

EXPERT
REVIEWS

Differences among group A streptococcus epidemiological landscapes: consequences for M protein-based vaccines?

Expert Rev. Vaccines 8(12), 1705–1720 (2009)

Pierre R Smeesters,
David J McMillan[†],
Kadaba S Sriprakash
and Melina M
Georgousakis

[†]Author for correspondence
Bacterial Pathogenesis
Laboratory, Queensland Institute
of Medical Research, Brisbane,
Queensland 4029, Australia
Tel.: +61 073 845 3698
david.mcmillan@qimr.edu.au

Group A streptococcus (GAS) is a bacterial pathogen responsible for a wide array of disease pathologies in humans. GAS surface M protein plays multiple key roles in pathogenesis, and serves as a target for typing and vaccine development. In this review, we have compiled GAS epidemiological studies from several countries around the world to highlight the consequences on the theoretical efficacy of two different M protein-based vaccine strategies.

KEYWORDS: group A streptococcus • M protein • M protein vaccine • *Streptococcus pyogenes*

Group A streptococcus (GAS) colonizes and infects the upper respiratory tract and skin of humans, resulting in a broad spectrum of diseases. As elegantly described by Carapetis *et al.*, the burden of GAS-related diseases predominantly occurs in settings of poverty where systems for collection of accurate disease-burden data are usually absent [1]. The most recent estimates suggest that GAS is responsible for more than 500,000 deaths per year [1]. This places GAS among the major human pathogens, with attributable mortality by a single species of pathogen only exceeded by HIV, *Mycobacterium tuberculosis*, *Plasmodium falciparum* and *Streptococcus pneumoniae* [1]. The most common GAS disease following colonization of the respiratory tract is pharyngitis. More than 600 million cases are estimated to occur each year [1]. Similarly, it is estimated that more than 111 million children under the age of 15 years in developing countries have pyoderma of any etiology [1]. While pharyngeal and pyodermal GAS infections are noninvasive and usually self-limiting, they too require significant healthcare attention. The costs of antibiotic treatment and time away from school or work for children and parents are a formidable financial burden [2]. After disseminating from primary colonization sites to otherwise sterile compartments of the body, GAS can cause life-threatening invasive diseases such as necrotizing fasciitis and streptococcal toxic

shock syndrome (TSS). Invasive GAS infections kill at least 163,000 people each year [1]. The incidence of invasive diseases has appeared to have increased over the last 20 years [3–5]. Most importantly, GAS has the ability to cause the nonsuppurative sequelae: acute poststreptococcal glomerulonephritis, rheumatic fever (RF) and rheumatic heart disease (RHD) [6]. While most industrialized countries now report a low incidence of RF and RHD [7], these diseases are endemic in developing countries and indigenous groups within developed countries [8–10]. Globally, it is estimated that RHD is responsible for 233,000–294,000 deaths per year [1]. In addition to the greater disease burden in developing countries, the spectrum of GAS diseases also differs between developed and developing countries. For example, GAS skin infections are more prevalent in tropical developing countries, whereas infections of the throat are common in industrialized countries [11].

The mechanism behind the different disease phenotypes and disease severity in different locations around the world is still undefined. It is believed that many factors, including the genetic susceptibility of the host, the environmental factors and ease of access to healthcare, play a role in disease progression and severity. However, there is no doubt that differences in the repertoire of virulence factors play a critical role in the pathogenesis of individual GAS

strains. In particular, the genetic diversity of the highly expressed M protein is believed to play an important role in the observed epidemiological differences.

The M protein

Discovery

The M protein is believed to be the major virulence determinant of GAS [12]. Discovered by Rebecca Lancefield in the early part of the 20th Century, the protein is found on the surface of all GAS isolates. M protein or related M-like proteins can also be found in the closely related *Streptococcus dysgalactiae* subsp. *equisimilis* (group G streptococcus [GGS]), *Streptococcus equi* spp. [13] and *Streptococcus iniae* [14], the latter two being pathogens of farm or domestic animals and fish. Within the GAS genome, the *emm* gene is found as part of the *mga* regulon, which also includes a transcriptional regulator (*mga*), the *emm* gene, *emm*-like genes and *scpA* [15,16]. In GGS, however, the genetic architecture is somewhat different [17]. These findings are consistent with evidence [18] suggesting that the *emm* gene was once part of a large mobile genetic element that was acquired by progenitors of GAS and GGS prior to subspeciation, that is, the ancient parental *emm* gene evolved outside of GAS. Subsequent selection of distinct *emm*-types is probably driven by immune pressure targeting the N-terminus. Recombination between the *emm* and *emm*-like genes within a strain, as well as interstrain *emm* gene transfers [19,20], have all contributed to the diversity of modern *emm* genes we see today.

Typing

The M protein exists as a coiled-coil dimer [21] extending from the surface of the bacteria to beyond the peptidoglycan layer. The protein is divided into a number of specific regions that have unique sequence, conformational and functional attributes (FIGURE 1). The amino acid sequence of the N-terminal region of the mature M protein is hypervariable, and antibodies raised against this region of the M protein are serotype specific and provide protection against that strain only. This had been the basis of the typing scheme for GAS for decades [22,23]. More recently, serotyping has been replaced by nucleotide sequence-based typing methods that target the DNA encoding for the hypervariable regions. Using this molecular typing method, more than 200 GAS *emm*-types have been reported [24,25].

Why is the M protein so important?

The major function of the M protein is to prevent opsonization of GAS by phagocytes of the host. The M protein achieves this by inhibiting the deposition of active complement molecules on the bacterial surface. Intriguingly, the M protein has developed a number of independent strategies to achieve this goal. The redundancy in function and evolution of unique anticomplement strategies by the different M proteins highlights the importance of this activity for the survival of GAS [26]. A common feature of all these mechanisms is the recruitment of host proteins to the bacterial surface that either actively inhibit complement activation or passively prevent complement deposition on the bacterial surface.

For example, FHL-1 and C4BP, host proteins that regulate complement activation, bind to regions of the M protein [26]. These molecules may also compete with serotype-specific antibodies for binding to the hypervariable region. A second strategy utilized by the M protein is 'passive' evasion of complement and other immune molecules. This is achieved by binding of host plasma proteins to the bacterial surface, thereby preventing the pathogen from being recognized for complement deposition. Perhaps the best examples for this are fibrinogen and albumin, which bind to the B- and C repeats of the M protein, respectively [27–31]. In the absence of antibody, fibrinogen–M protein complexes can reduce complement deposition by the classical pathway, possibly through inhibition of the convertase [32,33]. Both fibrinogen and albumin also block antibody binding to the conserved repeat region (CRR) [34], preventing antibody-mediated opsonization. The latter observation highlights the importance of the serotype-specific regions in promoting antibody-mediated opsonization in natural infections. Recent findings have also demonstrated a role of anti-M protein antibodies in disease pathogenesis by promoting a pathologic inflammatory response as well as platelet aggregation [35,36].

An additional role for the M protein in GAS virulence is in host cell adherence and cellular invasion. The adhesion of GAS to epithelial cells is the first stage of bacterial colonization of both the throat and/or the skin, and is an essential step to GAS infection. Primarily, it is the interaction between the M protein and the keratinocyte cell marker CD46 and fibronectin that promote GAS adherence [37]. Although GAS is considered an extracellular pathogen, recent evidence confirms that it can invade and reside within host cells [37]. The role of the M protein in this process has been confirmed by numerous researchers and is also believed to be mediated through the binding of fibronectin [38–40]. Whether this ability to invade host cells is a strategy to evade host defense mechanisms or a prerequisite for invasive disease is still to be established. In addition to understanding GAS pathogenesis, determining the mechanism the M protein employs to promote cell adherence and invasion may also provide information for vaccine design based on anti-attachment/dissemination strategies.

Roles of M protein in disease

In addition to its role in preventing opsonophagocytosis, the M protein is thought to be directly involved in a number of streptococcal diseases. Chiefly, the M protein is thought to play an integral role in RF and RHD. RF and RHD are autoimmune diseases in which antibodies and immune cells raised against GAS are redirected to attack host proteins, cells and tissues. In the case of RHD, these immune molecules attack heart tissue [41]. These diseases occur several weeks after a streptococcal infection. The postinfectious nature of primary cases of RF/RHD makes antibiotic treatment of the disease ineffective. However, ongoing antibiotic prophylaxis is recommended as a measure to prevent the future re-occurrence of RF/RHD. Molecular mimicry between the M protein and several host proteins, including myosin, keratin and vimentin, has been postulated as a leading cause of RHD [42,43]. Similar to the M protein, these proteins

also have a coiled-coil structure [41,44]. The epitopes that contribute to RF/RHF have been mapped to the central region of the M protein [45]. Thus, anti-GAS vaccines should not include this part of the protein. In fact, there is some evidence from an early vaccine study that administration of a whole M protein vaccine contributed to the development of RF in volunteers [46]. The results from this study obviously had a major impact on later GAS vaccine design.

Historically, certain GAS types have been classed as 'rheumatogenic' due to their association with RF (e.g., *emm*-types 1, 3, 5, 6, 18, 19 or 24) [10,12]. These strains are predominantly found in the throat rather than the skin. Thus, RF/RHD is normally considered to follow throat infections. However, more recent epidemiological studies in geographic regions where RF/RHD and streptococcal infections are endemic have failed to find significant numbers of these rheumatogenic strains [47,48]. In fact, the data emerging from the epidemiological studies in tropical regions challenge the accepted link between 'rheumatogenic GAS', pharyngitis and RF/RHD. Epidemiological studies in these regions tend to highlight the diversity of circulating *emm*-types. Rheumatogenic *emm*-types appear to be absent or rare. In these locations, the incidence of GAS impetigo is high and possibly involved directly or indirectly in the development of RF in some communities [48–51]. Other studies describe the recovery rate of GGS rather than GAS in the throat in these settings [52]. At present, a link between GGS and RF/RHD is tenuous but has been suggested in a number of studies [52,53].

