



## Review

# Host Responses to Group A Streptococcus: Cell Death and Inflammation

James A. Tsatsaronis<sup>1</sup>, Mark J. Walker<sup>2</sup>, Martina L. Sanderson-Smith<sup>1\*</sup>

**1** Illawarra Health and Medical Research Institute (IHMRI), School of Biological Sciences, University of Wollongong, Wollongong, New South Wales, Australia, **2** Australian Infectious Diseases Research Centre, School of Chemistry and Molecular Biosciences, University of Queensland, St. Lucia, Queensland, Australia

**Abstract:** Infections caused by group A *Streptococcus* (GAS) are characterized by robust inflammatory responses and can rapidly lead to life-threatening disease manifestations. However, host mechanisms that respond to GAS, which may influence disease pathology, are understudied. Recent works indicate that GAS infection is recognized by multiple extracellular and intracellular receptors and activates cell signalling via discrete pathways. Host leukocyte receptor binding to GAS-derived products mediates release of inflammatory mediators associated with severe GAS disease. GAS induces divergent phagocyte programmed cell death responses and has inflammatory implications. Epithelial cell apoptotic and autophagic components are mobilized by GAS infection, but can be subverted to ensure bacterial survival. Examination of host interactions with GAS and consequences of GAS infection in the context of cellular receptors responsible for GAS recognition, inflammatory mediator responses, and cell death mechanisms, highlights potential avenues for diagnostic and therapeutic intervention. Understanding the molecular and cellular basis of host symptoms during severe GAS disease will assist the development of improved treatment regimens for this formidable pathogen.

## Introduction

Group A *Streptococcus* (GAS; *Streptococcus pyogenes*) is a clinically important bacterial pathogen responsible for many severe human diseases. Whilst GAS is a frequent agent of self-limited pharyngitis and uncomplicated impetiginous infections, penetration of GAS into deeper tissues, trauma, or adverse progression of superficial tissue infections can result in devastating invasive infections, such as the “flesh-eating” syndrome, necrotising fasciitis (NF), or more rarely, myonecrosis [1,2]. Systemic manifestations of GAS disease, sepsis, or streptococcal toxic shock syndrome (STSS), when accompanying invasive cutaneous infections, strongly contribute to poor patient prognosis [3]. Conservative estimates of the global burden of severe GAS diseases account for 663,000 cases, with 32% of NF cases resulting in patient mortality within seven days in developed countries, rising to a 50% case mortality rate in NF episodes with associated STSS [4,5]. The destructive nature, rapid onset, and high mortality rate of severe GAS diseases, such as NF and STSS, despite prompt medical care and antimicrobial therapy, necessitates an improved understanding of the mechanisms underlying these pathologies. Whilst mechanisms by which GAS evades elements of the innate immune response have been the focus of intense investigation [6], comparatively less attention has been given to the host processes underlying the pathophysiology of GAS infections. Notably, the roles of cellular host mediators in the context of GAS infection are poorly understood. Here, we integrate recent works describing

cellular recognition of GAS infection, signalling pathways leading to inflammatory mediator release and consequent programmed cell death mechanisms. A holistic perspective, incorporating the role of host factors in GAS diseases, should enable the development of more efficacious and tailored treatment options.

## Leukocyte Recognition of GAS and Signalling Pathways

### Toll-like receptor recognition

Cellular surveillance and recognition of foreign agents by the innate immune system is an essential prerequisite to effector recruitment and induction of appropriate responses. One class of cell receptors involved in recognition of highly conserved microbial components are the Toll-like receptors (TLRs). There are currently more than 10 known TLRs, which recognize multiple pathogen-associated molecular patterns (PAMPs), including lipoteichoic acid (LTA), peptidoglycan (TLR2), lipopolysaccharide (TLR4), and unmethylated bacterial CpG DNA (TLR9) [7]. Studies investigating receptors involved in recognition of GAS infection have shown that TLR signalling, particularly through the MyD88 adapter molecule, plays a vital role in induction of host defences and inflammation (Figure 1) [8,9]. Given that TLR2 recognises multiple gram-positive bacterial ligands, it is likely this TLR plays a major role in GAS recognition and responses. Indeed, induction of streptococcal cell wall extract-induced joint inflammation is dependent on TLR2/MyD88 signalling [10].

