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Review article

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Pathogenesis, epidemiology and control of Group A *Streptococcus* infection

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Abstract

Streptococcus pyogenes (Group A *Streptococcus*; GAS) is exquisitely adapted to the human host, resulting in asymptomatic infection, pharyngitis, pyoderma, scarlet fever or invasive diseases, with potential for triggering post-infection immune sequelae. GAS deploys a range of virulence determinants to allow colonization, dissemination within the host and transmission, disrupting both innate and adaptive immune responses to infection. Fluctuating global GAS epidemiology is characterized by the emergence of new GAS clones, often associated with the acquisition of new virulence or antimicrobial determinants that are better adapted to the infection niche or averting host immunity. The recent identification of clinical GAS isolates with reduced penicillin sensitivity and increasing macrolide resistance threatens both frontline and penicillin-adjunctive antibiotic treatment. The World Health Organization (WHO) has developed a GAS research and technology road map and has outlined preferred vaccine characteristics, stimulating renewed interest in the development of safe and effective GAS vaccines.

Sections

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Introduction

Streptococcus pyogenes (Group A *Streptococcus*; GAS) is a Gram-positive host-adapted bacterial pathogen causing benign human infections such as pharyngitis and impetigo, through to rare yet severe invasive diseases such as septicaemia, streptococcal toxic shock-like syndrome (STSS) and necrotizing fasciitis. Repeated GAS infections may trigger autoimmune sequelae including rheumatic fever that can lead to rheumatic heart disease (RHD)[1]. Epidemiologically, GAS can be classified into more than 220 *emm* types[2] (based on the gene sequence of the amino terminal of the surface-exposed M protein) which show differing patterns of regional and global distribution[3]. Recent epidemiological investigation has detected multiclonal outbreaks of scarlet fever in Asia and the United Kingdom[4–7], with the UK outbreak paralleling a surge in invasive infections[4].

As a host-adapted human pathogen, GAS survival requires an unbroken cycle of transmission, adherence to the primary infection site (skin or throat), colonization and proliferation, defence against both innate and adaptive immune systems, and subsequent dissemination to a new host. New virulence strategies employed by GAS to manipulate host defence mechanisms are being discovered. For example, the cleavage of Gasdermin A (GSDMA) by the GAS protease streptococcal pyrogenic exotoxin B (SpeB) has been shown to trigger host cell pyroptosis[8,9], whereas mucosal-associated invariant T cells (MAIT cells) have been recently identified as highly activated in patients with STSS, and as primary contributors to the cytokine storm associated with this disease[10].

In the absence of a commercial GAS vaccine, medical intervention against GAS revolves around the use of antibiotics to treat or prevent infection. However, GAS antibiotic resistance is on the rise and the first mutations that confer reduced penicillin sensitivity have been reported[11–15]; nonetheless, GAS remains susceptible to β-lactam antibiotics. To expedite GAS vaccine development, the World Health Organization (WHO) has developed a GAS research and technology road map and has outlined preferred product characteristics[16]. Large-scale genomics has been applied to define global GAS population structure and predict vaccine antigen coverage[17]. New GAS vaccine formulations directed against M protein and non-M protein antigens are in development[18]. The non-human primate model of GAS pharyngitis has recently been used to assess GAS vaccine efficacy[19], and the development of a controlled human infection model (CHIM) of GAS pharyngitis[20] provides a future opportunity for assessment of vaccine efficacy in the human host.

The past decade has witnessed great advances in the field of GAS research, but even with the ongoing development of new experimental infection models and treatment strategies, a reinvigorated vaccine development effort and active surveillance efforts, the global burden of GAS diseases remains an unmet public health challenge. The emergence and dissemination of both multidrug-resistant strains and new toxigenic GAS clones underscore the urgent need to improve public health strategies to prevent or treat human GAS infections. As addressing all of the epidemiological, clinical and molecular aspects of GAS infections is beyond the scope of this Review, here we focus on the most recent research developments and advances.

Diseases caused by GAS

As an exquisitely human adapted pathogen, GAS can cause a broad spectrum of disease manifestations. Table 1 describes the most common diseases associated with GAS, but other associated diseases include otitis media, sinusitis, meningitis, endocarditis, pneumonia, peritonitis and osteomyelitis[1]. It is estimated that GAS accounts for half a million deaths annually, with RHD and invasive infections responsible for most deaths[21]. Recent estimates have stressed the significant health burden caused by GAS infections, suggesting that RHD is responsible for more than 100 million disability-adjusted life-years with 0.1% being attributed to GAS pharyngitis in children[22]. These estimates have not been determined for other GAS diseases, and epidemiological data, particularly in low and middle-income countries, remain scarce. Studies from Australia and New Zealand indicate that cellulitis is responsible for the highest health and economic burden of all GAS diseases in these settings[23], even above RHD. Collectively, global estimates for the health and economic burden of all GAS-related diseases remain poorly understood, highlighting the urgent need for better burden of disease data in order to understand the impact of this pathogen worldwide.

Over the past decade, an important advocacy movement by the WHO has raised awareness about RHD and its contribution to the global burden of disease, and the deepening of social inequalities in already vulnerable populations[24,25]. In addition, studies from the United States and Israel have highlighted that RHD is still an important public health problem even in high-income countries[26]. Nevertheless, there are still important gaps in our knowledge of this disease. Scientific efforts are ongoing to generate robust evidence that supports the hypothesis of a link between concurrent skin infections and development of immune sequelae[27,28].

Scarlet fever, a disease that had practically disappeared by the end of the twentieth century, has re-emerged recently with outbreaks reported in China, Hong Kong, South Korea, Singapore and the United Kingdom[4,5,29–31]. To date, outbreak strains are predominantly multiclonal and linked with distinct epidemiological markers such as carriage of mobile genetic elements that contain exotoxins and confer multidrug resistance to tetracycline and macrolides[6], particularly in Asia. Scarlet fever-like epidemic clones have also been detected in several other geographical regions[32,33]. It remains critical to have access to better local and global surveillance systems for tracking GAS diseases, given that studies have shown that vulnerable close contacts of patients with mild diseases are at greater risk of invasive infections[34]. In addition, a significant increase in the incidence of invasive GAS disease has been documented in several countries, particularly in disadvantaged and vulnerable populations[4,35–37], again highlighting the importance of closely monitoring GAS epidemiology. It should be noted that improved centralized healthcare reporting systems might also have contributed to the detected increase.

GAS infection, virulence factors and mechanisms

The process of human infection by GAS is complex and multifactorial, involving both host and bacterial factors that contribute to the pathogenesis of infection. GAS produces a large number of cell wall-associated and secreted virulence factors that have various effects on tissues, cells and components of the immune response (Fig. 1), which have been extensively reviewed elsewhere[1]. Here, we focus on key virulence factors that are important for the colonization of epithelial tissues and the progression of invasive disease, highlighting the most recent advances in this area.

Surface-bound virulence factors

M protein. GAS is classified based on the sequence of the 5′ end of the gene encoding the M protein (*emm*). More than 220 *emm* genotypes have been identified[2]. The M protein is a dimeric coiled-coil fibrillar protein that extends from the bacterial cell wall[38]. It consists of a conserved

