

MINI-REVIEW

Interferon-inducible guanylate-binding proteins at the interface of cell-autonomous immunity and inflammasome activation

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Summary sentence (25 max): Review of how the interferon-inducible GTPases called guanylate-binding proteins (GBPs) attack pathogens and activate inflammasomes, cell death and innate immunity.

Keywords (6 max): Caspase-1, caspase-11, pyroptosis, GBPs, bacteria, viruses

Word count: 3,460

ABBREVIATIONS

ASC, apoptosis-associated speck-like protein containing a caspase recruitment domain

BMDMs, Bone marrow-derived macrophages

CARD, caspase recruitment domain

CSFV, classical swine fever virus

EMCV, encephalomyocarditis virus

GBPs, guanylate-binding proteins

HCV, hepatitis C virus

IAV, influenza virus

IFN, interferons

IRGs, immunity-related GTPases

LPS, Lipopolysaccharide

NLRs, nucleotide-binding domain, leucine-rich repeat containing proteins

NS1, nonstructural protein 1

VSV, vesicular stomatitis virus

ABSTRACT

Guanylate-binding proteins (GBPs) are essential components of cell-autonomous immunity. In response to interferon signaling, GBPs are expressed in the cytoplasm of immune and non-immune cells, where they unleash their antimicrobial activity towards intracellular bacteria, viruses and parasites. Recent studies have revealed that GBPs are essential for mediating activation of the caspase-1 inflammasome in response to the Gram-negative bacteria *Salmonella enterica* serovar Typhimurium, *Francisella novicida*, *Chlamydia muridarum*, *Chlamydia trachomatis*, *Legionella pneumophila*, *Vibrio cholerae*, *Enterobacter cloacae* and *Citrobacter koseri*. During infection with vacuolar-restricted Gram-negative bacteria, GBPs disrupt the vacuolar membrane to assure liberation of LPS for cytoplasmic detection by caspase-11 and the non-canonical NLRP3 inflammasome. In response to certain cytosolic bacteria, GBPs liberate microbial DNA for activation of the DNA-sensing AIM2 inflammasome. GBPs also promote the recruitment of antimicrobial proteins, including NADPH oxidase subunits and autophagy-associated proteins to the *Mycobacterium*-containing vacuole to mediate intracellular bacterial killing. Here, we provide an overview on the emerging relationship between GBPs and activation of the inflammasome in innate immunity to microbial pathogens.

INTRODUCTION

Cell-autonomous immunity is a part of the innate immune system specialized in host defense against intracellular pathogens. Production of type I interferons (IFNs, including IFN- α subtypes and IFN- β) and type II IFN (IFN- γ) upon recognition of pathogens engages cell-autonomous immunity, which is required for restriction of microbial replication [1]. IFN signaling induced by pathogens results in the expression of hundreds of genes which contribute to innate and adaptive immune responses in both immune and non-immune cells [2, 3]. Many of the genes induced by IFNs encode potent effectors of cell-autonomous immunity, including the 65-67 kDa proteins called guanylate-binding proteins (GBPs). IFN- γ is a more potent inducer of GBPs than type I IFNs, and most of the work investigating cell-autonomous effects of GBPs to pathogens involves pre-activation of host cells with IFN- γ [4].

GBPs comprise a family of highly conserved IFN-inducible GTPases. They were originally identified as 67 kDa proteins in human and mouse cell lines treated with IFNs [5]. Two of these proteins were later isolated from IFN-treated human diploid fibroblastic cells and were found to have guanylate-binding activity [6]. The GBP family now consists of 7 human GBPs, 11 murine GBPs, and two mouse pseudogenes encoding GBPs (**Figure 1**) [4, 7-10]. While all human GBPs are located in a single cluster on chromosome 1, murine GBPs have been mapped to two chromosomal clusters, one on chromosome 3 and the other on chromosome 5 (**Figure 1**) [11, 12]. Genomic analysis of murine GBPs revealed the presence of *Gbp1*, *Gbp2*, *Gbp3*, *Gbp5*, *Gbp7* and one pseudogene on chromosome 3, whereas *Gbp4*, *Gbp6*, *Gbp8*, *Gbp9*, *Gbp10*, *Gbp11* and the second pseudogene are located on chromosome 5 [12].

