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Group A streptococcal vaccine development: current status and issues of relevance to less developed countries

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Executive summary

In light of the current lack of a clear strategy for primary prevention of GAS infections, there is definitely a place for a safe, effective, affordable and practical GAS vaccine. Based on the epidemiology of GAS diseases in less developed countries, there is some concern that the vaccine most advanced in development – a multivalent, type-specific vaccine – may not provide sufficient and long-lasting protection in countries with highly endemic GAS diseases. As a minimum, the efficacy of this and other GAS vaccines should be assessed in less developed country settings prior to advocating their widespread use. There is an urgent need for oversight and coordination of GAS vaccine development on a global scale. The priorities must be to ensure that a vaccine becomes available specifically for the prevention of GAS diseases in less developed countries, that any vaccines are therefore tested for safety and efficacy in less developed countries (or similar settings) using ARF, APSGN and skin infections as primary endpoints, and that preparations begin now for compiling important regional disease burden data that will be used in making decisions about introducing a GAS vaccine.

AN ACTION PLAN FOR GAS VACCINE DEVELOPMENT

Collaboration and coordination

Presently each research group operates independently, without much evidence of collaboration between groups. The progress of a number of the candidate vaccines is not known. WHO, NIH, and GAVI are the 3 organisations best placed to influence the process.

A meeting of experts

This meeting should include experts in GAS vaccines, epidemiology and control, and in vaccine development in general. The aims of the meeting would be to:

- provide updates on the current status of each candidate vaccine.
- identify obstacles that each group is facing.
- clarify the target groups for each vaccine; i.e. what disease(s) is the vaccine being primarily aimed at preventing, at what age will it be administered, and in what geographic region(s)?
- discuss the likelihood of each vaccine being efficacious, affordable and available for the prevention of the most important GAS diseases in developing countries (i.e. ARF, APSGN, invasive disease).
- determine the level of planning for downstream clinical trials of each vaccine; i.e. what immunological, safety and disease endpoints will be used, and in what population(s)?
- conduct a global discussion about GAS vaccines, including target groups for vaccination, obstacles and downstream clinical trials.
- clarify the regulatory and licensing issues involved for GAS vaccines.
- determine whether current understanding of immunological correlates of protection is sufficient, and if appropriate animal models of GAS diseases exist.
- consider if new vaccine antigens, adjuvants or modes of antigen presentation may be needed.

- discuss possible collaborations between research groups, including combinations of different vaccine antigens, adjuvants and/or modes of antigen presentation.
- determine the role and interest of the pharmaceutical industry in GAS vaccines, and ensure that industry is aware of the true public health role of a future GAS vaccine and the endpoints against which it should be assessed.
- discuss possible ways in which groups like WHO, GAVI, NIH and others may improve the likelihood that a GAS vaccine may become available that is of proven efficacy against the major GAS diseases in less developed country settings.

Preparation of key field sites

This is linked to the findings of the separate disease burden review compiled as part of this same consultancy, in which it was recommended that field sites be established for GAS disease burden studies in less developed countries. Any of these sites may then be suitable for clinical trials of GAS vaccines. The most critical requirement is that each new vaccine have at least one high-quality efficacy trial using ARF and APSGN as primary endpoints.

Further activities

- The meeting of experts will hopefully identify a list of other priority research questions and other activities for attention.
- In view of the orphan status of GAS vaccine development, there will be an important role for advocacy with funding bodies, industry, GAVI, governments and other organisations.
- There is a clear need for ongoing oversight of GAS vaccine development, to ensure that the needs of the less developed country market are always being catered for.

Introduction

This document will not present a comprehensive overview of GAS vaccine development. A number of reviews have been prepared on this topic, and the reader is referred to the following references. (1-8) Moreover, a chapter about GAS vaccine development co-authored by Drs Good, Cleary, Dale, Fischetti, Fuchs, Savharwal, and Zabriskie will soon be published in a new edition of "New Generation Vaccines", edited by Dr Mike Levine. This will be a valuable reference on this topic. Instead, this review will briefly summarise the current status of efforts to develop a GAS vaccine, and highlight important issues relating to the potential for a vaccine to become available for use in less developed countries.

Antibodies directed against the surface M protein of GAS are able to opsonize and lead to phagocytic clearance of GAS. (9) This antibody response is type-specific; it is directed against the hyper-variable amino terminal region of the streptococcal M protein, and protects against only GAS organisms of the same M serotype. (10, 11) The antibody response has been demonstrated to persist up to 32 years in some individuals, but in others there appears to be no persistent protective response. (12) The persistence of type-specific antibodies is not correlated with the severity of the initial infection or the GAS serotype. (12) These type-specific anti-M protein antibodies have been considered by many to be the basis for passive protective immunity from GAS infection, although others argue that antibodies to the conserved region of the M protein or to GAS carbohydrate may be more important for passive immunity. (13-15)

Because it may be the target of naturally-occurring protective immune responses, confers protection to GAS against complement-mediated phagocytosis, and is considered to be the main virulence factor of GAS, (4, 16, 17) the M protein has also been considered a major candidate for a GAS vaccine. M protein-based approaches fall into two categories: those based on type-specific epitopes present at the amino terminal of the protein, and those based on epitopes conserved among all or most GAS strains, present in the C-repeat region towards the carboxy terminal of the protein.