A preferential association between certain *emm*-types and the development of invasive diseases has also been described. As an example, *emm* 1, as well as *emm* 3, have been predominantly associated with invasive infections [54,55]. Moreover, a recent study demonstrated that the M1 protein itself is a superantigen [56] hence possibly explaining the particular predominance of *emm*-type 1 among TSS cases. Moreover, multiple horizontal gene transfer events might also have contributed to the emergence of successful GAS clones [57,58]. However, strains expressing major virulence genes are also commonly found in

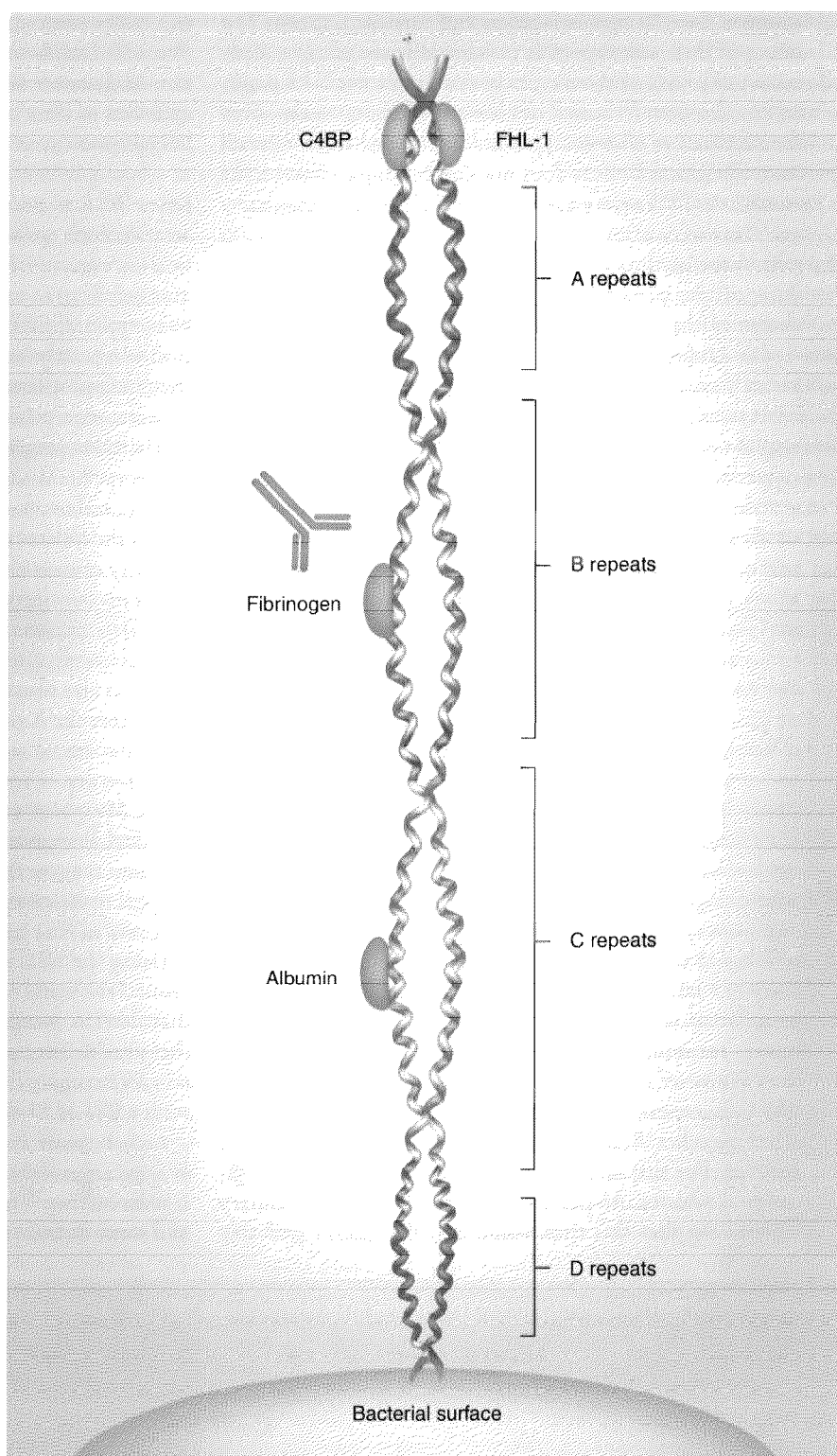


Figure 1. M proteins. Individual M proteins form coiled coils, which extend from the bacterial surface. Two coils wind around each other, forming a dimer that is stabilized by intermolecular interactions between amino acids in both strands. The hypervariable N-terminal region, lacking helical structure, is followed by the A, B, C and D repeat regions. Different regions of the protein also bind a number of different host molecules, assisting *Streptococcus pyogenes* to avoid opsonophagocytosis. FHL: Factor H-like protein.

GAS isolated from benign infections and carriage [54,59,60]. The prevalence of these *emm*-types in invasive diseases might simply reflect the prevalence of these types in the circulating GAS population [61], rather than increased virulence of these organisms. This diverse distribution of *emm*-types among strains from a broad spectrum of clinical sources does not enable simple causal links to be established between *emm*-types and specific pathologies.

M protein molecular epidemiology

The aim of the present section is to describe our current knowledge of the geographic distribution of circulating GAS *emm*-types. Unfortunately, epidemiological information is unavailable or incomplete for many countries. The parameters of specific epidemiological surveys (e.g., invasive infections only, pharyngitis only, carriage only or all clinical manifestations) can skew apparent *emm*-type distribution data in specific locations. Other criteria, such as the methods used to identify cases, the characteristics of the population studied (in terms of age, setting, size and inclusion criteria) and the length of the study, could also affect the apparent *emm*-type distribution in individual studies. Changes in epidemiology over time are not available for many countries. In order to overcome these problems and still allow strain comparison at a global level, we have developed and applied guidelines for the inclusion of epidemiological studies in our assessment. As highlighted in the previous section, the link between a defined *emm*-type and pathology is not universal. We therefore assume that the concept of the circulating *emm*-types, although clearly imperfect, is probably the best strategy for understanding the global picture. Hence, we have included all epidemiological studies to obtain a representation of the 'circulating *emm*-types', irrespective of their associated pathology. As the focus of this review is to emphasize the GAS M-protein epidemiology relative to vaccine development, we believe that the compilation of studies on a national level is mandatory as vaccination recommendations are most often also developed on this level. However, for reasons of clarity, we have combined data from the 11 European countries involved in the StrepEuro project [62] with three other Western European countries (Norway, Spain and Austria). For this review, we only considered epidemiological studies in which GAS isolates were recovered after 1 January 1988, assuming that this time window (1988–2007) probably

represents contemporary GAS M-protein epidemiology. Studies that relied solely on serological typing methods or on hybridization diagnostics without a complementary sequencing step were excluded as they probably underestimate the diversity of circulating *emm*-types. Some studies could not be included owing to missing information. We have used the Simpson's reciprocal index (SRI) to measure GAS strain diversity. The SRI takes into account both species richness and evenness. In this context, richness is a measure of the number of *emm*-types present in a defined area, while evenness represents the relative abundance of these *emm*-types. A SRI value of 1 corresponds to a theoretical situation where only one *emm*-type has been recovered, representing the minimal diversity possible. A value of 250 would correspond to a situation where all known *emm*-types would be recovered at a similar proportion, illustrating the maximal diversity of *emm*-types possible.

To examine whether the diversity of *emm*-types differ depending on the clinical manifestation being assessed, we compared the diversity of *emm*-types recovered from either pharyngitis, cutaneous or invasive infections in Fiji, a country where a high diversity of *emm*-type has been described [63]. TABLE 1 shows that, irrespective of the associated clinical manifestations, the SRI were similar [23–29]. This was also observed when comparing the distribution of *emm*-types from the American GAS pharyngitis and invasive infections surveillances [55,64], with both clinical pathologies displaying a low diversity of *emm*-types with a clear predominance of a few types. Moreover, seven of the ten most prevalent *emm*-types were identical, irrespective of clinical manifestations [55,64]. These comparisons suggest that the *emm*-type diversity observed within a clinical manifestation probably represents the overall *emm*-type diversity in that geographic location.

Using the SRI, we observed that the diversity in GAS strains around the world range from eight to 50 and presented a bimodal distribution (FIGURE 2). For the purpose of this review, we have therefore defined a low diversity of *emm*-types as corresponding to a SRI ranging from 8 to 13 and a high diversity of *emm*-types with a SRI of 27–50.

FIGURE 3 clearly shows that our knowledge of GAS epidemiology of some areas of the world where streptococcal diseases are endemic is sadly lacking. This is particularly true of South America, Africa and some locations in Asia (FIGURE 3). The predominance of GAS

Table 1. Comparison of *emm*-type diversity according to the associated pathology in Fiji.

Associated pathology	Isolates (n)	<i>emm</i> -types (n)	Simpson's reciprocal index (95% CI)	Cumulative frequency of the ten most prevalent <i>emm</i> -types (95% CI)
Pharyngitis	61	36	25.3 (19.6–35.4)	52% (39–66)
Impetigo	379	52	23.2 (20.3–27.1)	55% (42–68)
Invasive infections	55	37	27.7 (21.4–39.3)	49% (36–62)
Total	495	66	28.8 (25.5–33)	49% (44–53)

The Simpson's index (D) has been calculated with the formula: $D = \sum (n/N)^2$, where n is the total number of isolates from one *emm*-type and N is the total number of isolates of all the *emm*-types recovered in an area [119]. Simpson's reciprocal index (1/D) has been used in this review because it better illustrates our data than the classical Simpson's index of diversity (1-D). While the value of the Simpson's index of diversity only varies from 0 to 1, the value of the Simpson's reciprocal index varies in our case from 1 to 250. Confidence intervals (in brackets) have been calculated as previously described [120]. Based on [63] and [STEER A, PERS. COMM.].

research in Western countries clearly shows that the epidemiological information is not in proportion to the burden of disease, which mainly lies in developing nations. TABLE 2 shows that some *emm*-types were frequently recovered from nearly all the countries involved. *emm*-type 1 and 12, for example, were among the ten most prevalent *emm*-types in most countries.

When considering both the GAS M-protein diversity and the relatedness of circulating *emm*-types within the different countries, as seen in both TABLE 2 and FIGURE 3, it becomes possible to categorize them into three different groups. The first group includes Canada, the USA, Mexico, Western Europe, Korea and Japan. The *emm*-type diversity observed in these countries is relatively low (SRI 8–13) with only a few predominant *emm*-types. Moreover, the predominant *emm*-types are quite consistent between each of the countries, although some geographical differences can be noted. For example, *emm*-types 87, 83 or 81 are within the top ten most frequent *emm*-types recovered in Europe; however, they are less frequently (*emm* 87) or rarely (*emm* 83 or *emm* 81) recovered in North America.

The second group, which includes countries from Eastern Europe such as Serbia and Poland, but also interestingly China, also display a relatively low diversity of *emm*-types (SRI 10–12) but the predominant circulating *emm*-types are different to those found in the countries of group 1. Chinese data remain to be confirmed with larger epidemiological collections, but the diversity of circulating *emm*-types is apparently as low as in Europe. However, *emm*-types 60, 63, 58, 8 and 95 seem to be predominant in this geographical region, while they are not frequently recovered in the other regions.

The third group of countries includes Brazil, Ethiopia, Israel, India, Nepal, Australia and Fiji. The GAS strains recovered from these countries display a high diversity of *emm*-types (SRI 27–50). In comparison with groups 1 and 2, no real predominant *emm*-types can be observed, as shown by the low cumulative frequency of the ten most prevalent *emm*-types (34–49%). In addition, the *emm*-types circulating in these countries are quite different to the ones present in group 1 and 2 but also between each of the countries within group 3. For example, only a third of the *emm*-types recovered in Ethiopia are present in Fiji.

