Interferon-induced GTPases orchestrate host cell-autonomous defence against bacterial pathogens

Heike L. Rafeld1,2,3, Waldemar Kolanus3, Ian R. van Driel4 and Elizabeth L. Hartland1,5

1Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Clayton, Victoria, Australia; 2Department of Microbiology and Immunology, University of Melbourne at the Peter Doherty Institute for Infection and Immunity, Melbourne, Victoria, Australia; 3Life and Medical Sciences Institute (LIMES), Molecular Immunology and Cell Biology, University of Bonn, Bonn, Germany; 4Department of Biochemistry and Pharmacology, Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Melbourne, Victoria, Australia; 5Department of Molecular and Translational Science, Monash University, Clayton, Victoria, Australia

Correspondence: Ian R. van Driel (i.vandriel@unimelb.edu.au) or Elizabeth L. Hartland (elizabeth.hartland@hudson.org.au)

Interferon (IFN)-induced guanosine triphosphate hydrolysing enzymes (GTPases) have been identified as cornerstones of IFN-mediated cell-autonomous defence. Upon IFN stimulation, these GTPases are highly expressed in various host cells, where they orchestrate anti-microbial activities against a diverse range of pathogens such as bacteria, protozoan and viruses. IFN-induced GTPases have been shown to interact with various host pathways and proteins mediating pathogen control via inflammasome activation, destabilising pathogen compartments and membranes, orchestrating destruction via autophagy and the production of reactive oxygen species as well as inhibiting pathogen mobility. In this mini-review, we provide an update on how the IFN-induced GTPases target pathogens and mediate host defence, emphasising findings on protection against bacterial pathogens.

IFN signalling and induction of IFN-stimulated genes

Exposure of cells to interferons (IFN) results in the induction of a network of genes that combat infections, leading to so-called IFN-mediated cell-autonomous defence [1–5]. This network is a finely tuned mechanism to balance launching an efficient pathogen control while preventing collateral tissue damage. In the last two decades, IFN-induced GTPases have become a focus of attention as key mediators of IFN-mediated host defence.

There is abundant evidence for the vital role of IFN in combating an array of pathogens, including key roles in defence against bacteria [2,6–22]. Ten mammalian IFNs are known, with seven found in humans [23,24]. Based on genetic loci, homology in amino acid sequence and receptor binding, IFNs are currently divided into three groups, namely type I, II and III [1,25].

Upon binding their specific receptors, IFNs activate signal transduction via the JAK/STAT pathway which leads to the formation of the transcription factor complex IFN-stimulated gene factor 3 (ISGF3), consisting of phosphorylated STAT1/STAT2 and IRF9, for type I and type III IFNs and the transcription factor gamma-activated factor (GAF), a homodimer of phosphorylated STAT1, for type II IFN-specific signalling [26–28]. These activated transcription factors translocate into the nucleus and bind to their specific promoter elements, IFN-stimulated response element (ISRE) and gamma-activated sequence (GAS) for type I/III and type II, respectively [26–28]. The binding of these transcription factors can modulate the transcription of up to 2000 IFN-stimulated genes (ISGs) [1–3], resulting in immunomodulatory, anti-proliferative and anti-pathogenic consequences [2,29]. Even though these ISGs possess distinct receptors, transcription factors and promotor binding sites, the activation of ISGs via IFNs is complex. All types of IFN show non-canonical signalling, some ISGs are also controlled by IFN regulatory factors (IRFs); which in turn are also ISGs; other ISGs are constitutively expressed at low levels in addition to being IFN-inducible and another portion ISGs are also induced by NF-κB signalling [28,30–35].
Families of IFN-induced GTPases

GTPases induced by IFN have been identified as crucial effectors in IFN-mediated pathogen control [36–56]. These large GTPases can be divided into four subfamilies based on their paralogy and molecular mass [57]. The four subfamilies are the 21–47 kDa immunity-related GTPases (IRGs), the 65–73 kDa guanylate-binding proteins (GBPs), the 72–82 kDa myxoma (MX) resistance proteins and the 200–285 kDa very large inducible GTPases (VLIGs/GVINs) [58–60]. In the following, we will mainly focus on IRG and GBP GTPases and their functions in cell-autonomous defence against bacteria.

