tuberculosis [6], Mycobacterium avium [7], Chlamydia trachomatis [8], and Salmonella typhimurium [9]. These are all intracellular but otherwise very different organisms—some are protozoa, some Gram-negative bacteria, some Gram-positive, some living inside a vacuole or phagosome, and some free in the cytosol. Thus, Irgm1 appears to have exceptional properties of disease resistance not shared by other members of the IRG family.

Specific cell-autonomous resistance mechanisms have been attributed to Irgm1 in the context of mycobacterial resistance. Irgm1 has been considered to act by associating with the mycobacterial phagosomal membrane and accelerating lysosomal fusion [6] (Figure 1). There have also been suggestions that under certain conditions the protein can enhance the formation of autophagosomes that in turn control the pathogen [10–12]. These activities, related but distinct, could both be attractive candidates for a relatively direct mode of action of Irgm1 as a resistance protein.

These mechanisms for Irgm1 function are widely accepted, perhaps partly because of the importance of the diseases that they are supposed to control, but also because, right or wrong, they are immediately appreciable, plausible, proximal, cell-autonomous effects on the pathogen. However, optimism that there may be such direct explanations for the loss of mycobacterial resistance as a result of the loss of Irgm1 has apparently obscured an important literature on Irgm1 deficiency and activity that points in an entirely different direction.

Irgm1-deficient mice become strikingly leukopenic when infected with mycobacteria. Alan Sher and colleagues reported some years ago that the blood picture of young Irgm1-deficient adults is pretty normal, but collapses during infection [7]. They subsequently observed the same phenomenon for Trypanosoma infection [4]. A complete catalog has not yet been made, but we may infer that leukopenia is the rule when Irgm1-deficient mice are infected with any immunostimulatory pathogen. Indeed, induced leishmaniasis seems also to arise following non-pathogenic immune stimuli since induction of experimental allergic encephalitis in Irgm1-deficient mice with myelin basic protein peptides, a well-established model for multiple sclerosis, resulted in similar leukocytic defects, in this case with a beneficial outcome for the disease [13]. Thus we ask, does susceptibility to mycobacteria (or T. cruzi or Salmonella) really have something to do with the proposed cell-autonomous mechanisms, autophagocytosis or reduced vacuole acidification, or is it due to the profound and generalized immunodeficiency that these organisms induce in Irgm1-deficient hosts?

Gregory Taylor and colleagues showed recently that mice that are not only Irgm1- but also Irgm3-deficient (that is, they have a doubly deficient IRG system) are no longer susceptible to Salmonella [14] (see Table 1). Furthermore, the authors cite a personal communication from John MacMicking that the mycobacterial susceptibility phenotype of Irgm1-single-deficient mice is also reversed in the same double knock-out. Thus the absence of Irgm1 cannot be the direct cause of the susceptibility in either of these cases. There must be a more complex explanation.

Why do Irgm1-deficient animals rapidly develop a lymphomyeloid deficiency after infection or autoimmune stimulation? There seems to be reduced proliferative potential in the lymphomyeloid system that becomes acute after immune activation. It was shown recently that this affects the hematopoietic stem cell (HSC) as well as more peripheral lymphoid compartments [15]. The functional impairment depends absolutely on the presence of IFNγ and the integrity of its signal transduction pathway. If these are impaired or impeded, the Irgm1-dependent hematopoietic and lymphopoietic failures are reversed, as is susceptibility to infection by mycobacteria [16,17]; Margaret Goodell, personal communication). Thus, absence of Irgm1 is not in itself responsible for the hematopoietic and immune failures. Rather, it is the rest of the IFN response that is causing the problem in the absence of Irgm1. Stressing this point, Irgm1-deficient mice infected with a pathogen that stimulates only a Th2 response (Schistosoma mansoni), and therefore essentially no IFNγ production, show normal resistance and no lymphoid abnormalities [16]. Which of the thousand or so IFNγ-regulated transcripts is responsible for this mysterious effect? The double knock-out
of Irgm3 and Irgm1 seems to tell us the interesting answer, that the problem with Irgm1 deficiency is connected with the presence of the rest of the IRG family of proteins. What can that problem be?