GBPs are classified under the dynamin superfamily of GTPases based on their structural and biochemical properties [4, 13]. Similar to other dynamin family members, GBPs

undergo nucleotide-dependent oligomerization and can hydrolyze GTP to GDP and GMP [14-17]. Structural analysis of GBPs revealed the presence of an N-terminal globular GTP-binding domain (also known as the large G or LG domain) and a C-terminal α -helical GTPase effector domain [18]. These two domains are interconnected by a short middle region consisting of an α -helical domain and two β -sheets [18]. The amphipathic α -helices in the C-terminal region facilitate protein-protein and protein-lipid interactions [13]. In addition, the CaaX sequence ('aa' indicates two aliphatic residues and 'X' indicates any amino acid) at the C-terminus of murine and human GBP1, GBP2 and GBP5 promotes isoprenylation to mediate both protein-protein interactions and targeting of proteins to membranous compartments [4, 7, 19-21]. Regardless of protein modifications, GBPs are predominantly cytoplasmic (mouse GBP5) or within vesicle-like structures (mouse GBP1, GBP2, GBP3 and GBP6) in the absence of an infection [20-22]. The ability of GBPs to associate with other proteins and to anchor to membranes contributes to their role in pathogen restriction [3, 13, 23, 24]. For example, mouse GBP7 can recruit components of the NADPH oxidase subunits and the autophagy-associated protein ATG4B to the *Mycobacterium*-containing vacuole to promote bacterial killing [25].

Although characterized in the early 1980s, the function of GBPs in host defense has remained relatively obscure until now. Recent studies have unraveled a requirement for GBPs in cell-autonomous immunity against bacterial, viral and protozoan pathogens [13, 23, 26]. Moreover, emerging studies elucidated a specific role for GBPs in the activation of the inflammasome, an innate immune caspase-1-containing protein complex which drives the release of the proinflammatory cytokines IL-1 β and IL-18, and the induction of pyroptosis [27]. In this review, we provide an overview of the role of GBPs at the intersection of cell-autonomous immunity and activation of the inflammasome.

Emerging roles for GBPs in activation of the inflammasome

The inflammasome is a multi-protein innate immune complex composed of an inflammasome-initiating sensor, the adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC) and the cysteine protease caspase-1 [27]. Inflammasome-initiating sensors include members of the nucleotide-binding domain, leucine-rich repeat containing (NLRs; for example, NLRP1, NLRP3 and NLRC4), the pyrin and HIN domain-containing (also known as PYHIN, AIM2-like receptors or ALRs; for example, AIM2) or the TRIM (for example, pyrin) family [28]. Previous studies in the inflammasome field have identified a crucial role for type I IFN signaling in the activation of the AIM2 and NLRP3 inflammasomes in response to bacterial infection [29-34]. How type I IFN signaling regulated activation of these inflammasomes was unknown. Recent studies have now identified GBPs as a missing link between IFN signaling and activation of the inflammasome.

In 2012, Shenoy and colleagues searched protein databases and found GBP-like proteins in lower organisms *Danio* and *Branchiostoma*, which contain a caspase recruitment domain (CARD) resembling those of inflammasome proteins [35]. Based on this observation, they hypothesized that GBP proteins could have a role in activation of the inflammasome. They generated a mouse strain lacking GBP5 and demonstrated that this GBP was required for activation of the NLRP3 inflammasome in IFN- γ -primed mouse bone marrow-derived macrophages (BMDMs) treated with LPS and the NLRP3 activators ATP or nigericin (**Figure 2**) [35]. IFN- γ priming was not necessary, but potentiated activation of the NLRP3 inflammasome by LPS plus ATP [35].