Mice have 23 IRGs and this family of genes has been mostly lost in humans, apart from IRGM1 and IRGC [61,62]. The IRGs can be divided into two classes; the primarily cytosolic ‘GKS’ IRGs which possess a conserved canonical GX4GKS sequence in the first nucleotide-binding motif (G1) and the predominantly membrane-bound 'GMS' IRGs which possess the non-canonical GX4GMS sequence in their G1 nucleotide-binding motif [60,61]. The ‘GMS’ IRGs control the activity of ‘GKS’ IRGs by controlling the GDP to GTP switch, thus acting as guanosine dissociation inhibitors (GDIs) [59,63]. In the absence of ‘GMS’ IRGs, ‘GKS’ IRGs are constitutively active, form cytoplasmic aggregates and fail to localise to their respective cellular compartment, Toxoplasma gondii parasitophorous vacuole and Chlamydia trachomatis inclusions [59,63,64].

Thus far, 7 human GBP (hGBP) genes (GBP1–GBP7) located on chromosome 1 and 11 mouse GBPs (mGBPs) (Gbp2b-Gbp11) have been identified [49,65–67]. The mGBPs are organised in clusters on chromosome 3 (Gbp2b, Gbp2, Gbp3, Gbp5, Gbp7 and a pseudoglob Gbp2b) and chromosome 5 (Gbp4, Gbp6, Gbp8, Gbp9, Gbp10, Gbp11 and a pseudoglob Gbp2) [68]. Transcription of human and mGBPs can be triggered by type 1 and 2 IFN as well as other inflammatory cytokines and TLR ligands, although the quantitative responses vary substantially between the different GBPs and cytokines [49,69,70].

IFN-induced GTPases belong to the dynamin-protein family as judged by structural similarities and shared biochemical characteristics [57,71,72]. As members of the dynamin protein family, they possess a large GTPase domain (∼300 amino acids), a middle domain and a GTPase effector domain (GED) [73]. In addition to these three domains, many IFN-induced GTPases also possess other domains and motifs for protein–protein and protein–membrane interactions [44,73–75]. In contrast with dynamin, at least some IFN-induced GBPs can hydrolyse GTP to GDP and GDP to GMP, though their GTPase activation is still dependent on oligomerisation [73,76,77]. These dynamin-related characteristics enable IFN-induced GTPases to operate either as mechanoenzymes or as an assembly platform to coordinate diverse functions [57]. For instance, they govern vesicular trafficking and the coordination of protein complex assembly to stimulate autophagic, membranolytic, oxidative and inflammasome-related anti-microbial activities upon cytosolic bacteria as well as on pathogen containing vacuoles [2,57,78–81].

Mechanisms of host defence by IFN-induced GTPases

Targeting of specific pathogens by GBPs and IRGs

To execute anti-microbial functions, GBPs and IRGs co-localise with pathogens invading the host cell. GBPs and IRGs are typically found in the cytosol, in vesicle-like structures and on endomembranes, but translocate to pathogen compartments and cytosolic bacteria which have escaped from the phagosome (Figure 1) [70,75,82,83]. Bacteria shown to interact with GBPs and IRGs include Listeria monocytogenes, Legionella pneumophila, Shigella flexneri, Mycobacterium bovis BCG, Chlamydia trachomatis, Francisella novicida, Salmonella typhimurium, Brucella abortus, Yersinia pseudotuberculosis and Burkholderia thailandensis [2,38–40,42–44,47,56,65,69,84–96].

Even though the exact molecular mechanism which enables them to target and destroy pathogens is not fully understood, it has been shown that human and mGBPs form homo- and hetero- polymers to fulfil their anti-microbial function [50,97]. Kravets et al. [50] showed that mGBPs accumulate on T. gondii vacuoles in densely packed multimers consisting of several thousand monomers. Furthermore, these proteins seem to locate to the pathogen and associated membranes in a hierarchical manner, with GBP1, GBP2 and GBP5 leading the way due to a CaaX prenylation motif at the C-terminus of the protein, which enables them to bind to membranes and to recruit non-prenylated GBPs to their location [50,82]. In addition to targeting various pathogens and their vacuoles directly, in mouse cells ‘GMS’ IRGs have been suggested to influence the localisation and activation of GBPs and ‘GKS’ IRGs on target membranes via a ‘missing-self’ signal [64]. This control of GBP and IRG activation and aggregation on host membranes via the ‘GMS’ IRG family proteins (IRGM), is further supported by the targeting of GBPs and IRGs onto lipid droplets from which IRGM1 and IRGM3 have been removed independently of infection [64]. Based on this observation, it was suggested that a lack of IRGM proteins and therefore the mistargeting of self-membranes through activated GBPs and IRGs as well as the
formation of cytosolic clusters leads to a diminished pool of available GBPs and IRGs which could effectively target *C. trachomatis* and *T. gondii* [64]. It should be noted that there is some data that is not consistent with the ‘missing-self’ hypothesis [37,44,67], although some of this was refuted in later publications [98,99].

