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Review

The burden of group A *Streptococcus* (GAS) infections: The challenge continues in the twenty-first century

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https://doi.org/10.1016/j.isci.2024.111677

SUMMARY

Streptococcus pyogenes is a Gram-positive bacterium, also known as Group A *Streptococcus* (GAS), that has become a significant threat to the healthcare system, infecting more than 18 million people and resulting in more than 500,000 deaths annually worldwide. GAS infection rates decreased gradually during the 20th century in Western countries, largely due to improved living conditions and access to antibiotics. However, post-COVID-19, the situation has led to a steep increase in GAS infection rates in Europe, the United States, Australia, and New Zealand, which triggers a global concern. GAS infections are normally moderate, with symptoms of fever, pharyngitis, and pyoderma; nevertheless, if left untreated or with continued exposure to GAS or with recurring infections it can result in fatal outcomes. GAS produces a variety of virulence factors and exotoxins that can lead to deadly infections such as necrotizing fasciitis, impetigo, cellulitis, pneumonia, empyema, streptococcal toxic shock syndrome, bacteremia, and puerperal sepsis. In addition, post-immune mediated disorders such as post-streptococcal glomerulonephritis, acute rheumatic fever, and rheumatic heart disease contribute to extremely high death rates in developing nations. Despite substantial research on GAS infections, it is still unclear what molecular pathways are responsible for their emergence and how to best manage them. This review thus provides insights into the most recent research on the pathogenesis, virulence, resistance, and host interaction mechanisms of GAS, as well as novel management options to assist scientific communities in combating GAS infections.

INTRODUCTION

Group A *Streptococcus* (GAS) is a significant human pathogen that causes infections in humans with varied clinical presentations.[1] GAS is capable of causing a wide range of clinical infections, from minor diseases like pharyngitis and impetigo to serious invasive infections (iGAS) like sepsis, streptococcal toxic shock syndrome (STSS) and necrotizing fasciitis. Erysipelas, glomerulonephritis, suppurative tonsillitis, scarlet fever, and rheumatic fever are other illnesses caused by GAS.[2] iGAS infections tend to keep progressing, and prompt treatment processes are necessary to mitigate morbidity and mortality among patients.[3] Thus, GAS-related illnesses have major implications in many fields of medicine. Every year, there are approximately 616 million cases of GAS pharyngitis worldwide, of which 17,800 are new infections. Over 517,000 people die from severe GAS infections annually.[4] The global alarm has been raised by the rise in GAS invasive infections and scarlet fever in the UK and other European nations in 2022, mostly affecting children below the age of 10.[5–9] Further, it is important to note the incidence of GAS diseases varies with the season and geographical location. Despite seasonal changes, outbreaks of non-iGAS infections are common worldwide, whereas outbreaks of iGAS are relatively rare.[10]

Numerous virulence factors are linked to the intricate and multifaceted mechanisms of GAS infection. The production of exotoxins and specific surface proteins such as M-proteins that are encoded by the *emm* gene are associated with major

virulence factors of GAS.[11] The *emm* genotyping lays the foundations for understanding epidemic outbreaks and the severity of the infections caused by GAS. For instance, certain *emm* strains, particularly *emm*1, have been associated with iGAS cases such as necrotizing fasciitis and STSS with worsening clinical manifestations. Further, GAS is associated with several superantigens (T cell superantigens) virulence factors, some of which are found on chromosomes and others that are associated with prophages that could contribute to SSTS.[12]

Studies on antimicrobial resistance in GAS are crucial. Nearly all Gram-positive bacteria are resistant to penicillin, making penicillin resistance a severe global issue. Fortunately, no naturally occurring GAS strain has been identified that is penicillin-resistant, and GAS is still sensitive to β-lactam antibiotics. It is unclear as to why penicillin, which has been used extensively for 80 years, is still effective against GAS, whereas other Gram-positive bacteria are gaining resistance to these antibiotics.[13] It is speculated that 8% of individuals have a penicillin allergy, but only one in twenty people experience an IgE-mediated reaction that necessitates switching to macrolides and clindamycin treatment during GAS infections. However, clindamycin and macrolides are no longer effective against isolates linked to outbreaks of severe illnesses, making management of GAS infections a tedious process in patients allergic to penicillin.[13] Understanding the need for treatment strategies in such circumstances, pharmaceutical giants are on the run for GAS vaccines, which are yet to reach FDA approvals.[9] Antibiotics are consequently essential for effective therapy in the interim, and drug resistance in GAS requires global attention before it becomes difficult to manage. As the number of GAS infections has increased since the COVID-19 era, this review aims to raise awareness of GAS infections worldwide by offering comprehensive narrative information on epidemiology, treatment challenges, resistance and virulence mechanisms, clinical manifestations, management strategies, and vaccine development.

EPIDEMIOLOGY OF GAS INFECTIONS

GAS infection epidemiology is complicated and dynamic, with changing patterns of disease frequency and severity among locations and people. GAS infections began to decline in the mid-twentieth century. However, by the late 1980s, severe GAS infections had resurfaced[14] Over the last two decades, both non-suppurative (without pus formation) and suppurative (with pus formation) complications of GAS infections have increased. Additionally, changes in the virulence of GAS strains and the emergence of antibiotic resistance among GAS have contributed to the resurgence of GAS infections and their complications, increasing disease burden and challenges in managing infections.

The prevalence and incidence rates of GAS infections can vary by region and population. GAS infections can affect individuals of all ages, but certain age groups, particularly those with the type of disease, are more susceptible. A higher percentage of fatalities is associated with the more severe cases, which target people with weakened immunity, including the elderly and immunocompromised individuals. Population studies also revealed that GAS infections are more common among communities that are habituated to poor hygienic practices, particularly in low-income and crowded ones.[3] GAS infections and their complications differ across low-income and high-income countries. ARF and RHD are frequent diseases in low- and middle-income countries, as well as are reported among indigenous populations of high-income countries like Australia and New Zealand.[15,16] Untreated GAS infections, particularly streptococcal pharyngitis, can cause RHD. In contrast, mortality from iGAS infections, which can cause severe diseases such as necrotizing fasciitis and STSS, is more common in wealthy nations.

Scarlet fever caused higher rates of death and morbidity in the 19th century, but these incidences rapidly plummeted in the 20th century.[17] However, an unexpected spike in the incidence of scarlet fever was reported in mainland China and Hong Kong in 2011.[18] A prophage harboring superantigens (SSA and SpeC) and DNase (Spd1) in macrolide-resistant *emm*12 strain was identified as a notable outbreak isolate of scarlet fever in these regions. Further epidemiological surveillance in China unraveled an uptrend in the presentation of *emm*1 isolates from 3.8% to 48.5% between 2011 and 2014, which holds 94–100% sequence similarity with *emm*12 strain, providing evidence to suggest that prophages containing superantigens can be transposed by mobile genetic elements (MGE).[19] Likewise, a superantigen overexpressing *emm*1 strain designated as $M1_{UK}$, identified as an outbreak strain in England in 2016, is currently on the rise in European countries and regions of northern America, Australia, New Zealand, Canada, and Taiwan.[20–23] Notably, prophage-containing superantigens were present in 26% of the $M1_{UK}$ lineage scarlet fever isolates in Australia,[22] further demonstrating MGE as a substantial risk factor for upcoming rises in GAS infections.

An International Surveillance Network, the Strep-EURO program, was established to identify severe GAS infections.[14] By building the first international surveillance network dedicated to these diseases, the program achieved a key milestone of identifying almost 5,000 cases, each with extensive clinical and microbiological data, revealing light on the complex epidemiology of severe GAS infections. Recently, in England, the UK Health Security Agency released the results of a report on the GAS seasonal activity 2022–2023.[24] According to the report, as of the 29th of June 2023, the number of notifications and GP consultations for scarlet fever and iGAS infections in England has shown a marked and concerning increase.[25] Scarlet fever notifications have been consistently higher than expected, with over 4,600 cases significantly surpassing the average of the previous five years. Notifications of iGAS infections have followed a similar pattern, with 50,910 cases reported so far, well above the typical range of the past five seasons. Notably, these trends are more pronounced in children, raising concerns about their vulnerability.

GAS INFECTIONS AND COVID-19

Several European countries, including France, Ireland, the Netherlands, Sweden, and the UK, have reported a rise in GAS infection cases during 2022, particularly in the latter half of the year.[26–28] In the UK, there was a noticeable increase in pediatric cases of iGAS during the pandemic when compared to the pre-pandemic period. One of the major reasons was cited to be the

lack of exposure to GAS strains due to the quarantine period, which led to a weaker immune tactic. It was also vocalized that the increase in GAS infections could be due to the factors of secondary or co-infections by other viruses (influenza, respiratory syncytial virus (RSV), and COVID-19). The severity of GAS infections increases when it occurs simultaneously with other viral infections, and thus, it seems to be a plausible theory on how the spread of COVID-19 could easily create co-infections with iGAS, leading to the alarming increase in iGAS infections during the pandemic. However, this is highly debatable, with individuals arguing over misdiagnosis between the two infections due to a high level of similarity in the symptoms between them.

DRUG-RESISTANT IN GAS

The constant exploitation of antibiotics over the decades has led to the evolution of several drug-resistant GAS strains, which continue to run rampant around the world, raising global concerns. Due to the prevalence of such resistant strains, it is important to understand the mechanisms behind their functions to help create novel drugs to combat the persistent diseases that they cause. While GAS is usually susceptible to antibiotics such as penicillin and vancomycin, current trends have displayed a concerning pattern of drug resistance. Recent studies show that erythromycin resistance was found in 53% of isolates with inducible macrolide and lacosamide, while streptogramin and clindamycin resistance was seen in 33% of isolates with all of them exhibiting inducible resistance.[29] A deeper understanding of the various drug resistance mechanisms employed by GAS strain will facilitate further exploration of these areas. Such rising concerns are addressed with the need for epidemiological vigilance to ensure that the treatment matches the antibiotic sensitivity profile of these ever-resistant GAS strains. Therefore, it is evident that extensive scientific advancement and research are needed for better understanding and development of more effective antibiotics for prophylaxis.