The GBP5-dependent response appeared to be specific to ATP and nigericin because GBP5 was dispensable for inflammasome activation induced by the NLRP3 activators alum and monosodium urate, and the activator of the AIM2 inflammasome poly(dA:dT)

dsDNA, or the activator of the NLRC4 inflammasome, *Salmonella* flagellin [35]. Experiments using small hairpin RNAs and small interfering RNAs to knockdown the expression of GBP5 in a human macrophage cell line further validated the requirement for GBP5 in activation of the NLRP3 inflammasome in response to LPS plus ATP [35]. Using an overexpression system, the authors found that tetramerization of GBP5 promoted assembly of the NLRP3 inflammasome [35]. Tetramerized GBP5 mediated interaction between the GTPase domain of GBP5 and the pyrin domain of NLRP3 via an unknown mechanism [35].

Although this study demonstrated that GBP5 is involved in the activation of the NLRP3 inflammasome induced by soluble (ATP) but not crystalline (alum and monosodium urate) NLRP3 inflammasome activators, it is somewhat surprising that GBP5 is exclusively involved in the activation of the NLRP3 inflammasome induced by a subset of NLRP3 inflammasome activators. Phagosomal rupture and destabilization is a molecular hallmark by which crystalline substances engage activation of the NLRP3 inflammasome [36]. Owing to its ability to target cellular membranes, it is reasonable to speculate that GBP5 may also be involved in mediating rupture of phagosomes containing crystals. Nevertheless, the study by Shenoy and colleagues unveiled that the function of GBPs and inflammasomes could intersect in higher species to mediate innate immune responses.

Following the initial study by Shenoy et al., Meunier and colleagues generated two independent lines of GBP5-deficient mouse strains and found that the involvement of GBP5 in the activation of the NLRP3 inflammasome is not always apparent. They found that GBP5 was not required for activation of the NLRP3 inflammasome in LPS-primed BMDMs stimulated with ATP or nigericin [37]. Our lab was also unable to observe a requirement for GBP5 for the activation of the NLRP3 inflammasome in LPS-primed BMDMs stimulated with ATP [38]. Furthermore, analysis of inflammasome activation

performed by Meunier and colleagues using a mouse strain lacking GBPs encoded on chromosome 3 (GBP1, GBP2, GBP3, GBP5 and GBP7) also revealed that these GBPs are dispensable for the activation of the NLRP3 inflammasome induced by soluble or crystalline activators [37]. These observations are in line with a previous study showing no role for type I IFN signaling in the secretion of IL-1 β in BMDMs treated with LPS plus ATP [29], suggesting that GBPs – whose expression depends on type I IFN signaling – are dispensable in this process. Why the role for GBP5 in the activation of the canonical NLRP3 inflammasome induced by soluble activators is not consistently apparent has remained unclear. It is possible that prolonged priming by type I or type II IFNs, or LPS to induce robust levels of GBP5 is required to fully reveal its role for activation of the NLRP3 inflammasome by soluble NLRP3 inflammasome activators. A more likely explanation is offered by Pilla-Moffett and colleagues, who highlighted that the *Gbp5*^{-/-} mouse line used by Shenoy et al., was generated using 129 embryonic stem cells, resulting in a backcrossed C57BL/6 *Gbp5*^{-/-} mouse line carrying the 129 alleles of the adjacent *Gbp1*, *Gbp2*, *Gbp3*, and *Gbp7* genes [24]. The C57BL/6 *Gbp5*^{-/-} mouse lines used by Man et al. and Meunier et al. were generated using C57BL/6 embryonic stem cells, which carry the C57BL/6 alleles of *Gbp1*, *Gbp2*, *Gbp3*, and *Gbp7* genes [24]. Further studies are required to untangle the function of GBP5 in the activation of the canonical NLRP3 inflammasome.