We showed that the IRG proteins fall into two groups, the GKS and the GMS sub-families, based on the sequence of their nucleotide binding domains [18]. More recently, we showed that the three GMS proteins, Irgm1 (LRG-47), Irgm2 (GTPi), and Irgm3 (IGTP), are essential regulators of the GTPase cycle of the GKS proteins, binding to these in the GDP-bound, non-functional aggregates (red dots) in IFN-γ-induced cells [14] with striking cytopathic effects, especially on cells of the lymphomyeloid system [7,15]. We argue that this, rather than loss of Irgm1 from the mycobacterial phagosome, is the main reason for the dramatic immune impairment of Irgm1-deficient mice, including loss of mycobacterial resistance. doi:10.1371/journal.ppat.1001008.g001

stability even when expressing very low levels of the protein. Constitutive expression of Irgb6 in cells in the absence of IFNγ led to the formation of protein aggregates associated with marked pathological expansion of the endoplasmic reticulum lumen, though apparently without interfering with cell proliferation of mouse 3T3 fibroblasts [19]. It is worth mentioning that expression of individual GMS proteins has no detectable cytotoxic or cytopathic effect on cells growing in culture ([19] and J. Hunn, S. Konen-Waismans, J. Howard, unpublished data).

We can therefore propose the following preliminary scenario for the Irgm1-deficient mouse. In the absence of induced IFNγ production, the mouse appears relatively normal. However, for unclear reasons, there is constitutive expression of many IRG genes in HSCs [23–25]. In the absence of Irgm1, this expression would be expected to result in unregulated cytoplasmic aggregates of GKS proteins. These are presumably cytostatic or cytopathic in the HSC population, resulting in continuous turnover and concomitant near exhaustion of the stem cell pool, leaving little residual potential to respond to hematopoietic stress [15]. In the periphery, infection rapidly induces IFNγ, which in turn induces the IRG protein response in lymphoid and other cells. As in HSC, Irgm1 deficiency results in the formation of intracellular aggregates of unregulated GKS proteins [14,19,20]. These aggregates are presumably cytostatic or cytopathic for cells of the lymphomyeloid system, perhaps especially for replicating cells through inhibition of the ubiquitin-proteasome system [26], resulting in the observed infection-induced leukaemia and a generalized immunodeficiency. It seems that IRG aggregate formation must also be toxic for interphase lymphocytes to explain the generalized lymphopenia. We would argue that the deposition of aggregates in IFNγ-induced cells is responsible for the autophagic anomalies observed in Irgm1-deficient T lymphocytes [16].

Consistent with this scenario, Taylor and colleagues showed aggregates of GKS proteins (Irgb6 and Irga6) in bone marrow-derived macrophages from Irgm1 knock-out mice after in vitro stimulation with IFNγ [14]. However, they also observed aggregates in IFNγ-induced cells from Irgm3 knock-outs and Irgm1/Irgm3 double knock-outs, neither of which show a significant lymphopenia nor susceptibility phenotype to Salmonella or mycobacteria infection [6,9]. At first glance, this latter observation seems to argue against the idea that protein aggregates are
Incomplete regulation of induced effector IRG proteins

Taylor and colleagues also mentioned GMS deficiencies depending on the different cytopathic phenotypes for different IRG proteins. Such distinctions may well result in the extent by individual GMS proteins [19].

Irgm1 deficiency may lead preferentially to GKS aggregate formation on Golgi and endolysosomal membranes, where Irgm1 is normally localized [27,28], while aggregates due to Irgm3 deficiency form preferentially on endoplasmic reticulum membranes, where Irgm3 is normally localized [29]. There is already evidence that Irga6 and Irgb6 may be preferentially regulated to a different extent by individual GMS proteins [19]. Such distinctions may well result in different cytopathic phenotypes for different GMS deficiencies depending on the level and subcellular localization of dysregulation. Taylor and colleagues also noticed that there was a reduced amount of GKS IRG proteins in IFNγ-induced Irgm3-deficient macrophages compared with the wild-type or Irgm1-deficient cells [14]. In the Irgm1/1rgm3 double-deficient cells the causal chain heading towards cytopathy is truncated by the rapid clearance of the Irg protein aggregates. The consequences of Irgm1 deficiency are cellular as well as systemic and result in whole-animal immune failure. The range of pathogens genuinely controlled by the IRG system of mice is unclear. At present, T. gondii and C. trachomatis stand out, but it is not known what these two pathogens have in common that renders them susceptible to IRG-mediated immunity, nor what the other organisms lack or possess that renders them resistant.

do:10.1371/journal.ppat.1001008.t001

Table 1. Summary of cellular and systemic consequences of IRGM knock-outs.

