Virulence

ISSN: 2150-5594 (Print) 2150-5608 (Online) Journal homepage: www.tandfonline.com/journals/kvir20

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To cite this article: Helena Bergsten & Victor Nizet (2024) The intricate pathogenicity of Group A *Streptococcus*: A comprehensive update, Virulence, 15:1, 2412745, DOI: 10.1080/21505594.2024.2412745

To link to this article: https://doi.org/10.1080/21505594.2024.2412745

VIRULENCE
2024, VOL. 15, NO. 1, 2412745
https://doi.org/10.1080/21505594.2024.2412745

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The intricate pathogenicity of Group A *Streptococcus*: A comprehensive update

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ABSTRACT

Group A *Streptococcus* (GAS) is a versatile pathogen that targets human lymphoid, decidual, skin, and soft tissues. Recent advancements have shed light on its airborne transmission, lymphatic spread, and interactions with neuronal systems. GAS promotes severe inflammation through mechanisms involving inflammasomes, IL-1β, and T-cell hyperactivation. Additionally, it secretes factors that directly induce skin necrosis via Gasdermin activation and sustains survival and replication in human blood through sophisticated immune evasion strategies. These include lysis of erythrocytes, using red cell membranes for camouflage, resisting antimicrobial peptides, evading phagocytosis, escaping from neutrophil extracellular traps (NETs), inactivating chemokines, and cleaving targeted antibodies. GAS also employs molecular mimicry to traverse connective tissues undetected and exploits the host's fibrinolytic system, which contributes to its stealth and potential for causing autoimmune conditions after repeated infections. Secreted toxins disrupt host cell membranes, enhancing intracellular survival and directly activating nociceptor neurons to induce pain. Remarkably, GAS possesses mechanisms for precise genome editing to defend against phages, and its fibrinolytic capabilities have found applications in medicine. Immune responses to GAS are paradoxical: robust responses to its virulence factors correlate with more severe disease, whereas recurrent infections often show diminished immune reactions. This review focuses on the multifaceted virulence of GAS and introduces novel concepts in understanding its pathogenicity.

ARTICLE HISTORY
Received 4 June 2024
Revised 22 August 2024
Accepted 5 September 2024

KEYWORDS
Group A *Streptococcus*; *Streptococcus* pyogenes; bacterial pathogenesis; virulence; necrotizing fasciitis; toxins

Introduction

Group A *Streptococcus* (GAS, *S. pyogenes)* is frequently carried asymptomatically by school-aged children. It ranks among the top ten causes of infectious disease mortality worldwide [1], exerting a particularly severe impact in developing countries [2]. In wealthier regions, GAS remains a significant health concern due to its capacity to cause a large volume of mild diseases and occasional acute, life-threatening infections in otherwise healthy individuals. Although over a century of research on GAS has elucidated numerous specific virulence factors, it has yet to yield an approved vaccine. While the fundamental clinical features and disease mechanisms of GAS have been extensively reviewed [3,4]; this contribution will focus primarily on recent advancements in understanding the pathogenesis of acute, invasive GAS infections.

Clinical features

Most children and adults are familiar with strep throat or GAS pharyngotonsillitis, which typically presents as a mild and self-limiting infection that often resolves on its own [5,6]. Similarly, the superficial skin infection impetigo – caused by GAS, *Staphylococcus aureus* (*S. aureus*), or both – shares these characteristics. Antibiotic therapy for strep throat is recommended to reduce complications and contagiousness, though it only shortens symptoms by around 16 hours [7].

In many parts of the developing world, recurrent episodes of strep throat significantly increase the risk of acute rheumatic fever (ARF) [8], a condition closely linked to poverty, overcrowded living conditions, and the lack of antibiotic treatment due to insufficient healthcare infrastructure [9]. ARF generally follows strep throat but perhaps also GAS skin infections [10]. Indigenous populations often bear a disproportionate burden of ARF [11]. Recurring ARF episodes heighten the risk of developing rheumatic heart disease (RHD) [8]. RHD remains the principal cause of GAS-related mortality worldwide, affecting 1% of the population in sub-saharan Africa and 1,5% in Oceania, in the final stages requiring surgery to replace or repair heart-valves to prevent heart

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/21505594.2024.2412745

failure, stroke and death [12]. Glomerulonephritis, an immune complex-mediated condition that can arise from GAS infections of the skin or throat, typically resolves if acute renal complications like fluid overload and hypertension are managed effectively [13].

A less well understood condition linked to GAS is Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal Infections (PANDAS). Here, neuroinflammation following GAS infections is proposed to trigger symptoms like obsessive-compulsive disorder, tics, and Tourette's syndrome in children [14]. This disorder shares features with Sydenham's chorea – affecting 20–30% of ARF patients – which is characterized by involuntary movements and emotional disturbances, and for which a rat model has demonstrated the involvement of antibodies against GAS and the basal ganglia [15]. Recent studies have shown GAS-specific Th17 cells infiltrating the brain in mice after repeated intranasal infections [16], but the association of the symptoms to GAS is still debated. Some argue that Sydenham's chorea and PANDAS are not separate entities but instead should be termed "cerebral rheumatic fever" [17].

GAS can cause severe necrotizing soft tissue infections (NSTI), including necrotizing fasciitis, leading to rapid tissue necrosis and life-threatening conditions. About 30% of NSTI cases occur in previously healthy individuals, with 89% requiring mechanical ventilation within 24 hours due to severe septic shock [18]. GAS is the primary pathogen in monomicrobial NSTI, while other bacteria may cause polymicrobial infections like Fournier's gangrene. NSTI often results in streptococcal toxic shock syndrome (STSS), characterized by septic shock, multiorgan failure, and toxin effects on skin or mucosal surfaces. Both NSTI and STSS can severely affect children, who often require more intensive care compared to *S. aureus*-induced toxic shock [19]. Despite treatments including surgical debridement, antibiotics, intensive care, IVIG, and hyperbaric oxygen therapy, NSTI remains highly lethal, with an 18% mortality rate and a 22% amputation rate in the largest study to date [18].

In the 19th century, GAS infections were responsible for two out of every three postpartum deaths [20]. Despite advancements in healthcare, GAS puerperal fever remains the leading cause of infection-related death in pregnancy and the puerperium worldwide [21–24]. GAS also significantly affects child health, particularly during scarlet fever epidemics [25], which have seen a resurgence in regions like the UK, Australia [26], China, and Hong Kong [27]. Scarlet fever, a GAS disease characterized by fever, a skin rash (exanthem), and a distinctive bright red "strawberry tongue" [25], was once a leading cause of death among young children. Its impact had diminished in many areas until its recent re-emergence.

GAS infections can manifest in atypical ways that do not align with classical definitions of invasive disease. For example, a notable case involved a young man who developed non-rheumatic GAS myocarditis following a knee abscess. His condition became so severe that it required extracorporeal membrane oxygenation (ECMO) to sustain life [28]. Another unusual case featured a previously healthy pregnant woman who was diagnosed with sinusitis and a subdural empyema, complicated by preeclampsia. After undergoing a cesarean section, she experienced seizures and fell into a coma upon emerging from anesthesia [29].

Over the past decade, there has been a significant increase in scientific research and public health advocacy focused on developing an effective vaccine against GAS [30–33]. Recent advances in GAS research, including a human challenge model for strep throat, are providing crucial insights into pathogenesis and supporting the development of effective vaccines [34,35].

Prevalence of GAS diseases

GAS is estimated to cause > 517,000 deaths and > 720 million cases of superficial infections annually worldwide [1]. Estimates of incidence and prevalence of GAS diseases and infection outcomes are found in (Figure 1).

Acquisition of infection

GAS, a Gram-positive, β-hemolytic, chain-forming bacterium, is primarily a human pathogen with a unique restriction to our species [47]. It is asymptomatically carried in the pharynx by approximately 3% of adults and 8% of school-age children, with these rates exhibiting seasonal peaks during winter. This is when outbreaks frequently occur in schools, with up to 50% of children potentially carrying the outbreak strain without showing symptoms. Notably, children in high-income countries may exhibit the highest rates of GAS carriage [48]. One in every three children experience sore throat every year, and GAS pharyngotonsillitis, or strep throat, account for one in four of those experiences [36]. GAS accounts for 4–10% of pharyngitis cases in adults [1].

