

BACTERIAL PATHOGENESIS

Streptococci target inflammasome

The *Streptococcus pyogenes* surface M protein is a critical multifunctional virulence factor. Recent work sheds light on a new unexpected function of the M protein in activating the host inflammasome to induce macrophage cell death and promote infection.

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Infections with *Streptococcus pyogenes*, better known as group A streptococci (GAS), commonly cause pharyngitis but are notorious for severe life-threatening complications such as sepsis, pneumonia,

toxic streptococcal syndrome and necrotizing fasciitis. The major GAS surface M protein is known to carry out a variety of virulence-associated functions^{1–3}, including preventing

opsonization and phagocytosis early in infection and activating neutrophils, aggregating platelets, inactivating complement, and inducing vascular leakage and tissue injury⁴.

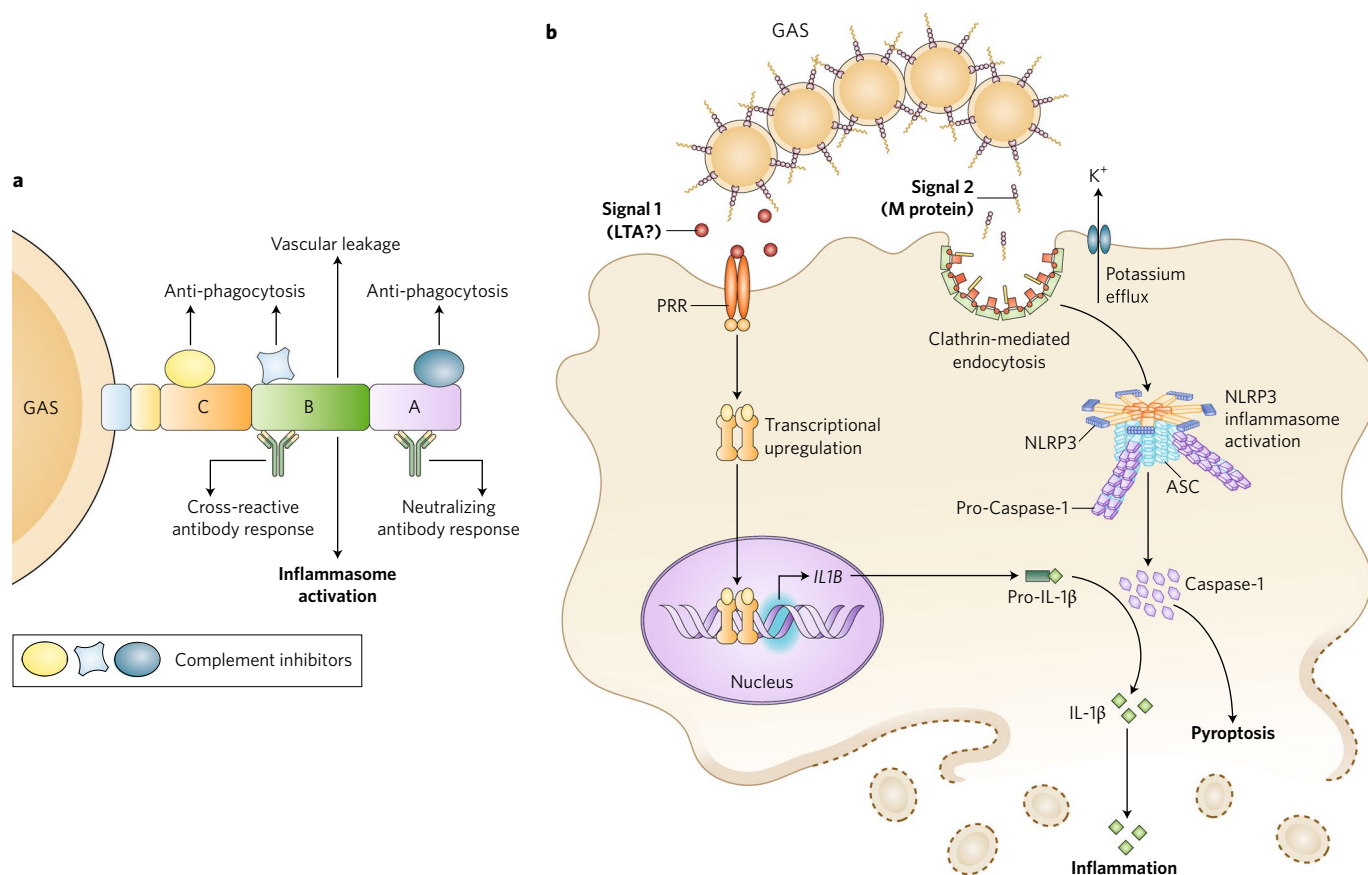


Fig. 1 | M-protein-mediated GAS virulence strategies. **a**, Cell-wall-anchored M protein is comprised of three repeat regions (A, B, C). The A-repeat region interacts with fibrinogen, which blocks the activation of complement via the alternate pathway, reduces complement C3b deposition and thereby phagocytosis. Similarly, factor H and C4-binding-protein recruitment to region C also inhibits complement activation and bacterial opsonization. Repeat region B is also involved in neutrophil activation and enhancement of vascular leakage by interacting with fibrinogen. Antibody responses to B-repeat regions can be cross-reactive with human cardiac proteins and potentially lead to autoimmune rheumatic fever sequelae seen in some GAS infections. **b**, A model for GAS inflammasome activation suggests that bacterial structures such as lipoteichoic acid (LTA) may provide the first signal for pattern recognition receptor (PRR) engagement, which leads to transcriptional upregulation of inflammasome and cytokine genes. Soluble M protein provides a second signal for inflammasome assembly after being endocytosed through a clathrin-dependent mechanism and inducing potassium efflux. Inflammasome assembly activation leads to caspase-1 activation, which cleaves pro-IL-1 β and also drives pyroptotic cell death. Cell rupture and death releases IL-1 β to drive inflammatory responses, which help promote GAS pathogenesis. The NLRP3 inflammasome in Fig. 1b is courtesy of Debbie Maizels.

