# Accelerating the development of a group A *Streptococcus* vaccine: an urgent public health need

**Jean-Louis Excler, Jerome H. Kim**

International Vaccine Institute, Seoul, Korea

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Corresponding author: Jean-Louis Excler, MD
International Vaccine Institute, SNU Research
Park, 1 Gwanak-ro, Gwanak-gu, Seoul 08826,
Korea
Tel: +82-2-881-1176, Fax: +82-2-881-1164
E-mail: JeanLouis.Excler@ivi.int

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Group A *Streptococcus* (GAS) infections cause substantial worldwide morbidity and mortality, mostly associated with suppurative complications such as pharyngitis, impetigo, and non-suppurative immune syndromes such as acute rheumatic fever, rheumatic heart disease, and acute post-streptococcal glomerulonephritis. Deaths occur mostly in children, adolescents, and young adults in particular pregnant women in low- and middle-income countries. GAS strains are highly variable, and a GAS vaccine would need to overcome the issue of multiple strains. Several approaches have been used multivalent vaccines using N-terminal polypeptides of different M protein; conserved M protein vaccines with antigens from the conserved C-repeat portion of the M protein; incorporation selected T- and B-cell epitopes from the C-repeat region in a synthetic polypeptide or shorter single minimal B-cell epitopes from this same region; and non-M protein approaches utilizing highly conserved motives of streptococcal C5a peptidase, GAS carbohydrate and streptococcal fibronectin-binding proteins. A GAS vaccine represents urgent need for this neglected disease and should therefore deserve the greatest attention of international organizations, donors, and vaccine manufacturers.

**Keywords:** Group A Streptococcus, Vaccines, Rheumatic heart disease, M protein, Low- and middle-income countries

## Introduction

The group A *Streptococcus* (GAS) bacterium—is one of the top-10 infectious causes of deaths in the world [1]. According to the Global Burden of Disease 2010 study, this disease affects more than 34 million people, causing >345,000 deaths and 10 million disability-adjusted life years (DALYs) lost per year, almost all in low- and middle-income countries (LMIC) [2,3]. Most cases and deaths occur in children, adolescents, and young adults, depriving developing countries of many young people. In particular GAS, through the progressive cardiac condition known as rheumatic heart disease, causes substantial morbidity and mortality in pregnancy—with consequences for the expectant mother and her child.

GAS infections cause substantial worldwide morbidity and mortality—the combined mortality associated with rheumatic heart disease (RHD) and invasive GAS exceeds all other causes of infectious disease death excluding human immunodeficiency virus, tuberculosis, malaria, and *S. pneumonia* [3,4]. GAS nosology includes suppurative complications (pharyngitis, invasive GAS disease, and impetigo), non-suppurative (immune)

syndromes (acute rheumatic fever [ARF], RHD, and acute post-streptococcal glomerulonephritis), and toxin-mediated diseases such as scarlet fever and streptococcal toxic shock syndrome. A consequence of ARF, RHD is responsible for progressive valvular heart disease (VHD) such as mitral stenosis and mitral regurgitation, and in resource limited settings pregnant women suffer a marked increase in maternal morbidity and unfavorable fetal outcomes, which are related to severity of disease [5]. While RHD has become relatively rare in developed countries, it remains quite common in the developing world where 90% of all heart disorders in women of childbearing age are rheumatic in origin. Although accurate statistics are lacking, the estimated incidence of rheumatic fever in sub-Saharan Africa is approximately 13 cases per 100,000 per year based on clinical screening, while estimations between 21.5 and 30.4 per 1,000 have been reported for example in Cambodia and Mozambique when using echocardiographic screening. Besides its high prevalence, RHD in developing countries is characterized by the occurrence of severe VHD at a younger age than in developed countries [6,7]. ARF, the condition that precedes RHD, is believed to be a form of autoimmunity mediated by the similarity between the GAS coiled M protein and GAS polysaccharides to human myosin and between GAS cell wall and carbohydrates on heart muscle [8]. An alternate explanation is an interaction between M protein and type IV valvular collagen, inducing an immune response to collagen [8-10]. Antibodies found in ARF recognize valvular endothelium structural proteins such as collagen and elastin, and similarly also recognize N-acetylglucosamine and target dopamine receptors in the brain, possibly explaining the choreiform movements found in ARF [10,11].