Penicillin sensitivity

For over a decade, penicillin had been the foremost Achilles heel for the treatment of GAS diseases. Penicillin confers protection by first targeting penicillin-binding proteins (PBPs) to restrict peptidoglycan synthesis, eventually leading to cell death. Recent experiments have discovered rare GAS strains possessing a chimeric penicillin-binding protein 2X (PBP2X) having a recombinant segment from *Streptococcus dysgalactiae* subspecies *equisimilis* (SDSE), which decreases their susceptibility to the antibiotic activity expressed by penicillin.[30] This has led to the characterization of a new strain marked by high fitness, virulence, and decreased susceptibility to penicillin. A novel study found two clonally related strains of rare type *emm*43.4 showing a lower susceptibility to various antibiotics. These two strains held identical nonsynonymous mutations in the *pbp2x* gene that encodes for PBP2X. This mutation incorporates a threonine-to-lysine replacement at amino acid 553 (Thr553Lys) (Figure 1A), which was not found in susceptible strains of type *emm*43.4.[31] This discovery has been hailed to be the grounds for the development of beta-lactam resistance, which has been a primary cause of concern for the medical community. Penicillin sensitivity is still relatively low among various strains, but supplementary data should be collected for developing future strategies.

Macrolide resistance

Macrolides are antibiotics with a distinct macrocyclic lactone ring attached to deoxy sugars. A few of the commonly used macrolides include erythromycin, azithromycin, and telithromycin. These exhibit a wider attack spectrum and are often administered to patients allergic to penicillin. In addition to their bacteriostatic properties, macrolides are well known for their mechanism of inhibiting bacterial protein synthesis by preventing the transfer of peptidyl transferase between amino acids. In an extensive study conducted by Tsai et al.[32] it was observed that there was a sharp increase in GAS macrolide resistance from 18.1% to 19.3% between the years 2000–2009 and from 58.4% to 61.0% in the following decade. A post-COVID study showed that erythromycin resistance had increased from 6% in 2020 to 25% during the years 2021–2022, with 13% of the isolates being erythromycin resistant.[33] This has led to the subsequent emergence of macrolide-resistant Group-A *Streptococcus* (MRGAS). Resistance to macrolides in GAS is influenced by three different mechanisms: ribosomal post and pre-transcriptional modifications (methylation), active expulsion of the antibiotic by efflux pumps, and target protection.[34] Transcriptional modifications are done by erythromycin-resistant proteins (ERM), which supply a point mutation in the ribosomal sequence by methylation of the adenyl residue on the 23S rRNA strand of GAS strains. The main genes offering macrolide resistance include *ermB*, *ermT*, and *ermTR* (Figure 1B).[35] Commonly resistant *emm* types were found to be *emm*92, *emm*11, and *emm*83, along with their respective mutated strains.[36] The US Centers for Disease Control and Prevention (CDC) active bacterial core surveillance program reported an increase from 11.9% to 24.7% and from 8.9% to 23.8% of erythromycin and clindamycin-resistant strains of GAS bacteria, respectively, which was due to the expression of types *emm*77, *emm*58, *emm*11, *emm*83, and *emm*92.[37] Another major resistance mechanism includes the usage of macrolide efflux pumps run by the *mef(A)* gene (Figure 1B).[38] Originally, it was thought that *mef(A)* was the gene responsible for supplying macrolide resistance to GAS, but a new gene, namely *msr(D)* was discovered alongside *mef(A)* that also helped in providing macrolide resistance to GAS strains.[39] The ever-increasing macrolide resistance marked in these novel GAS strains must be assessed with further research to aid in the proper placement of precautions that can be taken to protect vulnerable populations from iGAS infections.

Tetracycline resistance

Tetracycline is an oral antibiotic derived from the bacterial genus Streptomyces, which combats bacterial infections by inhibiting the process of protein translation in virulent bacteria. A couple of the renowned antibiotics of the tetracycline lineage include doxycycline and eravacycline. Tetracycline resistance was not a prominent occurrence in GAS until the early 21st century. In the previously cited study conducted by Tsai et al. it was observed that 12.3%, 99.2%, and 13.1% of tetracycline-resistant GAS strains were found to harbor *tetO*, *tetM*, and *tetK* genes (Figure 1C) respectively between the years 2000–2019.[32] These

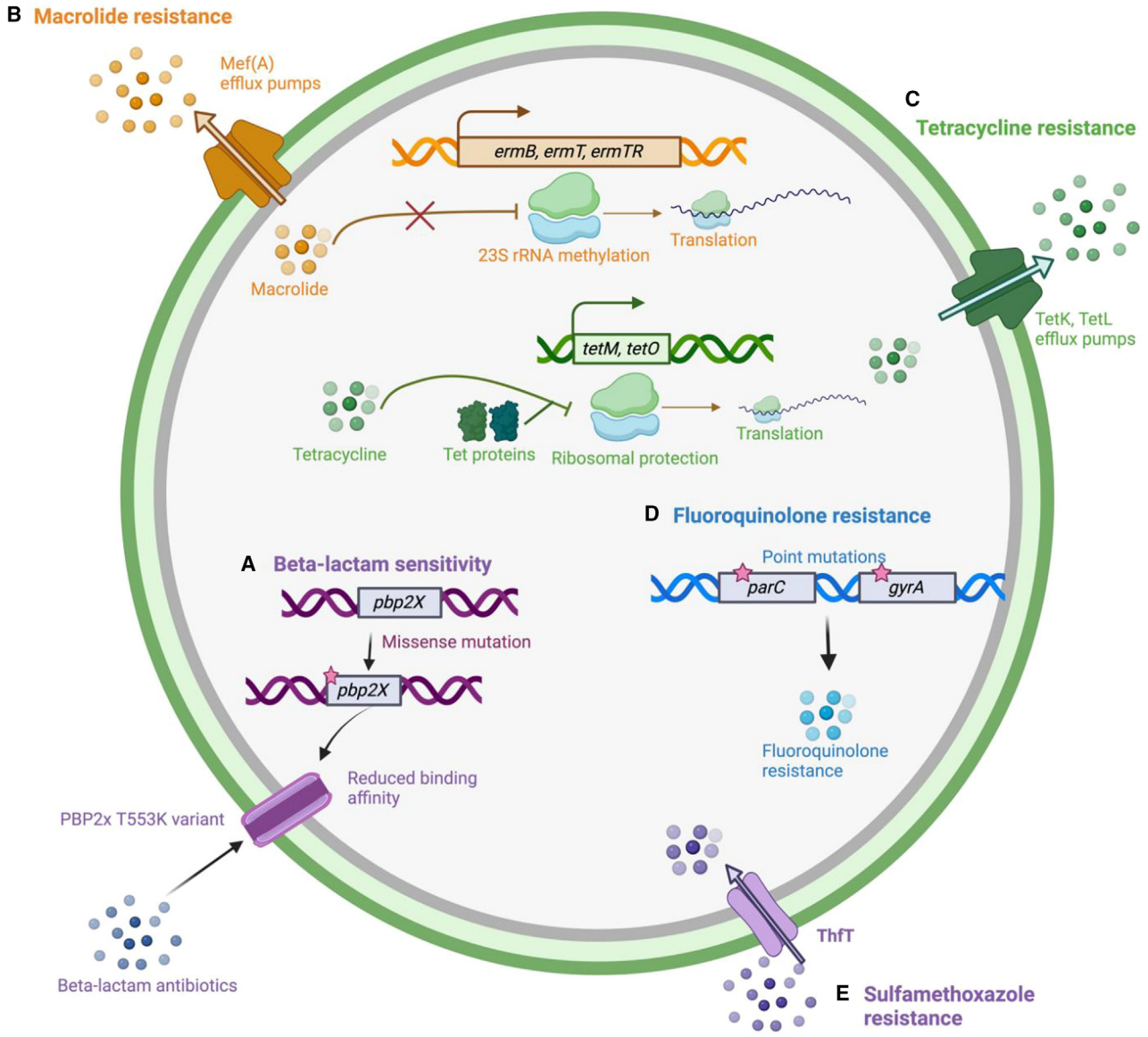


Figure 1. Diversity in the antimicrobial resistances exhibited by GAS

(A) The PBP2x-T553K variant diminishes penicillin's binding affinity and leads to reduced Beta-lactam susceptibility in GAS.

(B) Ribosomal methylation of the 23S ribosomal RNA by *erm* genes inhibits translation, thereby conferring resistance to macrolides. In parallel *mef(A)*gene *expression* confers macrolide resistance through efflux activation.

(C) During GAS infection, TetM and TetO, ribosomal protective proteins, displace tetracyclines from the 30S ribosomal binding site, whereas TetK and TetL enhance active efflux of tetracyclines from the cytosol.

(D) Point mutations in *parC and gyrA* gene leads to suppression of topoisomerases and DNA gyrase respectively, leading to fluoroquinolone resistance in GAS.

(E) The *ThfT* gene aids in the acquisition of folate compounds from the host leading to sulfamethoxazole resistance in GAS.

genes help to confer resistance toward tetracycline. A Malaysian study helped in the analysis of a few tetracycline-resistant GAS strains that carried the *tetM* gene and discovered that the *prtF1* gene used to produce fibronectin-binding protein F1 for cell adhesion was found in 60% of tetracycline-resistant strains when compared to the 28% found in tetracycline susceptible strains. This pronounced increase in tetracycline resistance was again replicated by a Spanish study where 61 isolates (6.8%) holding *tetO* and *tetM* genes were found to be tetracycline resistant. Most GAS strains that are resistant to tetracycline are also resistant to erythromycin (macrolides). The relationship between the two genes conferring their respective resistances was found in an intense study on the fragment ICESp2905 in the gene *erm(TR)* which confers erythromycin resistance, where fragments of the *erm* gene and the *tetO* gene were inserted in a clostridial scaffold.[40] Efflux pumps also form a crucial part of tetracycline resistance in GAS strains, as these pumps are membrane-bound and are mostly run by plasmid-encoded *tetK* and

tetL genes (Figure 1C). These genes actively work together to pump out excess tetracycline from their cytoplasm and slow down its accumulation, thereby conferring tetracycline resistance.[41] A large array of tetracycline-resistant variants is yet to be studied, this is a pressing topic that calls for attention to conduct further research and analysis.