However, not all vaccine candidates are based on M protein antigens. A number of groups are working with GAS antigens other than M protein for two reasons. The first reason is that these antigens are conserved among all GAS strains, so it may be possible to develop a vaccine against all GAS rather than a limited number of serotypes (the same argument used by those working on conserved M protein antigens). The second relates to the safety of GAS vaccines. The M protein has long been considered to harbour epitopes that cross-react with human tissues and therefore are the basis for the autoimmune response seen in ARF. By avoiding M protein antigens entirely, these groups argue that they avoid the possibility of a vaccine inducing ARF, or sensitising vaccinees to later ARF.

These two issues, vaccine safety and type-specific versus non type-specific protection, require some further attention:

Vaccine safety

A report from 1969 described the administration of a vaccine made of partially purified type 3 M protein to 21 healthy siblings of ARF patients. (18) During follow-up, there were 18 proven GAS infections (none due to type 3),

and following GAS infections two vaccinees developed definite ARF and one developed probable ARF. The authors contrasted this rate with the much lower incidence of 5 cases of ARF following 447 streptococcal infections in unimmunised siblings. This study raised the possibility that a GAS vaccine may have the potential to predispose recipients to developing ARF following subsequent GAS infections. There is considerable doubt that the vaccine played a role in the ARF cases described by Massell, and there have been many other people immunised with M protein-containing GAS vaccines with no other cases of ARF described. Regardless, it is now incumbent on researchers developing GAS vaccines to seek antigens that have minimal chance of inducing immunological cross-reactivity, and to prove the safety of candidate vaccines in as many ways as possible. The US FDA will require extensive in vitro and animal data to prove absence of potential cross-reactivity before sanctioning human studies. During clinical trials, participants will need intensive screening for development of cross-reactive antibodies, and echocardiography to detect the appearance of subclinical carditis.

Type specific versus non-type specific protection

The amino terminal of the M protein is highly variable and frequently undergoes genetic recombination that can lead to loss in opsonizing ability of type-specific antibodies. (19, 20) In populations with high prevalence of GAS infection and carriage, there is very rapid turnover of GAS strains. In one small Australian Aboriginal community of only 250 people, GAS strains belonging to 31 different *emm* types were isolated over a 25-month period, (21) up to 11 different types were present at any one time, and almost all *emm* types present at the start of the study had been replaced by others 2 years later. Over the past 10 years, almost 100 different Vir types (closely linked to *emm* type) have been isolated from Aboriginal people in the Top End of the Northern Territory of Australia (Personal communication, B Currie). Even if a multivalent, type-specific GAS vaccine could be developed that offered protection against such an enormous number of *emm* types, the ecological pressure induced by introduction of the vaccine may promote the emergence of new *emm* types. The ability of the N terminal region of the M protein to vary its antigenic characteristics was demonstrated with *emm* sequence typing of 53 GAS isolates from northern Thailand, which revealed 13 previously uncharacterised *emm* types and a wide variety of point mutations, small deletions, and insertions in the hypervariable region compared to published *emm* sequences. (22) Even in affluent, temperate climate populations there is rapid turnover of GAS serotypes. In one community in the USA, the predominant M serotype causing pharyngitis changed completely within a 12-month period, in the absence of any known precipitant. (23)

A recent study of GAS isolates from Aboriginal Australians gave some hope to the multivalent N-terminal vaccine strategy. Thirty-nine N terminal peptides were raised from GAS isolates endemic in this population, which induced opsonic antibodies in mice after covalent linkage to tetanus toxoid and the addition of complete Freund's adjuvant. (24) Encouragingly, some peptides induced antibodies cross-reactive with up to six other peptides. Although this study raises the possibility that the number of *emm* types effectively covered by a type-specific vaccine may be increased because of immunological cross-reactivity between strains, it also suggests that detailed knowledge of circulating GAS strains will be needed from each population in which the vaccine may be used. It also raises the prospect that individual vaccines may need to be tailored to particular regions, which is not likely to be a practical option for vaccine manufacturers.

GAS vaccines currently in development

The following list is based on literature searches and the author's personal knowledge of GAS vaccine development. Although it may be incomplete, this list covers the GAS vaccine candidates most advanced in development.

M PROTEIN BASED VACCINES

Multivalent, type-specific vaccine

Dr Jim Dale leads this vaccine program, in collaboration with Canadian biotechnology company ID Biomedical. The trade name of the product in development is StreptAvax. The vaccine consists of a sequence of short peptides from the N terminal region of multiple different GAS *emm* type strains in tandem, linked together using unique restriction sites. Earlier versions had a small number of *emm* types represented, but the version presently in phase II trials in Canada consists of peptides from 26 different *emm* types. (3, 25-27) The 26 types represent the most common from US invasive disease and pharyngitis. Detailed sequence analysis of 1064 consecutive invasive GAS isolates received by the Active Bacterial Core Surveillance network in the USA suggested that the 26 types in this vaccine represent 83% of US invasive isolates.(28) The vaccine also includes one conserved epitope; a protective antigen (Spa) that is expressed in several serotypes. (29) The vaccine contains alum adjuvant and in pre-clinical studies in rabbits induced ^{3}4-fold rise in titre for 25 of the 26 serotypes.

The advantage of this vaccine is that it uses a known, highly-immunogenic epitope of the M protein and does not contain any known cross-reactive epitopes. The disadvantages, as outlined above, are that even a 26-valent vaccine may be insufficient to protect against the wide variety of GAS strains in highly endemic populations, and the potential that any protection may be short-lived as new *emm* types emerge that are not included in the vaccine.