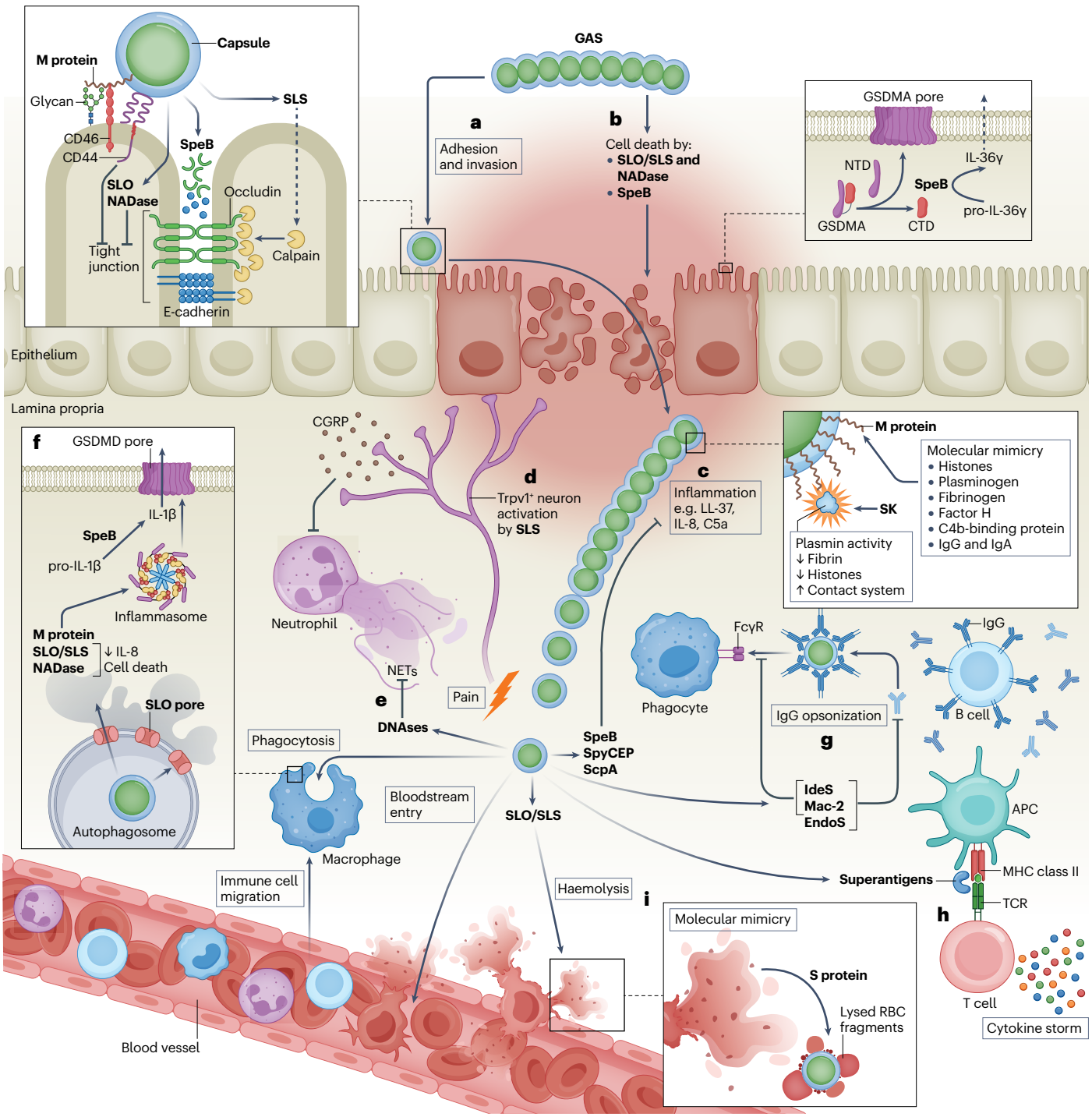
Table 1 | Diseases caused by GAS infection

Disease	Signature clinical symptoms	Associated M types	Treatment	Estimated burden of disease	Refs.
Superficial					
Pharyngitis	Sore throat, fever, tonsillopharyngeal inflammation, patchy tonsillopharyngeal exudates, palatal petechiae, anterior cervical adenitis	1, 2, 3, 4[a], 5, 6, 12, 28[a], 75, 89[a]	Oral antibiotics	288.6 million cases per year (children aged 4–15 years) and 0.1 million disability-adjusted life-years $224–539 million (in the United States only)	21,22,199,200
Scarlet fever	Maculopapular rash, exudative pharyngitis, 'strawberry tongue'	1, 3, 4[a], 12	Oral antibiotics	Unknown	21,199,201
Impetigo	Honey-coloured crusts most commonly on the face and extremities	33, 41, 42, 52, 53, 70	Topical, oral or systemic antibiotics depending on disease severity	111 million prevalent cases	168,201,202
Sequelae					
Acute rheumatic fever (ARF)	Fever, arthritis, carditis, chorea	1, 5, 6, 14, 18, 24	Oral or systemic antibiotics to prevent recurrent infections and supportive treatment for joint manifestations, carditis and chorea	5–51 per 100,000 population	201,203,204
Rheumatic heart disease (RHD)	Shortness of breath, mitral and/or aortic regurgitation, subsequent mitral stenosis	Unknown	Antibiotic secondary prophylaxis, medical heart failure treatment and interventional treatment	>40 million prevalent cases >300 deaths per year >10 million disability-adjusted life-years	21,204,205
Acute post-streptococcal glomerulonephritis	Facial oedema, hypertension, haematuria, complement deficiency	1, 4[a], 12, 49, 55, 57, 60	Treatment of clinical symptoms	>470,000 cases 5,000 deaths per year	21,201,206
Invasive					
Bacteraemia	High fever, nausea, vomiting	1, 3, 6, 12, 28[a], 53, 68, 81, 89[a]	Intravenous antibiotics, IVIG	>600,000 cases 160,000 deaths per year (all invasive disease)	202,207–209
Cellulitis	Erythema, oedema, warmth and tenderness	Unknown	Oral or systemic antibiotics depending on disease severity	Unknown	210
Puerperal sepsis	Fever, chills, pain, purulent vaginal discharge in pregnant or recent postpartum women	1, 4[a], 11, 12, 13, 28[a]	Intravenous antibiotics, surgical intervention if required	Unknown	211,212
Necrotizing fasciitis	Fever, malaise, local erythema, swelling, myalgias, abdominal pain	1, 3, 28[a]	Intravenous antibiotics and surgical debridement and/or amputations	Unknown	21,201
Streptococcal toxic shock syndrome (STSS)	Fever, rash, hypotension, end organ failure	1, 3	Intravenous fluids and antibiotics, IVIG	Unknown	201,213

GAS, Group A *Streptococcus*; IVIG, intravenous immunoglobulin. [a]Capsule-negative *emm* types[51].

carboxy terminal that confers covalent attachment of M protein to the cell wall and a hypervariable surface-exposed N terminal which contains the M type-defining 50 amino acids, that exhibits considerable antigenic diversity[39]. The contribution of M proteins to GAS virulence is attributed primarily to their immune-modulatory effects. They can directly bind to and recruit numerous host components, including plasmin(ogen) and fibrinogen, to the streptococcal surface, thereby conferring resistance against innate and adaptive immune responses[1]. M proteins also trigger programmed cell death in macrophages by inducing the NLRP3 inflammasome machinery, leading to the processing and secretion of the pro-inflammatory cytokines interleukin-1β (IL-1β) and IL-18 (refs. [40,41]), albeit in an M type-specific manner. Numerous studies have provided evidence that M proteins also contribute to host colonization through adhesive interaction with epithelial cell receptors, such as the membrane cofactor protein (MCP; also known as CD46)[42] and cell-surface glycans[43,44], although serotype-specific differences in these interactions have been reported[45].

Hyaluronic acid capsule. The hyaluronic acid capsule of GAS is composed of repeating disaccharide units of glucuronic acid and *N*-acetylglucosamine and confers the characteristic wet mucoid colony morphology. The GAS capsule is structurally identical to human hyaluronic acid, a major component of extracellular matrices found in many body tissues including connective and epithelial tissues. The GAS capsule therefore acts to camouflage the pathogen from the host immune system. By directly binding to the human cell surface glycoprotein CD44, a primary receptor for human hyaluronic acid[46], the GAS capsule mediates adherence to epithelial cells of the pharynx and skin[47]. CD44-dependent binding further leads to the activation of cell signalling pathways that disrupt epithelial barrier integrity, thereby allowing GAS to penetrate into deeper underlying tissues[47]. GAS encapsulation was also shown to increase virulence and resistance to complement-mediated phagocytic killing[48]. However, loss of capsule production has been reported in both invasive and non-invasive strains from several different *emm* types that either lack the entire *hasABC* capsule gene operon (*emm*4, *emm*22



and *emm*89)[49,50] or harbour inactivating mutations within the *hasAB* genes (*emm*28 and *emm*87)[51,52]. The selective advantage conveyed by capsule loss in these genetic backgrounds is not fully understood.

S protein. GAS has evolved many ingenious strategies to avoid immune clearance. A new form of molecular mimicry has recently been described, in which a highly conserved surface-associated protein (S protein) was shown to selectively bind red blood cell membranes[53]. S protein-dependent membrane coating of the GAS cell surface protects against phagocytic killing, providing a critical link between the characteristic haemolytic activity of this pathogen and an immune camouflage strategy that might help facilitate blood survival and dissemination.