Fluoroquinolone resistance

Fluoroquinolones are a subclass of quinolone antibiotics, known for their fluorine atoms found in nalidixic acid. Fluoroquinolones are highly reactive and are rarely administered on a first-cure basis due to adverse side effects such as gastrointestinal and central nervous system toxicities. They are mostly used as a last-ditch effort to curb extreme GAS infections. Fluoroquinolones work by inhibiting two type-II DNA topoisomerases - DNA gyrases and topoisomerase IV, thus leading to a halt in DNA replication and consequentially cell death.[42] Fluoroquinolones commonly used in a clinical setting include ciprofloxacin and delafloxacin. There has been a massive influx of fluoroquinolone-resistant (FQ-resistant) GAS strains in recent years, resulting in the need for a deeper understanding of the mechanism involved in ascribing this resistance. A Japanese study on GAS strains collected from children with pharyngotonsillitis denoted an increase in antimicrobial resistance in Japan as results showed that 11% of the strains had fluoroquinolone resistance that gradually increased over the years.[43] A later study denoted the introduction of fluoroquinolone-resistant isolates with amino acid substitutions, bringing fluoroquinolone resistance to a staggering 14.5% in Japan mainly due to the spread of *emm*6 and *emm*11 types.[44] Similarly, during a survey conducted in Southern Hungary, 13.5% of the strains showed non-susceptibility to norfloxacin (fluoroquinolone).[45] Fluoroquinolone resistance is attributed to mutations in the amino acid sequences of ParC, ParE, and GyrA, with all isolates having at least one mutation in their ParC sequence (Figure 1D).[46] A further study showed that mutations in ParC S79F and S79Y by substitution facilitated increased fluoroquinolone resistance.[47] Increased administration of fluoroquinolone antibiotics has paved the way for increased proliferation of FQ-resistant GAS strains to develop, leading this issue to be of global importance. Researchers are still vague on the exact mechanisms of FQ-resistant GAS strains, further studies are being conducted to facilitate an in-depth understanding of these mechanisms.

Sulfamethoxazole resistance

Sulfamethoxazole and trimethoprim are bacteriostatic antibiotics, usually prescribed in response to respiratory diseases. They work by inhibiting the production of dihydrofolate, thus halting the production of folic acid in bacterial cells. Folate plays a significant role during DNA replication and an inhibition in the folic acid cycle inevitably leads to cell death. The genes contributing to this resistance are variants of the Dyr sequences, namely DfrF, DfrA, and DrfG that are dihydrofolate reductase.[48,49] Overuse of antibiotics has led to an increase in sulfamethoxazole resistance in GAS strains, which was demonstrated by a recent survey conducted by Iranian scientists where they found 82.8% of isolated GAS strains were resistant to sulfamethoxazole.[50] The main mechanism behind sulfamethoxazole resistance involves mutations in the *dyr* and its variants which eventually lead to a stop in the folate cycle. However, a recent study established that horizontal upregulation of an energy-coupling factor (ECF) transporter substrate-binding part *thfT* (Figure 1E), helped supply sulfamethoxazole resistance by accessing extracellular reduced tetrahydrofolate compounds from the host cells.[51] The study also showed that ThfT helped GAS to uptake one carbon metabolite from the intermediates of the folate cycle. Earlier known to be one of the most widely accepted drugs of choice for GAS infections after beta-lactams, sulfamethoxazoles are no longer in frequent use due to the development of various resistance mechanisms against them. Adequate trials and research should be conducted to bring them back into the sphere of commonly prescribed antibiotics.

Acquired resistance through HGT elements

All the aforementioned resistant mechanisms arise due to horizontal gene transfer (HGT) elements and their factors. Horizontal gene transfer, also known as lateral gene transfer, refers to the transference of genes from one organism to another unrelated organism through processes such as transformation, transduction, and conjugation. These elements responsible for the movement of genomic DNA through lateral gene transfer are called MGEs. This mechanism is evolutionarily important as it helps in the conferring of antimicrobial resistance among bacteria. This evolution trick undermines the effectiveness of modern antibiotics, causing widespread panic among researchers. Transformation is the process of uptake of DNA by the bacterium from the external environment and incorporating it into their genomic material. Natural genetic transformation is a rarity in GAS due to its type-1 restriction-modification system encoded by the hsdRSM locus.[52] The next common element is transduction, where a foreign genome is transferred between cells with the help of viral vectors usually bacteriophages, which helps to confer diversity in genetic virulence between various strains of GAS.[53] Various lytic and lysogenic phages such as A25 help in the transduction processes of GAS, which may vary due to factors like growth state and genetic background. They help supply resistance against tetracycline, clindamycin, sulfamethoxazole, and macrolides by carrying genes such as *tetA*, *tetW*, and many other genes. The third and final element is conjugation. Conjugation is the transfer of genetic material from one cell to another through bridge-like formations or direct physical contact using pili. Plasmids or transposons are usually shared between each other. Transposons confer most erythromycin, tetracycline, and macrolide resistance in GAS by conjugation with other bacteria, leading to an enhanced genetic mutation and resistance.[53] Horizontal gene transfer is a natural process of the biome that cannot be prevented. Further understanding of the various elements, factors, and their mechanisms is needed to aid in the evolution of future modern antibiotics.

THE HOST IMMUNE RESPONSE AGAINST GAS INFECTIONS

The inflammatory response against GAS infections

The detection of GAS strain in the human host triggers the immune system that further recruits or activates macrophages,

neutrophils, and dendritic cells to combat the pathogen. The triggering of the innate immune response to these GAS pathogens however depends on the interaction of GAS-PAMPs (pathogen-associated molecular patterns) with that of PRRs (pattern recognition receptors. TLRs (Toll-like receptors) are a well-studied class of PRRs that detect a variety of damage or PAMPs on cell surfaces and within endosomes. For instance, the extracellular components of GAS (lipopeptides, lipoteichoic acid and peptidoglycan) are sensed by TLR2. Further, the TLR8 and TLR9 of the endosomes and lysosomes further help to identify bacterial RNA and bacterial CpG DNA (unmethylated). Recognition of these ligands further activates PRR-mediated signaling that initiates a wide range of transcription factors that includes NF-κB (nuclear factor kappa B), which drives pro-inflammatory genes such as TNF (tumor necrosis factor), IL-6 and pro-IL-1β.[54] Activation of these genes further recruits and activates macrophages and neutrophils, which are involved in phagocytosis, triggering adaptive immunity, and causing an inflammatory response at the site of infection. Similarly, MyD88 is a key signaling component of TLRs (except TLR3) that plays a vital role in triggering MAPKs and NF-κB activation to induce proinflammatory cytokines. Furthermore, GAS is also capable of activating type I IFN through MyD88-independent signaling that does not require cytolysins SLO and SLS.[55] Further, the triggering of MyD88 rapidly upregulates neutrophils and macrophages along with other chemokines (CXCL9 and CXCL10).[56]

Neutrophils

The onset of GAS invasion activates the neutrophils that restrain GAS infections through the production of NETs (neutrophil extracellular traps), ROS (reactive oxygen species) and antimicrobial peptides. In case of serious iGAS infections neutrophils serve as a first line of defense in managing this infection. However, several GAS infections cause serious cases of neutropenia, which results in poor patient prognosis. Further, the administration of a neutrophil-depleting antibody has been studied to change GAS infection from a non-invasive to an invasive form in mice models.[57]

Macrophages

Further, the role of macrophages is vital role during GAS infections. It has been shown that macrophages respond to GAS by releasing several cytokines such as IL-6, IL-8, TNF-α and IFN-β. The macrophage-secreted TNF-α plays a vital role in recruiting monocytes to the site of GAS infections. Further, GAS-infected macrophages (humans) also trigger the activation of NLRP3 inflammasome resulting in IL-1 production.[58,59]

Mast cells

Early recognition of GAS pathogens is participated by Mast cells which plays a vital role during skin infections. Mast cells reportedly destroy the GAS pathogen because of their extracellular structural similarities to that of NETs.[59,60]

Dendritic cells

Dendritic cells play a major role in priming T cell response on the onset of GAS infection. *In vitro* studies on human and murine dendritic cells have identified GAS to induce the maturation of dendritic cells thereby inducing the production of IL-12 (Th1-polarizing cytokines).[59,61]

Recognition of GAS by inflammasomes

Cytosolic multiprotein complexes such as inflammasomes are crucial in triggering inflammatory protease caspase 1. Protease caspase-1 and adaptor protein with a caspase activation and recruiting domain are the typical constituents of inflammasomes with oligomerization capacities. Members of the NLR family (NLRP1, NLRP3, and NLRC4; PRRs family) also aid in the recruitment of pro-caspase-1 to the inflammasome complex (Figure 2).[62] However, NLRP3 is the only inflammasome that responds to an active infection caused by GAS with least evidence of GAS known to activate NLRP1 and NLRC4. A priming signal that triggers NF-κB mediated NLRP3 and pro-IL-1β upregulation and an activation signal triggering inflammasome complex assembly process that activates caspase-1 and IL-1β production are typically required for the activation of NLRP3 inflammasome (Figure 2).[63,64] After recruitment to an inflammasome, caspase-1 is activated through proximity-induced autocatalytic activation that helps in cleaving pro-IL-1β and pro-IL-18 to its active biological form. The active IL-1β then facilitates the introduction of immune cells to the site of tissue damage or infections.[65] Active caspase-1 also cleaves GSDMD (gasdermin D), thereby allowing the N-terminal domain of GSDMD to create openings in the plasma membrane, resulting in pro-inflammatory form of cell death (pyroptosis process). Finally, it is critical to understand that inflammasome activation and pyroptosis are not the only mechanisms involved in GAS-induced inflammation. As uncontrolled infection could trigger other inflammatory pathways, diminishing the effect of inflammasomes. Further, to avoid adverse outcomes in patients with severe GAS infections, the interaction of GAS with inflammasomes and IL-1β production needs careful research investigations.