Park et al. [100] proposed a ‘triple check’ model for targeting of mGBPs and IRGs to pathogen vacuoles. This model suggests that pathogen vacuoles are targeted by the autophagy conjugation system by depositing microtubule-associated protein light chain 3 (LC3) and its homologues on the pathogen vacuole. IFN-γ stimulation would ‘trigger’ LC3 on these membranes, either via posttranslational modifications or via the addition of factors such as ubiquitin, to act as a guanine nucleotide exchange factor (GEF) for GBPs and IRGs and activate them. Misguiding of GBPs to endomembranes would be avoided through the protective function of IRGM proteins, which act as GDIs for GBPs and IRGs [63,101]. How the LC3 conjugation system recognises pathogen vacuoles remains unknown. However, Brown et al. [102] have suggested that the autophagy conjugation...
complex or some upstream sensor of this complex recognises changes to the membranes occupied by pathogens, such as missing-self (e.g. lack of IRGM proteins), changed-self (e.g. rearranged protein and lipid composition) and non-self (e.g. pathogen effectors and secretion systems). It was also suggested that the binding of this complex to membranes might be facilitated via autophagy related-protein ATG5 [103], as ATG5 from the autophagy conjugation complex can bind membranes via an unknown lipid moiety [104]. This model is supported by the observations that ATG5 and LC3 are found on murine norovirus (MNV) membranous replication complexes [105] and also T. gondii vacuoles without prior IFN-γ stimulation in mouse macrophages [100,106]. Furthermore, GBPs and IRGs are unable to target pathogen containing vacuoles and aggregate in the cytosol in cells lacking all ATG5 or all LC3 homologues [102,106,107]. Whether this model applies to other pathogens and host species, especially with humans which lack most IRGs, remains to be investigated.

To what extent IRGs and GBPs co-operate in targeted co-localisation to pathogens or pathogen vacuoles remains unclear, as reciprocal dependence has been observed. For example, IRGM1 and 3 are needed for targeting of mGBPs to T. gondii vacuoles and pathogen control in MEFs, whereas they are dispensable for Leishmania donovani control [41,52]. On the other hand, the localisation of IRGs can also be dependent on GBPs, as the targeting of IRGB10 and IRGB6, to F. novicida, T. gondii and E. coli are dependent on GBPs from chromosome 3, as these IRG failed to co-localise with pathogen inclusions in cells lacking GBPs on mouse chromosome 3 [39,108].

Different GBPs and IRGs have been shown to target specific pathogens, though the underlying mechanisms for this specificity is only now being uncovered [42,52,65,94,109]. Kohler et al. [89] have suggested that changes in the C-terminal polybasic motif (PBM) in primate GBP1s are responsible for the pathogen specificity towards S. flexneri. In line with this, it was shown that the unique triple-arginine cassette in the PBM of hGBP1 is responsible for targeting S. flexneri [43]. The highly divergent C-terminal amino acid sequence in mGBPs might also indicate a non-redundant function in determining pathogen specificity [49]. In addition, alternative splicing variants of GBPs might play a role in specific pathogen targeting, since a splicing variant of mGBP5, mGBP5a, was present in L. monocytogenes infected mouse liver but absent from T. gondii infected liver [49]. Besides the co-localisation of GBPs and IRGs with particular pathogens, differences in GBP targeting of the same pathogens have also been observed in distinct cell types of the same host species. For example, hGBP1 co-localises with T. gondii in mesenchymal stromal cells and THP1 but not A549 cells [69,96,110,111]. This remarkable diversity of targeting strategies for specific pathogens might be due to the diverse genetic backgrounds and proteomes of different host species and cell types as well as pathogen-specific virulence factors and intracellular life cycles.

**Mechanisms of pathogen clearance by IFN-induced GTPases**

The anti-microbial mechanisms of IFN-induced GTPases that are discussed below are represented in Figure 1.