GAS is a neglected disease of poverty and social injustice [12] that can however be prevented, using one of the cheapest and oldest antibiotics known—penicillin. The urgency for disease control is yet to be recognized at the highest level as it has been largely ignored by international organizations and other key stakeholders. Few affected countries have any coordinated strategy to implement control programs [13]. A GAS vaccine represents and urgent need for this neglected disease and should therefore deserve the greatest attention of international organizations, donors and vaccine manufacturers.

## Epidemiology of GAS Infections and Implications for Vaccine Design

GAS strains are categorized by variation in the nucleotide sequence of the *emm* gene that encodes the variable M protein. Limited data on *emm* gene sequences are available from LMIC which, as with serotypes, seem to differ from high-income countries [14]. Africa and the Pacific are characterized by a wider diversity of emm types, and many of the common emm types found in industrialized countries are far less common (emm 1, 4, 6, and 12). One explanation provided is the high prevalence of GAS impetigo accompanied by large numbers of circulating GAS of multiple emm types that are readily transmitted and found in some resource-poor settings [15].

Like pneumococcal vaccines, a GAS vaccine would need to overcome the issue of multiple strains. Recent data from both whole M protein sequencing and multivalent vaccines suggest that M protein–based vaccines may evoke cross-protective antibodies that would broaden their potential efficacy and potentially mitigate any concerns about the emergence of new non-vaccine serotypes [16,17]. Some data are available for a vaccine (J8), which contains a common B-cell epitope of M protein whose structure seems highly conserved among GAS strains in a limited number of tropical settings [14]. Another 26-valent M protein-based vaccine, which contains N-terminal M peptides from 26 of the most common serotypes in North America, would likely provide good coverage in high-income countries, particularly United States, Canada, and Europe, but poor coverage in Africa and the Pacific and only intermediate coverage in Asia and the Middle East [18]. These data clearly have significant implications for multivalent M protein vaccines and are the subject of ongoing investigation.

## GAS Vaccine Candidates

Several arguments suggest that a GAS vaccine is feasible [19, 20]. GAS infection is common in childhood and uncommon in adulthood, suggesting that immunity is acquired through lifetime exposure. Longitudinal data showing the development of antibodies against common emm types in the United States support this hypothesis. Moreover, pre-clinical studies in animals show protection against challenge with GAS. Older studies of a GAS vaccine showed efficacy in a human challenge model against homologous *S. pyogenes* challenge [21].

Table 1 provides an overview of the vaccine candidates at various pre-clinical and clinical stages. To address strain diversity, several vaccine design approaches have been proposed using M protein– and non-M protein–based vaccine antigens.

**Table 1.** Current GAS vaccine candidate approaches

| Design | Construct | Reference |
|---|---|---|
| M protein variable region | 26- and 30-valent N-terminal fragments | [16,24-26] |
| M protein conserved region | StreptInCor: B- and T-cell epitopes from the 55 amino acids of the C-repeat region | [27,28] |
| | J8 and J14: single minimal B-cell epitopes of the C-repeat region | [31,32] |
| Non-M protein region | Streptococcal C5a peptidase | [33] |
| | GAS carbohydrate and fibronectin binding proteins | [34] |
| | Cell walls and secreted virulence factors: spy0516 (spyCEP), spy0167 (streptolysine O, SLO), and spy0269 (surface exclusion protein) | [35] |

GAS, group A *Streptococcus*.

## M protein–based vaccines

The M protein is a coiled-coil protein consisting of 3 domains: an A-repeat of the N-terminal domain, which is highly variable and used for epidemiologic molecular typing (emm typing); a B-repeat domain (antibodies against this region are not opsonic and some are cross-reactive with human tissues); and a conserved C-repeat domain. A logical approach is the development of vaccines containing multiple serotypes, similar to multivalent pneumococcal polysaccharide or pneumococcal conjugate vaccines [19,22,23].