Recognition of GAS by autophagosomes

Autophagosomes are vesicles that are formed when lysosomes fuse around damaged organelles in a cell. GAS through various virulence factors can impede autophagosome formation by targeting autophagy adaptors and may survive in the intracellular niche for a few days. For instance, autophagy adaptors can be broken down by the release of the cysteine protease SpeB. GAS infections producing cytolytic toxins such as streptolysin O (SLO) are capable of preventing the maturation of GAS-containing autophagosomes in keratinocytes.[66] Recently, capsule-deficient GAS strains were identified to evade autophagy-mediated killing in the macrophages further revealing the role of previously unknown aspects of the host's recognition of the GAS capsule in macrophages.[67]

FIGHTING HOST IMMUNE RESPONSE

Virulence factors associated with surface molecules

A highly versatile M protein molecule

The cell wall of GAS carries an extension of dimeric coiled fibrillar protein, the M protein, which is an important virulence factor in GAS (Figure 3). The M protein functions as an anti-phagocytosis barrier, thereby serving as a crucial element for

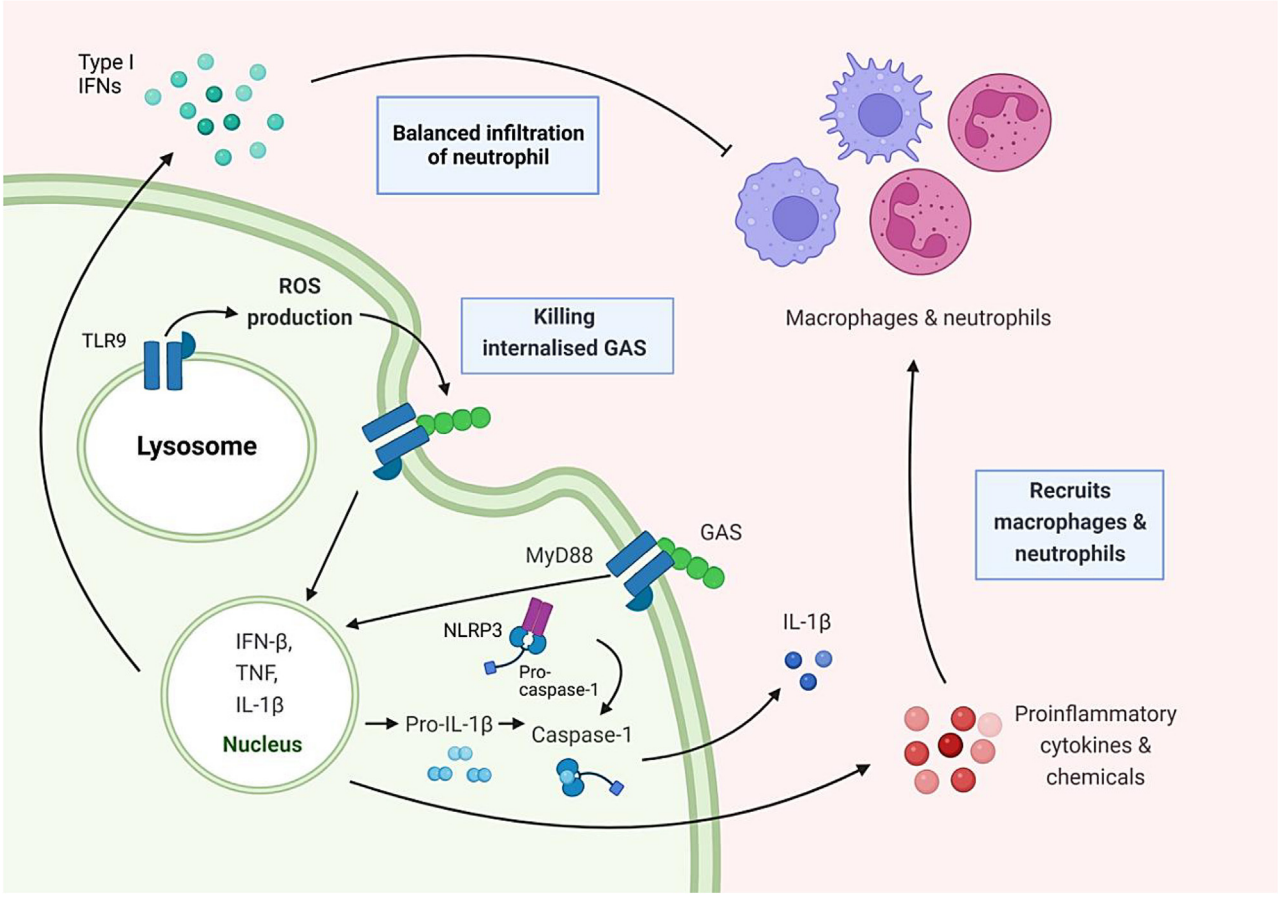


Figure 2. TLR and MyD88 signaling cascades stimulate the expression of IFN- and pro-inflammatory cytokines such as TNF and IL-6

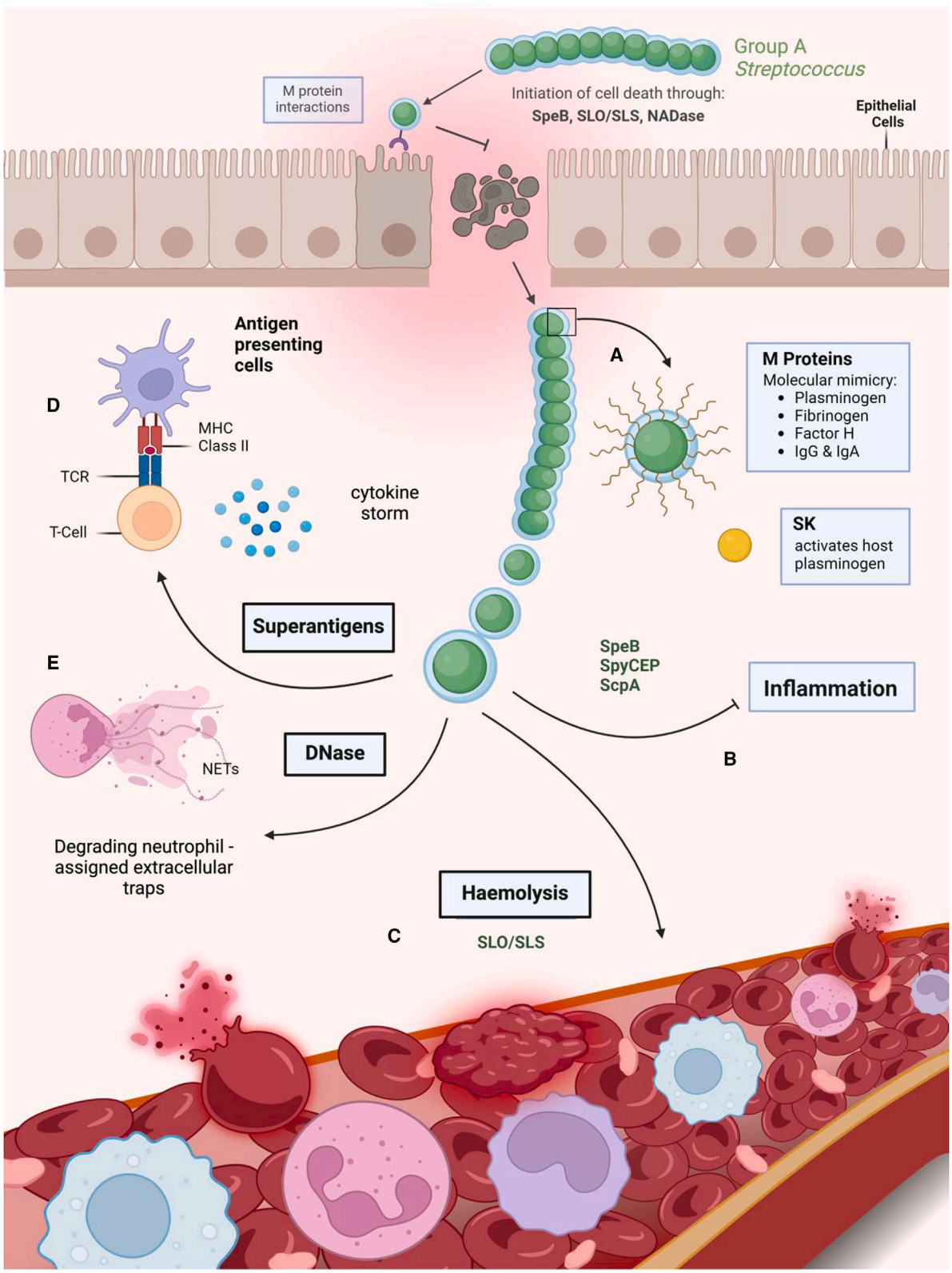
TNF encourages macrophage recruitment to the infection. Type I IFN signaling induced by IFN- and other type I IFNs initiates unidentified responses that end in balanced neutrophil infiltration and protective immune responses against GAS. TLR9 promotes GAS killing by ROS production. GAS induces IL-1β in an NLRP3-dependent manner.

survival in the host. The M protein is composed of a carboxy-terminal that is conserved and helps in cell wall attachment and a variable N terminal that contains the M-type defining sequences (50 amino acids, *emm* gene) that confers antigenic variations.[68] Because the N-terminal domain of GAS serotypes varies widely, it is classified as the hotspot region for *emm*-typing, which has identified ~250 antigenic variations.[69] However, only a few *emm*-types are widespread (Table 1), with the M1 type being the most prevalent in causing invasive infections, including toxic shock syndrome and necrotizing fasciitis.[63] M proteins are further capable of directly adhering to and recruiting numerous host components, including fibrinogen and plasminogen, to the surface of Streptococci, escaping both innate and adaptive immune responses.[70] Further, binding of M proteins to fibrinogen can activate platelets thereby activating neutrophils and monocytes, intensifying the pro-inflammatory response.[71] M1 proteins can induce the expression of cytokines interleukin (IL)-6, IL-1β and tumor necrosis factor-α while interacting with Toll-like receptors on human blood monocytes. Additionally, a recent study has shown that the M1 protein is a potent inducer of T cell proliferation.[72]