| Genotype | Normal expression and regulation of induced effector IRG proteins | No cytopathic consequences for cellular function | Heightened cell-autonomous immunity via IRG proteins | Resistance against a wide range of intracellular pathogens |
|----------|---------------------------------------------------------------|-------------------------------------------------|----------------------------------------------------|--------------------------------------------------------|
| Wt       |                                                               |                                                 |                                                     |                                                        |
| Irgm1−/− | Incomplete regulation of induced effector IRG proteins        | Cytosolic aggregates of IRG proteins with cytopathic consequences: | Stem cell exhaustion | Massive leukopenia |
|          |                                                               | Systemic immune deficiency                      |                                                                   |                                                          |
|          |                                                               | Macrophage dysfunction: reduced motility, impaired adhesiveness, |                                                                   |                                                          |
|          |                                                               | reduced phagosome acidification, multiple cell-autonomous immune deficiencies |                                                                   |                                                          |
|          |                                                               | Susceptibility to multiple pathogens including mycobacteria, |                                                                   |                                                          |
|          |                                                               | S. Typhimurium, and Leishmania in addition to C. trachomatis and T. gondii |                                                                   |                                                          |
| Irgm1/m3−/− | Incomplete regulation of induced effector IRG proteins       | Strongly reduced expression of effector IRG proteins | Cytosolic aggregates of IRG proteins with enhanced clearance and no cytopathic consequences: | Stem cell exhaustion | Leukopenia |
|          |                                                               | Systemic immune deficiency                      |                                                                   |                                                          |
|          |                                                               | No cell-autonomous dysfunction except loss of IRG-dependent immunity |                                                                   |                                                          |
|          |                                                               | Susceptibility only to T. gondii and C. trachomatis |                                                                   |                                                          |

This table summarizes the arguments presented, documented, and referenced in the accompanying article. Each panel can be read from top to bottom as a causal chain. Thus, Irgm1 deficiency results in incomplete regulation of induced effector GKS IRG proteins, which results in build up of cytosolic aggregates, and these in turn have cytopathic consequences. For Irgm1 deficiency, the causal chain is long and ends up with major systemic and cell-autonomous immunodeficiency. In wild-type cells, the causal chain is adaptive and leads to increased cell-autonomous immune competence, while in the Irgm1/Irgm3 double-deficient cells the causal chain heading towards cytopathy is truncated by the rapid clearance of the Irg protein aggregates. The consequences of Irgm1 deficiency are cellular as well as systemic and result in whole-animal immune failure. The range of pathogens genuinely controlled by the IRG system of mice is unclear. At present, T. gondii and C. trachomatis stand out, but it is not known what these two pathogens have in common that renders them susceptible to IRG-mediated immunity, nor what the other organisms lack or possess that renders them resistant.

responsible for the cytopathic sequelae of Irgm1 loss [14]. However, aggregates forming as a result of Irgm1 deficiency may well be qualitatively distinct from, and more cytotoxic than, those resulting from Irgm3 deficiency. We have shown that all three GMS regulators are required for complete GKS control and have hypothesized that each is required for GKS regulation on a different group of endomembranes [19]. Thus, Irgm1 deficiency may lead preferentially to GKS aggregation on Golgi and endolysosomal membranes, where Irgm1 is normally localized [27,28], while aggregates due to Irgm3 deficiency form preferentially on endoplasmic reticulum membranes, where Irgm3 is normally localized [29]. There is already evidence that Irga6 and Irgb6 may be preferentially regulated to a different extent by individual GMS proteins [19]. Such distinctions may well result in different cytopathic phenotypes for different GMS deficiencies depending on the level and subcellular localization of dysregulation. Taylor and colleagues also noticed that there was a reduced amount of GKS IRG proteins in IFNγ-induced Irgm3-deficient macrophages compared with the wild-type or Irgm1-deficient cells [14]. In the Irgm1/Irgm3 double-deficient cells the causal chain heading towards cytopathy is truncated by the rapid clearance of the Irg protein aggregates. The consequences of Irgm1 deficiency are cellular as well as systemic and result in whole-animal immune failure. The range of pathogens genuinely controlled by the IRG system of mice is unclear. At present, T. gondii and C. trachomatis stand out, but it is not known what these two pathogens have in common that renders them susceptible to IRG-mediated immunity, nor what the other organisms lack or possess that renders them resistant.