Current status: This is the only GAS vaccine currently in human trials. A dose escalating phase I study of the multivalent N terminal vaccine in adult volunteers has been completed at the University of Maryland. (26) Early indications are that the vaccine is well tolerated and immunogenic. A manuscript has been prepared but not yet submitted. The second clinical trial of the multivalent N terminal vaccine is a phase I/II study of the 26-valent construct, and is being conducted in Halifax, Novia Scotia in Canada. (27) The phase I component has enrolled 30 adult volunteers to receive 3 doses of vaccine. The phase II component plans to enrol 400 pre-school children to receive 3 doses of vaccine. The phase I component has been completed and the phase II component is underway.

Minimal conserved epitope in the C-repeat region of the M protein

Professor Michael Good's team in Australia initially identified a 20 amino acid peptide, peptide 145, located within the conserved, C-repeat region of the M protein. (30, 31) A high proportion of people living in areas with high rates of ARF and RHD had evidence of antibody to peptide 145 in their serum. A minimal B cell epitope has subsequently been identified within peptide 145 that does not contain any T cell epitopes. (32) Based on the theory that rheumatic valvulitis is predominantly T-cell mediated, (33-35) this approach should minimise the risk of inducing an autoimmune response. A construct was developed that displays the minimal peptide (both the J14 peptide and its closely related counterpart J8 have been used) in its normal alpha-helical structure. This vaccine

was combined with seven type-specific determinants on a polymer backbone, and induced opsonic antibodies and provided protection against lethal GAS challenge in mice. (36) Recent studies have concentrated on delivering the vaccine as either part of a self-adjuvanting lipid core peptide structure, or conjugated to diphtheria toxoid. (37-42)

Current status: This vaccine has completed pre-clinical evaluation and is now ready for phase I clinical trials (Personal communication, M Good). The major issue now is financial. Professor Good's team have had difficulty securing an industry partner willing to support the clinical evaluation.

A mucosal vaccine based on the conserved region of the M protein

Professor Vince Fischetti's team in New York noted that the relative resistance of adults to GAS pharyngitis could not be attributed to the presence of type-specific antibodies (as few adults have antibodies to multiple M types) but may be associated with the very high prevalence of antibodies to the C-terminal region of pepsin-cleaved M protein. ((14, 15) and unpublished data) Fischetti's team also demonstrated that secretory IgA passively protected mice from GAS infection and death, suggesting that a mucosal vaccine may not need to induce opsonic IgG in order to provide protection. (43) This was further demonstrated when C-region M protein peptides linked to cholera toxin B subunit provided significant protection from colonisation by homologous and heterologous M types of GAS in mice without evoking opsonic antibodies. (10, 14, 15)

After first demonstrating that vaccinia virus could be used as a vector for M protein, (44) this group has successfully expressed a surface-exposed fusion protein containing the C-terminal half of M protein in the human oral commensal, *Streptococcus gordonii*. This organism was able to colonize rabbits for up to 12 weeks, and the animals raised salivary IgA and serum IgG responses to the intact M protein (unpublished data).

The aim of this work is to produce a mucosal vaccine that would be cheap, easily administered, transported and stored in a lyophilised form (thus avoiding the need for a cold chain), and able to be transmitted to others in the population. However, there are two clear issues that may only be resolved if appropriate clinical trials are performed. The first relates to safety. This vaccine uses the entire C-terminal half of the M protein, including the whole C-repeat region. Although most M protein epitopes cross-reactive with human tissue have been found in the A and B-repeat regions (which are not included in this vaccine), there are epitopes in the C repeat region that react with anti-myosin antibody from ARF sera and induce anti-myosin antibodies in mice. (45, 46) Therefore, it will be critical to demonstrate that this vaccine does not induce autoimmunity. Initial studies in rabbits did not reveal any evidence of cross-reactivity with human heart tissue as determined by immunofluorescence assay (unpublished data). The second issue relates to the adequacy of mucosal immunity at providing protection from the diverse range of GAS infections. If, as suggested, this vaccine will not induce high levels of systemic opsonic antibodies, will it provide protection from invasive disease or skin infections? The latter is clearly important in developing countries, where skin infections are a major issue, underlie most cases of APSGN and invasive disease, and may even be linked to ARF pathogenesis (See Appendix 2).

Current status: This vaccine is being produced in collaboration with SIGA Technologies, based in New York. Safety trials of the *S gordonii* vector have been conducted on over 100 adult volunteers. The vector was well tolerated, no adverse events were noted, and the vector could be easily cleared with antibiotic treatment (unpublished data). Phase I human trials of the vaccine strain of *S gordonii* are expected to begin shortly.

NON-M PROTEIN VACCINES

Group A streptococcal carbohydrate

Antibodies to the GAS carbohydrate increase with age and are phagocytic for multiple types of GAS. (13) Professor John Zabriskie's team in New York has demonstrated that these antibodies provide passive protection in a mouse challenge model, and that mice immunised with GAS carbohydrate conjugated to tetanus toxoid were significantly protected from death when rechallenged with GAS strains from three different M serotypes (unpublished data). They have also shown that anti-GAS carbohydrate antibody increases with age and that titres correlate with the presence or absence of GAS colonisation in healthy Mexican children. Because of previous findings that antibodies to GAS carbohydrate may cross-react with human tissue, (47, 48) immunofluorescence and ELISA assays were used to demonstrate that anti-GAS carbohydrate antibodies did not appear to cross-react with frozen tissue from humans with autoimmune GAS diseases (unpublished data). More recently, a different group has synthesised a pentasaccharide corresponding to the terminal fragment of the GAS carbohydrate. (49)

Based on these data, the GAS carbohydrate may be a good vaccine candidate. The GAS carbohydrate is conserved among all GAS strains, so there are no problems with type-specificity.