Airborne transmission of GAS, particularly in schools, has been a significant concern since the 1930s-1940s [49,50]. During school-class outbreaks of scarlet fever in the UK, despite hygiene measures, isolation of index cases, and antibiotic treatment, the asymptomatic carriage of the outbreak strain in classroom contacts tripled from the first to the second

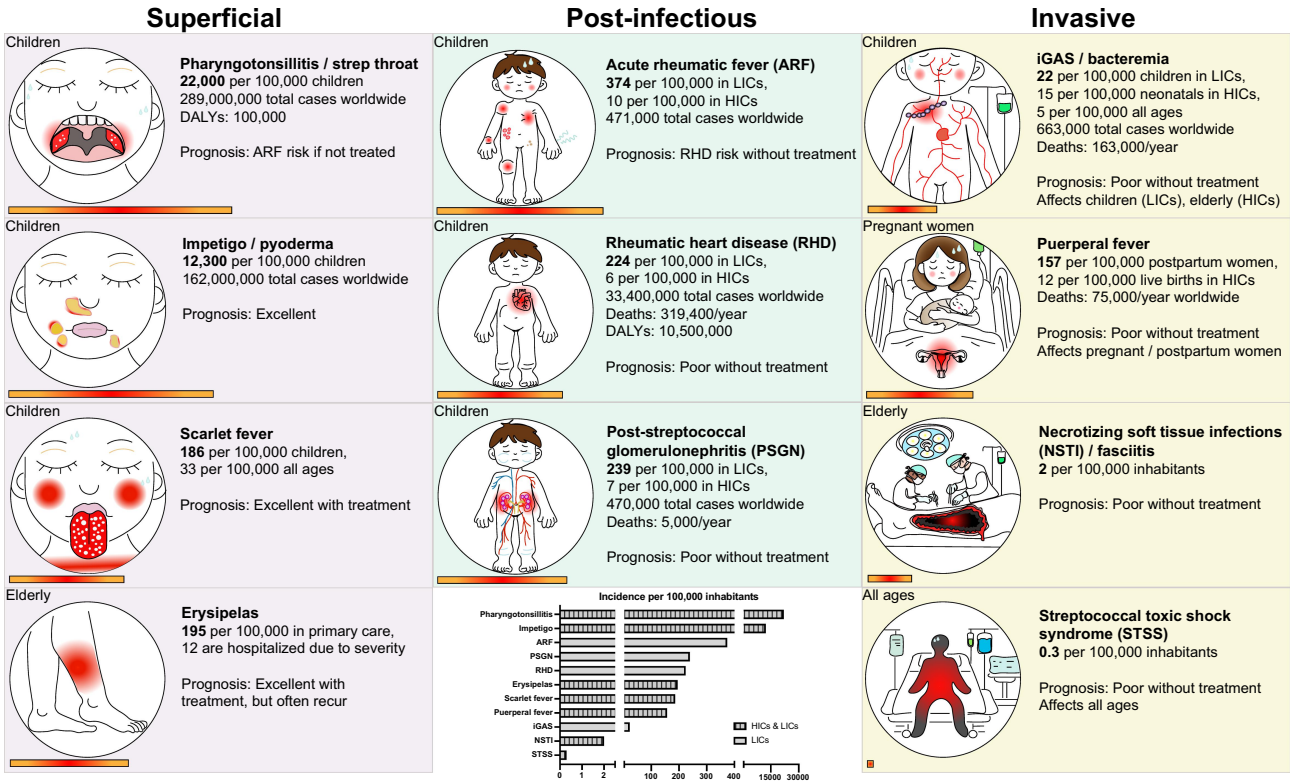

Figure 1. *Epidemiology of Group A Streptococcus (GAS)*. GAS infection induces many common diseases and infection outcomes. The figure indicates the estimated incidence per 100,000 inhabitants and for some diseases prevalence worldwide, deaths/year worldwide and disability-adjusted life years (DALYs)/year worldwide. Estimated incidence in the most affected risk group or setting is displayed. No incidence data was found for cellulitis, a more invasive form of erysipelas, hence not displayed. For NSTI, only GAS cases (30%) are indicated. Graph: note that the incidence scale has been cut to allow for comparisons between less common conditions. The bar below each illustration is proportional to the bars in the graph to allow for incidence comparisons. iGAS: invasive GAS infection, HICs: high-income countries, LICs: low-income countries. References: pharyngotonsillitis [36], impetigo [37], erysipelas [38,39], scarlet fever [25], ARF [1], RHD [12], PSGN [40], iGAS [1,23,24,41], puerperal fever [23,24,42], NSTI [18,43,44], STSS [45,46].

week [51]. By the third week, 17 to 50% of bacterial settle plates, placed at an elevated location in the classrooms, tested positive for the outbreak strain. Frequent surface disinfection proved ineffective in controlling the spread, as the children themselves were the primary sources of the bacteria. Evidence of heavy asymptomatic shedding (Figure 2), underscores the importance of physical distancing, enhanced respiratory hygiene, and improved ventilation in classrooms during outbreaks [51]. Although scarlet fever mortality can be managed with narrow-spectrum penicillins, the resurgence of the disease and challenges in controlling outbreaks, alongside a concurrent rise in invasive GAS infections, are concerning [52].

Invasive GAS infections can be devastating [41,43]. In the US, between 2005 and 2012, the mortality rates for invasive GAS diseases stood at 11.7%. These rates escalated dramatically for more severe conditions: 29% for NSTI, 38% for streptococcal toxic shock syndrome (STSS), and 45% for patients experiencing septic shock [53]. The reason why GAS leads to mild infections in some but life-threatening conditions in others has been the subject of extensive research. Studies suggest that a higher incidence of invasive disease among household contacts of affected individuals could be due to both the increased virulence of specific GAS strains and shared genetic susceptibility factors [54,55].

Particularly virulent clones, such as the M1T1 clone that arose and disseminated globally in the 1980s, and its sublineage the M1UK that emerged in 2015, are similar to other common GAS strains but have significant molecular advantages [56,57]. The M1T1 clone is an *emm1* (M1) type of GAS and had acquired bacteriophage-encoded DNAse, phage-encoded SpeA, increased expression of SLO and a cytotoxic NAD+ glycohydrolase [58]. The M1UK clone is responsible for the current resurgence of scarlet fever, due to

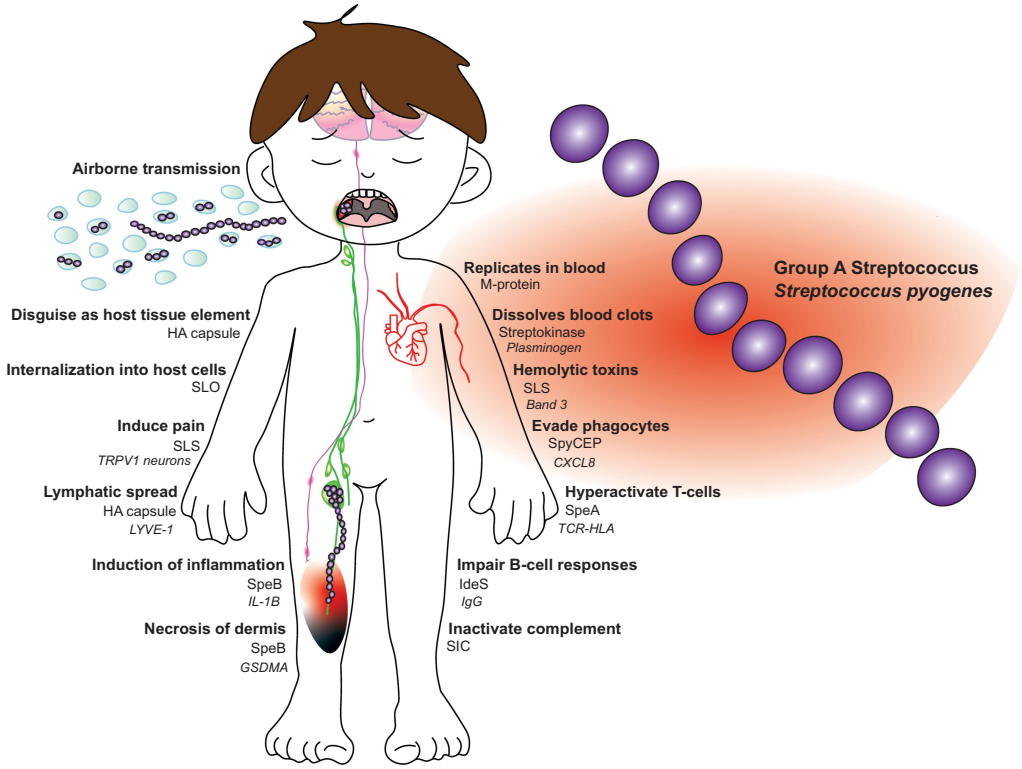

Figure 2. *Clinical features of Group A Streptococcus (GAS).* GAS exhibits a wide array of pathogenic effects in blood, skin- and soft tissues, the lymphatic system as well as on immune cells and neurons. Clinical aspects are visualized next to an example of a virulence factor with that property and, in some cases, the specific host cell receptor. GAS spread through airborne transmission from asymptomatic pharyngeal carriagers, often school-aged children. In blood, GAS causes hemolysis, replicates, dissolves clots, evades phagocytes, hyperactivates T-cells and impairs B-cell responses. GAS disguises as host tissue elements, internalizes through pore-forming toxins, induces inflammation, pain, dermal necrosis and spreads through lymphatic vessels.