### Leukocyte signaling events

Macrophages and dendritic cells (DCs) are commonly viewed as central coordinators of immune responses. This view has been supported by studies showing that macrophages and DCs are both essential for control of GAS infection [11,12], likely through secretion of multiple pro-inflammatory cytokines such as interleukin

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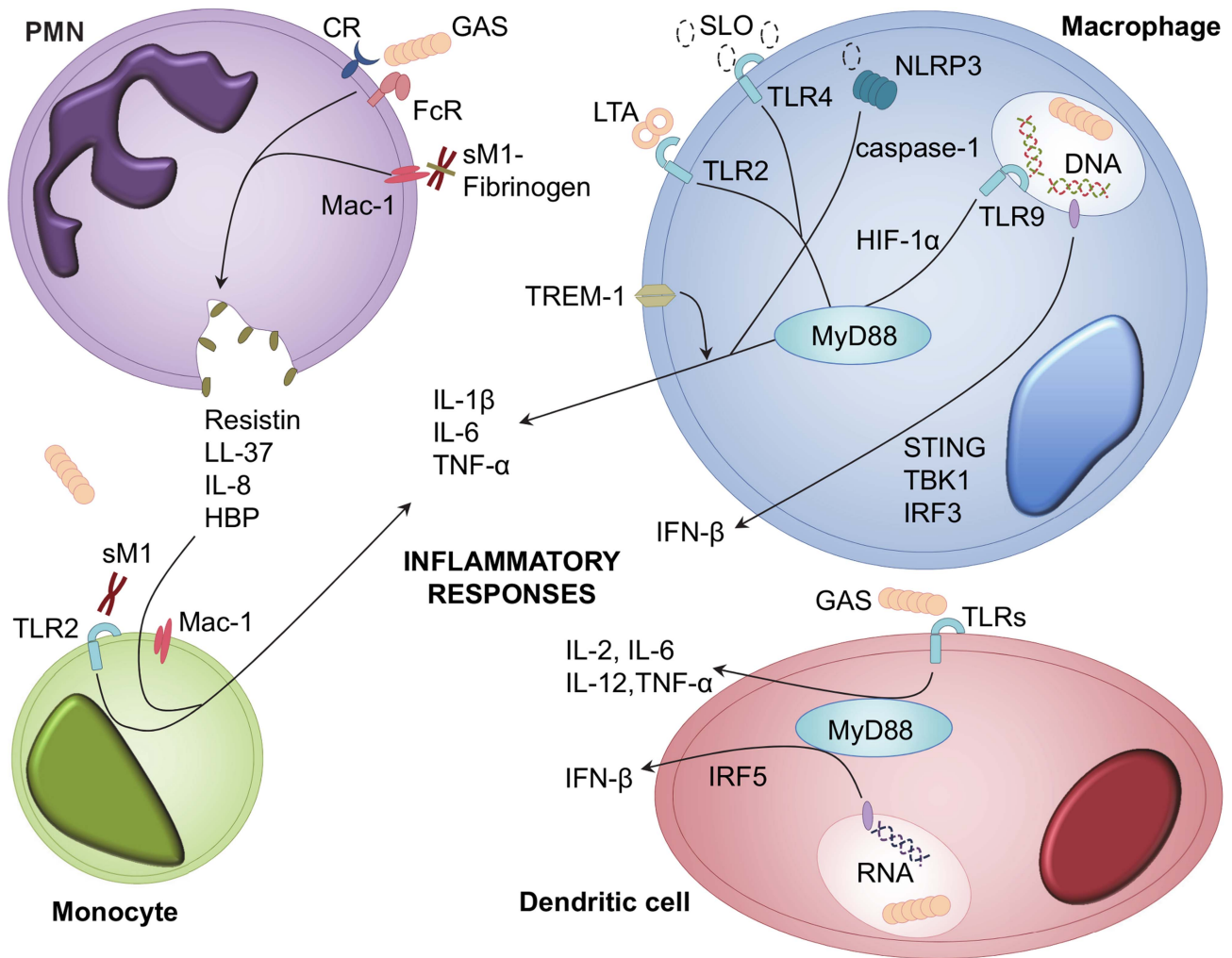
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\* Email: martina@uow.edu.au



**Figure 1. Cellular receptors and signalling pathways involved in GAS recognition and inflammatory mediator release.** Inflammatory mediators are released from multiple leukocyte types during GAS infection; including PMNs, monocytes, macrophages, and dendritic cells [12,13]. GAS and GAS-derived LTA, SLO, and soluble M1 protein (sM1), activate cellular responses to infection [14,26]. Receptors involved in recognition of GAS include TLRs, TREM-1, complement receptors (CR), immunoglobulin receptors (FcR), Mac-1, and NLRP3 [9,19,20,27]. Ligand binding to these receptors leads to downstream signalling via MyD88, HIF-1 $\alpha$ , STING, IRF3, IRF5, and TBK1 [8,16–18]. Recognition of GAS triggers release of interleukins, TNF- $\alpha$ , IFN- $\beta$ , HBP, resistin, and LL-37 [16,25]. doi:10.1371/journal.ppat.1004266.g001

