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Review

Human guanylate-binding proteins in intracellular pathogen detection, destruction, and host cell death induction

Yolanda Rivera-Cuevas, Barbara Clough and Eva-Maria Frickel

Cell-intrinsic defense is an essential part of the immune response against intracellular pathogens regulated by cytokine-induced proteins and pathways. One of the most upregulated families of proteins in this defense system are the guanylate-binding proteins (GBPs), large GTPases of the dynamin family, induced in response to interferon gamma. Human GBPs (hGBPs) exert their antimicrobial activity through detection of pathogen-associated molecular patterns and/or damage-associated molecular patterns to execute control mechanisms directed at the pathogen itself as well as the vacuolar compartments in which it resides. Consequently, hGBPs are also inducers of canonical and noncanonical inflammasome responses leading to host cell death. The mechanisms are both cell-type and pathogen-dependent with hGBP1 acting as a pioneer sensor for intracellular invaders. This review focuses on the most recent functional roles of hGBPs in pathways of pathogen detection, destruction, and host cell death induction.

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Introduction

Cells have intrinsic mechanisms for the *detection* of microbial infections through pattern recognition receptors (PRRs) that recognize pathogen-associated molecular patterns (PAMPs) resulting in the production of cytokines necessary to control the infection. Secretion of the inflammatory cytokine interferon gamma (IFNγ) enhances the expression of innate immunity-related proteins necessary to *detect* and *destroy* intracellular pathogens, a pathway that often results in cellular *death*. An important set of IFNγ-stimulated genes (ISG) encodes a group of guanylate-binding proteins (GBPs) that play a crucial role in the control of intracellular pathogens not only in mammals, but across the eukaryotic kingdom [1]. GBPs execute antimicrobial functions against viruses, numerous bacteria, including *Salmonella enterica*, *Francisella novicida*, *Neisseria meningitidis*, *Shigella flexneri*, *Chlamydia trachomatis*, *Yersinia enterocolitica*, *Legionella pneumophila*, *Burkholderia thailandensis*, *Moraxella catarrhalis*, *Listeria monocytogenes*, group-A *Streptococcus*, *and Mycobacterium bovis* and against the protozoan parasites *Toxoplasma gondii* and *Leishmania donovani* [2–7].

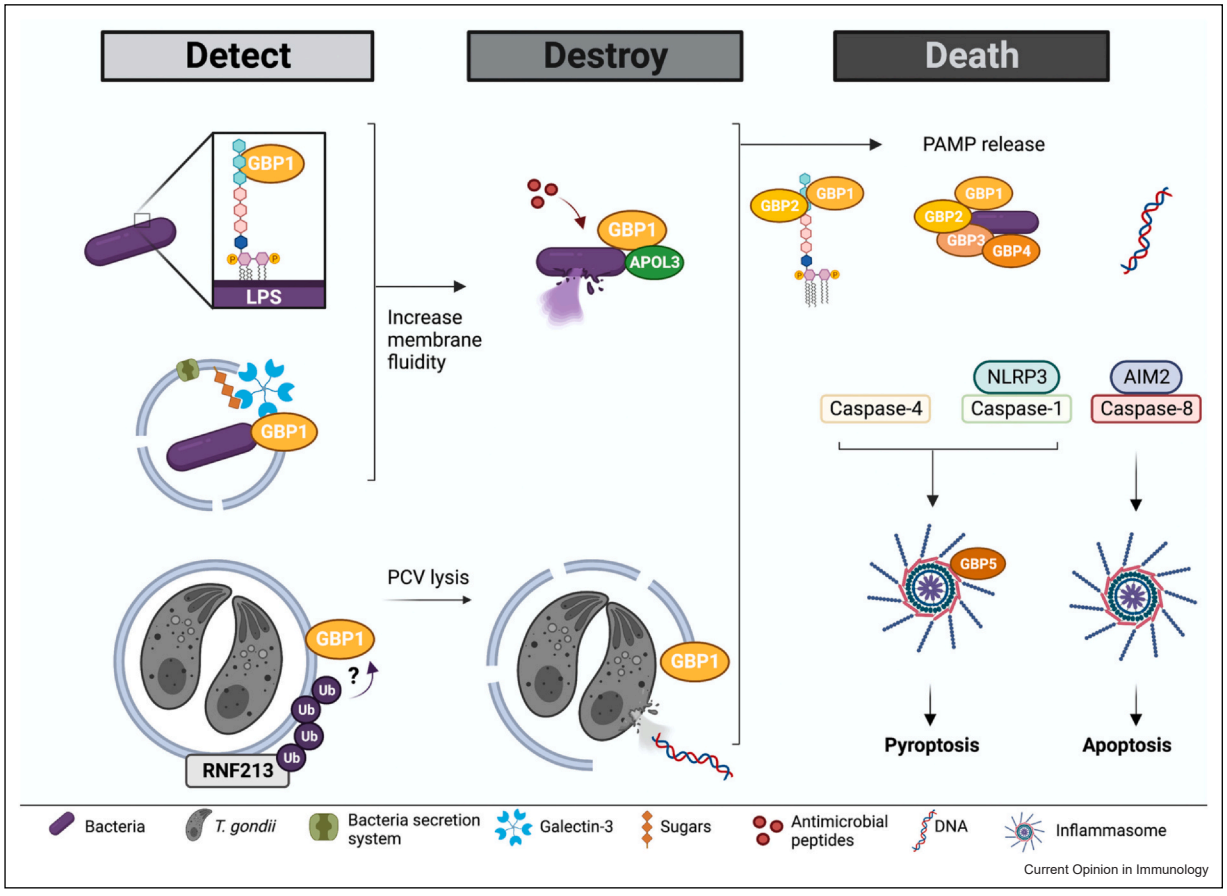
Humans encode 7 GBP isoforms (hGBPs) that are composed of three main domains: a large guanosine triphosphatase (GTPase) (LG) domain located at the N-terminus, followed by a middle domain, and finally a GTPase effector domain at the C-terminus [8,9]. Three of these isoforms, hGBP1, hGBP2, and hGBP5, contain a CaaX-prenylation site at their C-terminus that facilitates membrane association [10,11]. GBPs' ability to physically interact with microbial surfaces or their replication compartments depend on this post-translational modification. Furthermore, heterodimerization enables non-prenylated GBP isoforms to be recruited to the site of prenylated GBP in a hierarchical manner, initiated by hGBP1 [10]. The biochemical properties of hGBP function during infection are expertly reviewed in [2].