mutations that boosted SpeA expression 10-fold. M1UK swiftly disseminated through the UK [59], as well as in Canada [60] and the US [61], though not New Zealand [62]. In contrast, a scarlet fever outbreak in Hong Kong and China was initially attributed to an *emm12* clone, characterized by antibiotic resistance and a toxic prophage encoding SSA, SpeC, and Spd1 [27]. Subsequent research, however, indicated a significant rise in *emm1* clones, suggesting a more complex epidemiological pattern [63].

Research into the genetic predispositions to invasive streptococcal disease has highlighted the role of HLA alleles. Specific alleles such as *HLA-DRB11501* and *HLA-DQB10602* are associated with a protective effect against severe invasive GAS disease [64], RHD [65,66], and recurrent tonsillitis [67]. Conversely, alleles like HLA-DRB10101 and HLA-DRB10701 are linked to an increased risk of RHD [66,68] and recurrent tonsillitis [67]. A recent case-control study further identified HLA-DQA1 × 01:03 as doubling the risk of invasive GAS disease in otherwise healthy individuals [69]. The interactions between different HLA alleles and the GAS superantigen SpeA also significantly influence disease susceptibility [70]. For instance, HLA-DQA1 interactions with SpeA have been identified as a risk factor for infection [71], while HLA-DQ interactions with SpeA are linked to an increased risk of nasal colonization by GAS [72].

Invasive GAS disease is influenced by a wide array of risk factors. Advanced age may increase susceptibility due to diminished neutrophil responses [73]. Other factors include blunt trauma [74], obesity, diabetes [75], HIV infection, cardiovascular diseases, cancer, injection drug use, residency in long-term care facilities, homelessness, pregnancy, childbirth [22], and having recent influenza [76] or varicella zoster infections [77]. Exposure to children with sore throats also raises the risk [78], as do deficiencies in immune response molecules such as IL-1β [79], IL-6 [80], and IL-17D [81].

NSTI are more commonly associated with blunt trauma, absence of pre-existing skin lesions, and lower BMI compared to non-necrotizing cellulitis [82]. The post-COVID-19 context has also introduced

new hypotheses: some suggest that "immune exhaustion" following SARS-CoV-2 infection may predispose individuals to invasive GAS disease, while others attribute the surge in cases to "immune debt," a result of prolonged social distancing measures [58]. Interestingly, many individuals who develop invasive GAS disease are previously healthy with no known risk factors. Immunity from prior GAS infections can provide some protection, but those who have experienced invasive GAS infections often show lower antibody levels against the M1 protein and superantigens, increasing the risk of future episodes [83].

Establishment of infection

The establishment of a GAS infection is a complex process that begins with the bacterium attaching to pharyngeal and dermal epithelial cells. In the case of skin infections, areas with previous damage or injury can provide a conduit for GAS to penetrate the dermal barrier. GAS employs a variety of mechanisms to facilitate this attachment, making use of several key molecules (Appendix, Table A1). These include M protein and lipoteichoic acid, which are crucial for initial adherence. Additionally, the bacterium utilizes fibronectin and fibrinogen-binding proteins, collagen-binding proteins, and a hyaluronic acid capsule, all of which enhance its ability to bind firmly to human cells.

GAS exhibits significant genetic diversity, largely due to variations in the M protein, encoded by the *emm* gene. With over 200 distinct *emm* types identified [84], only a select few are responsible for the majority of human infections in high-income countries. GAS strains have historically been categorized as either throat-associated or skin-associated, based on the *emm* types they express. While there is some overlap in the types that cause strep throat and those that lead to invasive disease, there is notably less overlap between the types causing skin infections and those associated with invasive disease [85]. Invasive GAS diseases are most frequently associated with *emm* types 1, 3, 28, and 12, which are linked to severe outcomes like sepsis, STSS, NSTI, and an increased risk of death [53]. These patterns are consistent across different regions, including Europe, where similar *emm* types predominate [86,87]. In China, the most common *emm* types are 12, 1, 3, and 4 [63,88].

The M protein, a pivotal virulence and immunological factor in GAS, was first identified in studies conducted by Rebecca Lancefield a century ago [89]. As the most abundant protein on the GAS surface, it plays a crucial role in the establishment of infection by binding to keratinocytes via the CD46 receptor (Figure 3) [90]. Beyond this initial interaction, M protein significantly contributes to GAS pathogenicity through several mechanisms [86,87], including antigenic variation, inhibition of phagocytosis, blocking the activation of the alternative pathway of the complement system, and exerting a procoagulant effect by inducing the synthesis of tissue factor in endothelial and monocyte cells.

Most GAS strains produce a surface polysaccharide capsule made of hyaluronic acid (HA), which resembles human connective tissue. This capsule is antiphagocytic and aids in GAS attachment to keratinocytes and pharyngeal cells via the CD44 receptor (Figure 3), causing membrane ruffling and tight junction disruption that facilitate tissue penetration through a paracellular route [91–93]. Synthesis of the HA capsule is orchestrated by the *hasABC* synthase operon [91], which constructs the linear HA polymer by sequentially adding glucuronic acid and β1,3-linked N-acetylglucosamine residues [92,93]. Interestingly, certain invasive GAS serotypes, such as M4 and M12, lack the *hasABC* synthase operon and do not express an HA capsule. Instead, these strains produce HylA, an enzyme that degrades HA in mammalian connective tissues [94]. There is a mutually exclusive expression pattern between the HA capsule and hyaluronidase in GAS strains [94]. Moreover, loss of capsule expression, such as in the pandemic M1T1 clone, is associated with increased expression of an operon encoding toxins [56]. Additionally, the HA capsule contributes to pilus formation and biofilm development [95].

GAS can also co-opt the inflammatory response to its advantage in establishing pharyngeal infection. Specifically, the inflammation induced by interleukin-1 beta (IL-1β) plays a pivotal role by recruiting neutrophils to the nasopharynx. Research indicates that when IL-1β signaling is inhibited, the protease SpeB is neutralized, or neutrophils are depleted, the nasal carriage of GAS is significantly reduced [96]. This suggests that GAS benefits from the inflammatory response in the upper respiratory tract, possibly by disrupting the colonization resistance normally provided by the native microflora [96].