**Ubiquitination and lysosomal destruction mediated by GBPs and IRGs**

GBPs and IRGs can mediate pathogen control by induction of autophagy and ubiquitin-mediated destruction of pathogen vacuoles [112-114]. mGBP7 interacts with and recruits the autophagy protein ATG4B to *Mycobacterium*-containing vacuoles [65], which promotes the expansion of autophagic membranes around the bacteria and damaged bacterial compartments [2,57], leading to degradation of the pathogen via lysosome fusion [65]. Haldar et al. [41] demonstrated that IFN-γ-induced IRGM1 and IRGM3 control the recruitment of the E3 ligase tumour necrosis factor receptor-associated factor 6 (TRAF6) and subsequent ubiquitination of vacuoles of *T. gondii* and *C. trachomatis*. Following ubiquitination, GBPs co-localise with vacuoles in a sequestosome 1 (SQSTM1/p62)-dependent manner and mark these vacuoles for destruction [41]. IRGM-dependent autophagy was also shown for *Mycobacterium* infections though the exact mechanism remains unclear [45,114]. It seems likely that ‘GMS’ proteins IRGM1 and IRGM3 co-ordinate the localisation of other GKS IRGs to pathogen vacuoles, as virulent *T. gondii* strains and *C. muridarum* inhibit ‘GKS’ IRG activity and vacuole co-localisation of these IRG proteins thereby avoiding ubiquitination of the replicative niche [41,115-117].

In addition to mediating the ubiquitination of pathogen compartments and the subsequent lysosomal destruction via controlling the ‘GKS’ IRGs activity, IRGM1 has been shown to target *M. tuberculosis* vacuoles directly [37,65]. The recruitment of IRGM1 to pathogen containing vacuoles appears to facilitate fusion with lysosomes, as lysosomal fusion of *M. tuberculosis* vacuoles is impaired in *Irgm1*-deficient mutants [37]. The C-terminal amphipathic helix (αK) of IRGM1 binds to *Mycobacterium* vacuoles by interaction with phosphoinositide-3,4-bisphosphate (PtdIns[3,4]P2) and PtdIns[3,4,5]P3 [44].
For protection against the lung pathogen *L. pneumophila*, IRG-dependent as well as IRG-independent pathways have been described. Both IRGM1 and IRGM3, have been implicated in IFN-mediated control of *L. pneumophila* [9,36]. The binding of IRGM1 to the intracellular replicative niche of *L. pneumophila*, the Legionella-containing vacuole (LCV), results in the co-localisation of other IRG proteins and subsequent ubiquitination of the LCV, thereby leading to LCV degradation through autophagy [4]. GBP1 and GBP2 are involved in an IRGM-independent resistance against *L. pneumophila*, as the bacterial protein secretion system on the LCV is recognised as a PAMP, leading to binding of the cytosolic carbohydrate-binding protein galectin-3. The binding of galectin-3 to the LCV recruits GBP1 and GBP2 to the LCV, as well as subsequent ubiquitination and targeting by p62, which leads to the degradation of the bacteria via autophagy [93]. This IRGM-independent and GBP-dependent ubiquitination during Legionella infection is in contrast with the previously mentioned IRGM-dependent and GBP-independent ubiquitination of *T. gondii* vacuoles as well as *C. trachomatis* inclusions [41].

In most cases, the ubiquitination of pathogens and their compartments is a host-derived response which favours host survival and promotes pathogen control. In contrast with this, it was shown that the hGBP1-mediated, poly-ubiquitin coat on *S. flexneri* is not host-derived but mediated by a bacterial-derived E3 ubiquitin ligase IpaH9.8, which recognises, binds and ubiquitinates GBP1, GBP2 and GBP4 but not GBP3 and labels them for proteasome-mediated degradation [42,43,90]. This poly-ubiquitination reverses the GBP-mediated restriction and enables the bacteria to form actin tails and spread efficiently from cell to cell [42,43,90].

**GBP-mediated production of reactive oxygen species (ROS)**

Another host resistance pathway that mediates IFN-induced pathogen control is the production of ROS. NOX2 is an NADPH oxidase that is able to generate superoxide, which has microbicidal properties [118]. During *L. monocytogenes* and *M. bovis* BCG infection, mGBP7 binds the membrane-bound heterodimer gp91phox-p22phox (cytochrome b558) and cytosolic p67phox [65]. Thus, GBP7 acts as a linker between membrane-bound and cytosolic NOX2 components to assemble and activate the NOX2 holoenzyme on pathogen compartments after IFN-γ stimulation [65].