Secreted virulence factors

Chemokine degradation. Proteases are utilized by pathogenic bacteria to specifically cleave and neutralize key signalling molecules of

Fig. 1 | GAS virulence factors and their roles in cell adherence, invasion and immune evasion. a, Surface-expressed M protein and the capsule facilitate attachment of Group A *Streptococcus* (GAS) to epithelial cells via binding to CD46, specific glycan structures and CD44, respectively. Secretion of the toxins streptococcal pyrogenic exotoxin B (SpeB) and streptolysin S (SLS) at the epithelial surface destabilizes the junctional proteins of epithelial cells, resulting in the loss of cell–cell adhesion and translocation of GAS across the host epithelial barrier. Following bacterial invasion into epithelial cells, joint action of streptolysin O (SLO) and NAD glycohydrolase (NADase) leads to the disruption of the Golgi network, which further impairs epithelial barrier integrity. **b**, Invasion of deeper underlying tissue may also occur through streptolysin (SLS and SLO) and NADase-induced cell death, or via SpeB-induced, gasdermin A (GSDMA)-dependent pyroptotic cell death of epithelial cells. The resulting tissue damage mounts a robust inflammatory response characterized by abundant infiltration of innate and adaptive immunity cells, attracted by various stimuli, such as human cathelicidin LL-37, pro-inflammatory cytokine interleukin-8 (IL-8) and SpeB-activated IL-36γ. **c**, GAS has evolved multiple mechanisms to elude the host immune system. These include the degradation of LL-37 by SpeB, IL-8 cleavage by *S. pyogenes* cell envelope proteinase (SpyCEP) and cleavage of the complement component 5a (C5a) by a C5a peptidase (ScpA). Coating of the bacterial surface with host factors, such as histones, plasminogen and fibrinogen, via binding to surface-expressed M protein further prevents immune recognition. Host defence proteins, such as fibrin and histones, and the human contact system are also proteolytic target molecules for the surface-bound streptokinase (SK)–plasmin complex which assists bacterial dissemination. **d**, Streptolysin SLS activity triggers neural release of calcitonin gene-related peptide (CGRP) into infected tissues, suppressing neutrophil recruitment and bactericidal activity. **e**, Extracellular deoxyribonucleases (DNases) degrade the DNA backbone of neutrophil extracellular traps (NETs), allowing GAS to escape neutrophil killing. **f**, The coordinated activities of SLO and NADase further prevent maturation of phagolysosomes, inhibit IL-8 secretion and promote GAS survival in macrophages, where streptolysins SLO and SLS and M protein activate the inflammasome pathway to induce IL-1β production and pyroptosis. SpeB additionally amplifies inflammatory signalling by cleaving and activating pro-IL-1β in an inflammasome-independent manner. **g**, Survival strategies employed by GAS to escape adaptive immunity include the secretion of the IgG-degrading enzymes IdeS, Mac-2 and EndoS, which enables bacterial escape from IgG opsonization and recognition by FcγR on phagocytes. **h**, Superantigens, by contrast, cause excessive activation of the adaptive immune system by cross-linking MHC class II molecules on antigen-presenting cells (APCs) and T cell receptors (TCRs) in a nonspecific fashion leading to an event known as a 'cytokine storm'. **i**, Upon bloodstream entry, S protein-mediated coating of GAS cells with lysed red blood cell (RBC) fragments, such as those derived from SLO and SLS haemolytic activity, serves as an immune camouflage tactic which allows GAS to survive in and disseminate from blood vessels. CTD, carboxy-terminal domain; GSDMD, gasdermin D; NTD, amino-terminal domain.

the innate immune system[54]. GAS secretes two such proteases known as *S. pyogenes* cell envelope proteinase (SpyCEP) and C5a peptidase (ScpA) that cleave the chemokine IL-8 (also known as C–X–C motif chemokine ligand 8 (CXCL8)) and complement component 5a (C5a), respectively[55,56]. Cleavage of these potent chemoattractants impairs neutrophil infiltration and activation, a key defence mechanism of innate immunity.

Deoxyribonucleases. Various pathogenic streptococci produce extracellular deoxyribonucleases (DNases) to combat host immune defences[57]. All sequenced GAS strains contain at least one extracellular DNase[58]. In total, six prophage-encoded (*sda1*, *sda2*, *spd1*, *spd3*, *spd4* and *sdn*) and two chromosome-encoded (*spnA* and *spdB*) DNase genes have been identified in GAS[57]. Of these, SpnA is the only cell wall-anchored DNase containing the requisite sortase substrate LPXTG motif[59]. The primary functions of streptococcal DNases appear to be the degradation of the DNA framework of neutrophil extracellular traps (NETs) facilitating the release of entrapped bacteria[60–62], and autodegradation of bacterial DNA, thus suppressing TLR9-dependent recognition by immune cells[63]. Results from several infection models suggest a critical role for DNases in the pathogenesis of GAS disease[60–62].

Streptokinase. Streptokinase (SK) is a potent human-specific plasminogen-activating protein. Unlike other plasminogen activators, SK has no intrinsic enzymatic activity. The SK–plasminogen complex possesses plasmin-like activity and is critical to the pathogenesis of invasive GAS diseases, assisting bacterial dissemination via proteolysis of host defence proteins[64–67].

Immunoglobulin-degrading enzymes. To evade adaptive immunity, GAS secretes three immunoglobulin-degrading enzymes, known as IdeS/Mac-1, Mac-2 and EndoS, that specifically target opsonizing IgG antibodies. IdeS is a cysteine protease that cleaves the heavy chain of IgG[68]. Mac-2 is an allelic variant of IdeS with similar IgG endopeptidase activity[69]. Both proteins function as IgG endopeptidases; however, they also interact with Fcγ receptors of phagocytic cells, thus interfering with Fc-mediated host defence mechanisms. EndoS, by contrast, has endoglycosidase activity and specifically hydrolyses core glycans on human IgG antibodies, neutralizing antibody effector functions during infection[70].

SpeB. The broad substrate specificity of SpeB leads to cleavage of a wide range of host and bacterial proteins, including intercellular barrier proteins at epithelial junctions[71], host extracellular matrix proteins[72], complement factors[73], the cathelicidin-derived antimicrobial peptide LL-37 (ref. [74]), autophagy components[75] and chemokines[76]. SpeB also displays pro-inflammatory properties by directly cleaving and activating the precursors of IL-1β (ref. [77]) and epithelial IL-36γ (ref. [78]), two potent pro-inflammatory cytokines that are critical for host defence responses to infection and injury. Another recently discovered pro-inflammatory mechanism involves the cleavage and activation of pore-forming GSDMA in skin epithelial cells which triggers pyroptosis, a lytic form of inflammatory cell death[8,9]. Caspase-independent cleavage of GSDMA by SpeB is highly selective and requires SpeB to enter the cytosol of infected cells. Intriguingly, although SpeB is required during the early stages of the infection process, SpeB-negative variants frequently arise from immune selection during severe invasive infections in M1T1 GAS[79–81] and, to a lesser extent, in non-M1 GAS[82]. Loss of SpeB expression as a result of mutation in the *covR/S* regulatory system results in the accumulation of surface-bound plasmin activity which triggers the systemic dissemination of GAS in vivo[83].

Streptolysins and NAD glycohydrolase. Almost all clinical isolates of GAS secrete two potent cytolytic toxins, streptolysin S (SLS) and streptolysin O (SLO), that cause pore formation in eukaryotic cell membranes. Both cytolysins are cytotoxic against a wide range of host cells, including epithelial and immune cells. Various functions have been assigned to SLS and SLO, ranging from soft-tissue damage, tissue invasion and innate immune evasion to the activation of pro-inflammatory responses[41,84–88]. The peripheral nervous system is another specific

target for SLS, which activates sensory neurons to produce pain and suppress recruitment of immune cells, promoting bacterial survival during infection[89]. In GAS, activity of the cholesterol-dependent cytolysin SLO is functionally interdependent with the co-expressed toxin NAD glycohydrolase (NADase; also known as SPN or NGA)[90], which depletes host cells of cellular energy stores[91]. SLO and NADase physically interact and co-stabilize after secretion[92]. NADase-dependent membrane binding promotes pore formation by SLO[93], which conversely facilitates translocation of NADase into host cells[94]. In combination, SLO and its co-toxin NADase promote GAS intracellular survival and cytotoxicity in macrophages and epithelial cells[95,96], impair host defences in these cell types through Golgi fragmentation[97] and contribute to pathogenesis in vivo[98]. Streptococcal strain emergence and epidemicity has been associated with a high-activity promoter recombination event at the NADase–SLO locus which results in increased expression of the NADase and SLO toxins[50,52,99,100]. This recombination-related genome remodelling is often observed in acapsular isolates, suggesting that capsule production may be dispensable in high toxin-expressing strains[50,52,100], but the mechanistic basis for this relationship remains to be determined.

Superantigens. Superantigens, also commonly referred to as Spes, are potent exotoxins that crosslink the variable region of T cell receptor β-chains (TCR Vβ) with MHC class II molecules of antigen-presenting cells (APCs) in a non-antigen-specific manner, resulting in broad activation of T cells and uncontrolled cytokine responses[101]. Streptococcal superantigens have been implicated in a range of human diseases, most notably toxic shock syndrome and scarlet fever[101]. To date, 13 distinct superantigens have been identified in GAS (chromosome-encoded: *speG*, *speJ*, *speQ*, *speR* and *smeZ*; prophage-encoded: *speA*, *speC*, *speH*, *speI*, *speK–M* and *ssa*)[102]. Of these, three superantigens (SpeA, SpeC and SSA) have been linked with increased fitness and virulence of contemporary GAS strains causing scarlet fever and invasive disease[4,61,103]. Significant progress has been made in the field of superantigen biology using transgenic mice that express human leukocyte antigen (HLA) MHC class II molecules as a superantigen-sensitive infection model, which helped establish an important role for SpeA and SpeC in acute nasopharyngeal infection by GAS[61,103,104].