The M protein (encoded by *emm*) forms fibrils that may contribute to bacterial adhesion to host cell surfaces^{2,4,5} and is comprised of three repeat regions (A, B and C) that extend from the bacterial cell surface as an α -helical coiled-coil dimer^{1–4}. The A-repeat region is highly variable, is targeted by neutralizing host antibodies and confers serotype specificity to GAS (with over 100 serotypes and >200 *emm* genes identified⁶). The B region is somewhat less variable and is increasingly associated with multiple virulence mechanisms². In particular, the B and C regions bind human plasma proteins such as fibrinogen, factor H and C4-binding protein to inhibit complement activation and thereby prevent opsonization and downstream phagocytosis by polymorphonuclear leukocytes (Fig. 1a). The B-repeat region also has cross-reactivity with cardiac myosin and other heart proteins, which is probably responsible in part for GAS-associated autoimmune rheumatic fever and rheumatic heart disease^{1,2,7}.

In this issue of *Nature Microbiology*, Valderrama et al.⁸ identify a new function of the M protein in activating the host inflammasome to promote infection (Fig. 1b). The inflammasome is a large protein complex that assembles in response to pathogen detection, leading to cleavage and activation of caspase-1, which both induces pyroptotic host cell death and maturation of pro-inflammatory interleukin (IL)-1 β and IL-18⁹. Production of IL-1 β typically requires two signals: a first priming signal to induce cytokine and inflammasome gene expression and a second to mediate inflammasome assembly, activation of caspase-1 and cytokine maturation⁹. In many bacterial infections, the priming signal is immune recognition of pathogen structures by innate pattern recognition receptors, such as Toll-like receptors (TLRs). In GAS, previous work suggested that M proteins could activate TLR2, but Valderrama et al. found that recombinant M1 protein (from the M1 GAS serotype) did not induce IL-1 β transcription, suggesting that other GAS cellular structures (such as lipoteichoic acid) may provide the first signal to activate transcription. In contrast, soluble recombinant M1 protein was sufficient to provide a second signal for inflammasome activation and mediate IL-1 β

release and also induce pyroptotic cell death in both mouse and human macrophages. Using mutant murine macrophages and specific inhibitors, the authors show that macrophage killing required both NLRP3 (NA-CHT, LRR and pyrin domains-containing protein 3) and Caspase-1. Additionally, they found that IL-1 β secretion required clathrin-mediated endocytosis of M protein and potassium efflux (which has been suggested to be general feature of inflammasome activation).

Intriguingly, two other GAS factors — the toxin streptolysin O (SLO) and the ADP-ribosyltransferase SpyA — have been previously reported to induce NLRP3 inflammasome-mediated IL-1 β signalling and pyroptosis^{10,11}. While the authors found that both SLO and M1 GAS mutants had reduced IL-1 β release after macrophage infection, the SLO mutant did not have significantly different IL-1 β release in vivo compared to wildtype infection. In contrast, M1 mutants reduced IL-1 β release as well as the peritoneal bacteria load, and injection of purified M1 protein was sufficient to induce IL-1 β release in vivo, suggesting the M1 inflammasome signalling pathway may have greater relevance in promoting GAS pathogenesis during host infection.

While the work provides a relatively convincing case for M protein-mediated inflammasome activation in infection, several questions remain unanswered. First, while the M1 protein B-repeat regions were critical for inflammasome signalling, the exact mechanism by which M protein activates the complex remains unclear and deserves future elucidation. Second, while inflammasome induction could be damaging to tissues and infection with a M1 mutant shows reduced bacterial burden, it remains unclear how inflammasome signalling is specifically benefiting the pathogen during infection, especially as inflammasome activation is associated with host protection in a variety of other infections⁹.

As M1 did not cause cell death in human red blood cells, human peripheral blood mononuclear cells or human monocytes, further study of how GAS interacts with these important human cell types may help unravel the complex relationship between macrophage pyroptosis alongside other GAS virulence strategies in disparate cellular contexts^{3,4}. Finally, the streptococcal M1

clone is the most prevalent worldwide and has persisted as a leading cause of group A streptococcal severe infections⁶. While the authors show that M proteins from many serotypes possessed pyroptotic activity, whether the specific virulence and dominant emergence of the M1 clone can be attributed to the inflammasome modulating capacity of the M1 protein would be an interesting future avenue of study.

In conclusion, the GAS M protein is a perfect example of how a single protein can encode different mechanisms to subvert host innate and adaptive immunity. Its range of virulence appears in the earliest stages of infection where activating the NLRP3 inflammasome could be important in causing tissue damage by programmed macrophage cell death, while the same B-repeat region also promotes vascular leakage. M protein recruitment of complement factor H as well as fibrinogen further prevents complement deposition, opsonization and phagocytosis. Such multiplicity of virulence suggests more virulence factors and strategies are waiting to be characterized in the highly successful GAS pathogen. □

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Competing interests

The author declares financial interest in Moleculera Labs in Oklahoma City, OK, where she is Chief Scientific Officer.