**GBP-mediated inflammasome activation**

Recent work has linked IFN-induced GTPases with inflammasome activation in various host cells and in response to a diverse range of pathogens. IFN-induced GTPases appear to influence inflammasome activation by promoting inflammasome complex assembly and targeting pathogens and their compartments to increase the access of PAMPs to cytosolic inflammasome components. These two mechanisms of inflammasome activation can work in concert to achieve adequate inflammasome activation and thus host defence.

GBP5 is involved in the assembly of the NOD-, LRR- and pyrin domain-containing 3 (NLRP3) inflammasome during *Listeria* or *Salmonella* spp. infections via tetramerisation of GBP5 [119-121]. Assembly of the NLRP3 inflammasome leads to the induction of pyroptosis in order to control bacterial infections. Deletion of single mGBPs from chromosome 3 revealed unique functions for GBPs; namely, GBP5 binding to the pyrin domain of NLRP3 and GBP2 binding of apoptosis-associated speck-like protein containing a CARD (ASC) [121]. Due to their ability to form heterodimers, GBP2 and GBP5 thus facilitate the assembly of the NLRP3 inflammasome to activate caspase-1 [121].

Several observations have shown that GBPs activate inflammasomes by either directly sensing bacterial products or facilitating access to bacterial PAMPs. The induction of inflammasomes via GBPs and IRGs can result in canonical (caspase-1) or non-canonical (caspase-11, human caspase-4/5) mediated pyroptosis. It was shown that GBPs from mouse chromosome 3, namely GBP2, GBP5, as well as IRG10 are essential for the activation of the AIM2 inflammasome in *F. novicida* infected macrophages, as cells lacking these IFN-induced GTPases showed decreased inflammasome activation [39,87,88]. GBPs from chromosome 3 also control the non-canonical activation of caspase-11 in response to *L. pneumophila* as well as pathogenic and non-pathogenic *E. coli* outer membrane vesicles and free LPS injected into the cytosol [36,122,123]. GBPs from chromosome 3 were also essential for caspase-1 activation and IL1-α as well as IL-1β release in response to *B. abortus* and *Y. pseudotuberculosis* infections [47,92]. It has been suggested that GBPs might be influencing the membrane dynamics of outer membrane vesicles and the integrity of pathogen membranes due to their dynamin-like activities thus exposing lipid A of LPS and other PAMPs to the cytosol, hence making them accessible for inflammasome activation [39,122,124].
In line with these previous observations suggesting GBPs mediate LPS release and/or recognition by the inflammasome, hGBP1 was recently identified as a novel cytosolic LPS sensor [69,94-96]. hGBP1 binds to LPS via electrostatic interactions between the negatively charged LPS and positively charged amino acid residues of hGBP1 [94,97]. Detection of LPS via hGBP1 results in the recruitment of hGBP2-4 to cytosolic Salmonella, this GBP coat in turn recruits and activates caspase-4 [69,94,95]. Based on these observations and their own, Kutsch et al. [97] presented a model of hGBP1 acting as a detergent on the bacterial LPS layer. hGBP1 was identified as a LPS sensing and binding protein, which disrupts the O-antigen barrier of Gram-negative bacteria through the insertion of the farnesyl tail of hGBP1 molecules into this layer, thereby disrupting the interactions between LPS molecules mediated by the O-antigens [97]. A triple-arginine motif in the C-terminal end of GBP1 mediates the binding of hGBP1 to the pathogen LPS O-antigen [43]. The insertion of hGBP1 into the LPS layer seemingly changes the membrane stiffness and fluidity, thus making the bacteria more accessible to caspase-4 activation and more susceptible to the anti-microbial activity of polymyxin B, as well as potentially influencing the function of other pathogen proteins inserted into the outer membrane such as Shigella IcsA [97].

Using different GBP1 catalytic mutants, Xavier et al. [125] identified a novel pathway of NLRP3 activation mediated by hGBP1. This group discovered that hGBP1 recruitment to C. trachomatis inclusions activates GTP hydrolysis to GMP and the subsequent generation of uric acid activates the NLRP3 inflammasome [125]. This novel pathway suggests that, in contrast with previous findings [69,94–97], inflammasome activation can be independent of PAMP release in human cells, relying only on the hydrolytic activity of hGBP1 [125]. Whether this activation is unique to the Chlamydia inclusion or represents a more general response towards other pathogens, remains to be investigated.