Host responses to GAS infection

As a human-restricted pathogen, animal models of GAS diseases share limited fidelity with human disease, which is an impediment to mechanistic immunological studies. A CHIM for GAS pharyngitis has recently been developed, which provides an unparalleled opportunity to interrogate the cellular and humoral factors driving the early human immune response to superficial GAS infection[20,105]. Analysis of sera collected from CHIM volunteers revealed that the early systemic response is characterized by the elevation of IFNγ, IL-6, CXCL10 and IL-1Ra above baseline[105]. This was associated with a commensurate increase in IL-1Ra, IL-6, IFNγ and IP-10 above baseline in the saliva of patients who developed pharyngitis, which was less pronounced in patients who remained asymptomatic. The elevation of pro-inflammatory cytokines was associated with increased numbers of monocytes and dendritic cells, and by a decrease in conventional $CD4^+$ T cells (T follicular helper cells, T helper 17 cells (T_H17 cells), T_H1 cells) and B cells in the blood, as well as increased expression of activation markers by γδT cells. The rapid recruitment of T follicular helper cells and B cells to the site of infection in the CHIM is congruent with the finding that recurrent tonsillitis is an immunosusceptibility disease associated with defective T follicular helper cell and B cell function[106]. Critically, MAIT cells were activated after exposure to GAS, and IL-18, which activates MAIT cells, was elevated in the saliva of test subjects, which has not been reported from studies using mouse models of nasopharyngeal infection.

MAIT cells

Underscoring the need for careful interpretation of mechanistic insights gained from mouse models of GAS infections, MAIT cells have not been a focus in the context of GAS diseases. Further, murine MAIT cells were initially reported to not be activated by GAS[107] whereas human MAIT cells are activated by GAS via two distinct mechanisms[10,108,109].

MAIT cells were recently shown to be highly activated in patients with STSS, and were identified as primary contributors to the cytokine storm association with this disease[10]. Despite representing only 1–10% of the peripheral blood T cell population, during ex vivo stimulation of peripheral blood mononuclear cells from patients with STSS with GAS, MAIT cells represented 41% of IFNγ-producing and 15% of TNF-producing T cells, respectively. In some patients, MAIT cells represented nearly 60% of IFNγ-producing T-cells[10], and depletion of MAIT cells from peripheral blood mononuclear cells prior to stimulation with GAS reduced the production of IFNγ, IL-1β, IL-2 and TNF, which drive immunopathology during the STSS cytokine storm[110]. Similarly, MAIT cells are highly elevated in the blood of patients with active acute rheumatic fever (ARF) and in those who have recently been released from hospitalization due to ARF, compared with healthy individuals[111]. Additionally, MAIT cells from patients with ARF exhibit higher constitutive IFNγ and TNF production than those obtained from healthy individuals, which likely contributes to immunopathology[112,113]. These observations are consistent with an emerging paradigm which suggests that MAIT cells occupy a central pathological role in other autoimmune diseases including type 1 diabetes[114], ankylosing spondylotitis[115] and inflammatory bowel diseases[116]. Taken together, these findings implicate MAIT cells in the pathogenesis of pharyngitis, invasive GAS and ARF (Fig. 2), and although it remains hypothetical, it is tempting to speculate that therapies which selectively impair MAIT cell activity might have broad applicability as treatments for GAS diseases, particularly for STSS where mortality remains unacceptably high[117]. Although MAIT cell-directed immunotherapies are yet to enter the market, interventions against MAIT cells as treatments for other inflammatory diseases are under development[118]. However, our understanding of MAIT cell biology is still immature, and the exact contribution of individual MAIT cell subtypes to GAS diseases will need to be precisely elucidated.

Immunological insights into the pathogenesis of ARF and RHD

Animal models of ARF and RHD fail to recapitulate many of the cardinal features of disease pathophysiology, limiting their utility for interrogating the immunopathogenesis of these diseases. However, recent studies have provided mechanistic insights into the immunological processes which drive the pathogenesis of these diseases, namely the existence of an IL-1β–GM–CSF axis which might explain the selective trafficking of T_H1 cells to the mitral valves of the heart[119]. These cells are the major source of GM–CSF in humans among $CD4^+$ T cells[120], and are independently implicated in the pathogenesis of myocarditis[121,122]. Further, ligands for CXCR3 facilitate T cell recruitment to valvular tissue lesions associated with ARF progression to RHD[123]. The persistence of IL-1β release in peripheral blood mononuclear cells from patients with ARF or RHD suggests that dysregulated feedback inhibitory mechanisms may be a risk factor for the onset of both diseases, in addition to other GAS diseases such as necrotizing fasciitis in which a pathological role for excessive IL-1β production is well established[124].