The antiphagocytic capsules

During pharyngeal and invasive infections, GAS can produce hyaluronic acid (HA) capsule as a key virulence factor that is antiphagocytic. The *hasA* and *hasB* are critical genes for the biosynthesis of HA-capsules and are arranged in an operon along with *hasC*.[73] However, M4 and M22 serotype strains from pharyngitis and invasive infections do not harbor *hasABC* operon, thereby lacking HA-capsule production.[74] Similarly, rapid epidemiological surveillance has detected the emergence of a novel, genetically acapsular, hypervirulent strain of *emm89* linked to invasive infections, specifically being a dominant variant in the United Kingdom. Further, the fact that these genetic acapsular variants often express SLO and NADase is also concerning, since these variants may possess effective defense mechanisms against antibiotic treatment by facilitating bacterial internalization and intracellular survival within epithelial cells.[75]

Further, A two-component system, CovRS, is also a critical regulator of virulence factors including transcription of *has* operon in response to an environmental signal. Similarly, RocA, a regulatory protein is also known to interfere with capsule expression.[76] GAS HA-capsules and human-HA share structural



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similarities, allowing HA-capsules to infiltrate the host immune system. As a result, these HA-capsules can easily bind to CD 44 (human cell surface glycoprotein receptor) and connect to skin and pharyngeal epithelial cells. This connection stimulates other signaling pathways that may disrupt epithelial cell integrity, allowing GAS to cause deeper infections.[77]

The surface protein S

The hydrophobic properties of GAS are maintained partly by S proteins that help in the survival of GAS in the human bloodstream. To induce molecular mimicry, these S proteins recruit lysed RBCs as a coating on the GAS, boosting virulence. A further proteomic study has identified the S protein as a major virulence component, as S protein deletion changes extracellular protein integrity and reduces numerous virulence factors in GAS infections.[78]

The pili

GAS pili are multifunctional virulence factors that play a vital role in host colonization, biofilm formation, and modulation of host immune responses to GAS. It is important to note that fibronectin-binding, collagen-binding T antigen (FCT) regions are genetic loci within the pilus that contain genes for coding Lancefield T antigens, accessory proteins, pilus-associated sortases, and transcriptional regulators. Considering the gene organization and sequence variation of the *tee* gene, which codes for the T antigen, nine distinct FCT areas have been learnt in GAS. However, it is still unclear how T antigens in GAS of various pilus types operate biologically.[79]

Virulence factors associated with secretory molecules

SpeB (streptococcal pyrogenic exotoxin B)

Streptococcal pyrogenic exotoxin B, or SpeB, is one of the most important secretary virulent factors observed in GAS infections (Figure 3). Antibodies to these proteinases have been seen in patients with pharyngitis, invasive illness, and rheumatic fever. However, in fatal infections, the antibodies produced against this exotoxin are fairly modest.[80] These proteinases further induce pro-inflammatory properties that can alter IL-1β precursor to active IL-1β as well as activate epithelial IL-36γ which is very critical for host defense mechanisms in the event of infections.[81,82] Further, when vitronectin and fibronectin are being degraded by SpeB, endothelial cell matrix metalloprotease is activated.[83] SpeB are also capable of breaking down IgA, IgD, IgE, IgM and cleaves IgG into fragments of Fab and Fc. Similarly wide spectrum of chemokines that includes XCL1, CCL20, CXCL1-7, CXCL10-14, CXCL16, and CX3CL1 are also being degraded by SpeB..[84] Furthermore, this protease operates on the surface of mononuclear leukocytes by releasing the activator receptor of urokinase plasminogen, allowing physiologically active kinins to be released from their progenitors.[85] Further, SpeB has been recently identified to cleave several Complement component C3 (C3b) that contribute to evading host innate immunity.[86] According to recent studies, SpeB is the only known pathogenic protease that can trigger caspase-independent pyroptosis in keratinocytes by cleaving human gasdermin A.[87,88] The effects of SpeB on host proteins have been enlisted in Table 2. The two-component system CovR/S is thought to be a negative regulator of SpeB. However, investigations have shown that any spontaneous mutations in *covR* or *covS* result in the loss of SpeB expression. likewise, strains harboring *covR* mutation had no effect on SpeB expression, illustrating that a complex network might contribute to SpeB regulation and may differ on the strain type.[89]

Streptolysin O and NAD-glycohydrolase

GAS infections are also capable of producing cytolytic toxins such as streptolysin O (SLO), a pore-forming toxin belonging to the cholesterol-dependent cytolysins (CDCs) family. CDCs are capable of attaching to cholesterol-containing membranes, where they oligomerize and insert to generate enormous pores.[90,91] By interfering with the process of macrophage-mediated phagocytosis of GAS, SLO can cause both cause cytotoxic injury and contribute to the survival of GAS within the host (Figure 3). Similarly, SLO can also induce accelerated macrophage apoptosis.[92] In association to SLO, GAS also produces another cytolytic toxin, NAD-glycohydrolase (NADase), an enzyme that has the catalytic potential to convert NAD+ to adenosine diphosphoribose and nicotinamide.

SLO is intimately associated with another toxin, NAD-glycohydrolase (NADase), a secreted enzyme that catalyzes the hydrolysis of NAD+ to nicotinamide and adenosine diphosphoribose.[93] In an operon, *slo* gene is arranged together with *nga* which codes for NADase and *ifs* which codes for intracellular inhibitors (IFS). SLO is functionally connected to NADase, which together may aid in intracellular survival, enabling macrophagic cytotoxicity and epithelial damage, as well as Golgi fragmentation, culminating in pathogenesis.[94] Furthermore, disruption of the Golgi network disrupted not only the integrity of epithelial cells but also the release of IL-8 by macrophages in response to GAS infections.[95] It is worth noting, however, that many clinical isolates of GAS lack NADase activity, and these variants exhibit comparable cytotoxic properties to those of GAS with NADase activity.[96]

Superantigen (SAg genes)

GAS pathogens are capable of secreting exotoxins that are of superantigenic activity and are commonly referred to as Spes.

Figure 3. The M proteins that are surface expressed helps in initial attachment of GAS to epithelial cells

Secretory toxins such as SpeB, SLS/SLO and NADase helps in breaking the epithelial barriers thereby helping in the translocation of GAS to host cells.

(A) The M protein further prevents host recognition through molecular mimicry with host factors such as plasminogen and fibrinogen. Streptokinase (SK)–plasmin complex which assists bacterial dissemination.

(B) GAS has evolved a number of methods to avoid detection by the host immune system. These include SpeB-mediated LL-37 degradation, SpyCEP-mediated IL-8 cleavage, and ScpA-mediated cleavage of the complement component 5a (C5a) by a C5a peptidase.

(C) The hemolytic activity of SLO and SLS acts as an immunological camouflage technique, allowing GAS to live in and spread from blood vessels.

(D) Superantigens promotes excessive adaptive immune system activation by nonspecifically cross-linking MHC class II molecules on antigen-presenting cells (APCs) and T cell receptors (TCRs), resulting in a cytokine storm.

(E) DNases destroy the DNA backbone of neutrophil extracellular traps (NETs), allowing GAS to avoid neutrophil killing.

Table 1. M types and their clinical presentations

M Types	Presentation	References
M1, M3, M5, M6, M11, M12, M14, M17, M18, M19, M24, M27, M29, M30, M32, M41	Acute rheumatic fever	Metzgar and Zampolli[69] and Bessen et al.[191]
M1, M4, M12, M49, M55, M57, M60	Acute glomerulonephritis	Metzgar and Zampolli[69], Walker et al.[70] and Smeesters et al.[72]
M1, M12	Meningitis	Metzgar and Zampolli[69], Ho et al.[192] and Vlaminckx et al.[193]
M33, M41, M42, M52, M53, M70	Impetigo	Metzgar and Zampolli[69]
M1, M3, M5, M6, M12, M14, M17, M19, M24	Pharyngitis	Metzgar and Zampolli[69] and Osowicki et al.[194]
M28	Puerperal sepsis and bacteremia	Metzgar and Zampolli[69], Vlaminckx et al.[195] and Green et al.[196]
M1, M3	Streptococcal toxic shock syndrome	Metzgar and Zampolli[69], Vlaminckx et al.[195] and Vlaminckx et al.[197]
M1, M3, M28	Necrotizing fasciitis	Metzgar and Zampolli[69], Vlaminckx et al.[195] and Stetzner et al.[198]
M1, M3, M12, M28	Fatality outcomes	Metzgar and Zampolli[69], Zhang et al.[199] and Hollm-Delgado et al.[200]
M1	Global widespread	Metzgar and Zampolli[69] and Henningham et al.[201]

Spes as such can crosslink the T cell receptor β-chains in the variable region with that of the MHC class II molecules of APCs (antigen-presenting cells) in a non-antigen-specific fashion, thereby activating a large proportion of T cells, followed by a high-level of cytokine responses (Figure 3).[97] The superantigens identified in GAS are either chromosome encoded (*speG*, *speJ*, *speQ*, *speR*, streptococcal mitogenic exotoxin Z (*smeZ*)) or prophage encoded (*speA*, *speC*, *speH*, *speI*, *speK*, *speL*, *speM* and streptococcal superantigen (*ssa*)).[98–100] In clinical presentations of scarlet fever and invasive disease caused by GAS, SpeA, SpeC and SSA superantigens have been a major virulence factor.[101] Similarly, circulating superantigen have been detected in patient plasma samples with streptococcal toxic shock syndrome.[102]

Chemokine degradation

GAS pathogens are capable of producing proteases as a major virulence factor. For instance, GAS pathogens produce SpyCEP (*S. pyogenes* cell envelope proteinase) and ScpA (C5a peptidase) that can break down chemokine IL-8 and C5a (complement component 5a).[103] A transcriptional profiling of iGAS has shown SpyCEP as the second most highly upregulated gene with an expression of 25-fold.[104] An increase in SpyCEP expression has been found to correlate with disease severity associated with GAS infection.[105]