**IFN-induced GTPases and actin-based motility**

Recent findings have demonstrated that IFN-induced GBPs can inhibit the actin-based motility of intracellular bacteria. hGBPs target cytosolic S. flexneri after IFN-γ exposure and interfere with actin tail formation, which is required for cytosolic mobility and cell to cell spread [42,43]. GBP1 is essential for IFN-γ mediated inhibition of actin tail formation as well as recruitment of GBP2, 3 and 4 to the pathogen [42]. GBP-mediated inhibition of actin tails hindered the bacteria from spreading efficiently from cell to cell and resulted in large microcolonies forming in infected cells but significantly fewer cells becoming infected [42]. In addition to S. flexneri, hGBP1 also targets B. thailandensis through a C-terminal triple-arginine motif that binds O-antigen [43]. mGBPs also inhibit the formation of actin tails and the formation of multinucleated giant cells (MNGCs) during B. thailandensis infection by interfering with Arp2/3-mediated actin nucleation and cytoskeletal remodelling [86]. Cells lacking multiple GBPs from chromosome 3 as well as Gbp2−/− and Gbp5−/− cells showed an increased number of MNGCs and increased bacterial load [86].

**Perspectives**

**Importance of the field:** IFN-induced GTPases play a significant role in cell-autonomous defence against a wide variety of pathogens. They initiate and regulate a diverse range of host defence pathways and an appreciation of the roles of IFN-induced GTPases in host defence could lead to more effective anti-microbial treatments.

**Current thinking:** Individual IFN-induced GTPases possess unique functions that tailor the response to different pathogens and mediate their anti-microbial function by compromising the integrity of pathogen-related membranes, releasing PAMPS into the cytosol, inducing bactericidal small molecules, marking pathogens for destruction or inhibiting pathogen mobility.

**Future directions:** Identifying GTPase binding partners that mediate their specific function and regulate their activities, will be crucial in enhancing our understanding of how these GTPases mediate IFN-induced cell-autonomous defence against various pathogens.
Conflicts of interest
The authors have no conflicts of interest.

Competing Interests
The authors declare that there are no competing interests associated with the manuscript.

Funding
ELH and IVD are supported by the National Health and Medical Research Council of Australia APP1145244. This work was supported by DFG IRTG 2168. WK is funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany’s Excellence Strategy EXC2151 – 390873048.

Open Access
Open access for this article was enabled by the participation of the University of Melbourne in an all-inclusive Read & Publish pilot with Portland Press and the Biochemical Society under a transformative agreement with CAUL.

Author Contributions
Conception: H.L.R., I.V.D., E.L.H. Drafting: H.L.R., I.V.D., E.L.H., Revising and critiquing: H.L.R., W.K., I.V.D., ELH. Funding: W.K., I.V.D., E.L.H. All authors give final approval of the version to be published and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Abbreviations
GBPs, guanylate-binding proteins; GDIs, guanosine dissociation inhibitors; hGBP, human GBP; IFN, interferon; IRGs, immunity-related GTPases; ISGs, IFN-stimulated genes; LC3, microtubule-associated protein light chain 3; LCV, Legionella-containing vacuole; mGBPs, mouse GBP; MNGCs, multinucleated giant cells; PBM, polybasic motif; ROS, reactive oxygen species.