Plasticity is seen in several other GAS proteins than M protein. Historically, the T-antigen has been used as a supplementary serotyping tool to classify GAS strains into 21 T-types. T-antigens are highly variable molecules that make up the GAS pilus and are involved in adhesion, colonization and immune evasion [97]. The mechanisms behind GAS genetic diversity are homologous recombination and high levels of accessory gene plasticity [84]. The maintenance of many distinct genetic lineages of GAS not restricted to geographical boundaries is suggestive of rapid international spread followed by diversifying selection probably driven through immune selection and/or strain

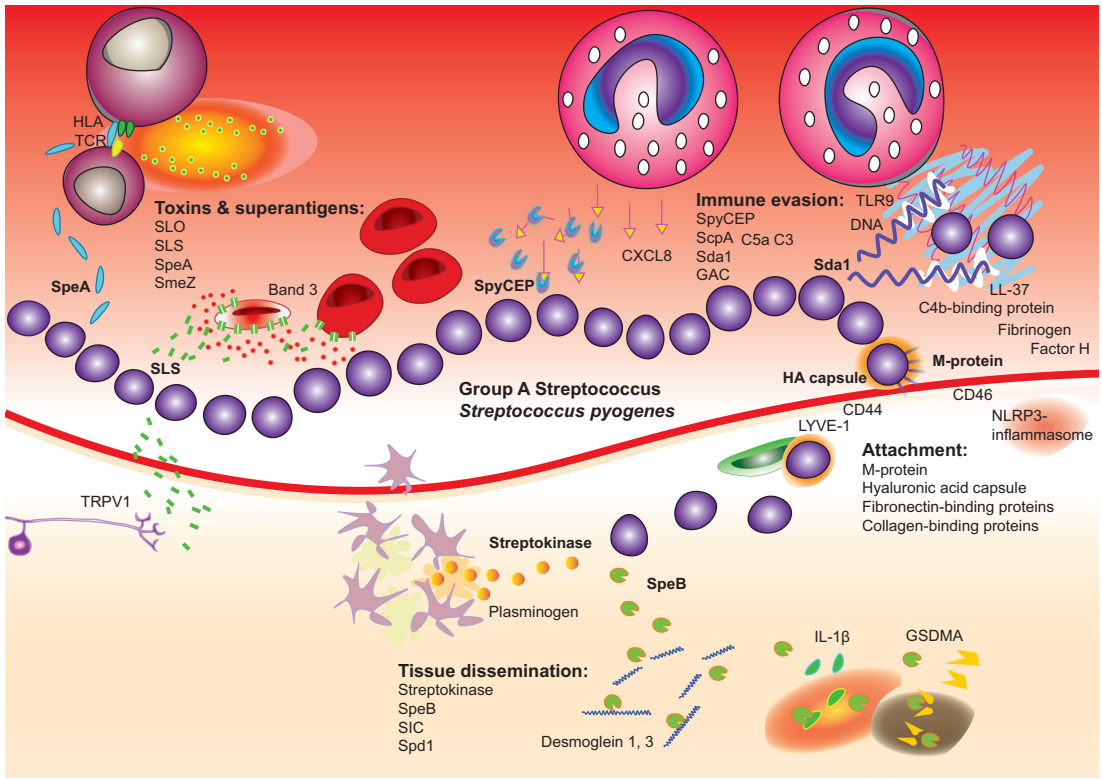

Figure 3. *Pathogenesis of Group A Streptococcus (GAS).* GAS induces pathology through the actions of toxins and superantigens, immune evasion, host cell attachment, and tissue dissemination. Specific examples of virulence factors in these categories are visualized and, in some cases, the specific host cell receptors. GAS lyses red blood cells, hyperactivate T-cells, evade phagocytes, degrade neutrophil extracellular traps, internalize into host cells and lymphatic vessels, activate the NLRP3 inflammasome and IL-1β, disseminate through host tissues by degrading host proteins through protease activity and fibrinolysis through plasminogen activation. Pain is induced through activation of nociceptor neurons and skin necrosis through gasdermin activation.

competition between phylogroups [84]. Approximately 50% of the accessory gene pool of GAS is phage related [84]. Recent studies of GAS pangenome has systematically analyzed GAS genes *in vitro* and *in vivo* and found that 24% of the genome is essential for GAS survival [98].

Recent studies in non-human primate (NHP) models have identified numerous GAS genes involved in pharyngitis [99], including M protein, ScpA, SOF, and *S. pyogenes* adhesion and division protein (SpyAD), the latter of which also contributed to colonization and disease in NHP studies of GAS genital tract infection [100].

Immune evasion

A distinctive characteristic of GAS, compared to other major human bacterial pathogens, is its ability to replicate in human blood. This capability is typically assessed using the Lancefield whole blood killing assay [101]. Researchers recognize that the bacterium's ability to resist opsonization and phagocytosis is a primary factor contributing to its virulence [102]; hence, this capacity remains a subject of extensive research over the years [103].

M protein plays a crucial role in the immune evasion strategies of GAS, primarily by inhibiting the complement system through two key mechanisms. It binds to factor H and fibrinogen (Figure 3), which significantly reduces the opsonization of GAS (Figure 2), thereby impairing the immune system's ability to mark the bacteria for destruction [104,105]. M protein also interacts with C4b-binding protein to inhibit the classical pathway of complement activation [106]. M protein has affinity for several other plasma proteins, including IgG, IgA and complement regulatory proteins [107]. Different M proteins have different preferences/specificities, e. g. M-type 1, 5 and 6 bind fibrinogen but not C4b-binding protein, which instead is bound by M4, M22 and M60 [108]. Without M protein, GAS becomes highly susceptible to rapid phagocytosis [109]. Immunity developed against a specific strain's M protein can protect against GAS infection by enhancing phagocytosis and bacterial killing. This has led to

the classification of GAS strains into specific M serotypes [110]. However, the immune response to M protein can also have detrimental effects. Antibodies generated against M protein may induce autoimmunity, as they can cross-react with human connective tissue antigens due to structural similarities [111,112]. Additionally, the M gene superfamily extends beyond M proteins to include M-related proteins and immunoglobulin-binding proteins, which also play roles in the bacterium's interaction with the host immune system [113–115].

The surface-expressed M1 protein of GAS plays a critical role in evading host defenses by interacting with key antimicrobial components. It can sequester and neutralize LL-37, a potent antimicrobial peptide, thereby preventing it from exerting its antibacterial activity [116]. Additionally, M1 protein exhibits resistance to the antimicrobial activity of histones, which are crucial components of neutrophil extracellular traps (NETs). This resistance helps GAS to survive and propagate even in the hostile environment created by neutrophil activation [117].

Invasive M1T1 GAS strains utilize several sophisticated mechanisms to evade neutrophil defenses. These strains express a phage-encoded DNAse, Sda1, which degrades neutrophil extracellular traps (NETs) and the bacterium's own CpG-rich DNA [118]. Sda1 is critical for promoting resistance to neutrophils and enhancing virulence, as demonstrated in murine models of necrotizing fasciitis [119]. It also suppresses macrophage activity and TLR9-mediated immune responses [119]. Additionally, the streptococcal collagen-like protein 1 (Scl-1), prominently expressed in the M1T1 clone, protects GAS from NET-associated antimicrobial peptides and inhibits the release of myeloperoxidase (MPO), thereby reducing NET formation [120]. GAS can further impair neutrophil defenses by engaging the inhibitory Siglec-9 receptor via its hyaluronic acid (HA) capsule, which blunts the oxidative burst, NET formation, and overall bactericidal activity of neutrophils [121]. The toxin streptolysin O (SLO) also plays a role by impairing neutrophil oxidative burst, degranulation, and NET formation at sublethal concentrations [122].

The classical Lancefield group A carbohydrate (GAC) antigen [123], a high molecular weight polymer consisting of rhamnose with an N-acetylglucosamine (GlcNAc) side chain, makes up about 40–50% of the GAS cell wall. This antigen is the species-defining marker used routinely for the rapid diagnosis of strep throat. Removal of the GlcNAc side chain increases GAS susceptibility to neutrophil killing (Figure 3), platelet-derived antimicrobials in serum, and the antimicrobial peptide LL-37, and results in attenuation in infection models [124]. The impact of the GAC GlcNAc side chain on GAS virulence may vary depending on the presence of other virulence factors within each strain [125]. It is important to note that 2–3% of streptococci that carry the group A antigen are not *S. pyogenes* but rather *Streptococcus dysgalactiae* subspecies *equisimilis* (typically carrying group G or C antigens) or *Streptococcus anginosus* (a commensal viridans streptococcus) [126]. Furthermore, not all M-types of GAS are virulent, and many group G streptococci, which can share pathogenic similarities with GAS, are capable of causing disease [127].

GAS chemokine-inactivating protein (SpyCEP), a surface-bound serine protease, plays a crucial role in immune evasion by targeting and degrading chemokines such as CXCL8 (Figure 3). This activity significantly hinders the recruitment of neutrophils to infection sites (Figure 2), thereby facilitating the persistence and spread of GAS [128,129]. Due to its pivotal role in immune modulation and high immunogenicity, SpyCEP is recognized as an attractive candidate for vaccine development [130]. RNAseq analysis of GAS infected tonsil epithelial cells showed that while GAS infection generally induces a pro-inflammatory response, SpyCEP specifically reduces the levels of CXCL8 post-transcriptionally, thus mitigating one of the body's key inflammatory reactions to infection [131].