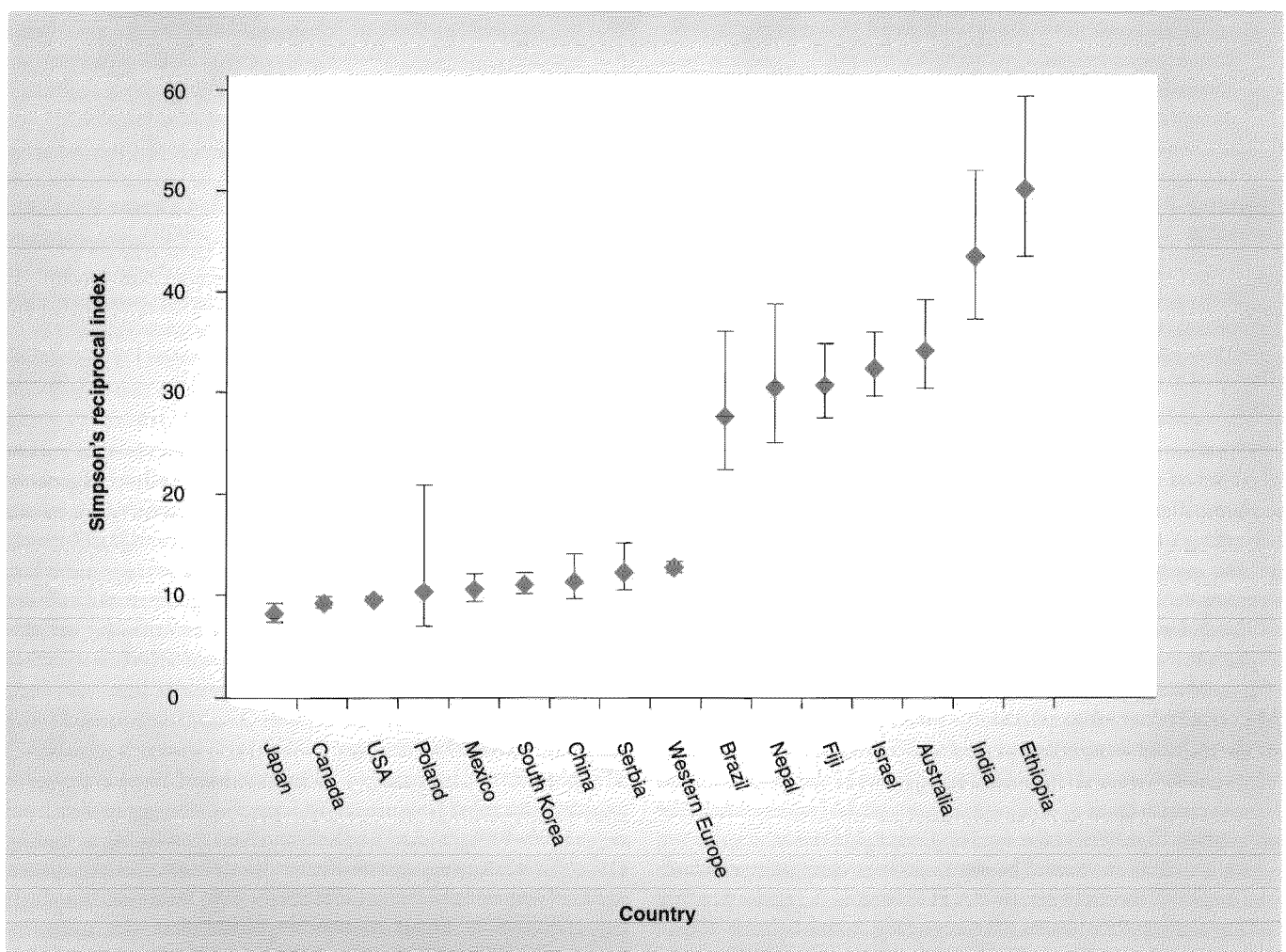


Figure 2. Bimodal distribution of Simpson's index among 16 countries. The value of the Simpson's reciprocal index (SRI) is shown per country, indicating the presence of two groups of countries with 'low' and 'high' SRI, respectively. Confidence intervals have been calculated as previously described [120].

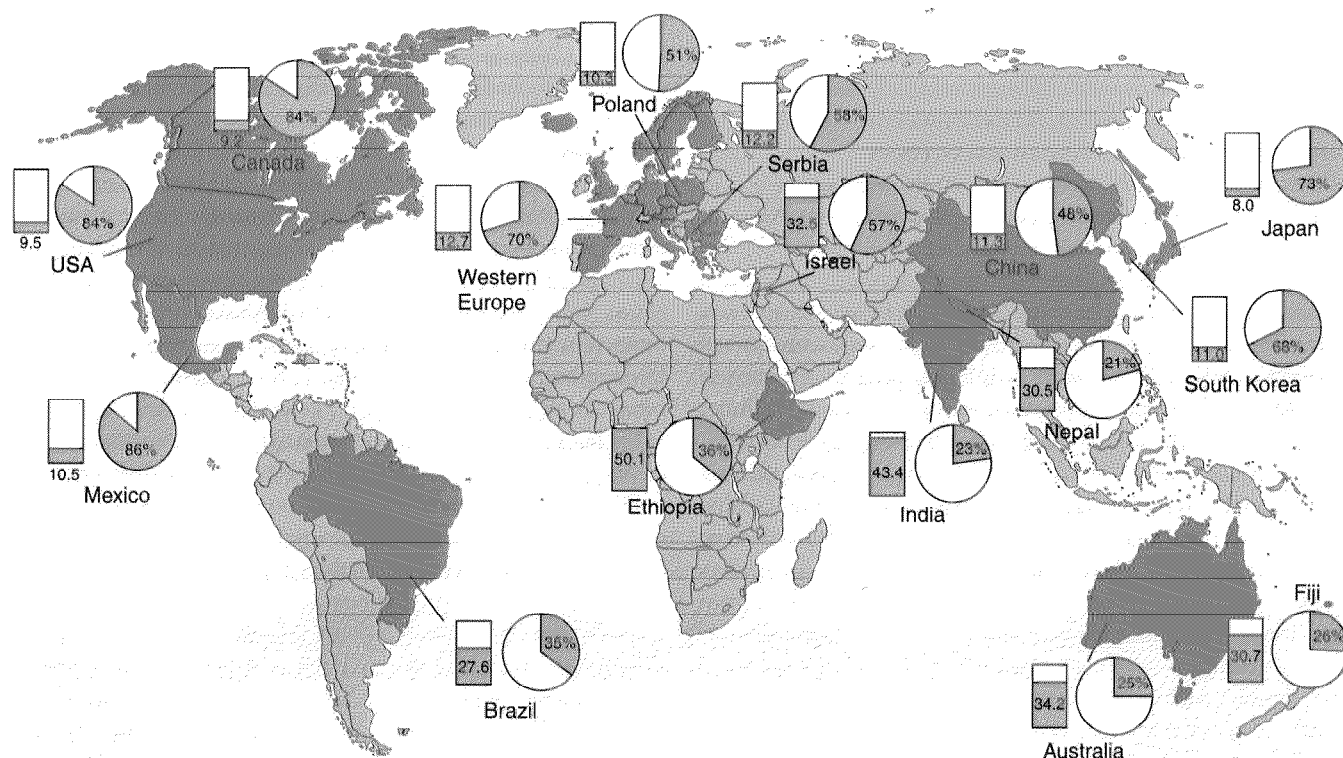


Figure 3. M-protein diversity and 26-valent theoretical coverage. The diversity of *emm*-types and the theoretical 26-valent vaccine coverage for 16 countries are both illustrated on this world map. The Simpson's reciprocal index of diversity for each country is represented by a square. The corresponding level of coverage theoretically provided by the 26-valent vaccine for the same country is represented by a pie chart. When calculating the theoretical 26-valent vaccine coverage, we assumed that protective cross-reactivity is achieved between an *emm*-type and its subtypes. Based on the GAS M-protein diversity and the relatedness of circulating *emm*-types, three different groups of countries were defined as further described in the text. Countries colored in orange, blue and purple belong to group 1, 2 and 3 countries, respectively. The Czech Republic and Romania, both members of the Strep-EURO project, could probably be included in group 2 instead of group 1. The level of 26-valent coverage in these countries are approximately 50 and 40%, respectively [120]. Australian data were only obtained in an indigenous population.

The selective pressures driving these differences in terms of the diversity of circulating *emm*-types remain to be found. Factors such as climate, primary care settings, antibiotic treatment, schooling, socioeconomic status, population immunity and host genetic susceptibilities might certainly influence the circulating *emm*-types. However, when looking at the data from all three of the defined groups, none of these factors seems sufficient to explain the observed differences.

M protein-based vaccines

As the major surface-expressed virulence factor and the determinant of naturally acquired immunity against GAS, the M protein has been the major focus of vaccine design [65,66]. Murine studies in the early 1900s highlighted the vaccine potential of the M protein [67], which was later confirmed by whole-protein vaccinations of both monkeys and then humans [68–70]. However, as the understanding of M protein-induced immunity progressed, it soon became evident that vaccine development would not be without hurdles. Primarily, the variability found at the N-termini of the M protein, responsible for type-specific immunity, would hinder the development of a vaccine targeting all circulating serotypes [24,71,72]. Second,

the presence of potentially human cross-reactive epitopes within the M protein prevented the development of the whole M-protein vaccine [73–75]. This initiated a large research effort focused on the identification of non-M-protein vaccine candidates [76,77]. However, it also resulted in the establishment of two main foci of M-protein vaccine design, each addressing safety and efficacy concerns. One strategy was directed solely towards the variable highly immunogenic N-terminus of the M protein, while the other focused on the conserved CRR of the M protein.

N-terminal vaccine design

The N-terminal approach to M protein-based vaccine design is arguably the most progressed GAS vaccine strategy to date, and is currently being assessed in human clinical trials [78,79]. Led by Dale, this strategy aims to create a multivalent vaccine incorporating the N-terminal subunits of multiple GAS serotypes in a single preparation. The initial concept was established with a tetravalent vaccine in which the N-terminal subunits from four different M serotypes were expressed recombinantly as a single-protein antigen [80]. Immunogenicity of each of the included subtypes was further optimized in a hexavalent construct in which the

M-type present at the C-terminus of the recombinant protein was duplicated at the N-terminus to increase its immunogenicity [81]. Both the tetra- and hexavalent vaccines successfully induced antibodies against each of the included M types, which resulted in opsonophagocytosis of the corresponding strains [80–82].

The N-terminal multivalent candidate that is currently in Phase II clinical trials is the 26-valent vaccine [83]. The 26-valent vaccine is comprised of four recombinant proteins, each containing the N-terminal domains of either six or seven different GAS serotypes [84]. A protective epitope from the GAS surface antigen, SPA, has also been included. This antigen also induces opsonic antibodies and was included in the vaccine to enhance cross-protection. Cross-reactivity within the subtypes of vaccine *emm*-type is usually the rule [85], although some exceptions have been noted [86,87]. Vaccine-induced cross-reactivity with nonvaccine *emm*-types may possibly occur [BATZLOFF M, PERS. COMM.]. The efficacy of the 26-valent vaccine has been tested in rabbits with the human-compatible adjuvant alum. Type-specific antibodies were induced to each of the included M-protein antigens. Combining data from two independent functionality assays, 24 out of the 26 serotypes tested were opsonized by the 26-valent specific sera, suggesting that the vaccine-specific response was functional [84]. Phase I clinical trials assessing the safety and immunogenicity of the 26-valent vaccine in 30 healthy volunteers produced promising results [79]. Each of the incorporated N-terminal peptides was immunogenic and, in contrast to the studies in rabbits, GAS strains representing all of the included M types were opsonized by the 26-valent-specific human sera in *in vitro* assays. In addition, no human tissue-reactive antibodies were detected. These results supported its progression into Phase II assessment.