(IL)-1 $\beta$ , IL-6, tumour necrosis factor (TNF)- $\alpha$ , and IL-12 [9,13]. Macrophage and DC recognition of GAS is partially mediated by TLRs, and interaction with multiple bacterial factors by different TLRs appears to provide immune redundancy in inducing cytokine responses. This redundancy is exemplified by the activation of macrophages via TLR4 signalling from nonstructural bacterial elements, such as GAS cytolysin streptolysin O (SLO), and the inability of singular TLR1, TLR2, TLR4, or TLR9 deficiencies to prevent stimulation of DC cytokine production [12,14]. However, downstream MyD88 signalling is critical for coordinated immune responses, an aspect prominent in clinical studies of patients lacking MyD88, who are highly predisposed to pyogenic infections [15]. MyD88 participates in macrophage and DC TLR-stimulated TNF- $\alpha$  production and type-1 interferon (IFN) responses [8,9,16,17]. GAS induction of IFN- $\beta$  is nucleic acid-dependent and requires interferon regulator factor (IRF) 3, IRF5, stimulator of interferon genes (STING), and TANK-binding kinase (TBK) 1 signalling via as-yet-undescribed receptors [16,17]. Although

GAS is typically considered an extracellular pathogen [1], recent studies indicate intracellular receptors, such as TLR9, are important for cytokine responses via hypoxia-inducible factor 1-alpha (HIF-1 $\alpha$ ) signalling, and also contribute to macrophage GAS killing and reactive oxygen species (ROS) production [18].

#### Non-TLR recognition of GAS

Other non-TLR receptors have been shown to respond to GAS infection. Secretion of SLO mediates activation of the intracellular NOD-like receptor (NLRP) inflammasome component NLRP3 and promotes macrophage caspase-1 mediated IL-1 $\beta$  secretion independently of both TLR and MyD88 signalling [19]. A recent report describes secondary modulation of macrophage cytokine responses by the triggering receptor expressed on myeloid cells (TREM)-1, which, when inhibited, has a therapeutic effect in murine models of GAS sepsis [20]. The recent work of Baruch et al. demonstrates SLO-mediated host signalling via induction of endoplasmic reticulum stress [21]. Adherence of *sil*-expressing GAS to epithelial cells and macrophages increases host asparagine

synthetase expression and asparagine production, driving GAS proliferation and virulence [21].

## Excessive Host Responses Contributing to GAS Disease Pathology

### Superantigenic stimulation of T-cell responses

Sufficient excitation of host defences is necessary to mount an adequate response to combat GAS infection. A large body of evidence suggests, however, that excessive and misdirected host responses underlie damaging pathologies of severe GAS infection [22]. GAS may produce one or more of a large variety of superantigens that increase host propensity to excessive immune activation. Superantigens circumvent conventional antigen presentation by binding simultaneously to both the major histocompatibility complex (MHC) class II and T-cell receptors outside of the peptide-binding area [23]. The resulting “cytokine storm”, driven by activated T-cells and antigen presenting cells, may underlie host hyperinflammatory responses [22]. While GAS superantigens play an important role in dictating immune responses, exhaustive discussion of these protein toxins is beyond the scope of this article, and the reader is directed to recent comprehensive reviews [22–24].