Streptococcal C5a peptidase (ScpA), a surface-bound endopeptidase, plays a critical role in GAS immune evasion by cleaving the complement-derived chemotaxin C5a at its PMN-binding site, thereby inhibiting the recruitment of phagocytic cells (Figure 3). Recent research has further uncovered that ScpA also targets C3, impairing neutrophil activation, phagocytosis, and chemotaxis [132]. Beyond its role in immune modulation, ScpA aids in GAS nasal colonization by adhering to epithelial and endothelial cells through mechanisms independent of the complement system. Moreover, intranasal immunization against ScpA has been shown to prevent GAS infection in murine nasal-associated lymphoid tissue (NALT), highlighting its potential as a target for vaccine development [133].

A recently identified immune evasion strategy employed by GAS involves the use of the surface-associated S protein to capture red blood cell fragments. This mechanism aids GAS survival by cloaking its opsonic targets under natural host cell components, effectively disguising the bacteria from the host immune system. The presence of S protein is crucial

for maintaining the virulence of GAS; its absence leads to reduced virulence and affects the development of immunological memory against the pathogen [134].

Although traditionally considered an extracellular pathogen, recent studies have explored the intracellular capabilities of GAS. GAS has demonstrated the ability to survive within macrophages, a process that relies on the presence of M1 protein [135], and is proposed to potentially account for the presence of viable bacteria in biopsies from patients undergoing antibiotic therapy [136]. GAS can replicate within viable human macrophages, indicating an active intracellular life cycle [137]. In the context of pharyngeal keratinocytes, GAS secretes SLO, which triggers autophagy – a process where the cell attempts to digest internalized material. However, the combined actions of SLO and NADase disrupt the maturation of autophagosomes, thereby prolonging GAS's intracellular survival (Figure 2) [138]. Additionally, GAS evades clearance by keratinocytes due to a lack of ubiquitination, which is essential for targeting bacteria for autophagy [139].

Beyond its classical interactions with phagocytes and lymphocytes, GAS also significantly impacts coagulation and thrombocytes. Severe infections often induce a pro-coagulant and pro-inflammatory state, with some effects directly linked to the actions of M protein [140]. Common complications in severe infections include deep venous thrombosis [141] and thrombocytopenia. These issues may arise from complement activation on the surface of activated thrombocytes, leading to their activation, aggregation into thrombi, and complex formation with neutrophils and monocytes. This process ultimately contributes to thrombocyte phagocytosis [142]. Similarly, the binding of fibrinogen and IgG by GAS in plasma triggers comparable effects, promoting coagulation disturbances [143].

Immunity to GAS is multifaceted and varies significantly with age. Adults generally experience fewer infections than children, partly due to higher antibody titers [144]. Additionally, complete immunity against scarlet fever typically develops over time, with anti-GAS antibodies potentially persisting for up to 45 years. This long duration supports the belief that immunity is primarily M-type specific [145,146]. However, recent studies indicate that invasive GAS infections can elicit both strain-specific and cross-strain specific opsonic antibodies [147]. GAS has evolved sophisticated mechanisms to evade humoral immune responses. For example, IdeS, a cysteine proteinase, specifically cleaves the heavy chain of Immunoglobulin G (IgG), impacting its functionality (Figure 2) [148,149]. Similarly, the endoglycosidase EndoS targets the Fc region of IgG, hydrolyzing it and impairing its effectiveness [150–152]. While IgG antibodies are crucial for protecting against GAS invasion, IgA antibodies play a key role in preventing adherence and colonization [153]. Similar to *S. aureus* protein A, M protein can reverse antibody orientation through Fc-binding, especially in saliva [154]. Additionally, GAS expresses IgA binding proteins that interfere with the effector functions of IgA, further complicating the immune response [155].

While GAS has known evasion strategies for phagocytes, its interactions with other leukocytes like lymphocytes are less documented. Lymphocytes typically mount a Th1-type pro-inflammatory response to GAS, characterized by the production of cytokines such as IL-1β, IL-6, TNF, IL-12, IFN-γ, and IL-18 [156]. Studies on human pharyngitis have shown that individuals who develop pharyngitis exhibit increased serum cytokines, a reduction in conventional lymphocytes, and activation of unconventional lymphocytes [35]. Specifically, after tonsillar challenge, elevated levels of IL-1β, IL-1Ra, IL-6, and IL-18 in saliva, along with IL-1Ra, IL-6, IFN-γ, IP-10, and IL-18 in serum, indicate strong local and systemic pro-inflammatory responses. Additionally, there are increases in classical monocytes and total dendritic cells in peripheral blood, with reductions in B-cells and CD4+ T-cells. While conventional peripheral T-cells show no activation, T-cells expressing γδTCR and Vδ2, as well as MAIT cells, are activated.

Tissue invasion

In murine models, the histopathology of streptococcal NSTI shows that while the epidermis remains intact initially, the underlying tissues exhibit significant inflammatory infiltrates, pronounced necrosis, and thrombosis, along with a massive bacterial burden, present both as aggregates and within cells [157]. Over time, necrosis progresses from the deeper tissues to the epidermis, demonstrating the GAS's capability to disseminate through deep layers of soft tissue and invade extensive areas, even with minor epidermal disruptions. The NHP model has been used to identify genes required for necrotizing myositis, and sequencing identified around 100 involved GAS genes [158].

GAS possesses several virulence factors that interact with plasminogen, converting it to plasmin [159,160]. This activation of the host's clot-dissolving system facilitates the bacterium's invasion and movement through tissue barriers. Plasmin aids GAS dissemination by proteolytically degrading host defense proteins. Key plasminogen-activating molecules include streptokinase, a well-known secreted

thrombolytic enzyme (Figure 2) [161], as well as plasminogen-associated M protein (PAM), alpha-enolase [162], and glyceraldehyde-3-phosphate dehydrogenase. In murine models, blocking streptokinase significantly improves survival rates, likely due to the inhibited ability of GAS to escape from blood clots [163]. Streptokinase activates plasminogen, promoting GAS dissemination at wound sites, as it facilitates the rapid dissolution of fibrin clots and the retraction of the keratinocyte wound layer, thus promoting bacterial spread [164].

Streptokinase is so efficient that it is used in medicine as treatment during thrombo-embolic events such as heart attacks, pulmonary embolisms and arterial clots. There is a production shortage of streptokinase, prompting efforts to genetically engineer less virulent streptococcal strains to increase availability [165]. Despite its significance, GAS can still acquire plasmin without streptokinase by utilizing host activators like urokinase plasminogen activator (uPA) [166], a capability recently demonstrated in a susceptible mouse model [167].

Initially mis-classified as a superantigen, streptococcal pyrogenic exotoxin B (SpeB) is an important virulence factor in GAS (Figure 2). SpeB is both a secreted, extracellular cysteine protease and a surface-bound adhesin with binding activity to laminin and other glycoproteins. The majority of pathogenic GAS strains secrete SpeB [168], which degrades nearly all proteins secreted by GAS, including other virulence factors. This protease plays a vital role in degrading the extracellular matrix, aiding colonization, and disrupting competitor bacteria such as *S. aureus* in biofilms [169]. It also cleaves desmoglein 1 and 3, exacerbating skin involvement in GAS infections [170], and neutralizes the signaling and antibacterial properties of chemokines from inflamed epithelium [171]. SpeB and SIC were among the streptococcal proteins identified in a comprehensive proteomic analysis of GAS infected human samples [172]. Furthermore, SpeB can directly activate IL-1β, bypassing canonical inflammasome pathways, enhancing immune responses that restrict GAS invasion (Figure 3) [79]. In some cases, invasive GAS strains may mutate to repress SpeB expression, helping them evade these immune responses.

Recent studies have linked GAS-induced endothelial apoptosis to both SpeB and the caspase pathway, although the exact mechanisms were initially unclear [173]. A significant breakthrough was the discovery that SpeB triggers epidermal pyroptosis, an inflammatory form of cell death, by cleaving gasdermin A (GSDMA). GSDMA acts as a sensor, substrate, and effector of pyroptosis, making it central to this process (Figure 3) [174,175]. GSDMA cleavage and activation is beneficial in severe GAS infections, as it leads to apoptosis in keratinocytes, protecting mice from widespread disease. Inhibitors of SpeB can enhance GAS clearance in the presence of human neutrophils [176].

The GAS extracellular nuclease Sda1 mediates M1 GAS escape from NETs (Figure 3), and its upregulation *in vivo* serves as a selective force for *covR/S* mutations associated with increased tissue dissemination [177]. Another DNAse, Spd1 (Figure 3), may contribute to nasopharyngeal shedding of GAS. Recent epidemic *emm3* genotype GAS strains are seen to have gained a prophage expressing Spd1 and superantigen SpeC [178].