GBPs and activation of the inflammasome by bacteria

In addition to GBP5, a role for other GBPs has also been identified in the activation of inflammasomes by intracellular bacteria. Intracellular bacteria have distinct lifestyles; some are vacuole-restricted, whereas others disrupt the vacuole and enter the cytoplasm for replication. Type I IFN signaling is essential for activation of the inflammasome by certain vacuolar-restricted and cytosolic bacteria [29, 32, 33].

Emerging studies have identified GBPs as a missing link between type I IFN signaling and activation of inflammasomes to certain pathogenic bacteria.

Vacuolar bacteria

In 2014, a study by Meunier and colleagues demonstrated that GBPs encoded on chromosome 3 are essential for activation of the caspase-11 non-canonical NLRP3 inflammasome in mouse BMDMs infected with the vacuolar-restricted Gram-negative bacteria *S. Typhimurium* mutant lacking the *Salmonella* pathogenicity island-2 type III secretion system (which preferentially activates caspase-11 rather than the NLRC4 inflammasome [39, 40]), *Vibrio cholerae*, *Enterobacter cloacae* and *Citrobacter koseri* [37]. Specifically, GBP2 is required for activation of the caspase-11-dependent NLRP3 inflammasome in response to these Gram-negative bacteria (**Figure 2**) [37]. In addition, Pillar and colleagues demonstrated the contribution from GBPs encoded on chromosome 3 in caspase-11-dependent pyroptosis in response to infection by the Gram-negative bacterium *Legionella pneumophila* [41]. Silencing of the expression of GBPs encoded on chromosome 3 by small hairpin RNAs revealed a partial role for GBP1, GBP2, GBP3 and GBP5 in driving pyroptosis induced by *L. pneumophila* [41]. A role for GBP2 was further confirmed in mouse BMDMs lacking GBP2 [41].

Further studies have found that GBPs encoded on chromosome 3 can partially promote pyroptosis in IFN- γ -primed BMDMs infected with the Gram-negative bacteria *C. trachomatis* or *C. muridarum* (**Figure 2**) [42]. GBPs encoded on chromosome 3 can also partially contribute to the secretion of IL-1 β and IL-18 in LPS-primed BMDMs infected with *C. trachomatis*, a pathogen which activates both the AIM2 and NLRP3 inflammasome [42].

The mechanism by which GBPs contribute to activation of the inflammasome by vacuolar-restricted bacteria – at least for *S. Typhimurium* – involves the ability of GBP2,

and to a smaller extent other GBPs on the chromosome 3 cluster, to target the pathogen-containing vacuole (**Figure 2**). BMDMs lacking GBPs encoded on chromosome 3 harbor less *S. Typhimurium* associated with galectin-8, a marker for lysed vacuoles, suggesting that GBP-mediated expulsion of the bacteria from the vacuole into the cytoplasm is the molecular basis for LPS release and sensing [37].

Interestingly, the autophagy marker LC3 was also recruited to *S. Typhimurium* in a GBP-dependent manner, however, pharmacological inhibition of autophagy or genetic deletion of ATG5 in BMDMs did not impair activation of the non-canonical NLRP3 inflammasome [37]. This finding suggests that autophagy is a downstream process independent of inflammasome signaling. This study by Meunier and colleagues provided the first mechanistic link to explain how GBPs govern activation of the non-canonical NLRP3 inflammasome by Gram-negative bacteria that are normally sequestered in the pathogen-containing vacuole.

Further development in the field has shown that inflammasome activation can occur independently of GBP recruitment to the pathogen. *C. muridarum* is resistant to cell-autonomous immunity mediated by GBPs and does not induce recruitment of GBPs to the pathogen-containing vacuole to drive activation of the inflammasomes (**Figure 2**) [42, 43]. How GBPs regulate inflammasome activation in this context requires further investigation.