### M1-mediated heparin binding protein release

Interactions of polymorphonuclear leukocytes (PMNs) and the classic GAS virulence factor, M protein, have been recently implicated in mediating excessive inflammatory responses [25]. As the major effector cell type of the innate immune system, activation of PMNs and the release of cytotoxic granules has implications for development of severe GAS diseases pathologies. Serotype M1 protein released from the GAS cell surface forms complexes with fibrinogen that bind to PMN Mac-1 receptor, both activating PMNs and triggering release of heparin binding protein (HBP) (Figure 1) [26]. HBP is a potent inducer of vascular permeability, and release of this protein elicits pulmonary lesion formation and vascular leakage [26]. PMN-mediated HBP release synergistically enhances inflammatory cytokine responses from M1 protein-stimulated peripheral blood monocytes during necrotizing infections [27]. Individuals with IgG antibodies directed towards the central region of the M1 protein elicit higher HBP release and are subsequently more susceptible to suffering a pathologically excessive inflammatory response to M1 protein–fibrinogen–IgG complexes [28]. Direct injection of purified M1 protein is sufficient to trigger PMN granule-mediated severe lung damage, which is markedly reduced in neutropenic mice [29]. The M1 protein structure plays an important role in triggering HBP-mediated lung injury, as mutated nonfibrinogen-binding M1 protein exhibits diminished ability to cause pulmonary haemorrhage [30]. The precise organisation of fibrinogen molecules into a supramolecular, cross-like network by M1 protein is essential for PMN activation and is conformationally distinct from normal fibrin clots [31]. A novel marker of septic shock severity, resistin, has also been found to predominately originate from M1 protein-activated PMNs during both systemic and localised severe GAS infection and contributes to local tissue damage [32].

### SLO-mediated inflammatory reactions

Other GAS virulence factors, particularly streptolysin O (SLO), have been found to modulate PMN responses and contribute to inflammation and tissue damage. Oligomerization of SLO in eukaryotic cell membranes forms large (~25–30 nm) pores and has been shown to disrupt membrane integrity in multiple cell types, including PMNs, macrophages, and epithelial cells [33,34].

SLO induces toxic PMN platelet aggregates during cutaneous infection that mediate progression of microvascular thrombosis and ischemic tissue necrosis [35]. SLO binding by PMNs has also been shown to mediate HBP, human cathelicidin (LL-37), alpha-defensin, and elastase release [36]. Multiple accounts indicate that SLO secretion also facilitates delivery of other GAS virulence factors. SLO-induced epithelial damage enhanced the penetration of the GAS SpeA superantigen in a vaginal mucosa model *ex vivo* [37]. SLO production also mediates translocation of GAS NAD-glycohydrolase into host keratinocytes, leading to intracellular signalling [38].

### Host genetic predisposition to excessive immune responses

The role of host genetic factors contributing to GAS disease has been investigated and found to play a decisive role in dictating host susceptibility to severe sepsis and STSS. Goldmann et al. utilised differential susceptibilities of distinct mouse strains to severe GAS infection to demonstrate that failure to control infection and excessive evocation of inflammatory responses by susceptible mice result in extensive tissue destruction [39]. This finding has been refined in studies ascribing differences in severity of STSS to host polymorphism in the human leukocyte antigen complex [13], [40]. Human MHC class II haplotype DRB\*1501/DQB1\*0602 is less commonly associated with STSS in the presence of NF, while DRB1\*07/DQB1\*0201 predisposes towards such disease manifestations [40]. A complementary systems genetics approach was recently utilized to identify a panel of host genes bestowing predisposition towards severe GAS sepsis [41]. Pathological levels of the product of one of these genes, Prostaglandin E, directly influences the severity of GAS infection [42].

### Host Leukocyte Cell Death Responses

Programmed cell death plays a decisive role in determining the outcome of microbial infections and inflammation. A variety of regulated cell death mechanisms have been described in response to infection, which have distinct morphological and molecular signatures (Box 1).

#### Apoptotic PMN cell death responses

Apoptosis limits the potential of damaged PMNs to ignite inflammatory responses [43]. Coordinated PMN apoptotic shutdown initiates a state of cellular torpor, and administration of apoptotic PMNs actively promotes anti-inflammatory responses [44]. Conversely, necrosis triggers a proinflammatory phenotype resulting in release of damage-associated molecular patterns (DAMPs) and prompts rapid immune responses [45]. In a landmark study by Kobayashi et al., phagocytosis-induced PMN transcriptional responses were analysed for a variety of bacterial pathogens, including GAS [46]. This study provided strong evidence of a common apoptotic program following bacterial uptake and ROS production. PMN apoptotic responses are accelerated in GAS and lead to rapid DNA fragmentation [46]. Although GAS-induced PMN apoptosis was associated with enhanced virulence potential, the virulence determinant(s) responsible for this activity were not elucidated. Later work by the same group indicated PMN apoptosis induced by phagocytosis of latex beads, as opposed to live bacteria, is associated with nullified inflammatory capacity and expedites resolution of inflammation [47].

#### GAS cytolysin-induced cell death

Several reports emphasize the dominant role of GAS cytolysins and, to a lesser extent, hyaluronic acid capsule in shaping host cell

### Box 1. Modes of Host Regulated Cell Death

Multiple pathways leading to host cell death have been described and exhibit characteristic morphological, molecular, and immunological features. Here we briefly summarize key aspects of host regulated cell death mechanisms (reviewed in detail in [78,79]).