GAS has a preference for inducing pathology in the lymphatic system and draining lymph nodes. Recent data have enhanced our understanding of GAS lymphatic spread (Figure 2). The lymphatic vessel endothelial receptor-1 (LYVE-1), sharing 41% amino acid sequence similarity with CD44, is identified as a critical host receptor for capsular HA (Figure 3) [179]. Non-encapsulated strains show reduced ability to disseminate to draining lymph nodes *in vivo*, while hyper-encapsulated (mucoid, often *covR/S* mutant) strains have a particular propensity for lymphatics. Recent findings demonstrate GAS spread through lymphatic metastasis: pathogenic spread through lymphatic vessels [180]. The bacteria can enter afferent lymphatics and reach lymph nodes, and use efferent lymphatics to enter the bloodstream [180]. Metastasizing bacteria are extracellular. Furthermore, mild blunt contusion of soft tissue enhances bacterial migration to the local draining lymph node from the site of contusion following GAS bacteremia [181].

Streptococcal inhibitor of complement (SIC) is a virulence factor expressed by M1 strains, known to inactivate components of the complement system (Figure 2, 3), inhibit host antimicrobial factors, and contribute to bacterial adherence to epithelial cells. Human antibodies to SIC are prevalent (around 40%), surpassing the frequency of M1 antibodies [182]. SIC immunization was found to protect mice from disseminating disease following intranasal or intramuscular infection. Notably, naturally occurring SIC antibodies did not provide protection against GAS growth in whole blood, whereas vaccine-induced antibodies did [183].

Toxins and superantigens

A severe form of streptococcal (or staphylococcal) septic shock is STSS, a state of multiorgan failure and signs of toxicity on mucosal surfaces following infection

[184]. STSS also occurs in young, immunocompetent individuals who rapidly deteriorate into life-threatening states with high mortality despite appropriate treatment. The extreme toxicity caused by GAS is likely due to a combination of toxins and superantigens [185]. Another significant manifestation of toxicity associated with GAS is scarlet fever, historically responsible for a significant portion of childhood mortality, now resurgent in many parts of the world.

The pore-forming streptolysin O (SLO) induces rapid, dose-dependent apoptosis in most human cells, particularly macrophages and neutrophils [186]. These cytolysins not only form pores in cholesterol-rich membranes but also exhibit high-affinity lectin activity [187]. SLO promotes survival, replication, and cytosolic growth in macrophages [137]. The recent emergence of pandemic clones of GAS with low capsule expression and high SLO expression has been noted [56]. In human decidual tissues, SLO and SpeB were identified as the main virulence factors [42]. Following intramuscular injections of SLO into rats, decreased tissue perfusion and occlusive intravascular complexes of platelets and neutrophils were observed, indicating that SLO may induce microvascular thrombosis leading to toxin-induced ischemia [188].

Another GAS pore-forming toxin, streptolysin S (SLS), induces a dramatic osmotic change in red blood cells, leading to cell lysis (Figure 2) [189]. Specific binding to ion channels on erythrocytes (Band 3) and keratinocytes (NBCn1) has recently been identified as important (Figure 3) [189,190]. SLS is required for the establishment of nasopharyngeal infections in HLA-transgenic mice and contributes to localized tissue destruction of nasal epithelium [191]. The "pain out of proportion" often described as a hallmark of necrotizing fasciitis may be explained through direct activation of TRPV1+ nociceptor neurons by SLS (Figure 2, 3) [192]. SLS-induced pain triggers the release of a neuropeptide that inhibits recruitment of neutrophils, a form of neuroimmune hijacking. This effect can be blocked both by antagonizing the neuropeptide and through local botulinum toxin (Botox) injection, reducing lesion development and infection-related morbidity.

GAS and *S. aureus* secrete toxins known as superantigens (Figure 3). These molecules cross-link the beta chain (Vβ) of the T-cell receptor with human MHC class II (HLA) expressed on antigen-presenting cells (B cells, monocytes, and dendritic cells). This leads to activation, excessive release of inflammatory cytokines, and proliferation of T-cells (Figure 2). The primary toxin implicated in scarlet fever is thought to be SpeA [25]. In addition to the classical GAS superantigens (SpeA, SpeC, SpeG-M, SmeZ, and SSA), two new superantigens have been recently described: SpeQ and SpeR [193]. These 13 superantigens are expressed in different combinations and quantities by different clinical isolates, as the majority of them are encoded on mobile genetic elements. A typical GAS isolate expresses 3–4 superantigens, with SpeA being the most common to find in NSTI or STSS isolates [194]. Even though superantigens are highly effective at low concentrations, the quantity of expression can matter. The expression of SpeA was 9 times higher in the M1UK clone than in comparable isolates [59]. However, no correlation has been demonstrated between the amount of superantigen expressed and disease severity; rather, the opposite is observed. Identical strains can cause both mild and severe invasive disease [195]. Individuals with a propensity to respond more strongly to superantigens develop more severe manifestations [196], and low humoral immunity confers susceptibility to severe disease [83].

In addition to severe disease and cytokine storms, superantigen function is linked to GAS colonization [72]. Nasopharyngeal infection by GAS requires superantigen-responsive Vβ-specific T cells, suggesting that GAS manipulate T-cells to establish nasopharyngeal infection [197]. Thus, superantigen interactions with host cells not only depend on HLA [64], but also the Vβ-profile of the host's T-cell repertoire. Typically, superantigen activation results in the expansion of T-cells with specific Vβ receptors in the acute phase, followed by depletion of that specific T-cell population [19,198]. Vβ activation in STSS correlates with the number of organ dysfunctions [19]. A newly described subset of T-cells, the mucosal-associated invariant T-cells (MAIT-cells), has been linked to superantigen activation. Although few in circulation, MAIT cells are the main responders among T-cells to superantigens and produce a majority of the cytokines [199]. MAIT cells in patients with STSS were activated and proliferated. In tonsillar tissue, the presence of SpeA and other superantigens resulted in B cell apoptosis and abrogation of total IgA, IgM, and IgG production [200]. The superantigens drove the follicular T-cells to a proliferating phenotype with the loss of tonsillar B-cells and antibody production.

M protein is anchored to the cell wall of GAS by sortase A [201]. While not generally considered a superantigen, M protein exhibits superantigen activity when in a highly purified soluble form lacking the membrane-spanning region [202]. In circulation, the virulence contributions of M protein expand from phagocyte evasion to additional potent proinflammatory effects. M protein released from the bacterial surface

forms pathological complexes with fibrinogen [83], leading to the activation of neutrophils through beta2 integrins [84]. This activation results in the release of heparin-binding protein, which induces vascular leakage and contributes to severe pulmonary damage and multi-organ failure, characteristics of STSS [84]. Released M1 protein also activates the NLRP3 inflammasome, leading to the release of IL-1β and macrophage programmed cell death [203].

In tissue-persistence: Intracellular survival, biofilm and immune modulation

Streptococci are well-known to respond appropriately to narrow-spectrum antibiotics, such as simple penicillins. However, recurrent disease, indicative of local persistence, is well-known clinically [204], and biopsies from severe tissue infections such as NSTIs contain a high bacterial burden despite prolonged antibiotic treatment [136]. Recurrence of erysipelas is around 16% [205]. Intracellular invasion by GAS, once considered an extracellular bacterium, was described long ago [153,206]. Tonsils excised from individuals after treatment failure of pharyngotonsillitis harbor intracellular GAS [207]. Fibronectin-binding proteins associated with internalization are similar between invasive and non-invasive isolates [208]. This internalization might represent successful containment by the host, but it could also lead to invasion of deeper tissues or constitute a pathogen reservoir with associated risk of recurrence. Another possible explanation for persistence in GAS infections is biofilm formation, identified in tissue biopsies from a third of NSTI patients [209]. Biofilm formation can be directly influenced by host and environmental factors [210].

GAS preferentially targets human tonsils, akin to nasal mucosa-associated lymphoid tissue (NALT) in mice [133]. GAS stimulation results in the expansion of CD4+ IL-17+ T-cells in NALT [211]. Exposure to saliva leads to GAS aggregation and inhibits binding to buccal epithelium [212]. Recurring tonsillitis, linked to immunosusceptibility involving HLA haplotypes and follicular T-cells, shows reduced germinal center size and fewer helper T-cells and B-cells, impairing antibody responses [67]. In recurrent tonsillitis, germinal center T-cells express granzyme B, leading to B-cell cytotoxicity, revealing a novel host-pathogen interaction mechanism.