Toxoplasma is completely lost in the Irgm1 knock-out [2], and this could of course as easily be due to the generalized immunodeficiency as to the loss of a key IRG protein function. However, resistance to Toxoplasma does not return in the Irgm1/Irgm3 double knock-out [14]. Furthermore, loss of Irgd or Irga6, both GKS proteins, also leads to a Toxoplasma susceptibility phenotype without any lymphopenia or generalized immunodeficiency [2,21] and O. Liesenfeld, I. Parvanova, J. Zerrahn, S-J. Han, F. Heinrich, et al., unpublished data). The conclusion is that the IRG proteins really do mediate resistance against Toxoplasma in mice. It is a good bet that the ability of multiple IRG proteins to relocalize to the T. gondii parasitophorous vacuole membrane, causing its disruption and killing the parasite [21,30], indicates the essential mechanism by which IRG proteins operate against this pathogen.

We conclude that the adaptive role of Irgm1 in mice is connected to its activity in the regulation of the GKS members of the IRG protein family. T. gondii is probably an important pathogen for mice because of the recent predominance of the domestic cat as definitive host, and it may therefore be that resistance to this parasite is driving the function of the IRG system in the mouse. Recent results from Jorn
Coers and colleagues show that the IRG system may also be directly active against *C. trachomatis* [8,31]. However, we consider it highly unlikely that Irgm1 has any adaptive function at all in resistance against *C. trachomatis* or any of the other pathogens attributed to it. Certainly mycobacteria and *Salmonella* can now be explicitly excluded [6,9], and there is every reason to suppose that most if not all the others except *T. gondii* and *C. trachomatis* will go the same way.

It is important to look back on the experiments that attributed specific cell-autonomous activities to Irgm1 to account for its role in resistance to mycobacteria. If resistance to mycobacteria or *Salmonella* really has nothing to do with IRG proteins, why does Irgm1 relocalize to the mycobacterial phagosome, and why would acidification of the phagosome be reduced in Irgm1-deficient cells [6] (see Figure 1)? Most of the relevant experiments were conducted on macrophages derived from the Irgm1-deficient strain, so it is the properties of macrophages that should be considered. As to the first point, it was shown some years ago that Irgm1 relocalizes to latex bead phagosomes in macrophages [27], so this step has nothing to do with IRG proteins, why does Irgm1 relocalize to phagosomes in macrophages derived from Irgm1-deficient mice [9,14,32]. These defects are completely reversed in the Irgm1/Irgm3 double knock-out [14].

In view of the hematopoietic abnormalities in the Irgm1-deficient mice, macrophage development and differentiation are probably also disturbed. Aggregate formation in Irgm1-deficient macrophages [14] may also have direct cytopathic consequences for many aspects of macrophage activity, including lysosomal function, perhaps as a result of autophagy, constitutively stimulated by the presence of IRG protein aggregates [33]. Therefore, a direct comparison between the cell-autonomous properties, such as phagocytic vacuole acidification and induction of autophagy, of Irgm1-deficient and wild-type macrophages is probably not valid. A direct analysis of phagosome and autophagosome function in the single and double GMS knock-outs would clarify whether some direct cell-autonomous function can be attributed to Irgm1.

It is also interesting to revisit the specificity control introduced by Taylor and colleagues to indicate that the immune deficiency due to Irgm1 was not universal, namely that resistance to mouse cytomegalovirus (MCMV) was normal [2,3]. Resistance to MCMV does not depend on T cells but is largely mediated by natural killer cells, which require cytokine-mediated activation to develop full functional activity [34,35]. This cell type may be less vulnerable to the cytopathic consequences of Irgm1 deficiency than T cells and HSCs.

It is important to emphasize that while the present view can account for much of the complexity of the observations on Irgm1 deficiency, it remains possible that Irgm1 may have additional “autonomous” activities of its own, perhaps in the control of autophagy. It now seems unlikely that this will be true for immunity against mycobacteria or *Salmonella* since this appears to be normal in the absence of Irgm1 so long as Irgm3 is missing too, but these, of course, do not exhaust the universe of intracellular pathogens. There is much experimental work left to do to assess the validity and completeness of this revision of view about how the IRG proteins fulfill their function. It is a complex argument, but it hangs together reasonably well and offers a broad and satisfying explanation for most, if not all, of the properties of the Irgm1-deficient mouse. Above all, however, the IRG system must be understood as a highly regulated, highly coordinated system of proteins where the properties of single-gene knock-outs may be misleading.