Deoxyribonucleases mediated escape

To withstand the human immunological response, GAS strains are capable of producing extracellular Deoxyribonucleases (Figure 3). DNases were among the first few secretary GAS proteins to be identified and thoroughly studied.[106] Currently by far eight DNases in GAS have been identified of which two of them are chromosomal encoded (*spnA* and *spdB*) and rest six of them are prophage associated DNase (*sda1*, *sda2*, *spd1*, *spd3*, *spd4* and *sdn*).[107] GAS DNases are capable of degrading neutrophil-assigned extracellular traps, ensuring bacterial survival. Furthermore, GAS DNase is capable of generating cytotoxic deoxyadenosine, which suppresses immunological response by limiting phagocytosis. In addition, prophage-mediated sda1 inhibits the innate immune response, while further hindering plasmacytoid dendritic cell recruitment by reducing type I interferon levels at the site of infection.[108] Additional Sda1 degradation of bacterial DNA may change how host innate immune cells recognize GAS through TLR9.[109] However, the role of DNases in infections is still undetermined and the possibility of DNases acting as virulence factors has just lately been investigated. *S. pyogenes* produces up to four different DNases, sometimes referred to as streptodornases.[110]

Streptokinase (SK)

GAS secrete streptokinase (SK), a powerful activator of host plasminogen known to be important in wound healing and angiogenesis.[111] Streptokinase released by GAS, converts the zymogen, human plasminogen (hPg), to the protease, human plasmin (hPm) by cleaving the $R^{561}V^{562}$ peptide link and removing a 77-residue activation peptide. The hPm further proteolyzes a large spectrum of substrates including proteins that holds the cellular structural integrity and fibrin clots.[112]

Evading adaptive immunity

Adaptive immunity against GAS is poorly understood, though recurring infections are common, especially in children with IgG antibodies rising toward GAS antigen. However, the lack of adaptive immune response against GAS antigens could be because of the variations of GAS serotypes and the surface antigens they exhibited. Additionally, GAS counterattacks the adaptive immunity by degrading IgG through IdeS, a cysteine protease that cleaves the IgG heavy chain. Further GAS can degrade IgGs through the secretion of endoglycosidase such as EndoS.[113]

CLINICAL MANIFESTATIONS

Pharyngitis

GAS causes bacterial pharyngitis in children aged 5 to 15 years roughly accounting for 15–30% of all cases of pharyngitis.[114–117] The pooled prevalence of GAS infections in children with pharyngitis (irrespective of age) was 37% (95% CI: 32%–43%). Children

Table 2. Host proteins cleaved by SpeB

Pre-SpeB cleavage	Post-SpeB cleavage	Effects	Reference
C3b	Breakdown	Escape phagocytosis	Terao et al.[86]
Fibronectin	Fragmented	Contribute to bacterial colonization and invasive infection	Natanson et al.[202]
Immunoglobulin (IgA, IgM, IgD, IgE, IgG)	Breakdown and cleavage into Fragments of Fab and Fc	Inhibit immunoglobulin-mediated opsonophagocytosis	Siemens and Lütticken[84]
Interleukin-1β precursor	active IL-1β	pro-inflammatory response	Kapur et al.[81] and Macleod et al.[82]
Kininogen	Bradykinin	Increasing vascular permeability; causing pain and fever	Nitzsche et al.[203]
Plasminogen	Breakdown	Reduce the activity of plasmin on the GAS surface.	Cole et al.[204]
Pro-matrix metalloprotease	Active-matrix metalloprotease	Increase bacterial infiltration and tissue damage	Burns et al.[83]
Vitronectin	Breakdown	Increase tissue damage	Kapur et al.[205]

below 5 years of age had a relatively lower prevalence of GAS infection 24% (95% CI: 21%–26%). Asymptomatic carriage in normal children with no clinical impression of pharyngitis was 12% (95% CI: 9%–14%).[118] Sudden onset of fever, sore throat, headache, and nausea along with exudative tonsillopharyngitis and tender anterior cervical lymphadenopathy are commonly observed in children above 3 years of age. Abdominal pain and vomiting along with a painful throat may lead to decreased intake.[119] Symptoms usually subside without requiring treatment in 3–5 days unless complications ensue.[114] Therapy with antimicrobials begun within 2 days of symptom onset reduces the duration and severity of symptoms by 1–2 days.[120] Antimicrobial therapy also prevents the spread of infection.[121] Below 3 years of age, symptoms are not very typical.[122] Protracted low-grade fever along with nasal congestion and cervical lymph node enlargement which is usually tender may be seen.[122]

Differential diagnosis of streptococcal pharyngitis will include a lot of other bacterial and viral infections. A throat culture or a rapid molecular test can help establish the diagnosis of streptococcal pharyngitis.[114,118] In one situation where there is asymptomatic carriage of GAS and an intercurrent viral pharyngitis, treatment response to streptococcus is not achieved. Complications of GAS pharyngitis may include the following.

Non-suppurative complications

Acute rheumatic fever (ARF)

ARF is one of the nonsuppurative sequelae. Recurrent ARF episodes can lead to rheumatic heart disease, which has its attendant morbidity and mortality. Following an episode of pharyngitis, there is a latent period (2–3 weeks) before any signs or symptoms of ARF may appear.[123] ARF can have major and minor manifestations. The major manifestations include; migratory polyarthritis involving large joints (60–80%), pancarditis and valvulitis (clinical/subclinical) (50–80%), central nervous system involvement (chorea) (10–30%), subcutaneous nodules (painless, over bony prominences and 0.5–2 cm) (0–10%) and erythema marginatum (transient and evanescent) (<6%).[124] Arthralgia (joint pain but no signs of inflammation on examination), fever and elevated acute phase reactants (ESR, CRP) are the minor manifestations of ARF.

Poststreptococcal reactive arthritis (PSRA)

PSRA is a reactive arthritis that involves one or more joints. This usually follows a pharyngeal GAS infection within a duration of one month.[125] There is usually no cardiac involvement and the criteria for ARF is not fulfilled.[126]

Scarlet fever

This is a diffuse erythematous rash occurring in association with GAS pharyngitis. The eruption develops if any prior exposure to GAS has happened and is due to delayed-type skin hypersensitivity reaction to pyrogenic exotoxins (erythrogenic toxins- A, B, C) produced by the organism. The rash has a sandpaper-like quality on the skin, accompanied by a strawberry tongue. There is a worsening of the rash in the skin folds, and this is called Pastia's lines. No treatment has been warranted for the skin rash.[114]

Streptococcal toxic shock syndrome (STSS)

STSS is a very rare complication of iGAS infections (pharyngitis, bacteremia, necrotizing soft tissue infections or pregnancy-associated infection). STSS occurs in roughly one-third of patients with any iGAS infections.[127] STSS is to be suspected in patients presenting with shock and multiorgan failure in the absence of a definitive etiology. The diagnosis is made on clinical criteria and culture findings. Clinical criteria for TSS are listed in Table 3. GAS isolation from a non-sterile location (throat, vagina, skin) combined with the preceding clinical criteria (Table 3) indicates probable STSS. Further GAS isolation from any sterile site (blood, CSF, joint fluid, wound tissue, peritoneal/pleural/pericardial fluid) in addition to the aforementioned clinical criteria (Table 3) concludes the conformation of STSS.

Post streptococcal glomerulonephritis (PSGN)

PSGN is caused by prior infection with GAS infection, especially nephritogenic strains. The incidence of clinically manifest PSGN in children during an epidemic is 5–10% with GAS pharyngitis and 25% with GAS skin infections.[128,129] Clinically, the patient may be completely asymptomatic. Sometimes, the patient may have microscopic hematuria or full-blown nephritic syndrome. Nephritic syndrome is manifested by red to brown urine (macroscopic), proteinuria, edema, systemic hypertension, and

Table 3. Clinical criteria for TSS

Clinical criteria	Condition
Hypotension	Defined as SBP $\leq$90 mmHg in adults or <5th percentile for age in children <16 years
Multiorgan involvement	Defined as 2 or more organs involved as below
Renal involvement	Defined as serum creatinine $\geq$2 mg/dL in adults and in children, $\geq$2 times the ULN for age. In those with renal disease, $\geq$2 times elevation of serum creatinine over baseline levels
Coagulopathy	Defined as platelet count of $\leq$100,000/mm^3 or DIC, defined by elevated clotting time, low fibrinogen levels, and elevated levels of FDP
Hepatic involvement	Defined by transaminases, or total bilirubin $\geq$2 times the ULN or in patients with liver disease, $\geq$2 times elevation over baseline levels
ARDS	Acute respiratory distress syndrome
Erythematous rash (macular and desquamation does occur)	–
Soft tissue necrosis	Necrotizing fasciitis or Myositis or Gangrene of limb

SBP: Systolic blood pressure, ULN: Upper limit Normal, DIC: Disseminated intravascular coagulation, FDP: Fibrin degradation products.

acute kidney injury.[130–132] Diagnosis of PSGN is based on acute nephritic syndrome and by documentation of a recent GAS infection (either by culture or serology).