**Apoptosis:** Also known as Type-I cell death, apoptosis is a non-inflammatory cell death mechanism. Apoptosis may be triggered via multiple external (extrinsic) or internal (intrinsic) stimuli. Extrinsic apoptosis follows extracellular signalling via ligands such as Fas/CD95 ligand, TNF- $\alpha$ , and TNF-related apoptosis inducing ligand (TRAIL) to specific death receptors. Intrinsic apoptosis can be induced by various intracellular stresses, including DNA damage and oxidative stress. Morphologically, apoptotic cells undergo cellular shrinkage, nuclear condensation, and blebbing. However, more stringent molecular identification of apoptosis considers dependency upon activation of distinct caspases (typically caspases-3, -6, and -7 for extrinsic apoptosis, and caspases-9 and -3 for intrinsic apoptosis), and the presence of biochemical features such as mitochondrial membrane depolarization, oligonucleosomal DNA fragmentation, ROS generation, exposure of phosphatidylserine, and cellular redistribution of factors such as cytochrome C and Bax. Apoptotic cells are generally phagocytosed by macrophages via efferocytosis; however, if this does not occur, cells may undergo secondary necrosis, whereby intact apoptotic cells lose plasma membrane integrity and undergo autolysis, triggering immune responses.

**Necrosis:** Originally designated “accidental cell death”, necrosis is a proinflammatory cell death mode. While historically regarded as unintentional cell demise in response to overwhelming stress, recent works indicate necrosis may proceed in a regulated fashion (also known as necroptosis or oncosis). Necrosis is exhibited morphologically via cell and organelle swelling, cytoplasmic vacuolization, eventual loss of plasma membrane integrity, and escape of cytoplasmic

content, leading to enhanced immune responses. In some instances, regulated necrosis has been linked to necrostatin-inhibitable RIP1-RIP3 activation and/or calpain-cathepsin activation. Most studies investigating programmed necrosis have been conducted using non-infectious disease models (e.g., ischemia-reperfusion); however, a mounting body of evidence suggests a role for this process in infectious disease pathogenesis [80,81], and warrants further study.

**Autophagic cell death:** Also known as Type-II cell death, autophagic cell death is a non-inflammatory cell death response. Cellular autophagy can be triggered by cells under specific stresses, including endoplasmic reticulum stress or starvation, and are distinguished via the formation of cytoplasmic double-membraned vesicles known as autophagosomes. Autophagosomes typically contain damaged organelles or cytoplasmic material, are labelled with LC3, and eventually fuse with lysosomes (acquiring the LAMP-1 marker), leading to degradation of autophagosomal contents. Autophagic responses that accompany cell death have been associated with a distinct programmed cell death routine; however, an increasing body of evidence supports the supposition that autophagy primarily exerts a protective response against cell demise in vivo.

**Pyroptosis:** Pyroptosis describes a specific form of pro-inflammatory cell death sharing characteristics of both apoptosis and necrosis. Pyroptotic activation of macrophages and other cell types by microbial and nonmicrobial agents is dependent on activation of caspase-1, which proteolytically drives maturation and release of IL-1 $\beta$  and IL-18. Caspase-1 activation is associated with the formation of large multiprotein complexes, either the inflammasome or pyroptosome, which involves signalling via NLRPs and the ASC adaptor protein. These events culminate in cytoplasmic swelling and osmotic lysis of pyroptotic cells, which combined with increased IL-1 $\beta$  and IL-18 release, enhances immune reactions.

death. Early studies of SLO and streptolysin S (SLS) function indicate both are capable of inducing plasma membrane permeabilisation, leading to host epithelial cell lysis [33,48]. GAS strains expressing high and low amounts of SLS were utilised to demonstrate that SLS expression is associated with caspase-7-dependent apoptosis of thioglycolate-induced murine PMNs [49]. However, the strains used in this study also differed significantly in capsule expression, a GAS virulence factor known to influence PMN phagocytosis. Capsule-deficient, GAS-expressing SLO are also more readily phagocytosed by DCs, preventing DC maturation by promoting caspase-dependent apoptosis [50]. Proinflammatory macrophage cell death consistent with regulated necrosis (Box 1) was reported by Goldmann et al. [34]. Macrophage necrosis was attributable to both SLO and SLS activities leading to glycine sensitive pore formation, loss of mitochondrial outer membrane potential ( $\psi_m$ ), ROS production, ATP depletion, and calpain activation [34]. Conversely, the work of Timmer et al. [51] found that phagocytosed GAS cytolysin expression also depolarises  $\psi_m$ ; however, this leads to DNA fragmentation, cytochrome C redistribution and caspase-3 and -7 dependent apoptotic cell death. Macrophages are principally responsible for the clearance of apoptotic PMNs, and either apoptotic or necrotic depletion of macrophages may exacerbate damage resulting from GAS-induced PMN cell death [52]. Apoptotic PMNs not phagocytosed by macrophages proceed to secondary necrosis and elicitation of