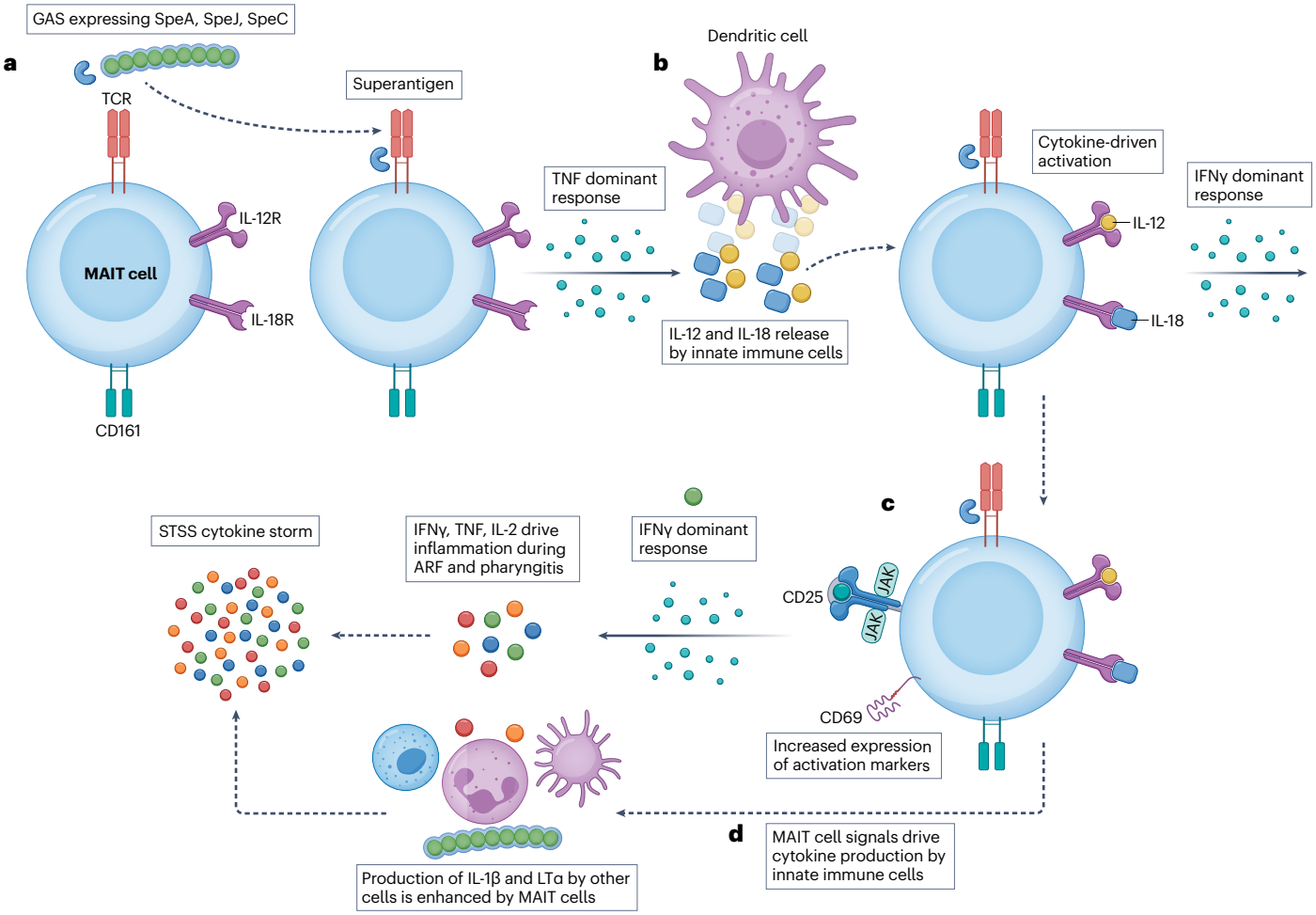


Fig. 2 | Overview of pathogenic mechanisms of MAIT cell activation during GAS infection. Mechanisms of mucosal-associated invariant T cell (MAIT cell) activation during Group A *Streptococcus* (GAS) infections. **a**, The first phase is driven by superantigens such as streptococcal pyrogenic exotoxin A (SpeA), SpeJ and SpeC interacting with the MAIT cell T cell receptor (TCR) in an MR1-independent manner. The activation of the MAIT cell TCR drives the rapid release of prodigious quantities of TNF, and low levels of IFNγ. **b**, In the second phase, interleukin-18 (IL-18) and IL-12, which are released by immune and epithelial cells, bind to their cognate receptors on the surface of MAIT cells. The activation of IL-12 and IL-18 receptors on the surface of MAIT cells results in an IFNγ-dominated cytokine response. **c**, MAIT cells from patients with streptococcal toxic shock-like syndrome (STSS), acute rheumatic fever (ARF) and pharyngitis are highly activated, exhibiting higher expression of CD25, CD69 and other activation markers compared with healthy individuals, which is associated with higher production of INFγ, TNF and IL-2. The increased production of INFγ, TNF and IL-2 contributes directly to immunopathology during GAS pharyngitis, and bouts of ARF. **d**, MAIT cell-derived pro-inflammatory cytokines also provide stimulatory signals to innate immune cells to enhance their production of other inflammatory cytokines. The release of IL-1β and lymphotoxin-α (LTα; formerly known as TNFβ) by innate immune cells and of INFγ, TNF and IL-2 by MAIT cells contributes to the cytokine storm which underpins the immunopathology associated with STSS. Aberrant cytokine response contributes to morbidity and mortality during STSS.

GAS epidemiology and evolution

The primary epidemiological marker of GAS is based on the immunodominant M protein which has been central in defining GAS strains over the last century. Originally developed as a serological method[125], the M-typing scheme became gene-based in the 1990s after molecular methods identified that the hypervariable N-terminal region of the *emm* gene conveyed M protein sero-specificity[126,127]. The global epidemiology of GAS on the basis of *emm* type was summarized in 2009 when a predominance of dominant GAS *emm* types was reported in high-income settings, which is in contrast to low-income settings (such as within Africa and the Pacific) where these GAS types are infrequently observed and there is a general lack of dominant GAS *emm* types in circulation[3]. Recently, whole genome-based approaches have been used to define relationships between GAS populations based on variation in both total gene content and associated sequence variation[17,128]. The correlation between epidemiological markers such as *emm*-type and whole-genome sequence clusters differs within a global context, yet gene-based methods such as *emm*-typing have proven effective for local, short time frame investigations. Recent review articles provide a comprehensive background to the intersection of genomics and GAS epidemiology[129–131], and here we focus on the latest advancements in GAS population biology. Continual knowledge advancements in these areas are providing new paradigms in pathogenesis, better frameworks for pathogen tracking, transmission dynamics and vaccine

advancement, which in turn will be used to improve clinical and public health control of GAS infections.

Population genomic studies have shown that the overall size of the GAS genome is relatively stable at 1.7–2.0 Mbp, encoding between 1,500 and 2,000 genes. Approximately 1,300 'core' genes are conserved in all GAS types, with an accumulated 'accessory' or variable gene content approximately 5 times larger than the core genome[17,129]. The central narrative of global GAS population genomics revolves around it being a genetically diverse pathogen with hundreds of co-evolving genome 'clusters' or 'lineages', with the relative abundance and fluctuation of these clusters differing substantially across both geography and time. Although these lineages are genetically distinct, their evolutionary trajectories are strongly influenced by homologous and non-homologous recombination events that play a major role in the evolutionary success of global GAS lineages. The contrasting population structure of GAS between diverse geographical settings is exemplified in Fig. 3, where alternating grey boxes represent ~300 evolutionary distinct GAS lineages as previously defined[17] and the geographical region where that lineage was reported is colour-coded. The lines connecting these two facets (geography and genomic lineage) indicate that although many lineages are globally dispersed, the Pacific and African geographical regions contain GAS lineages that are rarely observed in other locations. The contrasting population structure of GAS between diverse geographical settings is exemplified in preliminary findings from The Gambia[132], Kenya[133] and remote Australia[17,134] where the circulating GAS lineages are largely evolutionarily distinct from those originating from high-income settings. One interpretation of these data is that the frequency of GAS lineages differs globally, in which there is maintenance of higher numbers of GAS genotypes from geographical regions where the disease burden is highest. Although the driving forces for the maintenance of these temporal–spatial differences in population structure remain unclear, these dynamics are likely a complex interplay of differing transmission pathways, social-economic factors and pathogen/host gene selection events.

Genomic epidemiology has been pivotal in the identification and tracking of GAS strains within public health surveillance nodes, particularly in high-income jurisdictions where genome sequencing for selected notifiable pathogens is centralized and resourced. It is within these settings that a recently emerged GAS *emm*1 clone was identified (termed $M1_{UK}$) that differed from the progenitor M1 population by the presence of 27 single-nucleotide polymorphisms across the core (~1.7 Mbp) genome[4]. The 'rapid' spread of this variant of concern has been observed across other high-income surveillance nodes[135–137], highlighting the pandemic nature of this clone. Molecular events leading to selective replacement of GAS clones also include acquisition of mobile genetic elements carrying antimicrobial resistance markers and streptococcal superantigens, homologous recombination events associated with key virulence loci (particularly the NADase–*slo* locus) and variations in regulatory networks[50,52,129]. Although the underlying factors that influence the evolutionary trajectory of the GAS population are still being resolved, what is clear is that evolution is a dynamic and ongoing process, strongly influenced by temporal and spatial factors, which represents a challenge for global GAS surveillance and the design of therapeutic interventions. Despite this hurdle, population genomic frameworks have recently been used to support global GAS vaccine development through the identification of proposed GAS vaccine antigens that exhibit high global sequence coverage[17].

Recent insights have exemplified how the resolution afforded by whole-genome sequencing can shed new light on transmission pathways that would not readily be observed using traditional epidemiological tools. A study examining invasive disease outbreak clusters across several surveillance nodes in the United States found associations between transmission clusters primarily within populations of social disadvantage[138]. An important extension of this study was the observation that pharyngitis and invasive disease genomic clusters likely share the same transmission network[138,139]. Although the contribution of environmental and fomite transmission is less well characterized, recent invasive GAS outbreaks in subacute healthcare settings[140,141] and scarlet fever outbreaks in school-based surveillance settings[142] suggest that fomite-mediated, aerosol and household-mediated transmission contributes to the spread of disease, resulting in GAS clones which in some settings can persist and become dominant[141]. These findings indicate that GAS disease outbreaks are typically not of single point source, highlighting the need for intervention strategies that aim to reduce the GAS burden at the primary sites of infection (throat and skin) in addition to primordial prevention initiatives aimed at increasing health education, improving hygiene practices and improving housing conditions, especially within settings of social disadvantage[143].

Rise in antibiotic resistance

Antibiotic therapy remains an essential point of care for the treatment of both non-invasive and invasive GAS infection[144]. Although GAS remains universally sensitive to β-lactam antibiotics, mechanisms conferring resistance to first-in-line adjunctive and penicillin-alternate treatment regimens (that is, macrolide and lincosamide antibiotics) frequently result in recurrent infection, treatment failure and poor patient outcome[145–147] (Fig. 4). Additionally, the emergence of subclinical β-lactam resistance in GAS remains an ongoing concern[11–13].

Macrolide and lincosamide resistance

Ribosomal target site modification in GAS (that is, methylation of a single adenine in 23S ribosomal RNA (rRNA)), mediated by erythromycin resistance methylase (Erm) proteins, confers resistance to macrolides, lincosamides and streptogramin B, subsequently giving rise to the MLS_B phenotype. The MLS_B phenotype is frequently attributed to the constitutive or inducible expression of *ermB*, *ermTR* (an *ermA* gene subclass) or *ermT* methylase encoding genes[148]. The *ermB* gene is widely carried on transposons Tn*6002* and Tn*6003*, both derived from insertion of *ermB* in Tn*916*-family mobile genetic elements[149]. The integrative and mobilizable element IME*Sp*2907 is a primary carrier of *erm*TR[150]. Further, the plasmid-borne *ermT* gene – initially discovered in GAS in 2008 (ref. [151]) – has become a significant source of macrolide and clindamycin resistance in GAS[152]. During invasive GAS disease, inducible *erm* expression has been associated with high rates of clindamycin-treatment failure[13,153,154]. The *mefA* (macrolide efflux pump A) gene in GAS, which is frequently associated with prophage phage φ1207.3 (formerly Tn*1207.3*), confers resistance to 14 and 15 carbon-ring macrolides (that is, erythromycin and azithromycin)[155].