Acknowledgments

The authors gratefully acknowledge generous and wide-ranging discussions with the scientists who have done most to stimulate our present understanding of the role of Irgm1 in immunity: Gregory Taylor (Duke University), Jorn Coers (formerly Harvard University, now Duke University), Margaret Goodell (Baylor College of Medicine), and Carl Feng (National Institutes of Health). We are also indebted to the many members of our own lab who have contributed to the discussion of the Irgm1 problem and helped us to refine the arguments.

References

1. Bekpen C, Hunn JP, Rohde C, Parvanoa I, Guethlein L, et al. (2005) The interferon-inducible p47 (IRG) GTPases in vertebrates: loss of the cell-autonomous resistance mechanism in the human lineage. Genome Biol 6: R92.
2. Collazo CM, Yap GS, Sempowski GD, Lusby KC, Tesarollo L, et al. (2001) Inactivation of LRG-47 and IRG-47 reveals a family of interferon-gamma-inducible genes with essential, pathogen-specific roles in resistance to infection. J Exp Med 194: 181–188.
3. Taylor GA, Collazo CM, Yap GS, Nguyen K, Gregorio TA, et al. (2000) Pathogen-specific loss of host resistance in mice lacking the IFN-gamma-inducible gene IFGT. Proc Natl Acad Sci U S A 97: 751–755.
4. Santiago HC, Feng CG, Bafica A, Rolfe E, Arantes RM, et al. (2005) Mice deficient in LRG-47 display enhanced susceptibility to Trypanosoma cruzi infection associated with defective hemopoiesis and intracellular control of parasite growth. J Immunol 175: 8165–8172.
5. Taylor GA (2007) IRG proteins: key mediators of innate immunity. J Exp Med 194: 181–188.
6. Coers J, Jammes H, Grosky D, Parvanoa I, Howard JC, et al. (2008) Chlamydia muridarum evades growth restriction by the IFN-gamma-inducible host resistance factor Irgm1. J Immunol 180: 6257–6265.
7. Henry SC, Daniell XG, Burroughs AR, Taylor GA, Deretic V (2006) Interferon-gamma expression inhibits BCG and Mycobacterium muridarum evades growth restriction by the IFN-gamma-inducible host resistance factor Irgm1. J Immunol 179: 6963–6972.
8. Gutierrez MG, Master SS, Singh SB, Taylor GA, Colombo MI, et al. (2004) Autophagy is a defense mechanism inhibiting BCG and Mycobacterium tuberculosis survival in infected macrophages. Cell 119: 753–766.
9. Singh SB, Davis AS, Taylor GA, Deretic V (2006) Human IRGM induces autophagy to eliminate Intracellular Mycobacteria. Science 313: 1438–1441.
10. Ling YM, Shaw MH, Ayala C, Coppins I, Taylor GA, et al. (2006) Vacuolar and plasma membrane stripping and autophagic elimination of Toxoplasma gondii in primed effector macrophages. J Exp Med 203: 2603–2671.
11. Xu H, Weig H, Feng F, Gao L, Chen D, et al. (2010) Genetic deficiency of Irgm1 (LRG-47) suppresses induction of experimental autoimmune encephalomyelitis by promoting apoptosis of activated CD4+ T cells. Faseb J 24: 1583–1592.
12. Henry SC, Daniell XG, Burroughs AR, Indaram M, Howell DN, et al. (2009) Balance of IRgM protein activities determines IFN-gamma-induced host defense. J Leukoc Biol 85: 877–885.
13. Feng CG, Welsbeger DC, Taylor GA, Sher A, Goodell MA (2008) The p47 GTPase Lrg-47 (Irgm1) links host defense and hematopoietic stem cell proliferation. Cell Stem Cell 2: 83–89.
14. Feng CG, Zheng L, Jankovic D, Bafica A, Cannons JL, et al. (2008) The immunity-related GTPase Irgm1 promotes the expansion of activated CD4+ T cell populations by preventing interferon-gamma-induced cell death. Nat Immunol 9: 1279–1297.
15. Coers J, Zhang L, Lenardo MJ, Sher A (2009) Interferon-inducible immunity-related GTPase Irgm1 regulates IFN-gamma-dependent host defense, lymphocyte survival and autophagy. Autophagy 5: 232–234.
16. Boehm U, Guethlein L, Klamt T, Oztek K, Schaub A, et al. (1998) Two families of GTPases dominate the complex cellular response to IFN-gamma. J Immunol 161: 6715–6723.
17. Hunn JP, Koener-Waisman S, Papic N, Schroeder N, Pawloski N, et al. (2008) Regulatory interactions between IRG resistance GTPases in the cellular response to Toxoplasma gondii. Embry J 27: 2495–2509.
20. Papic N, Hunn JP, Pavadovski N, Zerrahn J, Howard JC (2008) Inactive and active states of the interferon-inducible resistance GTPase, Irg6, in vivo. J Biol Chem 283: 32143–32151.