In addition to GBP-mediated liberation of Gram-negative bacteria from the pathogen-containing vacuole, LPS can be delivered directly into the cytoplasm by transfection techniques or following pathogen-mediated disruption of phagosomes, resulting in activation of caspase-11. It is less clear whether GBPs are involved in mediating caspase-11 sensing of LPS in these scenarios where LPS is introduced to the cytoplasm independently of GBPs. Meunier and colleagues found no requirement for GBPs encoded on chromosome 3 in the secretion of IL-1 β and induction of cell death in

Pam3CSK4-primed BMDMs transfected with *E. coli* or *Salmonella minnesota* LPS [37]. In contrast, Pillar and colleagues identified a partial requirement for GBPs encoded on chromosome 3 in the induction of pyroptosis in IFN- γ -primed BMDMs transfected with LPS from *L. pneumophila*, *E. coli* or *S. minnesota* (**Figure 2**) [41]. It is possible that the subtle effect of GBPs can only be revealed when IFN- γ priming is used to induce abundant levels of GBPs in the cell.

Cytosolic bacteria

Unlike bacteria that reside within vacuolar compartments, certain bacteria escape from the vacuole into the cytoplasm for replication. GBPs are also essential for driving inflammasome responses to some of these cytosolic pathogens. The cytosolic Gram-negative bacterium *Francisella novicida* induces type I IFN production, triggering expression of GBPs via the transcription factor IRF1 [38]. Of the GBPs expressed during *F. novicida* infection, GBP2 and GBP5 are both recruited to the bacteria, where they contribute to the lysis of cytosolic bacteria and release of bacterial DNA [38, 44]. DNA is detected by AIM2, a cytoplasmic DNA sensor that activates the inflammasome (reviewed in [45, 46]). Importantly, both these GBPs are required for controlling *F. novicida* burden in BMDMs and in mice [38, 44]. The presence of both GBP2 and GBP5 is required to fully activate the AIM2 inflammasome, however, whether these two GBPs are recruited to the bacteria sequentially or whether both are recruited simultaneously has remained unclear. Further studies are required to unravel the mechanisms by which GBPs induce bacteriolysis.

Although GBPs are essential for inflammasome activation during infection with some cytosolic bacteria, the requirement for GBPs in the activation of inflammasomes in response to cytosolic bacteria is not universal. GBPs encoded on chromosome 3 did not appear to have a role for activation of the inflammasome in unprimed BMDMs infected

with *Burkholderia thailandensis*, a cytosolic Gram-negative bacterium known to activate caspase-11 [37]. In addition, GBPs encoded on chromosome 3 are dispensable for the activation of the non-canonical caspase-11 NLRP3 inflammasome in response to a genetically-engineered strain of *S. Typhimurium* which aberrantly enters the cytoplasm owing to a lack of the *Salmonella* vacuole-maintenance protein, *sifA* [37]. These studies suggest that although GBPs encoded on chromosome 3 contribute to activation of the non-canonical caspase-11 NLRP3 inflammasome in response to vacuolar-restricted Gram-negative bacteria, they are not necessary for inflammasome activation during infection with certain cytosolic Gram-negative bacteria that disrupt the vacuole and expose their LPS in the cytoplasm.

Interestingly, when BMDMs were primed with LPS or IFN- γ to induce robust expression of GBPs, a partial role for GBPs encoded on chromosome 3 for caspase-11-dependent pyroptosis in response to *S. Typhimurium* lacking *sifA* was revealed [37, 41]. In this case, whether GBPs contribute to the destabilization of the *Salmonella*-containing vacuole or enhance liberation of LPS by bacteriolysis for sensing by caspase-11 is not known. Overall, these studies have clearly demonstrated a requirement for GBPs in the activation of inflammasomes in response to certain vacuolar and cytosolic bacteria. However, evidence for the existence of this functional relationship during viral and parasitic infections has remained elusive.

Is there a role for GBPs in inflammasome activation during viral infection?