Globally, rates of erythromycin and clindamycin resistance vary greatly. Between 2011 and 2019, the US Centers for Disease Control and Prevention (CDC) Active Bacterial Core surveillance programme reported an increase from 11.9% to 24.7% and from 8.9% to 23.8% of invasive GAS isolates that were non-susceptible to erythromycin and clindamycin, respectively[156], which was largely driven by the expansion of types *emm*77, *emm*58, *emm*11, *emm*83 and *emm*92 (ref. [157]). Notably, in the United States, both erythromycin and clindamycin resistance has been identified as most frequent among persons experiencing homelessness, incarceration, drug use and long-term admission to

care facilities[157]. In China, GAS surveillance spanning the past three decades suggests that the incidence of both clindamycin and erythromycin non-susceptible *ermB* expressing GAS has been high since the 1990s (>95% in select geographical regions), reducing the clinical utility of clindamycin[154]. The integrative and conjugative element ICE-*emm*12 has been identified as a primary driver of macrolide resistance

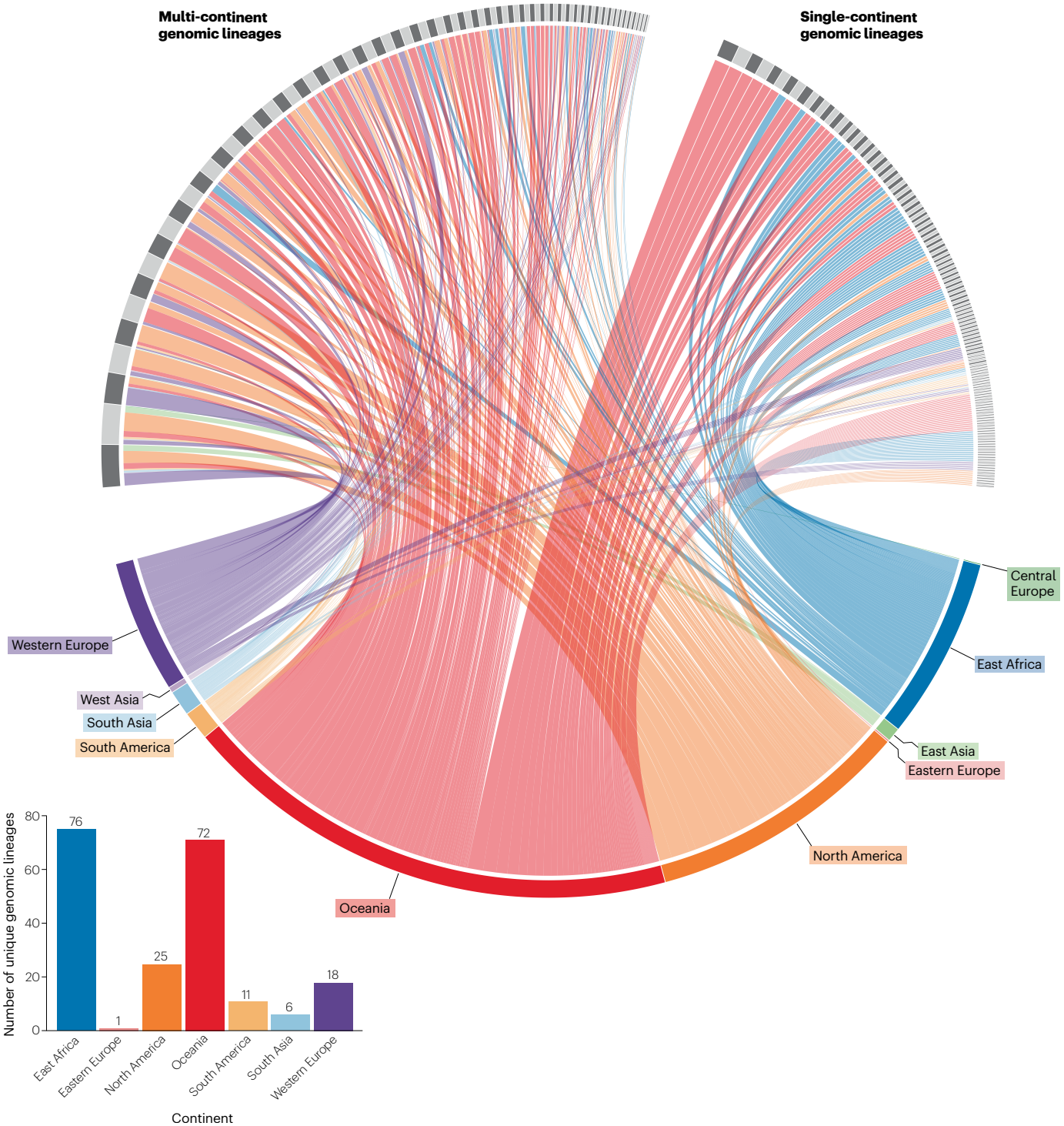


Fig. 3 | Global genetic diversity of GAS. CIRCOS plot of 299 global Group A *Streptococcus* (GAS) lineages based on 2,083 diverse GAS genome sequences[17] with connecting lines linking genomic lineages with the geographical region of isolation. Colours relate to the geographical region of isolation. Genomic lineages are represented in the upper hemisphere of the plot (alternating grey) and split into lineages that have been identified in multiple or single geographical regions. Bar plot represents the total number of 'unique' single-continent genomic lineages per geographical region (coloured).