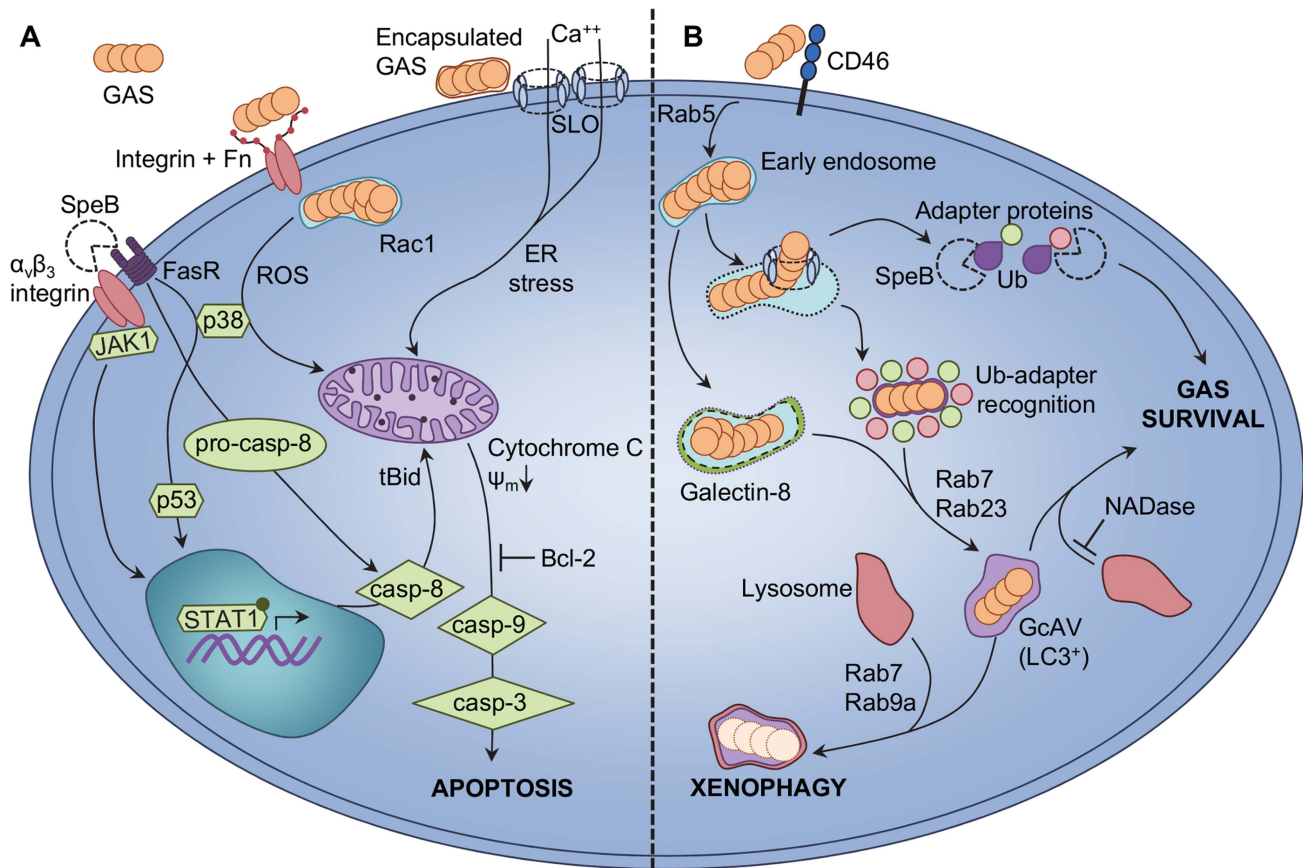
inflammatory responses [53]. These data support an emerging theme in bacterial pathogenesis [43], and we propose manipulation of phagocyte cell death responses has inflammatory implications for GAS disease.