Although the induction of an antiviral state is regarded as the hallmark of an IFN response, the importance of IFN-inducible GBPs in antiviral effector mechanisms has not been thoroughly explored. Earlier studies reported a role for human GBP1 in restricting viral replication and mediating cytopathic effects during infection with vesicular stomatitis virus (VSV) and encephalomyocarditis virus (EMCV) [47]. Indeed, HeLa cells

constitutively expressing human GBP1 produced a lower yield of progeny virions compared with cells which did not constitutively express GBP1 [47]. The antiviral effects of GBP1 were also observed in cells infected with hepatitis C virus (HCV), dengue virus, influenza virus (IAV) and classical swine fever virus (CSFV) [48-51]. GBP1 suppresses HCV replication in a GTPase-dependent manner [48]. However, the NS5B protein of HCV directly binds to GBP1 and inhibits its GTPase activity to counteract the antiviral effects of GBP1 [48]. Similar to the NS5B of HCV, the nonstructural protein 1 (NS1) of IAV as well as the NS5A protein of CSFV directly interacted with and inhibited the GTPase activity of GBP1 [50, 51]. The presence of these viral antagonists of GBP1 underscores the importance of this GTPase in anti-viral host defense.

In addition to human GBP1, mouse GBP2 has antiviral activities against VSV and EMCV [52]. The enzymatic activity of mouse GBP2 is required for restriction of EMCV, whereas VSV inhibition does not rely on the GTP binding motif of GBP2 [52]. Anti-IAV activity was also demonstrated for human GBP3 [53]. A splice variant of human GBP3 with a modified C-terminal α -helical domain, called GBP3- Δ C, confers substantial antiviral activity in epithelial cells by inhibiting the IAV RNA polymerase complex [53]. The antiviral activity of human GBP3- Δ C was dependent on GTP binding, but GTP hydrolysis was dispensable [53]. Unlike other GBPs with antiviral functions, mouse GBP4 disrupts TRAF6 and IRF7 interaction and negatively regulates type I IFN production and antiviral responses in cells infected with Sendai virus [54].

In addition to various RNA viruses, the antiviral activity of GBPs against retroviruses has also been demonstrated [55, 56]. Overexpression of human GBP5 in HEK293T cells reduces the yield of infectious HIV-1 NL4-3 [55]. Moreover, co-transfection of GBP5 with HIV and SIV proviral constructs reduces the yield of infectious virions [56]. GBP5 interferes with virion incorporation of mature envelope protein gp120, and thereby,

impairs the ability of virus to infect target cells [56].

Despite various reports demonstrating the importance of GBPs in inflammasome activation in response to canonical inflammasome activators and during bacterial infections, the role of GBPs in mediating inflammasome activation in response to virus infection is not known. A study by Krapp and colleagues found that silencing of GBP5 in human macrophages does not impair IL-1 β production in response to HIV [56]. This could be due to an increase in virus replication in the absence of GBP5 that amplifies the signals leading to inflammasome activation. Since GBPs are also important in restricting replication of other viruses including VSV, EMCV and IAV, which are known to activate inflammasome, further studies are warranted to investigate whether GBPs positively or negatively regulate inflammasome activation in response to viruses.

Is there a role for GBPs in inflammasome activation during *Toxoplasma gondii* infection?

Even though inflammasome activation is studied during parasitic infections, the link between inflammasome activation and GBPs has not been reported for any protozoan parasites. *Toxoplasma gondii* is one of the model pathogens commonly used to explore cell-autonomous immunity and the antimicrobial effects of GBPs. *T. gondii* is an obligate intracellular protozoan and the etiological agent of toxoplasmosis. The *Toxoplasma*-containing vacuole is targeted by multiple GBPs, including mouse GBP1, GBP2, GBP3, GBP5, GBP6, GBP7 and GBP9 [7, 57-59]. However, recruitment of human GBP1 to the *Toxoplasma*-containing vacuole is not required for restriction of the parasite in human epithelial cells [60]. Notably, members from a family of IFN-inducible GTPases closely related to the GBPs called immunity-related GTPases (IRGs) are also effectively recruited to the *Toxoplasma*-containing vacuole [61-67].