The rationale behind the choice of *emm*-types included in the 26-valent vaccine was the reduction of disease and economic burden associated with invasive disease and uncomplicated pharyngitis in Northern America and Western Europe [84]. In addition, serotypes historically associated with RF, such as M19 and M24, were also included as a precaution for the emergence of such strains under the selective pressure of a multivalent vaccine [88]. At the time of design, the 26-valent vaccine was predicted to provide protection against 85–90% of M types associated with pharyngitis cases and invasive diseases in the USA and Europe [89].

When assessed on a global scale, however, the level of M-type coverage provided by the current 26-valent vaccine is much lower than predicted in the USA. As described in the previous section and illustrated in FIGURE 1, there is a difference observed in both GAS strain diversity and prevalence in different countries. These differences would most probably impact vaccine efficacy. The level of coverage offered by the 26-valent vaccine is expected to be highest in group 1 countries with a low SRI; 68–86% of M types may be covered. The lower level of coverage predicted for Western Europe, Korea and Japan (~70%) is attributed to geographical differences in the circulating *emm*-types. As an example, although *emm*-types included in the multivalent vaccine, such as 1, 3 and 12, are highly prevalent in Western Europe, other *emm*-types, such as 87, 83 and 81, which are not included in the vaccine, are also prevalent.

Contrasting with group 1, the group 2 and 3 countries are not predicted to be significantly covered by the 26-valent vaccine. Group 2 countries, including Eastern Europe and China, mimic the limited strain diversity seen in group 1. However, the expected lack of vaccine coverage in these countries is attributed to the presence of different circulating *emm*-types. Hence, while the current 26-valent vaccine may not be suitable for group 2 countries, their relatively low *emm*-type diversity may support the development of their own group or country-specific multivalent N-terminal vaccine.

Group 3 countries clearly display a higher diversity of circulating *emm*-types with very little evidence of predominant strains [19,63,90,91]. Consequently, the coverage of a multivalent vaccine is predicted to be too low (between 21 and 57%) to have an efficient impact on the disease burden in these regions. There is also very little crossover between the prevalent *emm*-types in group 3 countries and that of the other groups. Furthermore, there is little consistency between the countries within group 3. One exception, however, is Israel, which, although having a high level of strain diversity (Simpson's index [SI] 32), is predicted to have a higher level of coverage by the 26-valent vaccine (57%) compared with the other group 3 countries (21–36%). However, the overall diversity of *emm*-types in Israel is still high compared with that of groups 2 and 1. It seems that an N-terminal-based multivalent vaccine approach would probably not be an effective option.

In addition to high strain diversity observed in the group 3 countries, an association between certain *emm*-types and particular disease propensities is unclear. As previously discussed, no obvious rheumatogenic or invasive types that may be specific targets for a multivalent vaccine are apparent [92]. This has been further complicated by observations of high pharyngeal carriage of human GGS compared with GAS in communities where RF and RHD are endemic [52]. Although GGS is generally thought of as a commensal organism, it has been suggested that these streptococci contribute significantly to disease burden, and possibly even to RF/RHD [93–95]. GGS also expresses the M protein; however, their N-terminal sequences are different again to that displayed by GAS, hence would not be covered by current N-terminal vaccines [96]. This evidence clearly suggests that an N-terminal M protein approach to GAS vaccine design would require tailoring the vaccine for different geographical locations. While this may be a feasible strategy for group 1 and 2 countries, it would be quite difficult for the group 3 countries; however, the exact cost:benefit ratio for tailoring the vaccine to the specific groups would need to be determined by independent economic analysis.

An additional concern of the multivalent approach to GAS vaccination is the threat of serotype replacement. If the 26-valent vaccine was to be released in the communities it was designed for, it could provide an immunological environment in the host that allows for the emergence of new virulent *emm*-types or an increased prevalence of serotypes that were not included in the multivalent vaccine. This situation is analogous to the release of the pneumococcal seven-valent vaccine. Experiences with this vaccine may inform us as to what we may expect upon release of a multivalent GAS vaccine [97–99]. The multivalent pneumococcal vaccine combines seven serotype antigens that cover 70–88% of invasive pneumococcal

Table 2. Group A streptococcus M-protein epidemiology.

Country	Associated pathology	Isolates (n)	Year of isolation	<i>emm</i> -types (n)	Simpson's reciprocal index (95% CI)	Cumulative frequency of the ten most prevalent <i>emm</i> -types (95% CI)
Canada	Pharyngitis	1434	2000–2007	33	9.2 (8.7–9.8)	88% (86–90)
USA	Pharyngitis	7040	2000–2007	56	9.5 (9.3–9.8)	89% (88–89)
Mexico	Pharyngitis	423	1991–2000	31	10.5 (9.3–12.1)	82% (79–86)
	Invasive Others*					
Brazil	Pharyngitis	128	2004	48	27.6 (22.3–36.1)	49% (41–58)
	Impetigo					
	Invasive					
	Sequelae					
Ethiopia	Pharyngitis	299	1990–2005	90	50.1 (43.4–59.3)	34% (28–39)
	Impetigo					
	Invasive					
	Sequelae					
	Throat carrier					
Western Europe	Invasive	4820	1988–2006	107	12.7 (12.2–13.3)	73% (71–74)
	Pharyngitis					
	Impetigo					
	Others*					
Serbia	Pharyngitis	145	2001–2007	31	12.2 (10.3–15.1)	79% (73–86)
	Impetigo					
	Invasive					
	Others*					
Poland	Invasive	41	1997–2005	23	10.3 (6.8–20.7)	68% (54–82)
Israel	Pharyngitis	819	1996–2005	71	32.5 (29.6–36)	43% (40–47)
	Invasive					
	Others*					
India	Pharyngitis	313	2000–2007	88	43.4 (37.2–52.1)	38% (33–43)
	Impetigo					
	Sequelae					
	Throat carrier					
Nepal	Impetigo	120	1998–1999	45	30.5 (25.1–38.8)	46% (37–55)
	Throat carrier					
China	Pharyngitis	261	1995–2005	46	11.3 (9.5–14)	72% (67–78)
	Impetigo					
	Invasive infections					
	Sequelae					
	Throat carrier					
South Korea	Pharyngitis	676	2001–2006	31	11.0 (10.1–12.2)	82% (79–85)
	Throat carrier					
Japan	Pharyngitis	712	1996–2006	36	8.0 (7.2–9)	85% (82–87)
	Invasive infections					
	Others*					
Fiji	Pharyngitis	535	2005–2007	67	30.7 (27.4–34.9)	46% (42–51)
	Throat carrier					
	Impetigo					
	Invasive infections					
Australia	Pharyngitis	547	1990–2006	69	34.2 (30.3–39.2)	42% (38–46)
	Impetigo					
	Throat carrier					

Others includes (depending on the studies): ears, vagina, cervix, vulva, catheter, eye and urine.

*Underlined *emm*-types are included in the 26-valent vaccine candidate.

Table 2. Group A streptococcus M-protein epidemiology (cont.).

Ten most prevalent <i>emm</i> -types*										26-valent theoretical coverage (95% CI)	Ref.
1	2	3	4	5	6	7	8	9	10		
12	1	28	4	3	2	77	89	6	75	84% (83–86)	[64]
1	12	28	4	3	2	6	75	89	77	84% (83–85)	[64]
1	12	75	2	3	6	4	22	77	9	86% (83–89)	[121]
22	53	49	58	83	8	44	59	12	33	35% (27–43)	[11]
12	1	3	74	st62	18	25	st463	5	28	36% (31–42)	[51,122]
1	28	3	89	12	87	4	83	81	77	70% (69–71)	[123–127]
6	12	1	58	3	4	50	28	53	82	58% (50–66)	[128]
1	12	81	44	85	4	5	8	11	28	51% (36–66)	[129]
1	81	14	89	5	28	4	12	18	75	57% (54–61)	[130]
4	1	49	74	81	11	71	77	42	75	23% (19–28)	[90,131] [BRAHMARI P, PERS. COMM.]
1	4	44	71	79	82	11	73	77	100	21% (14–28)	[132]
12	1	60	63	4	58	8	95	18	28	48% (42–54)	[133–135]
44	12	22	75	1	6	78	3	5	2	68% (64–71)	[136,137] [KIM S, PERS. COMM.]
1	12	4	28	89	3	11	6	49	58	73% (70–76)	[138,139]
70	25	33	11	93	76	69	56	73	89	26% (23–30)	[63]
55	100	11	22	44	71	103	78	91	STns1033	25% (22–29)	[50,140,141]

*Others' includes (depending on the studies): ears, vagina, cervix, vulva, catheter, eye and urine.

*Underlined *emm*-types are included in the 26-valent vaccine candidate.

disease in Europe, the USA and Canada. The release of this vaccine in the USA resulted in a reduction in the seven vaccine serotypes and associated invasive disease [100]. However, a large increase in the number of nonvaccine serotypes has since occurred, resulting in a 45% increase in nonvaccine serotype-related invasive diseases. This increase was reported to be due to the expansion of already established pneumococcal clones rather than the emergence of new clones [101]. Thus, the lack of association of some bacterial serotypes with disease in a population may not be a function of the pathogenicity of a particular strain; it is probably a function of the competition between strains for an appropriate niche in the host. Clearly, serotype replacement is a potential issue for any multivalent vaccine. If GAS strain replacement did occur postvaccination, effective coverage would require constant strain surveillance and the re-evaluation and construction of the multivalent vaccine so that the appropriate strains are incorporated. However, the cost of such constant vaccine scrutiny may outweigh the economic benefit of vaccine release in developed countries, while rendering vaccination completely unaffordable in developing countries.

Nevertheless, the pneumococcal vaccine has had a positive impact on disease reduction [102]. Although many considerations are still warranted with regards to the cost-benefit of the 26-valent vaccine, it is evident from the level of coverage provided by the 26-valent vaccine in group 1 countries that it would have a significant positive effect on the reduction of GAS disease burden and associated economic costs.

C-terminal vaccine design

In an effort to develop a vaccine that has the potential to provide protection against a majority of, if not all, circulating GAS strains, the highly conserved CRR of the M protein has been targeted by several groups [103–106]. Unlike the N-terminal and sub-N-terminal domains of the M protein, which presents high levels of variation between different and also the same *emm*-types, the CRR is present in all M proteins and its sequence is highly conserved. Although not responsible for naturally acquired serotype-specific immunity against GAS, antibodies specific for CRR have been found in the blood and also mucosal secretions of people from communities endemic for GAS disease [107,108]. These titers increase with age, which has been attributed to repeated GAS infections [109]. Owing to the numerous roles the CRR is believed to have in the pathogenesis, such as resistance to phagocytosis and also cellular adherence, it is thought that antibodies to this region would in turn reduce bacterial survival and protect against disease.