References
1 Rusinova, I., Forster, S., Yu, S., Kannan, A., Masse, M., Cumming, H. et al. (2013) INTERFEROME v2.0: an updated database of annotated interferon-regulated genes. Nucleic Acids Res. 41, D1040–D1046 https://doi.org/10.1093/nar/gks1215
2 MacMicking, J.D. (2012) Interferon-inducible effector mechanisms in cell-autonomous immunity. Nat. Rev. Immunol. 13, 367–382 https://doi.org/10.1038/nri3210
3 Shaw, A.E., Hughes, J., Gu, Q., Behdenna, A., Singer, J.B., Dennis, T. et al. (2017) Fundamental properties of the mammalian innate immune system revealed by multispecies comparison of type I interferon responses. PLoS Biol. 15, e2004086 https://doi.org/10.1371/journal.pbio.2004086
4 Pilla-Moffett, D., Barber, M.F., Taylor, G.A. and Coers, J. (2016) Interferon-inducible GTPases in host resistance, in Legionella, 3495–3515 https://doi.org/10.1016/j.jmb.2016.04.032
5 Huang, S., Meng, Q., Maminska, A. and MacMicking, J.D. (2019) Cell-autonomous immunity by IFN-induced GBPs in animals and plants. Nat. Rev. Immunol. 20, 67–80 https://doi.org/10.1038/s41568-019-0147-8
6 Lu, B., Ebensperger, C., Dembic, Z., Wang, Y., Kvatyuk, M., Lu, T. et al. (1998) Targeted disruption of the interferon-gamma receptor 2 gene results in severe immune defects in mice. Proc. Natl Acad. Sci. U.S.A. 95, 8233–8238 https://doi.org/10.1073/pnas.95.14.8233
7 Carrero, J.A. (2013) Confounding roles for type I interferons during bacterial and viral pathogenesis. Int. Immunol. 25, 663–669 https://doi.org/10.1093/intimm/dxt050
8 Banks, D.A., Ahlbrand, S.E., Hughitt, V.K., Shah, S., Mayer-Barber, K.D., Vogel, S.N. et al. (2019) Mycobacterium tuberculosis inhibits autocrine type I IFN signaling to increase intracellular survival. J. Immunol. 202, 2348–2359 https://doi.org/10.4049/jimmunol.1801303
9 Lipphardt, D., Müller, H.C., Naujoks, J., Tabeling, C., Shih, S., Witzenrath, M. et al. (2011) Dissection of a type I interferon pathway in controlling bacterial intracellular infection in mice. Cell. Microbiol. 13, 1668–1682 https://doi.org/10.1111/j.1462-5822.2011.01646.x
10 Oztürk, B., Vinczeng, M., Van Laak, V., Schneek, B., Heine, G., Günther, S. et al. (2006) Legionella pneumophila induces IFNβ in lung epithelial cells via IPS-1 and RIG3, which also control bacterial replication. J. Biol. Chem. 281, 36173–36179 https://doi.org/10.1074/jbc.M604638200
11 Flynn, J.J., Chan, J., Treibold, K.J., Dalton, D.K., Stewart, T.A. and Bloom, B.R. (1993) An essential role for interferon gamma in resistance to Mycobacterium tuberculosis infection. J. Exp. Med. 178, 2249–2254 https://doi.org/10.1084/jem.178.6.2249
12 Huang, S., Hendriks, W., Alttrage, A., Hemmi, S., Bluethmann, H., Kamijo, R. et al. (1993) Immune response in mice that lack the interferon-gamma receptor. Science 259, 1742–1745 https://doi.org/10.1126/science.8456301
13 Dussurget, O., Bieme, H. and Cossart, P. (2014) The bacterial pathogen Listeria monocytogenes and the interferon family: type I, type II and type III interferons. Front. Cell Infect. Microbiol. 4, 50 https://doi.org/10.3389/fcimb.2014.00050
14 Norazmi, M.N. (2017) Interferon-β controls non-tuberculous mycobacterial infection in mice. Virulence 8, 1085–1087 https://doi.org/10.1080/21505594.2017.1341035
15 Shtrichman, R. and Samuel, C.E. (2001) Review: the role of gamma interferon in antimicrobial immunity. Curr. Opin. Microbiol. 4, 251–259 https://doi.org/10.1016/S1369-5274(00)00199-5
© 2021 The Author(s). This is an open access article published by Portland Press Limited on behalf of the Biochemical Society and distributed under the Creative Commons Attribution License 4.0 (CC BY-NC-ND).
109 Jayakumar, A., Donovan, M.J., Tripathi, V., Ramahlo-Ortigao, M. and McDowell, M.A. (2008) Leishmaniasia major infection activates NF-kappaB and interferon regulatory factors 1 and 8 in human dendritic cells. *Infect. Immun.* **76**, 2138–2148. https://doi.org/10.1128/IAI.01252-07

110 Qin, A., Lai, D.H., Liu, Q., Huang, W., Wu, Y.P., Chen, X. et al. (2017) Guanylate-binding protein 1 (GBP1) contributes to the immunity of human mesenchymal stromal cells against *Toxoplasma gondii*. *Proc. Natl Acad. Sci. U.S.A.* **114**, 1365–1370. https://doi.org/10.1073/pnas.1619665114

111 Johnston, A.C., Piro, A., Clough, B., Siew, M., Vireire Winter, S., Coers, J. et al. (2016) Human GBP1 does not localize to pathogen vacuoles but restricts *Toxoplasma gondii*. *Cell Microbiol.* **18**, 1056–1064. https://doi.org/10.1111/cmi.12579