Current status: It is not clear if sufficient pre-clinical studies have been performed to enable this vaccine to proceed to human trials. The author is also uncertain about the current funding of this research and any involvement of industry.

C5a peptidase

Streptococcal C5a peptidase (SCPA) is a large surface protein expressed by most, if not all, GAS strains. It appears to be an important mediator of the resistance of GAS to phagocytosis, acting to destroy C5a chemotaxin, which is formed by activation of the complement pathway early in the inflammatory response. (50) SCPA also appears to be a mediator of invasion by GAS of epithelial cells. C5ase enzymes are also produced by groups B, C and G streptococci, and SCPB is 95-98% identical in amino acid sequence to SCPA, raising the possibility that a SCPA vaccine may provide cross protection against other beta-haemolytic streptococcal infections. (51, 52) Isogenic GAS strains not expressing SCPA are cleared more rapidly in mouse models of subdermal and nasopharyngeal infection than parents strains expressing SCPA, supporting its importance as an *in vivo* virulence factor. (53, 54) Following intranasal inoculation with unadjuvanted recombinant inactivated SCPA, mice produced strong serum IgA and IgM responses and SCPA-specific IgA in saliva. (55) Immunised animals cleared streptococci more rapidly than controls, and there was cross-protection across 4 different M serotypes. An injectable, adjuvanted formulation of SCPA induced high titres of SCPA-specific serum IgG in immunised mice, who also cleared intranasally-inoculated M type 1 or M type 49 GAS more efficiently than controls (unpublished data).

Current status: SCPA is being developed as a GAS vaccine by Dr Pat Cleary and colleagues. It is the only candidate GAS vaccine presently being developed with a large industry collaborator, Wyeth Lederle Vaccines. The current status of SCPA development as a GAS vaccine – in particular the prospect of human trials in the short term – is not known.

Cysteine protease

The gene for streptococcal cysteine protease, also known as Streptococcal pyrogenic exotoxin B (Spe B), is found in all GAS strains and expressed in most. A Spe B-negative mutant GAS strain was far less pathogenic to

mice than the parent strain, suggesting the role of Spe B as a virulence factor. (56) Professor Jim Musser's team demonstrated that passive immunisation using IgG from rabbits immunised with SpeB, and active immunisation with SpeB, prolonged the time to death in the mouse model, while not actually preventing death. (57)
Current status: Professor Musser has not published on SpeB as a vaccine candidate for a number of years. Therefore, the current status of SpeB vaccine development is not known.

Sfb1

The streptococcal adhesin, fibronectin-binding protein 1 (Sfb1) is being developed as a potential GAS vaccine by Professor Singh Chhatwal's group in Germany. Intranasal immunization of mice with either Sfb1 alone or coupled to cholera toxin B subunit (CTB) resulted in SfbI-specific IgG responses in serum and IgA in lung mucosa. Sfb1 immunised mice were 80-90% protected from death against challenge with homologous or heterlogous GAS strains compared to control mice immunised with CTB alone (90%-100% lethality). Seventy percent of GAS strains expressed Sfb1, and presence of Sfb1 correlated with GAS adherence to human epithelial cells, suggesting that strains expressing Sfb1 may be more likely to cause human upper respiratory infection. (58) Antibodies to Sfb1 did not react with heart or skeletal muscle myosin.

Current status: These results are very encouraging, but this vaccine candidate requires more pre-clinical development. The author is not aware of any involvement of an industry partner with Professor Chhatwal's work.

OTHER POTENTIAL GAS VACCINES

A number of other potential GAS vaccines have been explored in recent years. The author is not aware that any have made substantial progress towards clinical studies. They are presented here for completeness.

The Gram-positive adhesin, lipoteichoic acid (LTA), was administered as a vaccine to mice in combination with cholera toxin B subunit as an adjuvant. The serum anti-LTA IgG and pharyngeal anti-LTA IgA antibody levels of immunised mice were significantly higher than controls, and pre-treatment with pharyngeal washings from immunised mice provided passive protection from GAS adherence. (59)

The fibronectin binding protein, FBP54 is found in most, if not all, GAS strains. Mice immunised with FBP54 induced antigen specific IgG in sera and high levels of salivary IgA. Subcutaneous or oral immunisation with FBP54 resulted in significantly longer survival (but not protection from death) following GAS challenge than in unimmunised mice. (60)

A synthetic peptide containing 20 amino acid residues of the streptolysin S toxin induced antibodies in rabbits that could neutralise activity of the toxin. This peptide did not evoke opsonic antibodies, but enhanced phagocytosis induced by specific anti-M protein antibodies. (61) Therefore it has potential as a component of a multivalent, type-specific vaccine.

Mutant variants of Streptococcal pyrogenic exotoxins A (Spe A) and C (Spe C) were non-superantigenic and evoked protective antibody responses in rabbit models of streptococcal toxic shock syndrome (STSS) (62, 63) These toxoids may have a role in protection from toxin effects in STSS, but are unlikely to have roles as wider GAS vaccines.