Early vaccine studies used peptides spanning the entire CRR of the M protein, encompassing all three repeat domains [105,110]. When delivered intranasally in a murine model, these peptides stimulated both systemic and mucosal humoral responses, which could reduce colonization and also protect against disease. Similar results were also observed when the CRR from M6 was expressed on the surface of vaccinia virus and delivered to mice intranasally [65]. Further investigation of the CRR suggested that there may be safety risks associated with using the entire domain due to the presence of cross-reactive T-cell epitopes [111]. Subsequently, Hayman *et al.* identified a 20-mer

peptide (p145) spanning a region of the CRR that was capable of inducing opsonic antibodies, yet this epitope still reacted with T cells from RF patients. Further mapping of this peptide identified the specific B-cell epitope responsible for the protective immunity, which was termed J8-i, while omitting the potentially cross-reactive epitopes [112]. A longer homolog that encompasses J8-i was also identified termed J14-i. Initial studies with these peptides demonstrated that the protective B-cell epitope they contained was conformationally restricted; hence the peptides needed to be presented in their native α -helical conformation to induce a functional opsonic immune response. A novel strategy employed by Relf *et al.* was to position J8-i and J14-i within flanking regions of an α -helical peptide derived from yeast, CGN4, to produce the chimeric peptides J8 and J14, respectively [113]. Both chimeric peptide vaccine candidates have been predicted to be safe *in vitro* and, when coupled with diphtheria toxoid (DT), are immunogenic in mouse models, providing protection against systemic challenge with multiple GAS serotypes [114]. The mechanism of immunity induced by these peptides is eluded to be B-cell mediated, inducing opsonic antibody against GAS. In a mucosal GAS model [115], J14-DT provides protection against both colonization and systemic disease, suggesting a dual method of protection [116].

The conjugate vaccine J8-DT delivered with human-approved adjuvant alum is currently in preclinical assessment and expected to reach Phase I clinical trials in the near future [BATZLOFF M. PERS. COMM.]. Molecular epidemiological data acquired from two group 3 countries that are endemic for GAS (Fiji and India) are supportive of the potential of this CRR-derived vaccine candidate to provide protection against the majority of circulating GAS strains. In data collected from Fiji, 96% of strains contain either J14 or the closely related cross-reactive variant J14.1 in the C3 repeat. The remaining strains either contained new J14 types previously not encountered or a variant J14 type usually found in the downstream C repeats. How the presence of these new J14 types in the C3 repeat affects vaccine efficacy is yet to be determined through cross-reactivity studies with J14-specific antibodies. Similar data were also recorded in Brazil and Brussels, where 94% of 51 strains tested presented either J14 or J14.1 in the C3 repeat [19]. In addition, all of the M proteins sequenced contained at least two C repeats. Thus, this vaccine offers the possibility of providing equal levels of protection against the high diversity of circulating *emm*-types found in group 1, 2 and 3 countries.

While in theory J14 has great potential in the protection against GAS and associated diseases, there are two questions that are still to be addressed with regards to vaccine efficacy. First, what is the risk associated with relying on a single protective epitope in a protein that already displays some level of sequence divergence? The binding of the CRR to many host proteins such as factor H, IgG and albumin is thought to be dependent on the CRR, assuming its native α -helical conformation [117]. It has been hypothesized that due to these functionality constraints, the CRR may be resistant to amino acid changes normally acquired due to immune selective pressure. However, there are more than 59 J14 variants in the C-repeat regions; increased immune selection pressure may provide the impetus for faster evolution of the repeat

sequences. The recent identification of new J14 types present in the C3 repeats [63] suggests that this could be a possibility that may need to be considered and addressed. Hence, it is quite possible that the release of the conserved epitope-based peptide vaccine targeting the CRR may also require a form of bacterial surveillance to monitor evolution of the epitope following vaccine-induced immune pressure.

Second, what would be the consequences of complete eradication of GAS globally? If successful, a highly conserved vaccine could ultimately result in the eradication of GAS from the bacterial flora of the upper respiratory tract. If this does occur, what are the downstream effects of removing a well-described member of the pharyngeal flora on the remaining inhabitants? Will this new environmental niche provide an opportunity for colonization by new commensal or other pathogenic bacterial species? Or will GAS eradication result in the redistribution of existing bacteria in the upper respiratory tract? As highly conserved GAS vaccines are transitioning into clinical trials, the wider ramifications of the eradication of streptococci need to be contemplated.

Expert commentary

While the impact of morbidity and mortality due to GAS extends throughout the world, the severity is particularly felt in the populations of developing countries where the rates of RF and RHD are high. The stark contrast in the epidemiology of streptococcal research between the three cross-continental groups is probably due to complex interplay between the circulating M types, population density, infection rates, seasonal differences and horizontal cross-species genetic exchanges.

The contrasting features of epidemiology offer challenges for vaccine design. The immune response of the M molecule is known to be protective and some epitopes elicit cross-reactive antibodies responsible for autoimmune diseases. This has necessitated separation of these epitopes in the design of a safe vaccine candidate. In this article, two distinct approaches of M-based vaccine designs

are reviewed. While the multivalent N-terminal epitope vaccine has the advantages of a 'designer' vaccine by avoiding unnecessary immune responses, vaccine-induced GAS strain replacement may be a major drawback. On the other hand, there is already population-based evidence for the minimal effect of immune pressures due to herd immunity resulting from the vaccine based on the conserved epitopes. For instance, in GAS-endemic populations, the age-related acquisition of immunity to GAS infection is concordant with increased antibody titers against the conserved region. However, the quality of antibody response to the conserved region is different in the natural population compared with vaccinated individuals; the titers in natural population are low and acquired slowly in adults, while the vaccine is expected to elicit higher titers of antibody responses rapidly in the younger population. How does this affect pressures of herd immunity on strain replacement?

Five-year view

There is more optimism now than ever for a safe and effective vaccine against GAS. It is likely that the conserved epitope-based vaccine would cover both GAS and closely related commensal bacteria, such as GGS. However, the N-terminal vaccine has the distinct advantage of being a designer vaccine and in eliciting very high immune responses. If indeed both these candidates graduate to higher levels of clinical trials, it will be an obvious step to link both of these in one vaccine. In fact, a previous study attempted to achieve this with a limited number of N-terminal epitopes and showed protection in mouse model [118].

Acknowledgements

The authors sincerely acknowledge PV Brahmachari, Tdayoshi Ikebe, Sunjoo Kim, Vera Mijac, Stanford Shulman, Andrew Steer, Kimiko Ubukata and Haruo Watanabe for sharing details about their M-protein epidemiology studies. The authors also thank Michèle Dramaix for her valuable statistical support and Mimi Kersting for her excellent art design work.

Key issues

- Recent estimates place group A streptococcus (GAS) diseases in the top ten infectious disease burden; most cases occurring in settings of poverty.
- Sparse baseline epidemiological studies in countries where streptococcal disease is considered endemic may hinder advances in vaccine research and eventual uptake.
- Contrasting epidemiological features in tropical regions challenges the accepted association between pharyngitis resulting from 'rheumatogenic GAS' and rheumatic fever/rheumatic heart disease. In fact, the link between a defined *emm*-type and one disease pathology is not universal.
- Antigenic diversity of the major surface virulence protein, M protein, and its geographical distribution suggests that three groups of diversity exist.
- The selective pressures driving these differences in terms of the diversity of circulating *emm*-types remain to be found. Herd and protective immunities are primarily type-specific.
- The current 26-valent vaccine may be currently efficacious against GAS diseases in group 1 (i.e., Canada, the USA, Mexico, Western Europe, Korea and Japan) countries, but is not likely to be so in group 2 (i.e., countries from Eastern Europe such as Serbia and Poland, and China) and group 3 countries (i.e., Brazil, Ethiopia, Israel, India, Nepal, Australia and Fiji).
- The vaccine based on the conserved region could be effective against virtually all M types.
- We do not know the effect vaccine-generated immune pressure will have on the selection of novel variants. To determine this, we need baseline information on circulating GAS *emm*-types before and after the introduction of the vaccine.
- Will decreased GAS colonization rates significantly change nasopharyngeal flora?

Financial & competing interests disclosure

Pierre R Smeesters is a 'chargé de recherche' FNRS and has been supported by an ESPID Fellowship Award. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The authors have no other relevant affiliations or financial

involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

Papers of special note have been highlighted as:

• of interest

•• of considerable interest

- 1 Carapetis JR, Steer AC, Mulholland EK, Weber M. The global burden of group A streptococcal diseases. *Lancet Infect. Dis.* 5(11), 685–694 (2005).
- Excellent extensive review.
- 2 Pfoh E, Wessels MR, Goldmann D, Lee GM. Burden and economic cost of group A streptococcal pharyngitis. *Pediatrics* 121(2), 229–234 (2008).
- 3 Lamagni TL, Efstratiou A, Dennis J, Nair P, Kearney J, George R. Increase in invasive group A streptococcal infections in England, Wales and Northern Ireland, 2008–9. *Euro Surveill.* 14(5), pii, 19110 (2009).
- 4 Efstratiou A. Group A streptococci in the 1990s. *J. Antimicrob. Chemother.* 45(Suppl.), 3–12 (2000).
- 5 Aziz RK, Kotb M. Rise and persistence of global M1T1 clone of *Streptococcus pyogenes*. *Emerg. Infect. Dis.* 14(10), 1511–1517 (2008).
- 6 Cunningham MW. Pathogenesis of group A streptococcal infections. *Clin. Microbiol. Rev.* 13(3), 470–511 (2000).
- 7 Shulman ST, Stollerman G, Beall B, Dale JB, Tanz RR. Temporal changes in streptococcal M protein types and the near-disappearance of acute rheumatic fever in the United States. *Clin. Infect. Dis.* 42(4), 441–447 (2006).
- 8 Carapetis JR. Rheumatic heart disease in developing countries. *N. Engl. J. Med.* 357(5), 439–441 (2007).
- 9 Singh PI, Carapetis JR, Buadromo EM, Samberkar PN, Steer AC. The high burden of rheumatic heart disease found on autopsy in Fiji. *Cardiol. Young* 18(1), 62–69 (2008).
- 10 Stollerman GH. Rheumatic fever in the 21st Century. *Clin. Infect. Dis.* 33(6), 806–814 (2001).
- 11 Smeesters PR, Vergison A, Campos D, de Aguiar E, Deyi VY, Van Melder L. Differences between Belgian and Brazilian group A streptococcus epidemiologic landscape. *PLoS ONE* 1, e10 (2006).
- 12 Bisno AL, Brito MO, Collins CM. Molecular basis of group A streptococcal virulence. *Lancet Infect. Dis.* 3(4), 191–200 (2003).
- Comprehensive review of group A streptococcus (GAS) virulence factors.
- 13 Timoney JF, Artiushin SC, Boschwitz JS. Comparison of the sequences and functions of *Streptococcus equi* M-like proteins SeM and SzPSe. *Infect. Immun.* 65(9), 3600–3605 (1997).
- 14 Locke JB, Aziz RK, Vicknair MR, Nizet V, Buchanan JT. *Streptococcus iniae* M-like protein contributes to virulence in fish and is a target for live attenuated vaccine development. *PLoS ONE* 3(7), e2824 (2008).
- 15 Podbielski A. Three different types of organization of the vir regulon in group A streptococci. *Mol. Gen. Genet.* 237(1–2), 287–300 (1993).
- 16 Gardiner D, Hartas J, Currie B, Mathews JD, Kemp DJ, Sriprakash KS. Vir typing: a long-PCR typing method for group A streptococci. *PCR Methods Appl.* 4(5), 288–293 (1995).
- 17 Sriprakash KS, Hartas J. Lateral genetic transfers between group A and G streptococci for M-like genes are ongoing. *Microb. Pathog.* 20(5), 275–285 (1996).
- 18 Panchaud A, Guy L, Collyn F *et al.* M-protein and other intrinsic virulence factors of *Streptococcus pyogenes* are encoded on an ancient pathogenicity island. *BMC Genomics* 10(1), 198 (2009).
- 19 Smeesters PR, Mardulyn P, Vergison A, Leplae R, Van Melder L. Genetic diversity of group A streptococcus M protein: implications for typing and vaccine development. *Vaccine* 26(46), 5835–5842 (2008).
- 20 Whatmore AM, Kapur V, Musser JM, Sullivan DJ, Kehoe MA. Variation in *emm*-like gene sequences in the context of the population genetic structure of group A streptococci. *Dev. Biol. Stand.* 85, 159–162 (1995).
- 21 Phillips GN Jr, Flicker PF, Cohen C, Manjula BN, Fischetti VA. Streptococcal M protein: α -helical coiled-coil structure and arrangement on the cell surface. *Proc. Natl Acad. Sci. USA* 78(8), 4689–4693 (1981).
- 22 Lancefield RC. The antigenic complex of *Streptococcus hemolyticus*. I. Demonstration of a type-specific substance in extracts of *Streptococcus hemolyticus*. *J. Exp. Med.* 47, 9–10 (1928).
- 23 Lancefield RC. Current knowledge of type-specific M antigens of group A streptococci. *J. Immunol.* 89, 307–313 (1962).
- 24 Beall B, Facklam R, Thompson T. Sequencing *emm*-specific PCR products for routine and accurate typing of group A streptococci. *J. Clin. Microbiol.* 34(4), 953–958 (1996).
- 25 Facklam RF, Martin DR, Lovgren M *et al.* Extension of the Lancefield classification for group A streptococci by addition of 22 new M protein gene sequence types from clinical isolates: *emm103* to *emm124*. *Clin. Infect. Dis.* 34(1), 28–38 (2002).
- 26 Johnsson E, Berggard K, Kotarsky H *et al.* Role of the hypervariable region in streptococcal M proteins: binding of a human complement inhibitor. *J. Immunol.* 161(9), 4894–4901 (1998).
- 27 Retnoningrum DS, Cleary PP. M12 protein from *Streptococcus pyogenes* is a receptor for immunoglobulin G3 and human albumin. *Infect. Immun.* 62(6), 2387–2394 (1994).
- 28 Whitnack E, Beachey EH. Antiopsonic activity of fibrinogen bound to M protein on the surface of group A streptococci. *J. Clin. Invest.* 69(4), 1042–1045 (1982).
- 29 Whitnack E, Beachey EH. Biochemical and biological properties of the binding of human fibrinogen to M protein in group A streptococci. *J. Bacteriol.* 164(1), 350–358 (1985).
- 30 Akesson P, Schmidt KH, Cooney J, Bjorck L. M1 protein and protein H: IgGFC- and albumin-binding streptococcal surface proteins encoded by adjacent genes. *Biochem. J.* 300(Pt 3), 877–886 (1994).
- 31 Courtney HS, Hasty DL, Dale JB. Anti-phagocytic mechanisms of *Streptococcus pyogenes*: binding of fibrinogen to M-related protein. *Mol. Microbiol.* 59(3), 936–947 (2006).

- 32 Carlsson F, Sandin C, Lindahl G. Human fibrinogen bound to *Streptococcus pyogenes* M protein inhibits complement deposition via the classical pathway. *Mol. Microbiol.* 56(1), 28–39 (2005).
- 33 Ringdahl U, Svensson IIG, Kotarsky H, Gustafsson M, Weineisen M, Sjöbring U. A role for the fibrinogen-binding regions of streptococcal M proteins in phagocytosis resistance. *Mol. Microbiol.* 37(6), 1318–1326 (2000).
- 34 Sandin C, Carlsson F, Lindahl G. Binding of human plasma proteins to *Streptococcus pyogenes* M protein determines the location of opsonic and non-opsonic epitopes. *Mol. Microbiol.* 59(1), 20–30 (2006).
- 35 Pahlman LI, Olin AI, Darenberg J *et al.* Soluble M1 protein of *Streptococcus pyogenes* triggers potent T cell activation. *Cell. Microbiol.* 10(2), 404–414 (2008).
- 36 Kahn F, Morgelin M, Shannon O *et al.* Antibodies against a surface protein of *Streptococcus pyogenes* promote a pathological inflammatory response. *PLoS Pathog.* 4(9), e1000149 (2008).
- 37 Cortney HS, Hasty DL, Dale JB. Molecular mechanisms of adhesion, colonization, and invasion of group A streptococci. *Ann. Med.* 34(2), 77–87 (2002).
- 38 Osterlund A, Engstrand L. Intracellular penetration and survival of *Streptococcus pyogenes* in respiratory epithelial-cells *in vitro*. *Acta Oto Laryngologica* 115(5), 685–688 (1995).
- 39 LaPenta D, Rubens C, Chi E, Cleary PP. Group A streptococci efficiently invade human respiratory epithelial cells. *Proc. Natl Acad. Sci. USA* 91(25), 12115–12119 (1994).
- 40 Jadoun J, Burstein E, Hanski E, Sela S. Proteins M6 and F1 are required for efficient invasion of group A streptococci into cultured epithelial cells. *Adv. Exp. Med. Biol.* 418, 511–515 (1997).
- 41 Guilherme L, Kalil J, Cunningham M. Molecular mimicry in the autoimmune pathogenesis of rheumatic heart disease. *Autoimmunity* 39(1), 31–39 (2006).
- 42 Fae KC, Diefenbach da Silva D, Bilate AM *et al.* PDIA3, HSPA5 and vimentin, proteins identified by 2-DE in the valvular tissue, are the target antigens of peripheral and heart infiltrating T cells from chronic rheumatic heart disease patients. *J. Autoimmun.* 31(2), 136–141 (2008).
- 43 Gulizia JM, Cunningham M, McManus BM. Immunoreactivity of anti-streptococcal monoclonal antibodies to human heart valves. Evidence for multiple cross-reactive epitopes. *Am. J. Pathol.* 138, 285–301 (1991).
- 44 Manjula BN, Trus BL, Fischetti VA. Presence of two distinct regions in the coiled-coil structure of the streptococcal Pep M5 protein: relationship to mammalian coiled-coil proteins and implications to its biological properties. *Proc. Natl Acad. Sci. USA* 82(4), 1064–1068 (1985).
- 45 Cunningham MW. Pathogenesis of group A streptococcal infections and their sequelae. *Adv. Exp. Med. Biol.* 609, 29–42 (2008).
- 46 Massell BF, Honikman LH, Amezcua J. Rheumatic fever following streptococcal vaccination. Report of three cases. *JAMA* 207(6), 1115–1119 (1969).
- 47 Steer AC, Kado J, Jenney AW *et al.* Acute rheumatic fever and rheumatic heart disease in Fiji: prospective surveillance, 2005–2007. *Med. J. Aust.* 190(3), 133–135 (2009).
- 48 Bessen DE, Carapetis JR, Beall B *et al.* Contrasting molecular epidemiology of group A streptococci causing tropical and nontropical infections of the skin and throat. *J. Infect. Dis.* 182(4), 1109–1116 (2000).
- **Interesting comparison between throat and skin isolates from different locations.**
- 49 McDonald M, Currie BJ, Carapetis JR. Acute rheumatic fever: a chink in the chain that links the heart to the throat? *Lancet Infect. Dis.* 4(4), 240–245 (2004).
- **Original and argued question on the exclusive role of throat infections in the pathogenesis of rheumatic fever.**
- 50 McGregor KF, Bilek N, Bennett A *et al.* Group A streptococci from a remote community have novel multilocus genotypes but share emm types and housekeeping alleles with isolates from worldwide sources. *J. Infect. Dis.* 189(4), 717–723 (2004).
- 51 Tewodros W, Kronvall G. M protein gene (emm type) analysis of group A β -hemolytic streptococci from Ethiopia reveals unique patterns. *J. Clin. Microbiol.* 43(9), 4369–4376 (2005).
- 52 Haidan A, Talay SR, Rohde M, Sriprakash KS, Currie BJ, Chhatwal GS. Pharyngeal carriage of group C and group G streptococci and acute rheumatic fever in an Aboriginal population. *Lancet* 356(9236), 1167–1169 (2000).
- 53 Martin DR, Voss LM, Walker SJ, Lennon D. Acute rheumatic fever in Auckland, New Zealand: spectrum of associated group A streptococci different from expected. *Pediatr. Infect. Dis. J.* 13(4), 264–269 (1994).
- 54 Vlamincx BJ, Schuren FH, Montijn RC *et al.* Determination of the relationship between group A streptococcal genome content, M type, and toxic shock syndrome by a mixed genome microarray. *Infect. Immun.* 75(5), 2603–2611 (2007).
- 55 O'Loughlin RE, Roberson A, Cieslak PR *et al.* The epidemiology of invasive group A streptococcal infection and potential vaccine implications: United States, 2000–2004. *Clin. Infect. Dis.* 45(7), 853–862 (2007).
- **Large epidemiological surveillance.**
- 56 Pahlman LI, Olin AI, Darenberg J *et al.* Soluble M1 protein of *Streptococcus pyogenes* triggers potent T cell activation. *Cell Microbiol.* 10(2), 404–414 (2007).
- 57 Sumby P, Porcella SF, Madrigal AG *et al.* Evolutionary origin and emergence of a highly successful clone of serotype M1 group A streptococcus involved multiple horizontal gene transfer events. *J. Infect. Dis.* 192(5), 771–782 (2005).
- 58 Sitkiewicz I, Nagiec MJ, Sumby P, Butler SD, Cywes-Bentley C, Musser JM. Emergence of a bacterial clone with enhanced virulence by acquisition of a phage encoding a secreted phospholipase A2. *Proc. Natl Acad. Sci. USA* 103(43), 16009–16014 (2006).
- 59 Johnson DR, Wotton JT, Sher A, Kaplan EL. A comparison of group A streptococci from invasive and uncomplicated infections: are virulent clones responsible for serious streptococcal infections? *J. Infect. Dis.* 185(11), 1586–1595 (2002).
- 60 McMillan DJ, Beiko RG, Geffers R *et al.* Genes for the majority of group A streptococcal virulence factors and extracellular surface proteins do not confer an increased propensity to cause invasive disease. *Clin. Infect. Dis.* 43(7), 884–891 (2006).
- **Interesting study showing that the presence of most virulence factors does not increase *per se* the observed clinical virulence.**
- 61 Rogers S, Commons R, Danchin MH *et al.* Strain prevalence, rather than innate virulence potential, is the major factor responsible for an increase in serious group A streptococcus infections. *J. Infect. Dis.* 195(11), 1625–1633 (2007).