Both GBPs and IRGs mediate the rupture of the *Toxoplasma*-containing vacuole [61,

68], allowing *T. gondii* to become cytosolic for subsequent attack by GBP2 [22]. Liberation of the *T. gondii* into the cytoplasm ultimately results in the death of the parasite, and also causes pyronecrosis of the host cell, characterized by host cell membrane permeabilization and release of the alarmin HMGB1 [68]. Based on these observations, it is very likely that GBP-mediated cell-autonomous immunity to *T. gondii* is inherently linked to activation of inflammasomes.

It is known that *T. gondii* infection can induce IL-1 β secretion and cell death in macrophages by activating the mouse NLRP1, NLRP3 or rat NLRP1 inflammasomes [69-71]. Mice lacking the three *Nlrp1* genes, *Nlrp1a*, *Nlrp1b* and *Nlrp1c*, or the *Nlrp3* gene are more susceptible to *T. gondii* infection, show increased parasitic load and a reduction in serum IL-18 levels compared with infected WT mice [71], indicating an involvement for multiple inflammasomes in host defense against *T. gondii*. However, secretion of IL-1 β in mouse BMDMs infected with *T. gondii* is predominantly dependent on the NLRP3 inflammasome [71]. No published reports have examined the direct link between any IFN-inducible GTPases and activation of these various inflammasomes during *T. gondii* infection. Future studies are required to unravel any functional relationships that likely exist between GBPs and inflammasomes in the context of *T. gondii* and other parasitic infections.

Conclusions:

GBPs are major effectors of the IFN-mediated host defense to a whole range of intracellular pathogens. Recent studies have highlighted divergent roles of GBPs in cell-autonomous immunity as well as inflammasome activation, although these two functions are not mutually exclusive for some pathogens. The function of GBPs usually involves structural modifications of membranes that destroy pathogen-containing vacuoles or bacterial cell walls and membrane to release pathogen-associated molecular patterns for

detection by innate immune sensors to initiate an immune response, including activation of inflammasomes. Despite being an active area of research, the molecular mechanisms regulating activation of these GTPases and how these proteins combat against compartmentalized versus cytosolic pathogens is still unclear.

A number of important questions that could be a focus of future studies include: (1) Do GBPs target and destroy membranes of pathogen-containing vacuoles and those of the bacteria? Which components of the membrane are targets for GBPs? (2) What determines the selectivity of GBPs, specifically, why certain GBPs target bacteria and others do not? (3) GBPs are known to confer protection against viruses and protozoan parasites, but are they involved in mediating inflammasome activation in response to these pathogens? (4) How do GBPs coordinate with one another? (5) Do GBPs guide other unique cellular functions driving inflammasome activation? Unveiling novel regulatory functions and processes of GBPs will strengthen the tie between this important family of IFN-inducible GTPases and innate immune responses.

AUTHORSHIPS

All authors contributed to the writing of this review.

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DISCLOSURES

The authors declare no conflicts of interest

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FIGURE LEGENDS

Figure 1. Genomic and phylogenetic organization of GBPs. (A) Mouse and human GBP open reading frames organized by chromosome location based on NCBI annotations. (B) A phylogenetic tree was generated by amino acid multiple sequence alignments using the MUSCLE algorithm followed by the PhyML program (phylogeny.fr) with downstream visualization using FigTree v1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree>). Scale bar indicates the number of substitutions per site.