Genetics and the regulation of virulence

The GAS genome, spanning around 1.85 Mb and encompassing approximately 1,800 genes, contains many genes whose roles in pathogenesis are still not fully understood [213]. Key to the coordinated expression of these genes are several genetic regulatory systems, which include response regulators and two component signal transduction systems [214]. The response regulators, such as multiple gene regulator (mga), RofA-like protein (RALP), and Rgg/RopB, control expression of various virulence factors in a growth phase-dependent manner. The mga regulator, for instance, activates the transcription of multiple virulence factors such as M protein, ScpA, M-like proteins, serum opacity factor (SOF) and SIC [3]. Conversely, Nra (negative regulator of GAS) suppresses these and other genes, including mga itself [215]. Two-component systems such as CsrRS/CovRS, FasBCAX, and Ikk/Irr play crucial roles in modulating GAS pathogenicity. The CovRS system, in particular, represses the expression of about 15% of the GAS transcriptome, including many virulence factors [216], and its inactivation can lead to increased virulence. This system is a known hotspot for inactivating mutations that can enhance GAS pathogenicity [216]. The Ihk/irr two component system is upregulated during pharyngitis [217], promotes evasion of neutrophil phagocytosis, and is required for full virulence in a mouse infection model [218]. Ihk/Irr is likewise transiently upregulated in GAS shortly following intracellular uptake in macrophages; however, after several hours, up-regulation of the CovR/S system predominates [219].

A new quorum-sensing system, sil, present in a subpopulation of GAS strains, controls the expression of bacteriocins in response to host signals like asparagine [220]. This system can trigger an autoinduction mechanism that gives GAS a competitive advantage in polymicrobial environments. Additionally, GAS induces endoplasmic reticulum stress to acquire asparagine, a process that can be mitigated by PERK/ISR inhibitors, which have shown promise in reducing bacterial load and tissue damage in the infected host [221]. Furthermore, GAS adapts to various environmental stressors, such as glucose starvation, by upregulating the arginine deiminase pathway. This pathway enhances bacterial survival and virulence, partly by increasing the expression of exotoxins [222]. In polymicrobial environments, the Gram-negative metabolite Oxo-C12 has been shown to promote GAS adherence to host tissues and biofilm formation, highlighting the complex interplay between GAS and other microbial communities [223].

GAS is a natural source of Cas9 nuclease [224], often used today as a genome editing-tool for precise DNA targeting [225,226]. Cas9 is considered a bacterial

immune defense against phages and plasmids, but it is increasingly recognized as a GAS virulence factor. Cas9 mediates adherence, growth in human blood, and virulence in a murine NSTI model [227]. Most humans have antibodies [228] and T-cell responses to Cas9 [229]. The GAS CRISPR-Cas9 system prioritizes defense against the most recent invader [230]. Additionally, there are certain phages that neutralize this bacterial immune system [231,232].

Diagnostic issues of invasive disease

Diagnosing NSTI, including necrotizing fasciitis and myositis, presents challenges due to the absence of cardinal symptoms. The most common symptom observed is bruising of the skin in 51% of patients [18]. Other frequent symptoms include severe pain requiring opioids (42%), purple/black discoloration of the skin (32%), gas on radiology (30%), skin bullae (27%), crepitus (14%) and skin anaesthesia (6%). However, the majority of patients (87%) exhibit one or more of these findings. Although laboratory values suggest infection, no specific biomarker for NSTI has been identified.

The Laboratory Risk Indicator for Necrotizing Fasciitis (LRINEC) score was developed to predict NSTI risk early on [233], but its predictive value is limited [234]. Efforts to enhance the LRINEC score by emphasizing high CRP values and clinical features like pain out of proportion have been made [235]. Some advocate for rapid StrepA testing in NSTI cases [236] as in pharyngitis [237]. Other investigated biomarkers include the nitric oxide system [238] and inflammatory cytokines [234]. Pentraxin-3 has been associated with negative outcomes such as septic shock, amputation, and risk of death [239]. Thrombomodulin has been proposed as a biomarker for NSTI, showing promise in discriminating between NSTI and non-NSTI cases [240]. Additionally, a distinct biomarker profile distinguishing GAS NSTI from other types of NSTI has been identified, involving differential expression of IL-2, IL-10, IL-22, CXCL10, Fas-ligand, and MMP9.

Proteomic analysis of NSTI samples identified 19 GAS proteins, including SIC, trigger factor (TF), and phosphoglycerate kinase [172]. Among human proteins detected, 38% were neutrophil proteins, such as alpha enolase and lactotransferrin, proposed as biomarkers. Transcriptomic analysis revealed a strong interferon-related response specific to GAS NSTIs, with mediators CXCL9, CXCL10, and CXCL11 identified as potential diagnostic biomarkers [241]. Further technical advances promise to improve our understanding of the landscape of proteins at work during streptococcal infections. Detailed mass spectrometry of human plasma protein interactions with GAS found both already described virulence mechanisms and new interactions [242]. Detailed proteomics of mice infected by GAS found markers trackable in plasma samples of infected patients [243].

Diagnosing STSS can be challenging, despite consensus definitions [184]. It is possible that physicians frequently categorize STSS cases as septic shock or invasive GAS disease because of the overlap in presentation such as hypotension with multiple organ failure and isolation of GAS from a normally sterile site, and STSS could be 5.3 times more common than what is currently diagnosed by US physicians [45]. Importantly, there is no age restriction in the STSS criteria.

Treatment of invasive infections

Most GAS infections are treatable with penicillin [237], but treatment of invasive disease requires more complex management. A standard approach combines a β-lactam antibiotic with clindamycin to reduce toxin production, alongside surgical debridement. Despite these measures, mortality persists, leading physicians to consider additional treatments, though their efficacy is uncertain. In a large NSTI patient cohort, all 409 patients underwent surgery within a median of 19 hours of admission, with a median of 4 surgeries [18]. Most patients received combination antibiotics: 98% clindamycin, 87% a carbapenem, and 62% ciprofloxacin. Additionally, 80% received hyperbaric oxygen treatment (HBOT) and 58% received intravenous immunoglobulin (IVIG). The effectiveness of HBOT and IVIG in treating GAS NSTI remains debated.

The efficacy of adjunctive clindamycin treatment can be related to reduced Sda1 and SLO activity [244]. A retrospective cohort study of US hospitals showed that adjunctive clindamycin confers a mortality benefit [245]. The odds ratio for in-hospital mortality was 0.44 for clindamycin treated patients with invasive GAS infections, compared to non-clindamycin treated patients.

IVIG treatment confers inhibitory activity against superantigens [246–248], thus it was proposed that IVIG could enable a conservative surgical approach in combination with clindamycin [249]. A single-center randomized controlled study of adjunctive IVIG in NSTI of all microbiological etiologies showed no benefit [250]. However, in the subgroup of patients dominated by GAS infections, i.e. those patients with NSTI of the extremities, IVIG treatment was found to be beneficial. A single dose of 25 g IVIG is sufficient to

achieve superantigen neutralization, and there is a correlation between administered dose IVIG and superantigen protection [194]. IVIG was associated with survival in a cohort of 126 patients with GAS NSTI [82]. In STSS, IVIG is a clear survival factor, shown in this meta-analysis [251]. Recently, affinity purification of IVIG was shown to increase its effectiveness in promoting GAS opsonophagocytosis [252].

In the case of HBOT, a systematic review from 2005 failed to locate any relevant clinical evidence supporting or refuting its effectiveness in managing NSTI [253]. Randomized trials are sorely needed. However, a meta-analysis of non-randomized studies involving patients with NSTI of all microbiological etiologies showed a pooled odds ratio for in-hospital mortality of 0.44 in favor of HBOT [254]. Further, the odds ratio for amputation was 0.6 in favor of HBOT, and patients ineligible for HBOT (e.g. due to severe hemodynamic instability) showed decreased odds of survival [255]. An American nationwide retrospective study involving 60,481 patients with NSTI of all microbiological etiologies revealed that HBOT is associated with decreased mortality and amputations, despite the fact that only < 1% of patients received it between 2012 and 2020 [256].