112 Alonso, S., Pethe, K., Russell, D.G. and Purdy, G.E. (2007) Lysosomal killing of *Mycobacterium* mediated by ubiquitin-derived peptides is enhanced by autophagy. *Proc. Natl Acad. Sci. U.S.A.* **104**, 6031–6036. https://doi.org/10.1073/pnas.0700936104

113 Ponpuak, M., Davis, A.S., Roberts, E.A., Delgado, M.A., Dinkins, C., Zhao, Z. et al. (2010) Delivery of cytosolic components by autophagic adaptor protein p62 endows autophagosomes with unique antimicrobial properties. *Immunity* **32**, 329–341. https://doi.org/10.1016/j.immuni.2010.02.009

114 Gutierrez, M.G., Master, S.S., Singh, S.B., Taylor, G.A., Cokombo, M.I. and Deretic, V. (2004) Autophagy is a defense mechanism inhibiting *B*CG and *Mycobacterium tuberculosis* survival in infected macrophages. *Cell* **119**, 753–766. https://doi.org/10.1016/j.cell.2004.11.038

115 Coers, J., Bernstein-Hanley, I., Grotsky, D., Parvanova, I., Howard, J.C., Taylor, G.A. et al. (2008) Human GBP1 does not localize to pathogen vacuoles but restricts *Toxoplasma gondii*. *Cell Microbiol.* **10**, 6237–6245. https://doi.org/10.1111/j.1462-5822.2008.01417.x

116 Zhao, Y., Ferguson, D.J., Wilson, D.C., Howard, J.C., Sibley, L.D. and Yap, G.S. (2009) Virulent *Toxoplasma gondii* evades immunity-related GTPase-mediated parasite vacuole disruption within primed macrophages. *J. Immunol.* **182**, 3775–3781. https://doi.org/10.4049/jimmunol.0804190

117 Haldar, A.K., Piro, A.S., Finethy, R., Espenschied, S.T., Brown, H.E., Giebel, A.M. et al. (2016) *Chlamydia trachomatis* is resistant to inclusion ubiquitination and associated host defense in gamma interferon-primed human epithelial cells. *mBio* **7**, e01417-16. https://doi.org/10.1128/mBio.01417-16

118 Yang, Y., Bazhin, A.V., Werner, J. and Karakhanova, S. (2013) Reactive oxygen species in the immune system. *Int. Rev. Immunol.* **32**, 249–270. https://doi.org/10.3109/08830185.2012.755176

119 Shenoy, A.R., Wellington, D.A., Kumar, P., Kassa, H., Booth, C.J., Cresswell, P. et al. (2012) GBP5 promotes NLRP3 inflammasome assembly and immunity in mammals. *Science* **336**, 481–485. https://doi.org/10.1126/science.1217141

120 Caffrey, D.R. and Fitzgerald, K.A. (2012) Immunology. Select inflammasome assembly. *Science* **336**, 420–421. https://doi.org/10.1126/science.1223862

121 Kim, B.H., Chee, J.D., Bradfield, C.J., Park, E.S., Kumar, P. and MacMicking, J.D. (2016) Interferon-induced guanylate-binding proteins in inflammasome activation and host defense. *Nat. Immunol.* **17**, 481–489. https://doi.org/10.1038/ni.3440

122 Finethy, R., Luoma, S., Orench-Rivera, N., Feeley, E.M., Haldar, A.K., Yamamoto, M. et al. (2017) Inflammasome activation by bacterial outer membrane vesicles requires guanylate binding proteins. *mBio* **8**, e01188-17. https://doi.org/10.1128/mBio.01188-17

123 Santos, J.C., Dick, M.S., Lagrange, B., Degrandi, D., Pfeffer, K., Yamamoto, M. et al. (2018) LPS targets host guanylate-binding proteins to the bacterial outer membrane for non-canonical inflammasome activation. *EMBO J.* **37**, e98089. https://doi.org/10.15252/embj.201798089

124 Liu, B.C., Sarhan, J., Panda, A., Muendlein, H.I., Ilyukha, V., Coers, J. et al. (2018) Constitutive interferon maintains GBP expression required for release of bacterial components upstream of pyroptosis and anti-DNA responses. *Cell Rep.* **24**, 155–168.e5. https://doi.org/10.1016/j.celrep.2018.06.012

125 Xavier, A., Al-Zeer, M.A., Meyer, T.F. and Daumke, O. (2020) hGBP1 coordinates chlamydia restriction and inflammasome activation through sequential GTP hydrolysis. *Cell Rep.* **31**, 107667. https://doi.org/10.1016/j.celrep.2020.107667