Figure 2. The role of GBPs in the activation of inflammasomes

The Gram-negative bacterium *Salmonella* Typhimurium (*S. Typhimurium*) normally resides in a vacuole. Mouse GBP2 and other GBP members within the chromosome 3 cluster (GBPs on chr3) are recruited to the vacuole where they induce vacuolar rupture. This process allows the bacteria to become cytosolic, where LPS is sensed by caspase-11 to drive activation of the non-canonical NLRP3 inflammasome [37]. However, the Gram-negative bacterium *Francisella novicida* (*F. novicida*) is a cytosolic pathogen which directly escapes the vacuole and does not activate caspase-11. Mouse GBP2 and GBP5 directly target the bacteria to induce bacteriolysis and liberate bacterial DNA for sensing by the AIM2 inflammasome [38, 44]. Mouse GBPs encoded on chromosome 3 can partially promote pyroptosis in IFN- γ -primed BMDMs infected with the Gram-negative bacteria *Chlamydia trachomatis* or *Chlamydia muridarum* [42]. Recruitment of GBPs to the pathogen-containing vacuole is not necessary to drive inflammasome activation in response to *C. muridarum* infection. There is some evidence to suggest that GBPs encoded on chromosome 3 might also induce pyroptosis in IFN- γ -primed BMDMs transfected with LPS [41]. However, the mechanism regulating this process has remained unclear. Human and mouse GBP5 have been shown to undergo

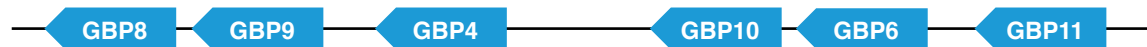
tetramerization and interact with the pyrin domain of NLRP3. Tetramerization of GBP5 might potentiate activation of the NLRP3 inflammasome in response to the soluble NLRP3 activators ATP and nigericin but not crystalline NLRP3 activators [35].

A**Mouse**

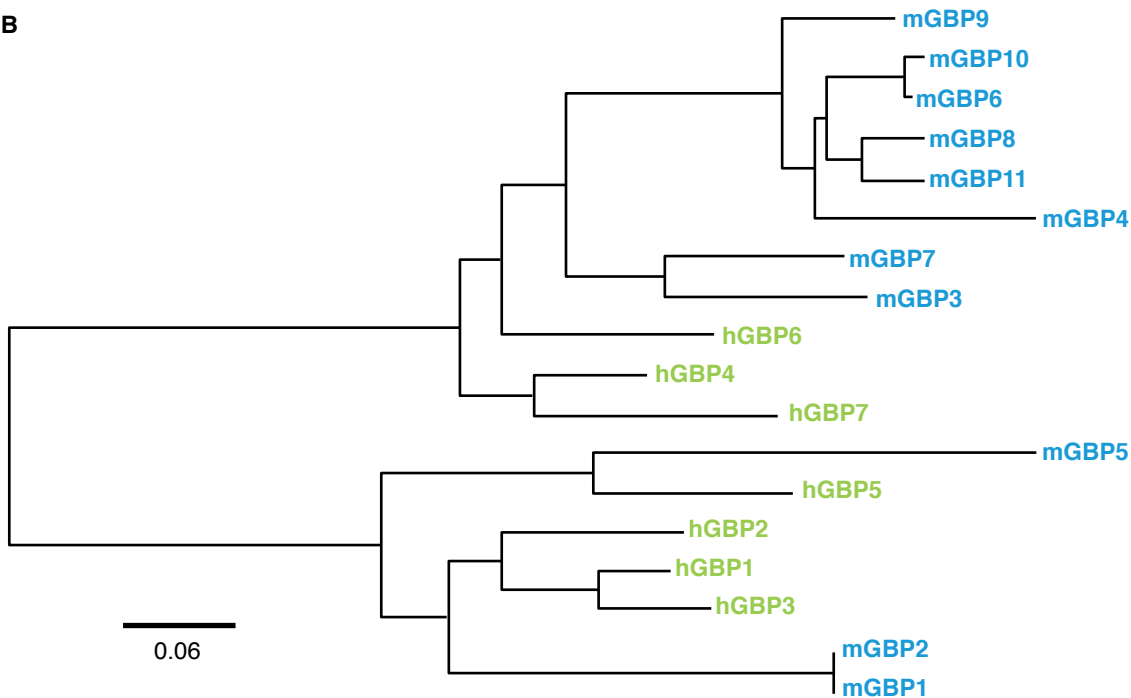
Chromosome 3



Chromosome 5

**Human**

Chromosome 1q22.2

**B**

S. Typhimurium

F. novicida

C. muridarum

Transfected LPS

ATP, nigericin