This review compiles the recent literature of hGBPs in bacterial and protozoan microbial control and induction of host cell death. We partially include studies of murine Gbps (mGbps) to contrast and better understand the function of these proteins in pathogen-targeting destruction and induction of host cell death.

Cytosolic pathogen detection and destruction by guanylate-binding proteins

At early stages of infection, intracellular pathogens enter the host cell and reside in a membrane-bound compartment, known as the pathogen-containing vacuole (PCV). As part of the host's intrinsic defenses, this compartment should be targeted to the lysosome for

Figure 1



Mechanisms for hGBP-driven pathogen detection, destruction, and cellular death. *Detect:* hGBP1 targets invading microbes present in the host cytosol, in broken PCV, or intact PCVs. hGBP1 can detect cytosolic bacteria via its ability to bind LPS and upon membrane damage, demarked by Galectin-3 recruitment. Exactly how hGBP1 recognizes intact PCVs remains to be determined; however, ubiquitination has been proposed to play a role in this process. *Destroy:* hGBP1 binding to bacteria LPS affects membrane fluidity, making the pathogen more susceptible to antimicrobial peptides and/or direct killing by the APOL3. hGBP1 can also actively lyse intact PCV via an unknown mechanism. *Death:* hGBP1 microbial targeting results in PAMP release or it can initiate the recruitment of other hGBPs to cytosolic bacteria to initiate a signaling platform for inflammasome activation. hGBP1 function can result in noncanonical inflammasome (Caspase-4 activation) or canonical inflammasome activation (Caspase-1 activation), which primarily leads to pyroptotic cell death. However, in the case of *T. gondii* infection, hGBP1 releases parasite DNA that is detected by Absent In Melanoma 2 resulting in Caspase-8-dependent apoptotic cell death.