- 62 Lamagni TL, Darenberg J, Luca-Harari B *et al.* Epidemiology of severe *Streptococcus pyogenes* disease in Europe. *J. Clin. Microbiol.* 46(7), 2359–2367 (2008).
- **Complete overview of the European epidemiology.**
- 63 Steer AC, Magor G, Jenney AW *et al.* *emm* and C-repeat region molecular typing of β -hemolytic streptococci in a tropical country: implications for vaccine development. *J. Clin. Microbiol.* 47(8), 2502–2509 (2009).
- **Large epidemiological study in a country with a high number of circulating *emm*-types.**
- 64 Shulman ST, Tanz RR, Dale JB *et al.* Seven-year surveillance of North American pediatric group A streptococcal pharyngitis isolates. *Clin. Infect. Dis.* 49(1), 78–84 (2009).
- **Recent and complete overview of North American pharyngitis epidemiology.**
- 65 Fischetti VA, Hodges WM, Hruby DE. Protection against streptococcal pharyngeal colonization with a vaccinia: M protein recombinant. *Science* 244(4911), 1487–1490 (1989).
- 66 Lancefield RC, Perlmann GE. Preparation and properties of type-specific M antigen isolated from a group A, type 1 hemolytic streptococcus. *J. Exp. Med.* 96(1), 71–82 (1952).
- 67 Dochez AR, Avery OT, Lancefield RC. Studies on the biology of streptococcus. I. Antigenic relationships between strains of *Streptococcus haemolyticus*. *J. Exp. Med.* 30(3), 179–213 (1919).
- 68 D'Alessandri R, Plotkin G, Kluge RM *et al.* Protective studies with group A streptococcal M protein vaccine. III. Challenge of volunteers after systemic or intranasal immunization with type 3 or type 12 group A streptococcus. *J. Infect. Dis.* 138(6), 712–718 (1978).
- 69 Fox EN, Waldman RH, Wittner MK, Mauceri AA, Dorfman A. Protective study with a group-A streptococcal-M protein vaccine – infectivity challenge of human volunteers. *J. Clin. Invest.* 52(8), 1885–1892 (1973).
- 70 Watson RF, Rothbard S, Swift HF. Type-specific protection and immunity following intranasal inoculation of monkeys with group-A hemolytic streptococci. *J. Exp. Med.* 84(2), 127–142 (1946).
- 71 Facklam RR, Moody MD. Production of streptococcal M-typing antisera. I. Antigenic response in different breeds of rabbits. *Appl. Microbiol.* 16(12), 1822–1825 (1968).
- 72 O'Brien KL, Beall B, Barrett NL *et al.* Epidemiology of invasive group A streptococcus disease in the United States, 1995–1999. *Clin. Infect. Dis.* 35(3), 268–276 (2002).
- 73 Krisher K, Cunningham MW. Myosin – a link between streptococci and heart. *Science* 227(4685), 413–415 (1985).
- 74 Fenderson PG, Fischetti VA, Cunningham MW. Tropomyosin shares immunologic epitopes with group A streptococcal M proteins. *J. Immunol.* 142(7), 2475–2481 (1989).
- 75 Ellis NM, Li Y, Hildebrand W, Fischetti VA, Cunningham MW. T cell mimicry and epitope specificity of cross-reactive T cell clones from rheumatic heart disease. *J. Immunol.* 175(8), 5448–5456 (2005).
- 76 Ji Y, McLandsborough L, Kondagunta A, Cleary PP. C5a peptidase alters clearance and trafficking of group A streptococci by infected mice. *Infect. Immun.* 64(2), 503–510 (1996).
- 77 Guzman CA, Talay SR, Molinari G, Medina E, Chhatwal GS. Protective immune response against *Streptococcus pyogenes* in mice after intranasal vaccination with the fibronectin binding protein SfbI. *J. Infect. Dis.* 179(4), 901–906 (1999).
- 78 Kotloff KL, Corretti M, Palmer K *et al.* Safety and immunogenicity of a recombinant multivalent group A streptococcal vaccine in healthy adults: Phase 1 trial. *JAMA* 292(6), 709–715 (2004).
- 79 McNeil SA, Halperin SA, Langley JM *et al.* Safety and immunogenicity of 26-valent group A streptococcus vaccine in healthy adult volunteers. *Clin. Infect. Dis.* 41(8), 1114–1122 (2005).
- **Results of the 26-valent Phase I clinical trial.**
- 80 Dale JB, Chiang EY, Lederer JW. Recombinant tetravalent group-A streptococcal M-protein vaccine. *J. Immunol.* 151(4), 2188–2194 (1993).
- 81 Dale JB. Multivalent group A streptococcal vaccine designed to optimize the immunogenicity of six tandem M protein fragments. *Vaccine* 17(2), 193–200 (1999).
- 82 Dale JB, Simmons M, Chiang EC, Chiang EY. Recombinant, octavalent group A streptococcal M protein vaccine. *Vaccine* 14(10), 944–948 (1996).
- 83 McMillan D. StreptAvax (ID Biomedical). *Curr. Opin. Investig. Drugs* 7(2), 186–190 (2006).
- 84 Hu MC, Walls MA, Stroop SD, Reddish MA, Beall B, Dale JB. Immunogenicity of a 26-valent group A streptococcal vaccine. *Infect. Immun.* 70(4), 2171–2177 (2002).
- 85 Dale JB, Penfound T, Chiang EY, Long V, Shulman ST, Beall B. Multivalent group A streptococcal vaccine elicits bactericidal antibodies against variant M subtypes. *Clin. Diagn. Lab. Immunol.* 12(7), 833–836 (2005).
- 86 Villasenor-Sierra A, McShan WM, Salmi D, Kaplan EL, Johnson DR, Stevens DL. Variable susceptibility to opsonophagocytosis of group A streptococcus M-1 strains by human immune sera. *J. Infect. Dis.* 180(6), 1921–1928 (1999).
- 87 Eriksson BK, Villasenor-Sierra A, Norgren M, Stevens DL. Opsonization of T1M1 group A streptococcus: dynamics of antibody production and strain specificity. *Clin. Infect. Dis.* 32(2), E24–E30 (2001).
- 88 Bisno AL. The concept of rheumatogenic and nonrheumatogenic group A streptococci. In: *Streptococcal Disease and the Immune Response*. Reed SE, Zabriskie JB (Eds). Academic Press, Inc., NY, USA, 789–803 (1980).
- 89 Schuchat A, Hilger T, Zell E *et al.* Active bacterial core surveillance of the emerging infections program network. *Emerg. Infect. Dis.* 7(1), 92–99 (2001).
- 90 Dey N, McMillan DJ, Yarwood PJ *et al.* High diversity of group A streptococcal *emm* types in an Indian community: the need to tailor multivalent vaccines. *Clin. Infect. Dis.* 40(1), 46–51 (2005).
- **Interesting paper illustrating the high number of circulating *emm*-types in India.**
- 91 McDonald MI, Towers RJ, Andrews RM, Bengier N, Currie BJ, Carapetis JR. Low rates of streptococcal pharyngitis and high rates of pyoderma in Australian aboriginal communities where acute rheumatic fever is hyperendemic. *Clin. Infect. Dis.* 43(6), 683–689 (2006).
- 92 Hartas J, Goodfellow AM, Currie BJ, Sriprakash KS. Characterisation of group A streptococcal isolates from tropical Australia with high prevalence of rheumatic fever: probing for signature sequences to identify members of the family of serotype 5. *Microb. Pathog.* 18(5), 345–354 (1995).
- 93 Wong SS, Lin YS, Mathew L, Rajagopal L, Sepkowitz D. Increase in group G Streptococcal infections in a community hospital, New York, USA. *Emerg. Infect. Dis.* 15(6), 991–993 (2009).