21. Martens S, Parvanova I, Zerrahn J, Griffiths G, Schell G, et al. (2005) Disruption of Toxoplasma gondii Parasitophorous Vacuoles by the Mouse p17-Resistance GTPases. PLoS Pathog 1: e24. doi:10.1371/journal.ppat.0010024.

22. Carlow DA, Teh SJ, Teh HS (1998) Specific antiviral activity demonstrated by TGTP, a member of a new family of interferon-induced GTPases. J Immunol 161: 2348–2353.

23. Advani AS, Dressman HK, Quiroz M, Taylor GA, Pendergast AM (2004) Elevated expression of a subset of interferon inducible genes in primary bone marrow cells expressing p185 Bcr-Abl versus p210 Bcr-Abl by DNA microarray analysis. Leuk Res 28: 285–294.

24. Terskikh AV, Easterday MC, Li I, Hood L, Kornblum HI, et al. (2001) From hematopoiesis to neuropoiesis: evidence of overlapping genetic programs. Proc Natl Acad Sci U S A 98: 7934–7939.

25. Venezia TA, Merchant AA, Ramos CA, Whitehouse NL, Young AS, et al. (2004) Molecular signatures of proliferation and quiescence in hematopoietic stem cells. PLoS Biol 2: e301. doi:10.1371/journal.pbio.0020301.

26. Bence NF, Sampat RM, Kopito RR (2001) Impairment of the ubiquitin-proteasome system by protein aggregation. Science 292: 1532–1535.

27. Martens S, Sabel K, Lange R, Uthaiah R, Wolf E, et al. (2004) Mechanisms Regulating the Positioning of Mouse p47 Resistance GTPases LRG-47 and IIGP1 on Cellular Membranes: Retargeting to Plasma Membrane Induced by Phagocytosis. J Immunol 173: 2594–2606.

28. Zhao YO, Koenen-Waisman S, Taylor GA, Martens S, Howard JC (2010) Localisation and mislocalisation of the interferon-inducible immunity-related GTPase, Irgm1 (LRG-47) in mouse cells. PLoS ONE 5: e8648. doi:10.1371/journal.pone.0008648.

29. Taylor GA, Stauber R, Rulong S, Hudson E, Pri V, et al. (1997) The inducibly expressed GTPase localizes to the endoplasmic reticulum, independently of GTP binding. J Biol Chem 272: 10639–10645.

30. Zhao YO, Khaminets A, Hunn JP, Howard JC (2009) Disruption of the Toxoplasma gondii parasitophorous vacuole by IFNgamma-inducible immunity-related GTPases (IRG proteins) triggers necrotic cell death. PLoS Pathog 5: e1000281. doi:10.1371/journal.ppat.1000281.

31. Bernstein-Hanley I, Coers J, Balsara ZR, Taylor GA, Starnbach MN, et al. (2006) The p47 GTPases Iqtp and Irgb10 map to the Chlamydia trachomatis susceptibility locus Cirq3 and mediate cellular resistance in mice. Proc Natl Acad Sci U S A 103: 14992–14997.

32. Henry SC, Traver M, Daniel X, Indaram M, Oliver T, et al. (2010) Regulation of macrophage motility by Irgm1. J Leukoc Biol 87: 333–343.

33. Rubinstein DC (2006) The roles of intracellular protein-degradation pathways in neurodegeneration. Nature 443: 780–786.

34. Biron CA, Nguyen KB, Pen GC, Cousens LP, Salazar-Mather TP (1999) Natural killer cells in antiviral defense: function and regulation by innate cytokines. Annu Rev Immunol 17: 189–220.

35. Yokoyama WM, Kim S, French AR (2004) The dynamic life of natural killer cells. Annu Rev Immunol 22: 405–429.

36. Twarri S, Choi HP, Matsuzawa T, Pypaert M, MacMicking JD (2009) Targeting of the GTPase Irgm1 to the phagosomal membrane via PtdIns(3,4,5)P(3) promotes immunity to mycobacteria. Nat Immunol 10: 907–917.