pathogen clearance. Pathogens often evade lysosome-mediated clearance by escaping from PCVs and establishing infection in the host cell cytosol. Emerging evidence uncovered a role for GBPs as cytosolic sensors to detect intracellular pathogens. During bacterial infection, hGBP1 binds to lipopolysaccharides (LPS) of Gram-negative bacteria exposed to the cell cytosol (Figure 1) [12–16]. The proposed model describes a detergent-like function of hGBP1 polymers that bind to LPS and subsequently diffuse from the polymer to the bacterial surface. This results in an increased outer membrane fluidity rendering the bacteria more susceptible to destruction by other immune-related effectors and/or antimicrobial peptides that otherwise would not have had access to the bacterial surface [2,14]. A recent study elucidated the contribution of hGBP1 in apolipoprotein L3 (APOL3)-dependent killing of *S. enterica* serovar Typhimurium (*S.* Typhimurium) [17]. hGBP1 is shown to promote outer membrane permeability when interacting with the bacterial LPS, thus facilitating APOL3 access to the bacterial inner membrane for its dissolution [17] (Figure 1). The same surfactant-like activity of hGBP1 comes into play when binding *S. flexneri* with the consequence of inhibiting its actin tail formation [18,19]. In addition to its role in membrane fluidity, recent studies demonstrate a novel role for hGBP1 in bacterial LPS shedding into the host cytosol [20,21]. Furthermore, mGbp1 itself encodes a functional antimicrobial peptide-like sequence ($mGbp1^{28–67}$) in its globular domain, capable of targeting *F. novicida* and *N. meningitidis* but no other Gram-negative bacteria, including *S.* Typhimurium [4]. These data

suggest selectivity in Gbp1-mediated killing. Although the GBP1$^{28–67}$ peptide is conserved across the murine and hGBPs [4], whether it functions similarly in hGBPs remains to be determined.

Recognition of broken and intact pathogen-containing vacuoles by guanylate-binding proteins

While recognition and clearance of cytosolic bacteria by GBP1 have undergone extensive study, less is known about the mechanisms for detection and control of intracellular pathogens encapsulated within a PCV. Currently, reports suggest sugar-binding galectins and/or ubiquitin as important contributors for GBP recruitment to the PCV [22–24] (Figure 1).

Galectins recognize sugar moieties present at the inner membrane leaflet that become exposed to the cell cytosol upon membrane damage, and PCV damage is often caused by the function of bacterial secretion systems [22,25]. Supporting this, it has been observed that recruitment of hGBP1 to *Y. enterocolitica*'s and group-A *Streptococcus*'s PCV occurs following membrane damage [7,26]. The damaged PCV, elicited by the bacterium itself through the function of its type-III secretion system (T3SS) or *Streptococcus* pore-forming protein, was recognized by the membrane damage sensor Galectin-3 and enabled hGBP1's access to the bacterial LPS [7,26]. Whether GBP1 also promotes membrane breakage in this scenario remains to be determined, however, hGBP1 was shown not to promote escape of *S.* Typhimurium from its PCV in both HeLa cells and macrophages [13,15]. Additionally, hGBP1 has been shown to detect sterile-damaged endosomes and this activity required the protein's GTPase activity, its farnesylation, and a triple-arginine polybasic motif at its C-terminus [19,24]. A potential role for Galectin-9 as an interactor of and contributor to mGbp2 recruitment to the *T. gondii* PCV has been proposed, but the precise state of the vacuole integrity at the time of recruitment of this complex is unclear [27].

GBPs also target pathogens that reside within intact PCVs, including *C. trachomatis* and *T. gondii* [23]. Whereas galectins seem to be contributing to the recruitment of GBPs toward damaged PCVs as a result of bacterial secretion systems [24], ubiquitination of the *C. trachomatis* and *T. gondii* PCV is an important marker for targeting the pathogens for clearance by cell-intrinsic defenses [23]. Interestingly, ubiquitination of *C. trachomatis* and *T. gondii* PCV has recently been demonstrated to be dependent on the E3 ubiquitin ligase RNF213 [28–30]. This provides a tool to assess whether ubiquitination plays a role upstream of hGBP1-mediated PCV detection and destruction (Figure 1). It is worthy to note that in HeLa cells, overexpressed hGBP1 targets latex bead-containing phagosomes without IFNγ stimulation [31], while in THP-1 macrophages, IFNγ stimulation is needed to drive overexpressed hGBP1 to the *T. gondii* PCV [32]. An important gap in our understanding of GBP1-dependent pathogen control is how it differentiates PCVs from host organelles to avoid destroying self-lipid membranes. Lipid composition and phosphoinositide signatures of the PCV in interferon-stimulated cells probably play a role in the recognition mechanism. Murine immunity-related GTPases (Irgs) bind to certain phosphoinositides, a property that is reported to aid in their recognition of pathogen-containing compartments [33–35]. Another attractive hypothesis is that PCVs lack a self-molecule that enables GBP recognition akin to the 'missing self' hypothesis of murine Irg recruitment, where self-membranes are protected by murine Irgm proteins [36]. Active detection of pathogen proteins localized to the PCV is another possibility to drive GBP recruitment, or inversely, the presence of a negative regulator or a guard system that controls the cellular localization of GBP1.