### GAS interaction with NETs and NETosis

PMNs are able to release extracellular traps (NETs) composed of DNA, histones, and granule components that ensnare and kill bacteria [54]. GAS and other bacterial pathogens have evolved specialized means of evading this host defence mechanism via secretion of extracellular DNases, which degrade NETs and enable bacterial survival [55]. In vitro studies have indicated PMNs undergo a distinct form of cell death following NET release, dubbed NETosis; however, the relevance of this process to host defence and bacterial pathogenesis has remained contentious [56,57]. Recent in vivo evidence obtained using intravital microscopy indicates that GAS and other gram-positive pathogens are ensnared by NETosing PMNs; however, these cells, in fact, remain viable and retain bactericidal capacity [58].

### Epithelial Cell Apoptotic Responses

Epithelial cells mediate the first interactions between host and bacteria. Adherence of GAS to epithelial receptors frequently precedes intracellular invasion of this bacterium [59], and multiple



**Figure 2. Epithelial cellular responses to GAS infection.** **A** GAS-induced apoptosis of epithelial cells is triggered via intrinsic and extrinsic pathways. SpeB binding to epithelial  $\alpha_v\beta_3$  integrins or the Fas receptor (FasR) triggers upregulation of caspase-8 (casp-8) via JAK2, p38, p53, and STAT1 signalling [62–64]. Pro-caspase-8 (pro-casp-8) is also activated directly due to FasR signalling, leading to truncation of cytosolic Bid (tBid) and translocation of tBid to the mitochondria. Extracellular GAS binding to host integrins is enabled via bridging molecules such as fibronectin (Fn), and enables Rac1-mediated internalization of GAS into host epithelial cells. GAS internalization and Rac1 activation facilitates production of ROS, leading to increased p38 phosphorylation [65,66]. Encapsulated extracellular GAS are not internalized by epithelial cells, and secrete the pore-forming toxin SLO. Integration of SLO into host cell membranes triggers net calcium (Ca<sup>++</sup>) flux into the cytosol and endoplasmic reticulum (ER) stress [67]. All three pathways elicit loss of mitochondrial outer membrane potential ( $\psi_m$ ) and release of cytochrome C from the mitochondria, which precedes activation of caspase-9 (casp-9), caspase-3 (casp-3), and apoptosis. Overexpression of the anti-apoptotic factor Bcl-2 by the host can inhibit epithelial cell apoptosis. **B** GAS evades xenophagic killing by epithelial cells. GAS binding to the CD46 receptor is an early signal to activate autophagic responses [72], and GAS are uptaken into early endosomes in a Rab5-dependent manner [74]; however, SLO expression allows GAS to escape from endosomes into the cytosol. GAS exposure to the cytosol is recognized via ubiquitin (Ub) adapter proteins that, in conjunction with Rab7 and Rab23, facilitate shuttling of GAS into GcAVs bearing the classic autophagy LC3 marker [71,73]. In the absence of SLO, streptolysin S is sufficient to damage endosomal vacuoles for targeting to GcAVs by Ub-independent, galectin-8-mediated autophagy [76]. Lysosomal fusion with GcAVs, via Rab7 and Rab9a, effects xenophagic destruction of intracellular GAS. Expression of SpeB by cytosolic GAS degrades Ub adapter proteins and prevents targeting of bacteria to GcAVs, enhancing intracellular GAS survival [77]. Secretion of GAS NADase also protects GAS from xenophagic killing by inhibiting fusion of GcAVs with lysosomes [76].  
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pathways of epithelial cell death have been reported following GAS infection (Figure 2A). GAS infection of A549 and HEP-2 cells was found to elicit morphological changes consistent with apoptosis, attributable to the activity of GAS cysteine protease SpeB [60]. The apoptotic Fas receptor (FasR) and  $\alpha_v\beta_3$  integrins are able to bind SpeB, leading to downstream caspase-8 activation and translocation of truncated-Bid (tBid) and Bax to mitochondria [61,62]. Binding of SpeB to host receptors also up-regulates caspase-8 and Bax via JAK-STAT, p38, and p53 signalling [63,64]. Internalization-dependent epithelial cell apoptosis in response to GAS infection has also been reported, whereby fibronectin-mediated binding of GAS to host integrins triggers actin rearrangement and Rac1 activation [65,66]. Rac1 activation is hypothesized to mediate epithelial cell ROS production, leading