Finally, interesting preclinical research is beginning to emerge in the realm of specific anti-virulence therapeutic strategies targeting GAS. For instance, treatment with a pan-caspase inhibitor reduced GAS skin lesion size and bacterial counts in mice [257], and similar reduction in skin lesion size occurred when GAS-induced pain was blocked by local Botox injection [192]. Additional examples include the broad-spectrum neutralization of GAS pore-forming toxins achieved with human erythrocyte membrane-coated nanoparticles [258], the development and characterization of a SpeB inhibitor [176], and monoclonal antibodies against SLO or M protein that reduced morbidity in GAS superinfection of influenza in a murine model [259].

Antibiotic resistance

A retrospective study of American NSTI patients between 2015–2018 found that clindamycin resistance was common (31%) in GAS NSTI isolates, and that this resistance was associated with more frequent need for amputations [260]. Adjunctive clindamycin may be replaced by linezolid as resistance to clindamycin increases [261]. Another alternative could be tedizolid (a newer oxazolidinone) that had comparable results to linezolid in a murine GAS model [262]. Additionally, adjunctive treatment with rifampicin could be added to the β-lactam and clindamycin regimen [263]. A GAS-infected skin tissue model showed surprisingly high bacterial counts after treatment with penicillin and clindamycin in high doses, while adjunctive rifampicin reduced bacterial counts and bacterial metabolism. Concerningly, the first step in developing β-lactam resistance has been discovered in two GAS strains [264]. A study of 7025 GAS genomes identified 137 strains with reduced β-lactam MICs [265]. This is a warning signal about a potential future where strep throats may not be so reliably treated with penicillin, with massive clinical implications.

Antibiotic resistance has also been described in GAS against commonly used antibiotics such as erythromycin, tetracycline and fluoroquinolone [266–268]. On a global scale, 38% of GAS strains are tetracycline resistant and 25% erythromycin resistant [267]. During a scarlet fever outbreak in Beijing, 96% of strains were erythromycin resistant, 94% tetracycline resistant and 79% were clindamycin resistant [266].

Progress in GAS vaccine development

Lack of relevant animal models, high genetic diversity of antigen targets, safety concerns, lack of consensus on clinical endpoints for establishment of proof of concept, and uncertain market incentives have created major impediments to progress in GAS vaccine development [269]. The pipeline of GAS vaccines remains relatively empty [270]. However, the field of GAS vaccine development has had a revival, especially since the 2018 WHO resolution on rheumatic fever and RHD.

The most advanced M protein (StreptAnova 30-valent, J8/S2 combivax, P * 17/S2 combivax, StreptInCor) and non-M protein (Combo4, VAX-A1, Combo5, TeeVax) vaccine candidates are reviewed in [270]. Successful early phase clinical trials in humans have been conducted without serious safety signals with 4 M protein vaccine candidates: a 6-valent, a 26-valent, a 30-valent (all N-terminal) and a conserved C-repeat region M protein vaccine [271–274]. The 6-valent vaccine provided the first evidence in humans that hybrid fusion M protein is a feasible strategy [271]. The 26-valent [272] and 30-valent [273] vaccine expanded the technique and the C-repeat region vaccine showed that immunity against more conserved regions of the M protein is possible and safe [274].

In the non-M protein based arena, the Combo5 vaccine uses antigens SLO, SpyCEP, ScpA, arginine deiminase (ADI), and TF. It has been tested in the NHP-model, where antibody responses against all antigens were detected in serum and immunized NHPs showed a reduction in pharyngitis and tonsillitis [275]. Another non-M protein vaccine candidate

named VAX-A1 uses modified GAC conjugated to SpyAD in combination with SLO and ScpA [276]. This has been developed further using non-native amino acid click-chemistry to conjugate GAC to SLO which successfully generated functional antibodies and protected mice against systemic GAS challenge [277]. The TeeVax vaccine candidate focus on T-antigens [97] and GlaxoSmithKline/GVGH has a non-M protein combination vaccine consisting of SpyCEP, SLO, SpyAD recombinant proteins and native GAC conjugated to a carrier protein [278].

Immunization to the conserved (J8) region of the M protein and to the superantigen SpeC protect mice against STSS [279]. Also, passive immunotherapy with antibodies to J8 could resolve established disease, which could be further enhanced by addition of SpeC antibodies.

An opsonophagocytic killing assay has been developed to measure serum protection against GAS without fresh neutrophils and complement, to reduce donor variability [280]. The assay utilizes human promyelocytic leukemia cells as a source of neutrophils and baby rabbit complement, giving the model a potential to provide a robust and reproducible platform to accelerate vaccine development. However, an opsonophagocytosis assay alone might be inadequate to understand protection after GAS vaccination, particularly considering protection from immune evasion factors [281]. A recent advancement has been the development of the human infection model of pharyngitis [282]. During 2018–2019 a total of 25 healthy adults were challenged with GAS and pharyngitis was diagnosed in 85% at the starting dose level (1-3×10^5 CFU/mL). Antibiotic treatment was started at diagnosis of pharyngitis or at 5 days post-challenge. This model can be used to establish immune correlates for protection to help vaccine development.

Outlook

GAS produces a broad repertoire of virulence factors, and we are getting closer to answering which of them are key to driving pathogenesis. However, this represents a race against the clock, since new strains arise and the molecular evolutionary events transpiring in just one bacterial cell can ultimately spread and produce millions of human infections worldwide [57]. During the initial year of the COVID-19 pandemic, invasive GAS infections decreased rapidly, showing that protection against disease is possible. Today though, invasive GAS disease is back, and we are seeing record-breaking numbers of infections in many countries, a critical opportunity to conduct patient-based research. When clinical awareness is high, and all available therapy is given, mortality in NSTI is still around 18% and the amputation rate is 22% [18]. There is a substantial burden of invasive GAS disease in pregnancy and young children in low-income countries [24] and 319,000 people die from RHD every year [12]. With new drugs, this could decrease, but the threat of antibiotic resistance is approaching and becoming real among additional streptococcal pathogens, indicating that clinical outcomes could actually deteriorate. For example, centers located in high clindamycin-resistant areas may be advised to adjust to linezolid as adjunctive treatment in NSTI. The future threat of β-lactam resistant GAS exists, but is balanced by the advances in vaccine development. Increasing numbers of potential therapeutics and vaccines are in the pipeline, yet while waiting, we should aim to reduce barriers to access primary healthcare [283].

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

H.B.is funded by the Swedish Society for Medical Research (SSMF) contract number PD21-0193. V.N.research on streptococcal infections is funded by NIH grants R01-AI173689, R01-AI154149, and the LeDucq Foundation PRIMA and iSpy Networks. Fondation Leducq [PRIMA, iSpy Networks]; National Institutes of Health [R01-AI173689, R01-AI154149]; Svenska Sällskapet för Medicinsk Forskning [PD21-0193]

Author contribution statement

H.B. and V.N. conceptualized and designed the review article. Analysis and interpretation of the published literature was performed by both authors. H. B. drafted the paper, V. N. revised it critically for intellectual content; both are responsible for the final version and accountable for all aspects of the work.

Data availability statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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A1 Appendix

Table A1. GAS virulence factors.

Virulence factor	Figure	References
M protein	1, 2	[45,76,108,109,127,128,132,134,135,195,274,284]
HA capsule	1, 2	[48,83,86,87,113,171–173,285]
Fibronectin-binding protein	2	[201]
Collagen-binding protein	2	
SLO	1, 2	[48,114,129,130,179–181]
SLS	1, 2	[182,183,185]
SpeA	1, 2	[64,75,187–190,192,193]
SpeB	1, 2	[71,88,160–164,166–168]
SpeC, SpeD, SpeF, SpeG, SpeH, SpeJ, SpeK, SpeL, SpeM, SpeQ, SpeR, SSA		[186]
SmeZ	2	
SpyCEP	1, 2	[120–123]
ScpA	2	[124,125]
SpnA		
Scl-1	2	[112]
Streptokinase	2	[151–153,155]
Sda1	2	[110,111]
Spd1	2	
DNAse		
NADase		[130,286]
LTA		
Fibronectin		
GAC	2	[116,117]
SIC	1, 2	[164,174,175]
S protein		[126]
IdeS	1	[140,141]
EndoS		[142–144]
Alpha-enolase		[154,164]
uPA		[158,159]
SpyAD		[91,92]
Sil		[213]
Oxo-C12		[216]
Arginine deaminase		