## Twenty six-valent and 30-valent M protein vaccines

Short peptides from the N-terminal region of M proteins from multiple different GAS emm types are fused together in tandem to form larger vaccine-antigen polypeptides [16,24,25]. In humans, the 26-valent vaccine was shown to be safe and immunogenic [24]. Functional opsonic antibodies were induced against all emm types of GAS present in the vaccine. Epidemiologic surveys suggest that the 26-valent vaccine would provide good coverage of circulating strains of GAS in industrialized countries (over 72%) but poor coverage in many developing countries (as low as 24% in the Pacific region) [14]. This 26-valent vaccine was therefore reformulated into a 30-valent vaccine to increase "coverage" of circulating emm types in the United States, Canada, and Europe as well as developing countries [19]. In preclinical studies, the 30-valent vaccine induced functional opsonic antibodies against all of the emm types represented in the vaccine [26]. Interestingly, the 30-valent vaccine antibodies cross-opsonized a proportion of non-vaccine emm types of GAS [26], suggesting that cross-protection may mitigate, to some extent, the limited coverage of the 30-valent vaccine in many tropical developing settings where GAS disease is endemic. A phase I clinical evaluation of the 30-valent vaccine in adult volunteers has now been initiated.

## Conserved M protein vaccines

These vaccines contain antigens from the conserved C-repeat portion of the M protein. The StreptInCor vaccine incorporates selected T- and B-cell epitopes from the C-repeat region in a synthetic 55 amino acid polypeptide, whereas the J8 and the conjugate version with diphtheria toxoid J8-DT and J14 vaccines contain shorter single minimal B-cell epitopes from this same region [27,28]. These vaccines comprise a minimal number of antigens, which may represent of benefit compared to the use of hypervariable M protein domains. Mouse studies, particularly of the J8-DT vaccine candidate, have shown that these antigens produce opsonic antibodies that protect against intraperitoneal challenge when the vaccine is administered parenterally and against intranasal challenge when the vaccine is administered intranasally [29,30]. In a murine model for infection that closely mimics human skin infection, J8-DT was able to protect against pyoderma and subsequent bacteremia caused by multiple GAS strains. The vaccine was however ineffective against a hypervirulent cluster of virulence responder/sensor mutant GAS strain; this correlated with the strain's ability to degrade CXC chemokines, thereby preventing neutrophil chemotaxis. By combining J8-DT with an inactive form of the streptococcal CXC protease, *S. pyogenes* cell envelope proteinase, the combination vaccine was highly effective in blocking CXC chemokine degradation and permit opsonic antibodies to kill the bacteria [31]. Limited epidemiological data available for the J8 peptide indicate that its sequence is highly conserved among multiple emm types of GAS and across regions [32]. The J8 vaccine entered a Phase 1 trial in adult volunteers in 2013, but the results are pending. The StreptinCor vaccine has been formulated into GMP StreptInCor plus alum with plans to enter Phase I clinical assays in healthy adult volunteers in Brazil.

## Non-M protein–based protein vaccines

Since several non-M proteins are highly conserved across

strains, another approach involves the use of conserved non-M protein GAS antigens for vaccine design by reverse vaccinology technology. Cell wall and secreted virulence factors, such as streptococcal C5a peptidase [33], GAS carbohydrate and streptococcal fibronectin-binding proteins are promising candidates although other approaches with GAS gene segments [34] tested in mice identified several known and new antigens including spy0516 (spyCEP), spy0167 (streptolysine O, SLO), and spy0269 (surface exclusion protein) [35]. When combined together, broad coverage of multiple GAS strains was achieved in CD1 mice. None of these approaches has entered yet clinical development.

## Vaccine Safety

Nineteen clinical studies of GAS vaccines have been conducted involving thousands of subjects. Candidate vaccines have generally been safe and well tolerated [19]. However, one major concern was the possible induction of ARF through autoimmune mechanisms triggered by vaccine components [36]. Following some initial unfortunate experiences in humans, the U.S. Food and Drug Administration issued a regulation preventing licensure of vaccines containing GAS or its derivatives; a restriction removed in 2006. It is now believed that immunogenic regions of the M protein could be distinguished from those sections believed to be rheumatogenic. Subsequent studies of M protein vaccines that do not include M protein components cross-reacting with human tissues suggest that these vaccines are safe.

## Vaccine Development Challenges

Table 2 summarizes the key scientific and strategic challenges to GAS vaccine development. The estimates of the global burden of disease, particularly the morbidity and mortality associated with ARF/RHD, acute post-streptococcal glomerulonephritis and invasive disease have been based on limited data from LMIC [37-40]. Deployment of vaccines into populations at highest risk for ARF/RHD will require additional surveillance and effectiveness studies to provide the data required to support policy decisions and effective implementation. There is also a considerable burden of GAS impetigo in tropical settings. There is a hypothetical link between GAS impetigo and ARF and RHD, although the pathogenetic link remains unknown [41]. Preventing skin infections would be highly desirable for a global GAS vaccine. However, little is known about immune protection against skin infections.