Whole cell vaccines have not been used frequently. One attempt involved constructing an M-negative mutant strain of an M28 parent isolate. (64) This vaccine offered protection from death in mice challenged with GAS strains belonging to three different M types. Another group immunised mice with heat-killed M50 or M55 GAS, or an M12 strain deficient in M-protein. (65) The mice were 82% to 88% protected against death following intranasal challenge with M50 GAS.

Practical issues in GAS vaccine development

GAS VACCINES ARE ORPHAN VACCINES

One reason for the slow progress in GAS vaccine development is that these vaccines have not been wholeheartedly embraced either by industry or the larger government and non-government research funding bodies. The reality is that this is an orphan vaccine program, but some groups do not support this contention. For example, an approach to the Bill and Melinda Gates Foundation two years ago brought the response that industry was already working on a GAS vaccine because GAS is a problem in affluent communities, so external funding was not necessary. As stated above, only one prospective GAS vaccine candidate is being developed with a large industry partner, and the current status of that vaccine is not known.

The major market for a GAS vaccine from industry's perspective is for prevention of GAS pharyngitis in children (and perhaps also for prevention of invasive disease) in more developed countries, particularly North America and Europe. For example, the SIGA Technologies website states that they are developing "a mucosal vaccine for strep throat" (see http://www.siga.com/corporate.html). As outlined in the companion document to this one, the global burden of GAS diseases is overwhelmingly concentrated in less developed countries. The epidemiology of these diseases is very different between less developed and more developed countries. In the former, the total burden of GAS infection is high, the turnover of strains is rapid, the major GAS diseases differ to those in more developed countries, and the most important GAS strains also differ (particularly in that skin-associated strains dominate the epidemiology in many tropical less developed regions). Therefore, it cannot be assumed that a vaccine that protects children living in affluent, temperate climate countries from GAS pharyngitis will also work in a tropical less developed country to prevent ARF, APSGN, invasive disease or skin infection.

DO WE HAVE THE RIGHT VACCINE(S) AVAILABLE AMONG THE CURRENT CANDIDATES?

This will not be known until the appropriate clinical trials are performed. The most advanced candidate – the multivalent type-specific vaccine – is not likely to be useful in many less developed countries for reasons outlined above. However, there is the potential to combine different antigens. For example, a combination of the conserved minimal B cell epitope with seven type-specific epitopes resulted in good immunogenicity and protection in mice. (66) Combining vaccine antigens will require high levels of cooperation between groups that may be in competition for similar markets.

Until there is a GAS vaccine of proven efficacy for the range of important GAS diseases, there will be room for new vaccine candidates. The sequencing of the GAS genome has made it possible to identify new candidate antigens. A number of these have already been identified and are being further investigated. (67-69)

WILL A GAS VACCINE EVER BE AFFORDABLE IN LESS DEVELOPED COUNTRIES?

The extremely high cost of conjugate pneumococcal vaccine (approx US $50 per dose) makes it unaffordable for all but the wealthiest of countries. It is yet to be seen if the pattern with that vaccine will follow the Hepatitis B vaccine example (where the initial high cost reduced over the ensuing decade to make it affordable by most

countries) or even *Haemophilus influenzae* type b vaccine (where the cost continues to reduce, although not yet to levels affordable by the poorest countries). Some of the technologies required to develop GAS vaccines are expensive, but others are not (e.g. the *S gordonii* vector expressing an M fusion protein).

PARALLELS WITH CONJUGATE PNEUMOCOCCAL VACCINE DEVELOPMENT

The situation with GAS vaccines has some similarities with these faced several years ago when conjugate pneumococcal vaccines were being developed. Industry wanted to produce a vaccine primarily aimed at more developed countries. However, groups including WHO and NIH became involved in the process, and influenced industry to include serotypes important in less developed countries. Moreover, these non-industry groups also worked with industry to establish phase III trials in less developed countries, so that the efficacy of conjugate pneumococcal vaccines against important endpoints for the majority of the world's children could be assessed. This approach acknowledged the different epidemiology of pneumococcal diseases in affluent and poor communities. Ongoing involvement of the Global Alliance for Vaccines and Immunization is ensuring that further questions specific to the use of pneumococcal vaccines in less developed countries are answered.

Conjugate pneumococcal vaccines could not be considered orphan vaccines (at least four major companies have conducted clinical trials of these vaccines). Yet key international organizations recognised the importance of taking an active role in the vaccine development process in collaboration with industry, so that the right vaccine was produced for the people who bear the major global burden of disease, so that sufficient information would become available to allow decision makers in less developed countries to make choices about vaccine introduction, and to try to make the vaccine affordable for less developed countries.

Priorities in developing a GAS vaccine for the world

- That vaccines are developed in the specific knowledge that they will prevent GAS diseases in less developed countries. Such vaccines are almost certain to be effective in preventing GAS diseases in more developed countries. The opposite may not be true (i.e. vaccines for more developed countries may not be effective at preventing GAS diseases in less developed countries).
  - This requires complete consideration of the epidemiology, immunology and pathogenesis of GAS diseases in all settings, including specifically in less developed countries.
- That any GAS vaccine be evaluated for its protective efficacy against ARF, APSGN and skin infection, and that safety in less developed country settings also be evaluated.
  - This requires developing the field sites now in less developed countries where such studies can take place in the near future.
- That consideration be given now to the likely availability and affordability by less developed countries of a GAS vaccine.
- That preparations are needed now to compile the regional disease burden data that countries will require when making decisions about introducing GAS vaccines.
  - This is addressed in the disease burden document prepared separately as part of this consultancy.