- 94 Zaoutis T, Attia M, Gross R, Klein J. The role of group C and group G streptococci in acute pharyngitis in children. *Clin. Microbiol. Infect.* 10(1), 37–40 (2004).
- 95 Davies MR, Tran TN, McMillan DJ, Gardiner DL, Currie BJ, Sriprakash KS. Inter-species genetic movement may blur the epidemiology of streptococcal diseases in endemic regions. *Microbes Infect.* 7(9–10), 1128–1138 (2005).
- 96 Alberti S, Garcia-Rey C, Garcia-Laorden MI, Dal-Re R, Garcia-de-Lomas J. Survey of *emm*-like gene sequences from pharyngeal isolates of group C and group G streptococci collected in Spain. *J. Clin. Microbiol.* 43(3), 1433–1436 (2005).
- 97 Ardanuy C, Tubau F, Pallares R *et al.* Epidemiology of invasive pneumococcal disease among adult patients in Barcelona before and after pediatric 7-valent pneumococcal conjugate vaccine introduction, 1997–2007. *Clin. Infect. Dis.* 48(1), 57–64 (2009).
- 98 Byington CL, Samore MH, Stoddard GJ *et al.* Temporal trends of invasive disease due to *Streptococcus pneumoniae* among children in the Intermountain West: emergence of nonvaccine serogroups. *Clin. Infect. Dis.* 41(1), 21–29 (2005).
- 99 Gonzalez BE, Hulten KG, Lamberth L, Kaplan SL, Mason EO; US Pediatric Multicenter Pneumococcal Surveillance Group. *Streptococcus pneumoniae* serogroups 15 and 33 – an increasing cause of pneumococcal infections in children in the United States after the introduction of the pneumococcal 7-valent conjugate vaccine. *Pediatr. Infect. Dis. J.* 25(4), 301–305 (2006).
- 100 Dobay O, Amycs SGB, Nagy K. The effect of the 7-valent conjugate vaccine (PCV7) on the incidence of pneumococcal infections world-wide. *Rev. Med. Microbiol.* 19(3), 79–85 (2008).
- 101 Beall B, McEllistrem MC, Gertz RE *et al.* Pre- and postvaccination clonal compositions of invasive pneumococcal serotypes for isolates collected in the United States in 1999, 2001, and 2002. *J. Clin. Microbiol.* 44(3), 999–1017 (2006).
- 102 Dinleyici EC, Yargic ZA. Pneumococcal conjugated vaccines: impact of PCV-7 and new achievements in the postvaccine era. *Expert Rev. Vaccines* 7(9), 1367–1394 (2008).
- 103 Pruksakorn S, Currie B, Brandt E *et al.* Towards a vaccine for rheumatic fever: identification of a conserved target epitope on M protein of group A streptococci. *Lancet* 344(8923), 639–642 (1994).
- 104 Bolken TC, Franke CA, Jones KF *et al.* Analysis of factors affecting surface expression and immunogenicity of recombinant proteins expressed by Gram-positive commensal vectors. *Infect. Immun.* 70(5), 2487–2491 (2002).
- 105 Bessen D, Fischetti VA. Synthetic peptide vaccine against mucosal colonization by group A streptococci. I. Protection against a heterologous M serotype with shared C repeat region epitopes. *J. Immunol.* 145(4), 1251–1256 (1990).
- 106 Guilherme L, Fae KC, Higa F *et al.* Towards a vaccine against rheumatic fever. *Clin. Dev. Immunol.* 13(2–4), 125–132 (2006).
- 107 Brandt ER, Hayman WA, Currie B *et al.* Functional analysis of IgA antibodies specific for a conserved epitope within the M protein of group A streptococci from Australian Aboriginal endemic communities. *Int. Immunol.* 11(4), 569–576 (1999).
- 108 Pruksakorn S, Currie B, Brandt E *et al.* Identification of T cell autoepitopes that cross-react with the C-terminal segment of the M protein of group A streptococci. *Int. Immunol.* 6(8), 1235–1244 (1994).
- 109 Brandt ER, Hayman WA, Currie B *et al.* Opsonic human antibodies from an endemic population specific for a conserved epitope on the M protein of group A streptococci. *Immunology* 89(3), 331–337 (1996).
- 110 Bessen D, Fischetti VA. Influence of intranasal immunization with synthetic peptides corresponding to conserved epitopes of M protein on mucosal colonization by group A streptococci. *Infect. Immun.* 56(10), 2666–2672 (1988).
- **Pioneering studies eluding to the potential of peptides encompassing conserved M-protein epitopes as mucosally delivered vaccine antigens.**
- 111 Pruksakorn S, Galbraith A, Houghten RA, Good MF. Conserved T and B cell epitopes on the M protein of group A streptococci. Induction of bactericidal antibodies. *J. Immunol.* 149(8), 2729–2735 (1992).
- 112 Hayman WA, Brandt ER, Relf WA, Cooper J, Saul A, Good MF. Mapping the minimal murine T cell and B cell epitopes within a peptide vaccine candidate from the conserved region of the M protein of group A streptococcus. *Int. Immunol.* 9(11), 1723–1733 (1997).
- 113 Relf WA, Cooper J, Brandt ER *et al.* Mapping a conserved conformational epitope from the M protein of group A streptococci. *Peptide Res.* 9(1), 12–20 (1996).
- **Describes identification of the minimal B-cell epitope within the conserved repeat region of the M protein and the strategies employed to maintain the conformation of this epitope to promote immunogenicity.**
- 114 Batzloff M, Yan H, Davies M, Hartas J, Good M. Preclinical evaluation of a vaccine based on conserved region of M protein that prevents group A streptococcal infection. *Indian J. Med. Res.* 119(Suppl.), 104–107 (2004).
- 115 Pandey M, Batzloff MR, Good MF. Mechanism of protection induced by group A streptococcus vaccine candidate J8-DT: contribution of B and T-cells towards protection. *PLoS ONE* 4(4), e5147 (2009).
- 116 Batzloff MR, Yan H, Davies MR *et al.* Toward the development of an antidisease, transmission-blocking intranasal vaccine for group A streptococcus. *J. Infect. Dis.* 192(8), 1450–1455 (2005).
- 117 Nilson BH, Frick IM, Akesson P *et al.* Structure and stability of protein H and the M1 protein from *Streptococcus pyogenes*. Implications for other surface proteins of Gram-positive bacteria. *Biochem.* 34(41), 13688–13698 (1995).
- 118 Brandt ER, Sriprakash KS, Hobb RI *et al.* New multi-determinant strategy for a group A streptococcal vaccine designed for the Australian Aboriginal population. *Nat. Med.* 6(4), 455–459 (2000).
- 119 Saikaly PE, Stroot PG, Oerther DB. Use of 16S rRNA gene terminal restriction fragment analysis to assess the impact of solids retention time on the bacterial diversity of activated sludge. *Appl. Environ. Microbiol.* 71(10), 5814–5822 (2005).
- 120 Grundmann H, Hori S, Tanner G. Determining confidence intervals when measuring genetic diversity and the discriminatory abilities of typing methods for microorganisms. *J. Clin. Microbiol.* 39(11), 4190–4192 (2001).
- 121 Espinosa LE, Li Z, Gomez Barreto D *et al.* M protein gene type distribution among group A streptococcal clinical isolates recovered in Mexico City, Mexico, from 1991 to 2000, and Durango, Mexico, from 1998 to 1999: overlap with type distribution within the United States. *J. Clin. Microbiol.* 41(1), 373–378 (2003).

- 122 Abdissa A, Asrat D, Kronvall G *et al.* High diversity of group A streptococcal *emm* types among healthy schoolchildren in Ethiopia. *Clin. Infect. Dis.* 42(10), 1362–1367 (2006).
- 123 Luca-Harari B, Darenberg J, Neal S *et al.* Clinical and microbiological characteristics of severe *Streptococcus pyogenes* disease in Europe. *J. Clin. Microbiol.* 47(4), 1155–1165 (2009).
- 124 Eisner A, Leitner E, Feierl G, Kessler HH, Marth E. Prevalence of *emm* types and antibiotic resistance of group A streptococci in Austria. *Diagn. Microbiol. Infect. Dis.* 55(4), 347–350 (2006).
- 125 Rivera A, Rebollo M, Miro E *et al.* Superantigen gene profile, *emm* type and antibiotic resistance genes among group A streptococcal isolates from Barcelona, Spain. *J. Med. Microbiol.* 55(Pt 8), 1115–1123 (2006).
- 126 Meisal R, Hoiby EA, Aaberge IS, Caugant DA. Sequence type and *emm* type diversity in *Streptococcus pyogenes* isolates causing invasive disease in Norway between 1988 and 2003. *J. Clin. Microbiol.* 46(6), 2102–2105 (2008).
- 127 Kittang BR, Langeland N, Mylvaganam H. Distribution of *emm* types and subtypes among noninvasive group A, C and G streptococcal isolates in western Norway. *APMIS* 116(6), 457–464 (2008).
- 128 Mijac V, Ranin L, Markovic M, Heeg C, Reinert RR, Opavski N. Distribution of *emm* types among group A streptococcal isolates from Serbia. *Clin. Microbiol. Infect.* (2009) (Epub ahead of print).
- 129 Szczypa K, Sadowy E, Izdebski R, Strakova L, Hryniewicz W. Group A streptococci from invasive-disease episodes in Poland are remarkably divergent at the molecular level. *J. Clin. Microbiol.* 44(11), 3975–3979 (2006).
- 130 Nir-Paz R, Korenman Z, Ron M *et al.* *Streptococcus pyogenes* *emm* and T types within a decade, 1996–2005: implications for epidemiology and future vaccines. *Epidemiol. Infect.* 1–8 (2009) (Epub ahead of print).
- Complete and interesting review of the epidemiological situation in Israel.
- 131 Sagar V, Bakshi DK, Nandi S, Ganguly NK, Kumar R, Chakraborti A. Molecular heterogeneity among north Indian isolates of group A streptococcus. *Lett. Appl. Microbiol.* 39(1), 84–88 (2004).
- 132 Sakota V, Fry AM, Lietman TM, Facklam RR, Li Z, Beall B. Genetically diverse group A streptococci from children in far-western Nepal share high genetic relatedness with isolates from other countries. *J. Clin. Microbiol.* 44(6), 2160–2166 (2006).
- 133 Jing HB, Ning BA, Hao HJ *et al.* Epidemiological analysis of group A streptococci recovered from patients in China. *J. Med. Microbiol.* 55(Pt 8), 1101–1107 (2006).
- 134 Ho PL, Johnson DR, Yue AW *et al.* Epidemiologic analysis of invasive and noninvasive group A streptococcal isolates in Hong Kong. *J. Clin. Microbiol.* 41(3), 937–942 (2003).
- 135 Zheng MH, Jiao ZQ, Zhang LJ *et al.* Genetic analysis of group A streptococcus isolates recovered during acute glomerulonephritis outbreaks in Guizhou Province of China. *J. Clin. Microbiol.* 47(3), 715–720 (2009).
- 136 Kim S, Yong Lee N. Antibiotic resistance and genotypic characteristics of group A streptococci associated with acute pharyngitis in Korea. *Microb. Drug Resist.* 10(4), 300–305 (2004).
- 137 Koh EH, Kim S. Distribution of T types and *emm* genotypes of *Streptococcus pyogenes* in Jinju, 2004. *Korean J. Lab. Med.* 26(4), 269–274 (2006).
- 138 Ikebe T, Hirasawa K, Suzuki R *et al.* Distribution of *emm* genotypes among group A streptococcus isolates from patients with severe invasive streptococcal infections in Japan, 2001–2005. *Epidemiol. Infect.* 135(7), 1227–1229 (2007).
- 139 Wajima T, Murayama SY, Sunaoshi K, Nakayama E, Sunakawa K, Ubukata K. Distribution of *emm* type and antibiotic susceptibility of group A streptococci causing invasive and noninvasive disease. *J. Med. Microbiol.* 57(Pt 11), 1383–1388 (2008).
- 140 McDonald MI, Towers RJ, Fagan P, Carapetis JR, Currie BJ. Molecular typing of *Streptococcus pyogenes* from remote Aboriginal communities where rheumatic fever is common and pyoderma is the predominant streptococcal infection. *Epidemiol. Infect.* 135(8), 1398–1405 (2007).
- 141 Valery PC, Wenitong M, Clements V *et al.* Skin infections among Indigenous Australians in an urban setting in far north Queensland. *Epidemiol. Infect.* 136(8), 1103–1108 (2008).

Affiliations

- Pierre R Smeesters, MD, PhD
Bacterial Pathogenesis Laboratory,
Queensland Institute of Medical Research,
Brisbane, Queensland 4029, Australia
and
Laboratoire de Génétique et Physiologie
Bactérienne, Institut de Biologie et de
Médecine Moléculaires, Faculté des
Sciences, Université Libre de Bruxelles,
Belgium
and
Infectious Diseases Department,
Hôpital Universitaire des Enfants Reine
Fabiola, Université Libre de Bruxelles,
Belgium
Tel.: +32 2650 9776
psmeeste@ulb.ac.be
- David J McMillan, PhD
Bacterial Pathogenesis Laboratory,
Queensland Institute of Medical Research,
Brisbane, Queensland 4029, Australia
and
Griffith Medical Research College,
Herston, Queensland, Australia
Tel.: +61 073 845 3698
david.mcmillan@qimr.edu.au
- Kadaba S Sriprakash, PhD
Bacterial Pathogenesis Laboratory,
Queensland Institute of Medical Research,
Brisbane, Queensland 4029, Australia
and
Griffith Medical Research College,
Herston, Queensland, Australia
sri.sriprakash@qimr.edu.au
- Melina M Georgousakis, PhD
Bacterial Pathogenesis Laboratory,
Queensland Institute of Medical Research,
Brisbane, Queensland 4029, Australia
melina.georgousakis@qimr.edu.au