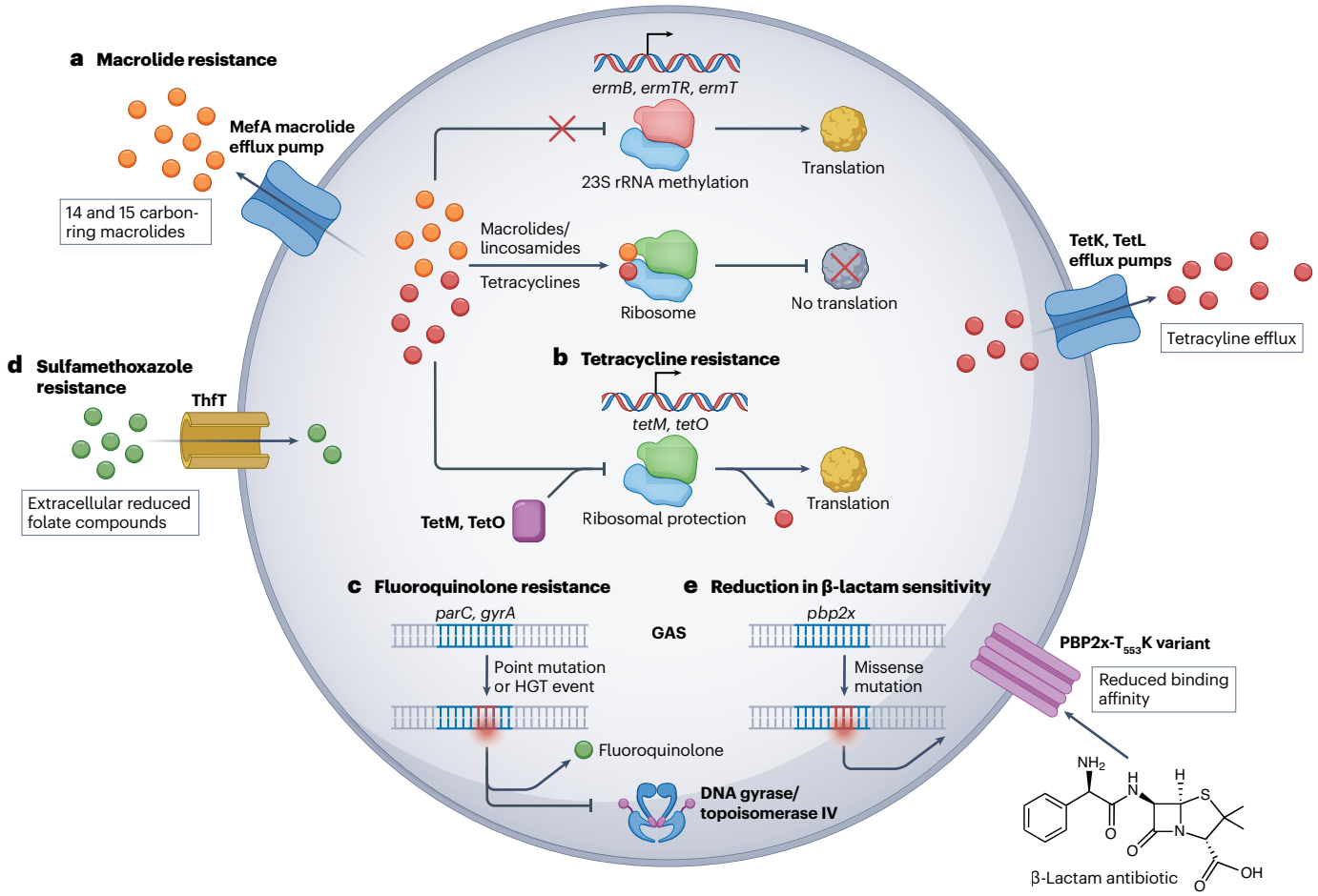


Fig. 4 | Mechanisms of GAS antibiotic resistance. a, Macrolide resistance: methylation of 23S ribosomal RNA (rRNA) by methylase encoding erythromycin resistance methylase (*erm*) genes mediates resistance to macrolides, lincosamides and streptogramin B, giving rise to the MLS_B resistance Group A *Streptococcus* (GAS) phenotype. Active macrolide efflux conferred by *mefA* (macrolide efflux protein A) gene expression drives resistance to 14 and 15 carbon-ring macrolides only. **b**, Tetracycline resistance: ribosomal protection proteins TetM and TetO displace tetracyclines from the 30S ribosomal binding site, whereas TetK and TetL expression mediates the active efflux of tetracycline from the GAS cytosol. **c**, Fluoroquinolone (FQ) resistance: mutations in *parC*, encoding topoisomerase IV, confer low-level FQ resistance in GAS, whereas additional stepwise mutations in *gyrA*, encoding DNA gyrase, lead to the high FQ resistance GAS phenotype. **d**, Sulfamethoxazole resistance: the horizontally acquired energy-coupling factor transporter S component (ThfT) sequesters extracellular folate intermediate compounds such as 5,6,7,8-tetrahydrofolate and 7,8-dihydrofolate which feed into the folate cycle, bypassing the inhibitory effects of sulfamethoxazole on folate synthesis. **e**, Reduction in β-lactam sensitivity: missense mutations in penicillin binding protein 2X (PBP2x) result in reduced GAS susceptibility to β-lactam antibiotics, amoxicillin and ampicillin below resistant minimum inhibitory concentration (MIC) breakpoints. HGT, horizontal gene transfer.

in *emm*12 scarlet fever outbreak isolates from this region[6]. A recent multicentre Northern European study identified that both erythromycin and clindamycin resistance ranges from 1% to 2% in patients presenting GAS necrotizing soft tissue infections[158]. Both global and national variances in erythromycin and clindamycin rates of resistance can often be attributed to the differences in geographic percentage of *mefA*-expressing relative to *erm*-expressing isolates which confer higher levels of clindamycin resistance[154]. The clonal and subclonal expansion of select resistant strains, as well as temporal variation of the *mefA*-encoded and *erm*-encoded phenotypes across and within *emm* types circulating in specific geographic regions, are all factors which drive the frequency of macrolide and lincosamide resistance in GAS[6,152,159,160].

Tetracycline resistance

In GAS, tetracycline resistance is conferred by the ribosomal protection genes *tetM* and *tetO*, and efflux pump system genes *tetK* or *tetL*[161]. Acquired via horizontal gene transfer, *tet* genes are typically presented on a wide range of mobile genetic elements, often co-located with *erm* and *mef* genes[6]. In a retrospective study carried out from 2000 to 2019 in Taiwan, 12.3%, 99.2% and 13.1% of macrolide-resistant GAS were found to harbour *tetO*, *tetM* and *tetK* genes, respectively[162]. Along with GAS clonal expansion, the usage of tetracycline class antibiotics has also been suggested to drive macrolide resistance, and vice versa[1]. As such, the acquisition of tetracycline resistance determinants warrants particular attention during ongoing and future epidemiological GAS surveillance studies.

Fluoroquinolone resistance

Although fluoroquinolones (FQs) are not regarded as a directed treatment for the management of GAS infection, low-level and high-level FQ resistance phenotypes in GAS occur with varying frequency[163]. Large-scale, up-to-date information on the global rates of GAS FQ resistance remains scarce. Two recent independent studies have identified that FQ non-susceptibility rates in Japan have ranged from 11.1% (between 2011 and 2013) to 14.3% (between 2012 and 2018), mainly attributed to the spread of *emm*6 and *emm*11 GAS[164,165]. Between 2011 and 2016, the frequency of GAS FQ non-susceptibility in Shanghai, China, was reported at 1.3%, with 80% of FQ non-susceptible isolates harbouring both *ermB* and *tetM* resistance determinants. In Shanghai, China, FQ non-susceptibility was attributed to the spread of *emm*1, *emm*6, *emm*11 and *emm*12 GAS[166]. Interestingly, topoisomerase IV ParC-S_{79}A mutations conferring low-level FQ resistance are frequently associated with the *emm6* GAS complex[13]. Exceptionally high rates of FQ consumption have been noted worldwide[167]. As a likely driver of FQ resistance in GAS, FQ antibiotic consumption combined with the emergence of FQ multidrug-resistant clones underscores the need for global improvements in FQ stewardship practices.

Sulfamethoxazole resistance

The combination of sulfamethoxazole and trimethoprim (forming co-trimoxazole) has been recently employed for the treatment of GAS skin infection in endemic settings[168]. Through targeting of the GAS folate cycle, co-trimoxazole inhibits both de novo folate synthesis and the folate cycle. GAS resistance to sulfamethoxazole and trimethoprim has been attributed to the mutation of the target enzymes FolP and Dyr, respectively, or the acquisition of trimethoprim-resistant variants of Dyr (DfrF and DrfG)[169,170]. Further, recent work has identified that the energy-coupling factor transporter S component gene (*thfT*) enables GAS to acquire extracellular reduced folate components directly from the host, bypassing the inhibition of folate biosynthesis by sulfamethoxazole[171]. ThtF requires host metabolites for activity; as such, standard minimum inhibitory concentration (MIC) testing is inadequate for the detection of ThtF-mediated sulfamethoxazole resistance. Although currently rare among global GAS isolates, it is now imperative to monitor the emergence and dissemination of *thfT*-positive GAS to guide appropriate patient treatment.

β-Lactam susceptibility

In streptococcal species, penicillin resistance is primarily mediated by mutations in penicillin-binding proteins (PBPs), the target site for β-lactam antibiotics. Although penicillin resistance above clinical thresholds in GAS is yet to be documented, a community GAS outbreak in Seattle (Washington, USA) led to the identification of two related clinical *emm*43.4 GAS isolates with eightfold-reduced susceptibility to both ampicillin and amoxicillin. Consistent with a first step in developing β-lactam resistance, missense mutations (T553K substitution) were identified in PBP2x (ref. [14]). In three subsequent independent studies, authors examined genome sequences of 7,025, 9,667 and 13,727 GAS isolates, respectively. In the first study, 137 out of 7,025 GAS strains were identified to contain non-synonymous mutations in 36 codons of *pbp2x* (ref. [11]). In the second study, 84 out of 9,667 strains carried PBP2x amino acid variations associated with tolerance to subclinical penicillin MICs[12]. In the third study, which examined invasive GAS isolates in the United States from 2015 to 2021, 388 PBP2x variants demonstrated elevated β-lactam MICs, with *emm*4/PBP2x-M593T/*ermT* being the predominate lineage; the previously described *emm*43.3/PBP2x-T553K variant was present in two isolates and demonstrated the highest subclinical ampicillin MIC[15]. In accordance with initial findings, only the latter study identified the presence of the T553K substitution in PBP2x in *emm*43.4 GAS, suggesting the occurrence of a recent antimicrobial selection event. Increased subclinical resistance to β-lactam antibiotics in *emm*43.3/PBP2x-T553K variants has been attributed to several non-PBP mutations present in this exceedingly rare phenotype[15].

Although mutations occasioning low-affinity PBPs were once thought to incur a fitness cost in GAS[172], the T553K substitution in PBP2x expressing GAS did not affect bacterial growth in vitro[14]. Further, isogenic mutant GAS isolates with PBP2x mutations (P601L) that confer reduced β-lactam susceptibility show no change in virulence in vivo but demonstrate enhanced growth in vitro[173]. These concerning reports underpin the vigilance required when monitoring β-lactam resistance phenotypes in GAS.