A WHO action plan for GAS vaccine development

COLLABORATION AND COORDINATION

Presently each research group operates independently, without much evidence of collaboration between groups. The progress of a number of the candidate vaccines is not known. WHO, NIH, and GAVI are the 3 organisations best placed to influence the process.

A MEETING OF EXPERTS

There is a good argument for bringing together the global expertise in GAS vaccines, epidemiology and control, and external expertise in vaccine development in general. The aims of such a meeting would be to:

- provide updates on the current status of each candidate vaccine.
- identify obstacles that each group is facing.
- clarify the target groups for each vaccine; i.e. what disease(s) is the vaccine being primarily aimed at preventing, at what age will it be administered, and in what geographic region(s)?
- discuss the likelihood of each vaccine being efficacious, affordable and available for the prevention of the most important GAS diseases in developing countries (i.e. ARF, APSGN, invasive disease).
- determine the level of planning for downstream clinical trials of each vaccine; i.e. what immunological, safety and disease endpoints will be used, and in what population(s)?
- conduct a global discussion about GAS vaccines, including target groups for vaccination, obstacles and downstream clinical trials.
- clarify the regulatory and licensing issues involved for GAS vaccines.
- determine whether current understanding of immunological correlates of protection is sufficient, and if appropriate animal models of GAS diseases exist.
- consider if new vaccine antigens, adjuvants or modes of antigen presentation may be needed.
- discuss possible collaborations between research groups, including combinations of different vaccine antigens, adjuvants and/or modes of antigen presentation.
- determine the role and interest of the pharmaceutical industry in GAS vaccines.
- discuss possible ways in which groups like WHO, GAVI, NIH and others may improve the likelihood that a GAS vaccine may become available that is of proven efficacy against the major GAS diseases in less developed country settings.

PREPARATION OF KEY FIELD SITES

This is linked to the findings of the separate disease burden review compiled as part of this same consultancy, in which it was recommended that field sites be established for GAS disease burden studies in less developed countries. Any of these sites may then be suitable for clinical trials of GAS vaccines. The most critical requirement is that each new vaccine have at least one high-quality efficacy trial using ARF and APSGN as primary endpoints.

FURTHER ACTIVITIES

- The meeting of experts will hopefully identify a list of other priority research questions and other activities for attention.
- In view of the orphan status of GAS vaccine development, there will be an important role for advocacy with funding bodies, industry, GAVI, governments and other organisations.
- There is a clear need for ongoing oversight of GAS vaccine development, to ensure that the needs of the less developed country market are always being catered for.

References

1. Medina E, Chhatwal GS. The potential for vaccine development against rheumatic fever. Indian Heart J 2002;54:93-8.
2. Good MF. Progress towards developing a vaccine for group A streptococcus based on the M protein. Intern Med J 2002;32:132-3.
3. Dale JB. Multivalent group A streptococcal vaccines. In: Stevens DL, Kaplan EL, eds. Streptococcal infections: clinical aspects, microbiology and molecular pathogenesis. New York: Oxford University Press, 2000:390-433.
4. Fischetti VA. Protection against group A streptococcal infection. In: Stevens DL, Kaplan EL, eds. Streptococcal infections: clinical aspects, microbiology and molecular pathogenesis. New York: Oxford University Press, 2000:371-389.
5. Brandt ER, Good MF. Vaccine strategies to prevent rheumatic fever. Immunol Res 1999;19:89-103.
6. Dale JB. Group A streptococcal vaccines. Infect Dis Clin North Am 1999;13:227-43, viii.
7. Dale JB. Group A streptococcal vaccines. Pediatr Ann 1998;27:301-8.
8. Dale JB, Cleary PP, Fischetti VA, Kasper DL, Musser JM, Zabriskie JB. Group A and group B streptococcal vaccine development. A round table presentation. Adv Exp Med Biol 1997;418:863-8.
9. Lancefield RC. The antigenic complex of *Streptococcus hemolyticus*. I. Demonstration of a type-specific substance in extracts of *Streptococcus hemolyticus*. J Exp Med 1928;47:91-103.
10. Jones KF, Fischetti VA. The importance of the location of antibody binding on the M6 protein for opsonization and phagocytosis of group A M6 streptococci. J Exp Med 1988;167:1114-1123.
11. Lancefield RC. Current knowledge of the type specific M antigens of group A streptococci. J Immunol 1962;89:307-13.
12. Lancefield RC. Persistence of type-specific antibodies in man following infection with group A streptococci. J Exper Med 1959;110:271-292.
13. Salvadori LG, Blake MS, McCarty M, Tai JY, Zabriskie JB. Group A streptococcus-liposome ELISA antibody titers to group A polysaccharide and opsonophagocytic capabilities of the antibodies. J Infect Dis 1995;171:593-600.
14. 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 1988;56:2666-72.
15. 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 1990;145:1251-6.
16. Fischetti VA. Streptococcal M protein: molecular design and biological behavior. Clin.Microbiol.Rev. 1989;2:285-314.