Pediatric autoimmune neuropsychiatric disorder associated with group A streptococci (PANDAS)

The diagnostic criteria for PANDAS include the following[133–135]: Obsessive-compulsive disorder and/or tic disorder, pediatric age onset (3 years to puberty), sudden onset and an episodic course, temporal correlation between GAS infection and the onset of symptoms or exacerbation and neurologic abnormalities (motoric hyperactivity, choreiform movements or tics during exacerbations).[136]

Suppurative complications

Local and distant suppurative complications may develop in patients with streptococcal pharyngitis and tonsillitis. Cellulitis/abscess can often be seen forming in the peritonsillar or retropharyngeal spaces after an infection with GAS tonsillopharyngitis. The infection is usually polymicrobial and GAS is one of the offending pathogens.[137] Similarly, otitis media can occur via direct extension of infection from the pharynx to the middle ear through the eustachian tube. The clinical manifestations are similar to any other etiological reason of otitis media. GAS is estimated to cause 2–3% of acute otitis media cases in children and accounts for 14% of hospitalized cases.[138] Sinusitis can occur via a direct extension of infection from the pharynx to the sinuses. The clinical manifestations are similar to any other etiological reason for sinusitis.[139]

Necrotizing fasciitis and pyomyositis are seen with iGAS infections. Skin and soft tissue infections are common following a breach in the skin or following the spread hematogenously from acute pharyngitis.[140,141] Any layer of skin, subcutaneous tissue/fascia or muscle can be involved. Presentation is usually over hours to days.[142] Clinical manifestations include erythema (72%), edema (75%), severe pain (disproportionate to signs; 72%), fever (60%), crepitus (50%), skin bullae, necrosis, or ecchymosis (38%), and hypotension and systemic toxicity (if left untreated).[143–145] Streptococcal bacteremia and metastatic infection to brain, or distant sites are rare complications of GAS pharyngitis. GAS pharyngitis may also complicate into septic thrombophlebitis of the internal jugular vein and may have a varied clinical course. High-grade fever and rigors are common. Breathlessness, pleurisy and hemoptysis can occur if there has been a septic embolus to the lungs.[146,147]

THE GOALS OF ANTIBIOTIC THERAPY

Antibiotic therapy has been shown to mitigate symptom severity and help in recovery in patients with GAS pharyngitis.[148–150] Suppurative complications associated with GAS often require antibiotic therapy. In a meta-analysis, antibiotic therapy was associated with a reduction in the incidence of acute otitis media and sinusitis.[149] ARF and RHD are the main indications for antibiotic therapy for GAS pharyngitis.[151] RHD is an important cause of cardiovascular mortality.[4] Further, in a meta-analysis, therapy with penicillin reduced the incidence of RHD by 67%.[149] In the case of scarlet fever, antibiotic treatment is generally not warranted. Similarly, antibiotic treatment to reduce the incidence of PSGN is uncertain.[152,153] Further in the case of PANDAS and PSRA, antibiotic treatment is not warranted unless active GAS infection is identified.

However, in the case of STSS, an aggressive management approach is needed to avoid serious complications. STSS is not readily apparent on the first presentation to emergency and needs symptomatic management of shock with intravenous fluids and vasopressor support. It is prudent to rule out other causes of shock like cardiogenic and obstructive shock. If there is an obvious source of infection in the skin or soft tissues, surgical debridement is often warranted to control the source of infection. Antibiotics need to be given after taking appropriate cultures and are often lifesaving in STSS. An empiric choice of antibiotic would be intravenous clindamycin along with intravenous Vancomycin and intravenous piperacillin-tazobactam or intravenous carbapenems.[154–158] Clindamycin is known to inhibit protein synthesis and helps reduce the toxin production by the bacteria. As the culture reports are made available, therapy can be tailored as per the culture sensitivity and clindamycin can be continued. There is a role of intravenous immune globulin therapy as an adjunct in treating STSS.[159] However, mortality rates of STSS are reported from 30 to 79%.[160–163] Common antibiotic regimens used for managing TSS are listed in Table 4. Secondary prophylaxis with antibiotics is indicated and in patients with acute rheumatic fever with carditis and already pre-existing rheumatic heart disease, therapy needs to be given for 10 years

Table 4. Antibiotics to manage TSS

Antibiotic class	Drugs	Duration	Dosing (adults)	References
Penicillin	Penicillin V	10 days	500 mg PO BD/TID	Gerber et al.[164], Shulman et al.[206] and Nishimura et al.[207]
	Amoxicillin	10 days	500 mg PO BD	
	Penicillin G benzathine	One single dose	1.2 million units IM	
Cephalosporins	Cephalexin	10 days	500 mg PO BD	Gerber et al.[164], Shulman et al.[206] and Nishimura et al.[207]
	Cefadroxil	10 days	1 g OD	
	Cefuroxime	10 days	250 mg PO BD	
	Cefpodoxime	5-10 days	100 mg PO BD	
	Cefdinir	5-10 days	300 mg PO BD or 600 mg PO OD	
	Cefixime	10 days	400 mg PO OD	
Macrolides	Azithromycin	5 days	500 mg PO OD	Gerber et al.[164], Shulman et al.[206] and Nishimura et al.[207]
	Clarithromycin	10 days	250 mg PO BD	
Lincosamides	Clindamycin	10 days	300 mg PO TID	Gerber et al.[164], Shulman et al.[206] and Nishimura et al.[207]

PO: Per oral, BD: Twice daily.

or until 40 years of age (whichever is the longer period). Sometimes lifelong prophylaxis may be advised. In patients with ARF carditis and no underlying rheumatic heart disease, therapy can be given for 10 years or until 21 years of age (whichever is the longer period). In patients with ARF and no carditis too, therapy can be given for 10 years or until 21 years of age (whichever is the longer period) as per guidelines.[164] Antibiotic treatment is not routinely given to chronic GAS carriers.[114]

GAS VACCINE DEVELOPMENT

Considering the rapidly increasing antibiotic resistance demonstrated against frequently used antibiotics, including clindamycin, penicillin, and macrolides, it is crucial to research and develop GAS vaccines.[165,166] The development and testing of potential GAS vaccines can be traced back to 1923.[167] In the quest for effective vaccines, two groups of potential candidates; M-protein-based vaccines and non-M protein-based vaccines are in a race to hit the global market. However, to date, no GAS vaccines have been approved for use on humans. Besides these conventional groups, a few novel approaches, such as the development of peptide-based vaccines and multiplex immunoassays, also hold promise as either potential vaccine candidates or to aid in the development of high-efficacy vaccines.[12,168]

M-protein vaccine candidates

M-protein vaccine candidates deliver immunity through two mechanisms; either by employing conserved C-terminal peptides that are common in all GAS strains or through multivalent vaccines that incorporate several N-terminal peptides from GAS isolates. Due to the extensive serotype diversity expressed by GAS strains, a potential solution to mitigate this issue is to develop vaccines effective against multiple serotypes, similar to the development of multivalent pneumococcal vaccines to combat *Streptococcus pneumoniae* infections.[169] To exploit the amino-terminus region of the M-protein, which is known for inducing type-specific opsonic antibodies to combat GAS infections, multivalent M-protein vaccines were developed. Initially, a 6-valent M-protein vaccine was drafted using six N-terminal peptides of the M-protein, after which a 26-valent M-protein vaccine was developed using N-terminal peptides from various GAS isolates associated with pharyngitis and rheumatic fever. After passing phase I and phase II clinical trials in human adults, results were promising as the vaccine did not induce cross-reactivity. One issue of primary concern was its limited serotype coverage in developing regions such as the Middle East or Africa in contrast to effective serotype coverage in developing countries such as North America and Europe.[168,170] A novel 30-valent M-protein-based vaccine was evaluated by a study conducted by Dale et al.[171] and concluded that such multivalent vaccines, which possess the conserved N-terminal peptide sequences seen in prevalent GAS strains, evoke a bactericidal response against numerous strains and could extend beyond the *emm*-types that are usually targeted by the vaccine, drastically increasing serological coverage.

Production of M-protein vaccines based on C-terminal peptides confers an advantage because they are effective against a broad spectrum of GAS strains. J8 C-terminal vaccine candidates are known to produce memory B-cells in mice which provides long-lasting immunity.[172] Their administration also has one additional advantage: they stimulate the production of cross-reactive antibodies, which have the potential to combat against infections induced by non-vaccine serotypes.[173]

Non-M protein vaccine candidates

Certain non-M protein antigens have been observed to be conserved across most GAS strains which can serve as a basis for vaccine development. Since non-M proteins have the potential to lower anti-GAS immune responses, these candidates make use of the peptides that are associated with them. These peptides are aimed to produce vaccines that serve the purpose of removing any antigenic material that does not contribute toward eliciting a proper immune response and also prevent short-term GAS infections and minimize the risk of developing autoimmune sequelae.[174,175] Surface-bound C5a peptidase

(ScpA), G-related α-2-macroglobulin binding protein (GRAB), superoxide dismutase (SOD), serum opacity factor (SOF) and streptococcal fibronectin-binding (SFb) proteins are a few antigens that are potential non-M protein vaccine candidates.[176–178] The Lancefield group-A carbohydrate (GAC) present across all clinically isolated GAS strains has been considered a potential candidate to be used for developing a vaccine. However, anti-GlcNAc monoclonal antibodies have been shown to recognize GlcNAc side chains present in GAC. They are responsible for inducing cross-reactivity in the myocardium and brain, which has subsequently halted its potential as a valid vaccine candidate.[179]

In a study conducted by Bensi et al.[180] mice were immunized with GAS gene segments, and an array of new antigens were identified, including Streptolysin-O (SLO), *Streptococcus pyogenes* cell envelope proteinase (SpyCEP), and spy0269 protease. All three antigens were incorporated to form a single vaccine popularly known as 'Combo', which promised to provide extensive serological coverage against multiple GAS strains in mice; Combo is yet to be approved for clinical trials. In a similar fashion, nonhuman primates (NHPs), namely populations of *Rhesus macaques* were used to model GAS infections to develop a vaccine to combat GAS-induced pharyngitis. This vaccine candidate, known as 'Combo5,' consists of five antigens; SLO, ScpA, SpyCEP, arginine deiminase (ADI), and trigger factor (TF). These antigens were chosen specifically to reduce instances of autoimmune sequelae. The immunized NHPs showed a reduced incidence of pharyngitis and tonsillitis in comparison to controls, and NHPs were demonstrated to have the potential as a viable model for studying GAS infections.[181]