to downstream apoptotic responses [65–66]. A third pathway triggered by extracellular GAS also elicits epithelial apoptosis via an SLO-dependent mechanism [67]. Secretion of SLO by encapsulated GAS triggers calcium flux into the cytosol of infected cells, leading to vacuolization of the endoplasmic reticulum and cell apoptosis [67]. Central to these pathways is the role of the mitochondria, as cytochrome c release and  $\psi_m$  depolarisation are described as key events, and that overexpression of the anti-apoptotic factor Bcl-2 can inhibit mitochondrial dysfunction. In many studies, both caspase-9 and caspase-3 are reported to mediate the final apoptotic cascade. Transcriptional analyses of epithelial cells also indicate overall apoptotic responses to GAS infection, and that GAS elicits up-regulation of caspases and calcium regulators [68,69]. It is important to note that different GAS

serotypes do not elicit uniform epithelial responses, and comparison of apoptotic induction by disparate GAS strains demonstrates multiple caspases are utilized [70].

## GAS-Induced Autophagy

Epithelial cell apoptosis has been suggested to protect host cells from infection; however, data validating this hypothesis is lacking, and contention exists regarding whether epithelial apoptotic cell death is, indeed, a protective host response or a pathogenic mechanism utilized by GAS [66,67]. An alternative epithelial cell defence mechanism against GAS infection has been recently described. Autophagy, a stress response wherein damaged cellular components are targeted to degradative endosomal vacuoles, can protect cells from intracellular GAS (Figure 2B). HeLa cells containing intracellular M6 serotype GAS recognize cytosol-exposed bacteria and target them for autophagic degradation via ubiquitinylation, LC3 labelling, and the formation of GAS-containing, autophagosome-like vacuoles (GcAVs) [71]. Expression of SLO by intracellular GAS is crucial for GAS escape from endosomes into the cytosol and subsequent targeting to GcAVs [71]. Binding of the cell surface CD46 receptor is an early trigger of GAS-mediated autophagy [72]. The Rab-family of G-proteins is responsible for multiple aspects of GcAV formation. Rab7 protein performs numerous roles, including targeting of GAS to GcAVs and initial GcAV formation, and autophagosomal maturation [73]. Other Rab-enzymes, Rab5, Rab23, and Rab9a, also play distinct roles, which include facilitating GAS uptake into endosomes, targeting of GAS to GcAVs, fusion of nascent GcAVs, and autophagosomal maturation [74,75]. Recent studies report that GAS is able to subvert autophagy-mediated bacterial destruction (also called xenophagy) for intracellular survival. SLO-mediated translocation of GAS NADase prevents efficient killing of serotype M6 GAS via inhibition of autophagosomal fusion with lysosomes [76]. A novel ubiquitin-independent pathway of GcAV formation was also described for primary

human keratinocytes in this study, mediated by SLS damage to endosomal vacuoles leading to galectin-8 labelling [76]. Infection of HEp-2 cells with M1T1 serotype GAS reveals significantly greater intracellular survival than by M6 GAS, with M1T1 GAS displaying exposure to the cell cytosol but lack of the LC3 autophagy marker [77]. Enhanced M1T1 GAS survival was found to be directly linked to SpeB activity, which degrades cytosolic ubiquitin and the adapter proteins NDP52, p62, and NBR1 to effect highly efficient evasion of cellular xenophagy [77].

## Discussion

Detailed understanding of the host cellular processes that precipitate severe GAS disease is vital to the development of improved treatment strategies. Host receptor binding to GAS-derived ligands activates PMNs and pro-inflammatory signalling cascades of other cell types, which result in the release of host molecules such as HBP, cytokines and resistin. Excessive exposure of these factors is associated with multiple hallmarks of severe GAS disease, including increased vascular permeability, systemic inflammatory responses and destruction of focal tissues. GAS and GAS cytolysin induction of regulated cell death of leukocytes, leading to increased DAMP release, may further contribute to the virulence and inflammatory potential of this pathogen. While GAS-induced, host leukocyte apoptotic, and regulated necrotic cell death may contribute to GAS disease pathologies, regulated epithelial cell death responses could serve a more protective role, as epithelial xenophagy directly counters GAS intracellular invasion. However, as recent studies illustrate, GAS is able to prevent intracellular killing, and so apoptosis of epithelial cells may serve as a host contingency response, as these cells will be taken up by nearby phagocytes. Thus, therapeutic interventions that reduce the exposure of inflammatory mediators and/or restore native cell death processes may help alleviate pathologies of severe GAS infection.

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