Human immune correlates of protection against GAS infection are not clearly defined. The focus for decades has been on the protective role of M protein antibodies in animal models of infection. For most of the potential GAS vaccine antigens, there are few data supporting their role in protection against natural infection in humans. The small animal models currently used to assess potential vaccine efficacy are considered to be of limited predictive value and could lead to the exclusion of potentially efficacious antigens [42]. There are limited criteria for selection of antigens to include in combination vaccines that would optimize vaccine efficacy. Vaccines containing M protein peptides evoke opsonic antibodies that promote bactericidal killing *in vitro*. C5a peptidase induces antibodies that neutralize the enzyme [43], thus preventing the degradation of this potent chemo-attractant. Adhesins evoke antibodies that block bacterial adherence [44]. Many of the non-M protein common antigens do not have associated functional *in vitro* assays that could be applied in pre-clinical or clinical studies.

The complex global epidemiology of GAS infections poses a challenge to the development of a single vaccine for the en-

**Table 2.** Key scientific and programmatic challenges to GAS vaccine development

| |
| --- |
| Scientific challenges |
|    Limited disease burden data associated with acute rheumatic fever and rheumatic heart disease in low- and middle-income countries |
|    Prevention of impetigo skin infection |
|    Human immune correlates of protection against GAS infection not clearly defined |
|    Small animal models for assessment of vaccine protection are of limited predictive value |
|    Complex global epidemiology of GAS infections and variability of emm types pose a challenge to the development of a single vaccine for the entire world |
| Strategic challenges |
|    No roadmap developed with vaccine developers, researchers, vaccine manufacturers, global health policy makers and donors |
|    Absence of industrial manufacturers and sufficient public/private funding |
|    International collaborative effort and leadership gathering key stakeholders urgently needed |
|    Strong advocacy effort needed by establishing and maintaining country-level dialogues to facilitate decision-making on GAS vaccine policy |

GAS, group A *Streptococcus*.

tire world [24,25]. Vaccines containing amino-terminal peptides of M proteins may or may not provide sufficient serotype coverage, or durable serotype coverage, in areas of the world where ARF is highly prevalent.

## Urgent Need for a Roadmap

World Health Organization (WHO) has made the development of GAS vaccines a priority. GAS vaccine development is mentioned in the Global Vaccine Action Plan 2010-2020, framework approved by the World Health Assembly in May 2012 to achieve the Decade of Vaccines vision by delivering universal access to immunization [45]. So far, no detailed plan has been developed with vaccine developers, researchers, vaccine manufacturers, global health policy makers, and donors. The strategic goal to accelerate the development and licensure of an effective and affordable GAS vaccine to prevent ARF and RHD as well as invasive infections in LMIC should receive utmost attention. Unfortunately, this effort seems, at least for the moment, precluded by the absence of industrial manufacturers and sufficient public/private funding. Funding for RHD prevention and GAS vaccines accounts for less than 0.1% of neglected tropical diseases global health funding [46].

The establishment of a roadmap implies an international collaborative effort, leadership, administrative and governance structures, gathering key stakeholders including public health and scientific experts on GAS disease pathogenicity and epidemiology, immunology, pre-clinical and clinical vaccinology, assay development, health policy and cost-effectiveness, vaccine manufacturers, private and public donors to secure sufficient funding for a comprehensive strategy. The WHO is positioned to provide leadership, and a funded product development partnership that is focused on the critical path to GAS vaccine development will be important to the success of this undertaking.

## Conclusion

GAS infection and its devastating morbidity and mortality remain a 'hidden' public health disease borne disproportionately in LMIC that received little attention. The scale and impact of GAS infections and the preliminary evidence that a vaccine may be successful make cogent and compelling case for work on a GAS vaccine. Importantly, engagement of major vaccine manufacturer would facilitate the successful development of this product, and there is experience in developing vaccines for global health needs that have used product development partnerships funded by private philanthropy. To achieve these goals, a strong advocacy effort is needed by establishing and maintaining country-level dialogues to facilitate decision-making on GAS vaccine policy that would benefit to LMIC populations most at risk for GAS infection.

## ORCID

Jean-Louis Excler  *http://orcid.org/0000-0002-6462-5101*
Jerome H. Kim  *http://orcid.org/0000-0003-0461-6438*

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