How does guanylate-binding protein 1 drive membrane damage of intact vacuoles?

GBPs are part of the dynamin-like superfamily proteins, a group of GTPases that function in membrane-remodeling events such as fission or fusion through GTP hydrolysis. So, unsurprisingly, the hGBP1 isoform and its ortholog mGbp2 are capable of mediating tethering of giant unilamellar vesicles (GUV); however, the biological relevance of this observation in the context of their antimicrobial function remains unknown [11,31,37]. Although lipid composition was shown to be important for mGbp2 membrane binding and hinge motion, it was not sufficient to cause membrane curvature or damage [37]. Equally, hGBP1 recruitment to GUVs did not cause membrane damage to those compartments [11]. Therefore, it is possible that GBPs by themselves are not capable of causing membrane damage and that other proteins might be necessary. This is consistent with the observation that hGBP1-triggered membrane damage of *T. gondii* PCV is dependent on IFNγ stimulation, suggesting that there could be an undiscovered cofactor that is important [13]. We know that hGBP1 lyses PCVs of the bacteria *L. pneumophila* and the protozoan parasite *T. gondii*, but it remains unclear exactly how GBP1 disrupts these membranes (Figure 1) [13,38]. During *T. gondii* infection, hGBP1 isoprenylation and GTPase activity are important for PCV targeting in macrophages [39]. As a result, GTPase- and lipidation-deficient hGBP1 fail to control *T. gondii* infection [39]. Furthermore, a constitutive dimeric mutant was able to control *T. gondii* infection as effectively as wild-type hGBP1 [39]. This is consistent with structural analysis showing that hGBP1 lipid binding is facilitated by the conformational change employed upon GTPase activity and the stabilization of

the open state through dimerization [11]. Even though these data highlight important functional features in hGBP1 necessary for vacuolar pathogen immune control, the mechanism and coplayers driving GBP-mediated PCV membrane disruption remain elusive.

Guanylate-binding protein-dependent growth control independent of pathogen targeting

GBP-dependent pathogen control can also be exerted without active recruitment to the PCV. This is exemplified during infection with another protozoan parasite *L. donovani* where hGBP1 is necessary for parasite control, independent of PCV targeting [40]. A similar discovery was reported for hGBP1 control of *T. gondii* in A549 epithelial cells, and hGBP2 and hGBP5 *T. gondii* control in macrophages [39,41]. In the context of *L. donovani* infection, it appears that hGBP1 functions in pathogen restriction by promoting lysosome fusion with the PCV [42]. Although this could suggest a novel role for GBP1-dependent pathogen control, further elucidation of the molecular mechanisms involved in this process is required and how this might resemble the role of hGBP2 and hGBP5 in distal growth restriction of *T. gondii*.

Guanylate-binding protein-triggered host cell death

GBPs engage in canonical and noncanonical inflammasome activation as part of their role in cell-autonomous innate immune response (Figure 1) [43]. Inflammasomes are supramolecular complexes made of PRRs and inflammatory chemicals that, when activated, trigger pyroptotic cell death [44]. Most of our understanding of the role of GBPs in inflammasome activation comes from mouse GBPs (see reviews [2,43,45,46]). Here, multiple studies have focused on assessing the contribution of different mGbp and hGBP isoforms in pathogen control through host cell death [4,5,12,15,18,47].

hGBP1 contributes to noncanonical inflammasome activation through its role as a cytosolic LPS sensor, which results in Caspase-4 activation [12–15,21,48]. During *S. flexneri* and *S.* Typhimurium infection, it was observed that hGBP2, hGBP3, and hGBP4 form a complex that could target cytosolic bacteria but, unlike mGbps, localization of the different hGBP isoforms to the bacteria followed the initial recruitment of hGBP1 [12,15,18]. Thus, at least in human epithelial cells, there appears to be a hierarchy for the assembly and function of these proteins onto the bacterial surface. hGBP1 is the pioneering protein, hGBP4 and possibly hGBP2 control recruitment of Caspase-4, and hGPB3 binds LPS and drives Caspase-4 activation [12,15]. Cryo-electron tomography revealed insertion of hGBP1 molecules into the bacterial OM mediated by hGBP1 farnesylation and a C-terminal polybasic motif triggered release of LPS leading to activation of Caspase-4 [20]. The hGBP1 orthologs, mGbp2, mGbp1, Gbp3, and Gbp5, are also recruited to the cytosolic Gram-negative bacteria *M. catarrhalis* and *F. novicida* and are important for inflammasome activation [4,5]. Interestingly, a recent study employing *S. flexneri* mutants defective in antagonizing Caspase-4 and GBP1 bacterial binding or bacterial LPS release still observed pyroptosis [21,48]. Overexpressing hGBP2 or an hGBP1 defective in bacterial targeting restored pyroptotic cell death upon *S. flexneri* infection in cells deficient for hGBP1 [48]. hGBP1 and hGBP2 are capable of binding free LPS and acting as surfactants, clustering LPS molecules, and stimulating Caspase-4 activation [48].