17. Cunningham MW. Pathogenesis of group A streptococcal infections. Clin.Microbiol.Rev. 2000;13:470-511.

18. Massell BF, Honikman LH, Amezcua J. Rheumatic fever following streptococcal vaccination: report of three cases. JAMA 1969;207:1115-1119.

19. Fischetti VA, Jones KF, Scott JR. Size variation of the M protein in group A streptococci. J Exp Med 1985;161:1384-1401.

20. Jones KF, Hollingshead SK, Scott JR, Fischetti VA. Spontaneous M6 protein size mutants of group A streptococci display variation in antigenic and opsonogenic epitopes. Proc Natl Acad Sci U S A 1988;85:8271-8275.

21. 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 2000;182:1109-16.

22. Pruksakorn S, Sittisombut N, Phornphutkul C, Pruksachatkunakorn C, Good MF, Brandt E. Epidemiological analysis of non-M-typeable group A Streptococcus isolates from a Thai population in northern Thailand. J Clin Microbiol 2000;38:1250-4.

23. Kaplan EL, Wotton JT, Johnson DR. Dynamic epidemiology of group A streptococcal serotypes associated with pharyngitis. Lancet 2001;358:1334-7.

24. Brandt ER, Teh T, Relf WA, Hobb RI, Good MF. Protective and nonprotective epitopes from amino termini of M proteins from Australian aboriginal isolates and reference strains of group A streptococci. Infect Immun 2000;68:6587-94.

25. Dale JB, Chiang EY, Lederer JW. Recombinant tetravalent group A streptococcal M protein vaccine. J Immunol 1993;151:2188-94.

26. Dale JB. Multivalent group A streptococcal vaccine designed to optimize the immunogenicity of six tandem M protein fragments. Vaccine 1999;17:193-200.

27. Hu MC, Walls MA, Stroop SD, Reddish MA, Beall B, Dale JB. Immunogenicity of a 26-valent group A streptococcal vaccine. Infect Immun 2002;70:2171-7.

28. Li Z, Sakota V, Jackson D, Franklin AR, Beall B. Array of M protein gene subtypes in 1064 recent invasive group A streptococcus isolates recovered from the active bacterial core surveillance. J Infect Dis 2003;188:1587-92.

29. Dale JB, Chiang EY, Liu S, Courtney HS, Hasty DL. New protective antigen of group A streptococci. J Clin Invest 1999;103:1261-8.

30. 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 1994;344:639-42.

31. 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 1996;89:331-7.

32. 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. 1997;9:1723-1733.

33. Quinn A, Kosanke S, Fischetti VA, Factor S, Cunningham MW. Induction of autoimmune valvular heart disease by recombinant streptococcal M protein. Infect Immun 2001;69:4072-4078.

34. Guilherme L, Oshiro SE, Fae KC, et al. T-cell reactivity against streptococcal antigens in the periphery mirrors reactivity of heart-infiltrating T lymphocytes in rheumatic heart disease patients. Infect Immun 2001;69:5345-5351.

35. Kemeny E, Grieve T, Marcus R, Sareli P, Zabriskie JB. Identification of mononuclear cells and T cell subsets in rheumatic valvulitis. Clin Immunol Immunopathol 1989;52:225-237.

36. 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. 2000;6:455-459.

37. Hayman WA, Toth I, Flinn N, Scanlon M, Good MF. Enhancing the immunogenicity and modulating the fine epitope recognition of antisera to a helical group A streptococcal peptide vaccine candidate from the M protein using lipid-core peptide technology. Immunol Cell Biol 2002;80:178-87.

38. Horvath A, Olive C, Wong A, et al. Lipoamino acid-based adjuvant carrier system: enhanced immunogenicity of group a streptococcal peptide epitopes. J Med Chem 2002;45:1387-90.

39. Olive C, Clair T, Yarwood P, Good MF. Protection of mice from group A streptococcal infection by intranasal immunisation with a peptide vaccine that contains a conserved M protein B cell epitope and lacks a T cell autoepitope. Vaccine 2002;20:2816-25.

40. Olive C, Batzloff MR, Horvath A, et al. A lipid core peptide construct containing a conserved region determinant of the group A streptococcal M protein elicits heterologous opsonic antibodies. Infect Immun 2002;70:2734-8.

41. Batzloff MR, Hayman WA, Davies MR, et al. Protection against group A streptococcus by immunization with J8-diphtheria toxoid: contribution of J8- and diphtheria toxoid-specific antibodies to protection. J Infect Dis 2003;187:1598-608.

42. Olive C, Batzloff M, Horvath A, et al. Potential of lipid core peptide technology as a novel self-adjuvanting vaccine delivery system for multiple different synthetic peptide immunogens. Infect Immun 2003;71:2373-83.

43. Bessen D, Fischetti VA. Passive acquired mucosal immunity to group A streptococci by secretory immunoglobulin A. J Exp Med 1988;167:1945-1950.

44. Hruby DE, Hodges WM, Wilson EM, Franke CA, Fischetti VA. Expression of streptococcal M protein in mammalian cells. Proc.Natl.Acad.Sci.U.S.A 1988;85:5714-5717.

45. Quinn A, Kent W, Fischetti VA, Hemric M, Cunningham MW. Immunological relationship between the class I epitope of streptococcal M protein and myosin. Infect Immun 1998;66:4418-4424.