Novel approaches toward GAS vaccine development

Most GAS strains possess a peptide antigen with a conserved B-cell epitope which can be used as a basis for vaccine discovery. Peptide-based vaccines have started to gain traction for their irrefutable efficacy against GAS infections; however, they require an adjuvant or a delivery system to stimulate an immune response. A promising peptide-based vaccine was developed by Nevagi et al.[182] by creating an adjuvant based on the ionic activity exhibited by cationic trimethyl chitosan (TMC) and the aforementioned peptide antigen conjugated with poly-α-L-glutamic acid (PGA) through the application of cycloaddition reactions. The resulting anionic nanoparticles (NP-1) produce serum antibodies that help in invading GAS bacteria' phagocytosis. Furthermore, immunized mice have been demonstrated to have a pronounced absence of bacteria in lymphoid tissues, nasal exudates and pharyngeal surfaces. Poly-hydrophobic amino acids (PHAAs) could also be a potential adjuvant for delivering peptide-based vaccines. PHAAs are self-adjuvants that help self-assemble the PHAA-peptide antigen conjugate into chain-like aggregates of nanoparticles (CLANs). These nanoparticles also aid in the production of serum antibodies which exhibit opsonic activity in mice.[183] In response to the limited serological coverage of the 30-valent M-protein vaccine, the peptides of three structurally similar families of M-related proteins (Mrp) were recombined into an antiserum in order to determine if its incorporation with the 30-valent M-protein vaccine could potentially expand serological coverage. Rabbits were immunized and it was observed that the combination of the trivalent recombinant Mrp (trMrp) and 30-valent M-protein vaccine resulted in an antiserum that demonstrated higher levels of opsonization of GAS more so than either performed when delivered on their own. This study in particular may prove to be useful in producing a vaccine that is able to express massive serological coverage.[184] An optimal assay is crucial for the development of a vaccine; in lieu of this, a recent study aimed at producing a multiplex immunoassay which would be helpful at quantifying antibody responses, more specifically the production of IgG antibodies toward several GAS antigens present in a blood sample. A total of eight antigens; DNase B, SLO, Spy0843, SpyCEP, ScpA, SpnA, GAC and SpyAD were made into spectral beads and a complex panel of an 8-plex assay was created. This assay was able to measure a spectrum of IgG titers from an individual serum sample; such an assay would be useful for testing GAS vaccines through its initial development phases.[185] M-protein vaccine candidates in general have the highest potential to enter the commercial market and become a universal vaccine for GAS infections. Owing to low serological coverage, most non-M protein vaccine candidates have only limited applications in treatment and further research needs to be facilitated for better high-efficacy vaccines (Table 5).

Challenges in GAS vaccine development

Currently, there are no GAS vaccines that are available in the commercial sphere due to multiple hurdles on the path toward vaccine development. These hurdles are complex as they interlace with each other and further complicate the development of a viable vaccine. Widely known vaccine development issues include; the exhibition of extensive serotype and genetic diversity, variation in GAS antigens and consequential immune sequelae that develop due to reoccurring GAS infections. Logistical and humanitarian issues such as safety and ethical concerns, knowledge gaps in the understanding of GAS immune responses, absence of epidemiological data and overall, less priority in the development of vaccines which take into consideration both conserved antigens and type-specific *emm* antigens also serve as a hindrance to the development of GAS vaccines.[186] A study conducted by Davies et al.[187] made possible by DNA sequencing technology has demonstrated that the overall structure of GAS populations shows substantial genomic heterogeneity due to homologous recombination layered with intricate gene plasticity. The very same property of homologous recombination is the key driving factor toward the evolution of GAS lineages more so than mutation. One of the foremost issues plaguing the development of GAS vaccines is their high cross-reactivity with organs such as cardiac tissue, skeletal myosin and keratin. M-proteins relative to non-M proteins show a higher degree of cross-reactivity, a few outliers include non-M proteins such as N-acetyl-β-D-glucosamine which is a cross-reactive antigen that affects cardiac valves and skin. This cross-reactivity serves as a barrier to the potential of M-proteins to act as a strong vaccine candidate.[188]

Further, understanding *emm* cluster typing is important as it dictates the reasons behind resistance between differing GAS strains conferred by M-protein vaccines. It also highlights the complicated serological diversity expressed by GAS pathogens.

Table 5. A table of well-documented GAS vaccine candidates with their respective developmental stages

Vaccine Classes	Vaccine Candidates	Antigens	Adjuvants	Developmental Stage	References
M-protein vaccines	J8 vaccine	Acetylated peptide antigen (J8)	Alum	Phase-I	Castro and Dorfmueller[165], Azuar et al.[176] and Steer et al.[186]
	6-valent vaccine	N-terminal peptides from M1, M3, M5, M6, M19 and M24 serotypes	Aluminum hydroxide	Phase-I	
	26-valent vaccine	N-terminal peptides from 26 M proteins	Alum	Phase-II	
	30-valent vaccine	N-terminal peptides from 30 M proteins	Alum	Approaching Phase-I	
Non-M protein vaccines	Surface-bound C5a peptidase (ScpA)	Recombinant ScpA-49 mutated proteins	Cholera toxin (CT)	Pre-clinical	Castro and Dorfmueller[165], Azuar et al.[176] and Steer et al.[186]
	Chemokine cleaving protease (SpyCEP)	J8 & SpyCEP epitopes (S1-S6)	Alum	Pre-clinical	
	Serum opacity factor (SOF)	SOF & SFbI	Cholera toxin B (CTB)	Pre-clinical	
	Streptococcal fibronectin-binding (SFb) proteins	J8/J14 and FNBR-B or FNBR-BT which contains B-cell and T cell epitopes	CTB	Pre-clinical	
	Group-A carbohydrate (GAC)	GAC isolated from D58X strain	Alum	Pre-clinical	
Combination vaccines	Combo vaccine	SLO, SpyCEP and Spy0269	Alum	Animal Study: Mice	Azuar et al.[176], Bensi et al.[180], Rivera-Hernandez et al.[181] and Courtney et al.[184]
	Combo5 vaccine	SLO, ScpA, SpyCEP, ADI and TF	Alum	Animal Study: *Rhesus macaques*	
	trMrp & 30-valent M-protein antisera vaccine	Peptides of MrpI, MrpII, and MrpIII & 30-valent vaccine	Alum	Animal Study: Rabbits	
Adjuvant delivery vaccines	Polyglutamic acid-trimethyl chitosan-based intranasal peptide nano-vaccine	B-cell epitope	PGA	Animal Study: Mice	Nevagi et al.[182] and Azuar et al.[183]
	Poly-hydrophobic amino acid based self-adjuvating vaccine	B-cell epitope	PHAAs	Animal Study: Mice	

Most GAS infections are limited to skin infections and non-invasive pharyngitis, but infection patterns are still poorly understood amongst strains that are characterized by broader *emm* clusters. Thus, the development of effective GAS vaccines also calls for further research into the study of accurate human infection models as evidenced.[189,190]

CONCLUSION AND FUTURE PERSPECTIVES

GAS infections remain a significant public health concern due to their diverse clinical presentations and potential for severe complications. Untreated or inadequately treated GAS infections can lead to rheumatic fever, an autoimmune disease affecting the heart, joints, skin, and nervous system. Rheumatic fever is now rare in developed countries. Still, it remains a concern in some parts of the world. Resistance to GAS pathogens may be caused by the indiscriminate use of antimicrobial agents at an inappropriate dosage. To create the most effective course of treatment, continuous national and worldwide susceptibility monitoring is required due to the evolution of drug-resistant species and MDR strains of Streptococcus species. Due to the overuse of antibiotics, AMR among Streptococcus species developed from earlier sensitive inhabitants, leading to parallel gene transfer or even point mutations in chromosomes.

To define the evolutionary developmental paths of pathogenic GAS populations, ongoing initiatives involving both research and public health laboratories are essential. GAS outbreaks continue to spread around the globe, producing significant illness incidence. Coordinated efforts to increase the capacity and surveillance nodes in low-resource settings are crucial for describing GAS transmission chains and providing a framework to evaluate the effectiveness of future preventative measures. Even though substantial research has been carried out explaining the virulence mechanisms of GAS, new host-pathogen interactions are being discovered. The direct examination of GAS-infected humans has opened up new perspectives, such as the function of mucosal-associated invariant T cells (MAIT cells) in STSS patients. It is obvious that additional research using human patient data is necessary and will offer helpful insights for creating new treatments and preventative measures. It is quite concerning that first-step PBP2x mutations in GAS have caused other streptococcal species to lack penicillin susceptibility.

Developing a reliable and efficient GAS vaccine to minimize the GAS disease burden is now firmly considered a priority by the WHO, vaccine developers and other important stakeholders. Further, since there is not always a direct link between resistance and medicine failure, everyone must understand the significance and impact of antimicrobial drug resistance on streptococcal

infections. Programs for knowledge sharing and professional healthcare recommendations should be supported to encourage efforts to reduce the use of antibiotics. Research to enhance new vaccine designs must be implemented to prevent the emergence of resistant strains. Additional therapeutic approaches beyond β-lactams are crucial for treating severe GAS infections. Clindamycin is one of the most effective treatments available alongside β-lactams for treating necrotizing fasciitis or STSS, even though resistance is rising globally. We lose this tool as resistance rises quickly, necessitating new treatment approaches. Similar to penicillin, surveillance is essential to identify recent trends in resistance.

Improved diagnostic tools, such as point-of-care tests with high sensitivity and specificity, could aid in the rapid and accurate diagnosis of GAS infections, allowing for timely treatment and better patient outcomes. Public awareness campaigns and education on the importance of seeking timely medical attention for symptoms of GAS infections can help reduce the spread of the bacterium and prevent complications. Despite the existence of possible pandemic pathogens like SARS-CoV-2, coupled GAS or secondary GAS infections require further attention. Clinicians should strive to diagnose and treat affected persons as quickly as feasible and avoid making incorrect diagnoses. Focus should be placed on the potential rise in invasive GAS infections, and clinicians should exercise extreme caution and offer the proper safety advice. This is important since early detection of GAS-infected patients and prompt beginning of supportive care can save lives.

ACKNOWLEDGMENTS

The authors extend their appreciation to the Deanship of Research and Graduate Studies at King Khalid University for funding this work through Large Research Project under grant number RGP2/399/45.

AUTHOR CONTRIBUTIONS

Conceptualization and methodology, A.T., and S.H.; Original draft preparation, subsection contributions, review, and editing, A.V., T.A., S.K. and N-M.G.B.; English review and editing, A.A.; Project administration and editing, A.P. All authors have read and agreed to the published version of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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