GAS vaccine development

The complexity of developing a safe and globally effective GAS vaccine is well recognized[18]. Despite more than a century of research, a GAS vaccine has not reached commercial use. GAS vaccine design and development must circumnavigate issues of extensive genetic diversity, potential autoimmune epitopes and the challenges of using animal models to assess protective efficacy against an exclusively human-adapted pathogen responsible for a diverse array of disease manifestations[18]. These scientific obstacles have been further compounded by historical regulatory and commercial barriers to GAS vaccine development. Undoubtedly the most significant of these barriers was a 25-year US Federal Drug Administration (FDA) ban on the administration of GAS and its products into humans, issued in response to fears surrounding the autoimmune potential of GAS antigens[174]. Although the ruling was revoked in 2005, only four vaccine candidates have since progressed to early-stage human trials (Table 2).

M-protein vaccine candidates

To date, all vaccine candidates in the clinical pipeline target the GAS M protein. M protein vaccines are specifically designed to exclude auto-epitopes and contain either a mixture of hypervariable N-terminal fragments from various clinically relevant M serotypes or conserved epitopes derived from the protein's C-repeat region. The most advanced multivalent N-terminal peptide-based candidate (StreptAnova) was well tolerated and immunogenic among participants in a 2019 phase I clinical trial[175]. StreptAnova was formulated based on the 30 M serotypes responsible for >90% of pharyngitis and invasive disease cases in North America and Europe[176], but vaccine antisera from rabbits cross-opsonize numerous structurally similar non-vaccine serotypes that dominate diverse geographic regions[176,177]. Although cross-opsonization of non-vaccine serotypes is predicted to increase coverage of the 30-valent vaccine among populations in both Mali (from 37% to 84%)[178] and South Africa (from 63% to >90%)[179], a recent analysis indicates coverage would still be insufficient among Northern Australian populations where RHD is endemic[180]. Targeting the highly conserved epitopes within the C-repeat region of M protein therefore has the significant advantage of conferring global protection regardless of current or future epidemiological trends. A phase I clinical trial of the MJ8VAX vaccine, containing the C-repeat region B cell epitope J8, demonstrated increased J8-specific antibody titres in vaccinated volunteers after a single intramuscular injection[181]. MJ8VAX has since been reformulated as MJ8CombiVax, with an additional modified epitope from SpyCEP that confers protection against hypervirulent *covR/S*

Table 2 | Clinical trials of GAS vaccine candidates (post 2004)

Vaccine	Year of publication	Phase	Clinical trial participants and location	Target antigen	Adjuvant	Study outcomes	Ref.
Hexavalent amino-terminal M protein polypeptide	2004	I	28 participants; United States	M protein	Alum	Post-vaccination antibody titres significantly increased for all vaccine antigens; 30% increase in post-vaccination serum bactericidal activity against vaccine M serotype strains	214
26-valent N-terminal M protein polypeptide+Spa N-terminal peptide (StreptAvax)	2005	I	30 participants; United States	M protein	Alum	Significant increase in post-vaccination antibody titres for 26/27 antigenic peptides and serum bactericidal activity against vaccine M serotype strains	215
	2006	II	90 participants; United States	M protein	Alum	Sero-responses induced for 23/27 vaccine antigens	216
Minimal B cell epitope J8 (MJ8VAX)	2018	I	12 participants; Australia	M protein	Alum	Increased vaccine antigen-specific antibodies post vaccination	181
30-valent N-terminal M protein polypeptide+Spa N-terminal peptide (StreptAnova)	2020	I	36 participants; Canada	M protein	Alum	Antibody titres against 25/31 vaccine antigens significantly increased post vaccination	175

GAS, Group A *Streptococcus*; Spa, streptococcal protective antigen.

mutants in a mouse model of GAS skin infection[182]. The StreptInCor and P*17 vaccines, both also based on the C-repeat region, stimulate protective responses in mouse GAS challenge models[183,184]. Extensive safety profiling of MJ8CombiVax and StreptInCor has been undertaken in rat and minipig models, respectively, in preparation for phase I trials. No evidence of vaccine-related autoimmunity or toxicity with either candidate was observed[185,186].

Non-M protein vaccine candidates

Numerous studies have identified non-M protein antigens that are protective against GAS challenge in animal models. Multicomponent formulations of selected antigens with high gene carriage and low sequence variation within the global GAS population can theoretically offer high vaccine coverage[17], and several experimental vaccines employing this strategy are efficacious in animal models. Leading candidates include the GlaxoSmithKline three-component vaccine (SLO, *S. pyogenes* adhesion and division protein (SpyAD) and SpyCEP)[187], Vaxcyte's VAX-A1 (ScpA, SLO and SpyAD conjugated to GAS cell wall carbohydrate containing only polyrhamnose (SpyAD-GAC^{PR})[188], Combo#5 (arginine deiminase (ADI), trigger factor (TF), SpyCEP, ScpA and SLO)[189,190], 5CP (Sortase A (SrtA), ScpA, SpyAD, SpyCEP and SLO)[191] and Spy7 (ScpA, SpyAD, oligopeptide-binding protein (OppA), pullulanase A (PulA), Spy1228, Spy1037 and Spy0843)[192], which when formulated with alum (or CpG oligodeoxynucleotides in the case of the 5CP vaccine) all stimulate protective immune responses in mouse models of GAS infection. Combo#5/alum vaccination also significantly reduces symptoms of pharyngitis and tonsillitis in non-human primates[19]. Another candidate, TeeVax, targets multiple T antigens of GAS pili using a multivalent approach analogous to the strategy employed for the StreptAnova vaccine. TeeVax/alum induces modest protection in an invasive GAS mouse model and antiserum from vaccinated rabbits reacts to all 21 T antigens included within the vaccine (representing >95% of all known *tee* serotypes) as well as three non-vaccine subtypes[193].

Outlook for GAS vaccine research and development

Recent years have seen revitalized efforts by key stakeholders to coordinate and guide GAS vaccine research. GAS vaccine research and development was declared a priority of the WHO 2018 global resolution on ARF and RHD[194], and is stated by the WHO as a key intervention against rising trends in invasive GAS infections and antibiotic overuse[16]. The WHO has now released a GAS Vaccine Development Technology Roadmap detailing preferred product characteristics and priority research activities to address scientific gaps, support clinical evaluation and guide policy decision-making[16]. GAS vaccine research and development has suffered from a lack of financial investment in the past, but a recent health-economic analysis estimates that a vaccine that meets the WHO preferred product characteristics would avert up to $1 billion in GAS-associated costs each year in the United States[195].

Advances in vaccine formulation and delivery are anticipated to improve GAS vaccination strategies. All GAS vaccine candidates tested in clinical trials to date have been formulated with alum and therefore favour T_H2 cell-type (antibody) responses, although recent preclinical studies with the experimental adjuvant CAF®01 and emulsions containing the saponin QS21 point to the importance of inducing both cellular (T_H1 cell) and antibody responses in GAS immunity[184,190]. Microarray patch vaccine delivery offers advantages of potential dose sparing with improved immunogenicity, longer shelf-life and ease of use compared with intramuscular vaccination. The J8-DT vaccine candidate was recently evaluated for efficacy using high-density microarray patch delivery, demonstrating T_H1 cell/T_H2 cell induction and superior protection over intramuscular vaccination against GAS skin infection in mice[196]. Although not perfect representations of human GAS disease, valuable animal models for studying GAS vaccine candidates have been established and standardized, including a humanized mouse model for assessing invasive GAS infection[197], a mouse skin infection model[198] and a non-human primate model of GAS pharyngitis[19]. Furthermore, a human GAS challenge model recently established by researchers in Australia is expected to reveal correlates of immune protection and accelerate clinical evaluation of current and future vaccines[20].

Conclusions and future perspectives

GAS outbreaks continue to emerge across the globe, causing significant disease incidence, requiring vigilant monitoring with ongoing efforts integrating both research and public health laboratories key to defining evolutionary trajectories of pathogenic GAS populations. Although

the epidemiology of GAS infection has shifted substantially in some developed countries over the last century in line with changing social-economic factors, coordinated efforts to build capacity and surveillance nodes in low-resource settings are essential to both defining GAS transmission chains and providing a framework to assess the impact of future preventative measures. Although a significant body of work describing the GAS virulence mechanisms exists, new host–pathogen interactions are being documented, such as the cleavage of the GSDMA pro-inflammatory mechanism by the GAS cysteine protease SpeB, triggering pyroptosis. The direct study of humans infected with GAS has provided new perspectives, such as the role of MAIT cells in patients with STSS. Further work using human patient material is clearly warranted and will provide valuable insights for the development of future therapeutics and prophylactics. The identification of first-step PBP2x mutations in GAS that have led to penicillin non-susceptibility in other streptococcal species is of considerable concern. The development of a safe and effective GAS vaccine to reduce the GAS disease burden is now clearly recognized as a priority by the WHO, vaccine developers and other key stakeholders. The commercialization, distribution and widespread uptake of such a vaccine would do much to reduce the GAS disease burden, the sum of which is a major cause of infectious disease deaths worldwide.

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