46. Cunningham MW, Antone SM, Smart M, Liu R, Kosanke S. Molecular analysis of human cardiac myosin-cross-reactive B- and T-cell epitopes of the group A streptococcal M5 protein. Infect Immun 1997;65:3913-3923.

47. Cunningham MW, McCormack JM, Talaber LR, et al. Human monoclonal antibodies reactive with antigens of the group A streptococcus and human heart. J Immunol 1988;141:2760-2766.

48. Dudding BA, Ayoub EM. Persistence of streptococcal group A antibody in patients with rheumatic valvular heart disease. J Exp Med 1968;128:1081-1098.

49. Hoog C, Rotondo A, Johnston BD, Pinto BM. Synthesis and conformational analysis of a pentasaccharide corresponding to the cell-wall polysaccharide of the Group A Streptococcus. Carbohydr Res 2002;337:2023-36.

50. Wexler D, Chenoweth ED, Cleary P. A streptococcal inactivator of chemotaxis: a new virulence factor specific to group A streptococci. Proc Natl Acad Sci U S A 1985;82:179-180.

51. Chmouryguina I, Suvorov A, Ferrieri P, Cleary PP. Conservation of the C5a peptidase genes in group A and B streptococci. Infect Immun 1996;64:2387-2390.

52. Cleary PP, Peterson J, Nelson C. Virulent human strains of group G streptococci express a C5a peptidase enzyme similar to that produced by group A streptococci. Infect Immun 1991;59:2305-2310.

53. Ji Y, McLandsborough L, Kondagunta A, Cleary PP. C5a peptidase alters clearance and trafficking of group A streptococci by infected mice. Infect Immun 1996;64:503-510.

54. Ji Y, Schnitzler N, De Master D, Cleary PP. Impact of M49, Mrp, Enn, and C5a peptidase proteins on colonization of the mouse oral mucosa by *Streptococcus pyogenes*. Infect Immun 1998;66:5399-5405.

55. Ji Y, Carlson B, Kondagunta A, Cleary PP. Intranasal immunization with C5a peptidase prevents nasopharyngeal colonization of mice by the group A Streptococcus. Infect Immun 1997;65:2080-7.

56. Lukomski S, Burns EH, Jr., Wyde PR, et al. Genetic inactivation of an extracellular cysteine protease (SpeB) expressed by Streptococcus pyogenes decreases resistance to phagocytosis and dissemination to organs. Infect Immun 1998;66:771-776.

57. Kapur V, Maffei JT, Greer RS, Li LL, Adams GJ, Musser JM. Vaccination with streptococcal extracellular cysteine protease (interleukin-1 beta convertase) protects mice against challenge with heterologous group A streptococci. Microb Pathog 1994;16:443-50.

58. Valentin-Weigand P, Talay SR, Kaufhold A, Timmis KN, Chhatwal GS. The fibronectin binding domain of the Sfb protein adhesin of Streptococcus pyogenes occurs in many group A streptococci and does not cross-react with heart myosin. Microb Pathog 1994;17:111-20.

59. Yokoyama Y, Harabuchi Y. Intranasal immunization with lipoteichoic acid and cholera toxin evokes specific pharyngeal IgA and systemic IgG responses and inhibits streptococcal adherence to pharyngeal epithelial cells in mice. Int J Pediatr Otorhinolaryngol 2002;63:235-41.

60. Kawabata S, Kunitomo E, Terao Y, et al. Systemic and mucosal immunizations with fibronectin-binding protein FBP54 induce protective immune responses against Streptococcus pyogenes challenge in mice. Infect Immun 2001;69:924-30.

61. Dale JB, Chiang EY, Hasty DL, Courtney HS. Antibodies against a synthetic peptide of SagA neutralize the cytolytic activity of streptolysin S from group A streptococci. Infect Immun 2002;70:2166-70.

62. Roggiani M, Stoehr JA, Olmsted SB, et al. Toxoids of streptococcal pyrogenic exotoxin A are protective in rabbit models of streptococcal toxic shock syndrome. Infect Immun 2000;68:5011-7.

63. McCormick JK, Tripp TJ, Olmsted SB, et al. Development of streptococcal pyrogenic exotoxin C vaccine toxoids that are protective in the rabbit model of toxic shock syndrome. J Immunol 2000;165:2306-12.

64. Chappell JD, Stuart JG. Demonstration of protection in mice from a lethal challenge of three M serotypes of Streptococcus pyogenes using an M-negative vaccine. Vaccine 1993;11:643-8.

65. Stjernquist-Desatnik A, Kurl DN, Schalen C, Christensen P. Protective effect of heterologous gram-positive vaccine against lethal upper respiratory tract infection with type M50 group A streptococci in mice. Vaccine 1990;8:150-2.

66. 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 2000;6:455-9.

67. Graham MR, Smoot LM, Lei B, Musser JM. Toward a genome-scale understanding of group A Streptococcus pathogenesis. Curr.Opin.Microbiol. 2001;4:65-70.

68. Reid SD, Green NM, Sylva GL, et al. Postgenomic analysis of four novel antigens of group a streptococcus: growth phase-dependent gene transcription and human serologic response. J Bacteriol 2002;184:6316-24.

69. Voyich JM, Sturdevant DE, Braughton KR, et al. Genome-wide protective response used by group A Streptococcus to evade destruction by human polymorphonuclear leukocytes. Proc Natl Acad Sci U S A 2003;100:1996-2001.