hGBP5 was the first Gbp family member (murine or human) found to contribute to inflammasome activation by directly interacting with the intracellular PRR NOD-like receptor 3 (Nucleotide-binding and oligomerization domain– Leucine-rich repeat and pyrin domain-containing protein 3) [49]. The role of hGBP5 needs further attention to elicit its exact inflammasome-inducing mechanism, also in light of the recent finding that GBP5 deficiency leads to decreased proinflammatory mediator transcripts and secreted chemokines and cytokines [50]. Furthermore, the inflammasome can be activated through a novel pathway during *C. trachomatis* infection that is dependent on the GTPase activity of hGBP1 [51]. This study showed that whereas GTP hydrolysis is important for pathogen growth restriction, GMP produced by hGBP1 is metabolized to uric acid that can activate the inflammasome [51]. Thus, hGBP1's sequential GTP hydrolysis can employ different functions, including pathogen restriction and induction of cell death. Depending on the pathogen, hGBP1 can also drive host cell death to apoptosis. hGBP1-dependent disruption of the PCV and parasite membrane of *T. gondii* releases parasite DNA that is recognized by Absent In Melanoma 2 resulting in apoptosis-associated speck-like protein containing a caspase-recruitment domain Caspase-8-dependent death of human macrophages [13,20,32,52]. *S.* Typhimurium, however, elicits the expected hGBP1- and Caspase-4-dependent pyroptosis in human macrophages, but this pathogen also activates Caspase-1. In this case, hGBP1 is cleaved by Caspase-1, thereby reducing its recruitment to *S.* Typhimurium resulting in less Caspase-4 activation and reduced pyroptosis.

Conclusions/outlook

GBPs are important innate immune components that contribute to microbial control through pathogen detection and destruction and host cellular death induction. Current work has shed light on these functionalities, yet to fully understand GBPs, we have to address microbial counterdefense to GBPs, their cellular regulation, and targeting specificity. For GBP microbial counterdefense, the *Shigella* E3 IpaH9.8 is the only

identified microbial hGBP antagonist, ubiquitinating hGBP1 for proteasomal degradation [18,19,53], but there must be others. For cellular GBP regulation, what other post-translational mechanisms besides farnesylation control GBP function? Is there a broader role for caspase-driven regulation of GBP function that might extend into the extracellular space [13,54]? What additional mechanisms of ubiquitination impact GBP function? Does phosphorylation play a role in GBP regulation? For GBP targeting, can other PAMPs besides LPS be recognized and different GBPs distinguish LPS structural types [16]? How does hGBP1 target pathogens encapsulated within an intact vacuole and what entities are recognized on this non-self-membrane? What are the mechanisms of hGBP1-driven membrane disruption? GBP5 is robustly localized to the Golgi, yet we only know of its function to inhibit furin-mediated processing of viral glycoprotein at that location [55]. Employing GBP2/5 chimeras, bacterial targeting versus intracellular targeting to the Golgi are independent features in hGBPs [16]. How GBP targeting to self-membranes is regulated and what other functions specifically GBP5 performs at the Golgi are intriguing questions. Additionally, we await discovery of more GBP-interacting proteins that direct their function for the apparent selectivity in the context of different microbial infections and host cell death induction.

Beyond these immediate questions, it is important to understand that hGBPs vary dramatically in expression level depending on tissue and cell type and inflammatory condition [6]. Thus, some hGBPs will have cell-type-specific functions even when probing with the same pathogen infection. It is noteworthy, for example, that even after IFNγ stimulation, GBP3 and 4 show minimal expression in different human macrophages [32]. It is intriguing to note that GBP2, 3, and 4 generally are highly expressed at homeostasis, with GBP1 being almost absent and GBP5 demonstrating intermediate expression levels. Upon infection and inflammation, GBP1 is most highly upregulated. Whether this broadly will mean GBP1 (and possibly GBP5) are responsible for infection control, while other GBPs also serve additional homeostatic functions important for infection outcome, still needs to be established.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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