HUMAN VACCINES & IMMUNOTHERAPEUTICS
2018, VOL. 14, NO. 8, 2034–2052
https://doi.org/10.1080/21645515.2018.1462427

Taylor & Francis
Taylor & Francis Group

REVIEW

OPEN ACCESS Check for updates

Contribution of cryptic epitopes in designing a group A streptococcal vaccine

Victoria Ozberk, Manisha Pandey*, and Michael F. Good*

Griffith University, Institute for Glycomics, Gold Coast Campus, Queensland, Australia

ABSTRACT

A successful vaccine needs to target multiple strains of an organism. *Streptococcus pyogenes* is an organism that utilizes antigenic strain variation as a successful defence mechanism to circumvent the host immune response. Despite numerous efforts, there is currently no vaccine available for this organism. Here we review and discuss the significant obstacles to vaccine development, with a focus on how cryptic epitopes may provide a strategy to circumvent the obstacles of antigenic variation.

ARTICLE HISTORY
Received 21 September 2017
Revised 22 March 2018
Accepted 3 April 2018

KEYWORDS
Streptococcus pyogenes; group A streptococcus (GAS); cryptic epitopes; vaccine; J8-DT; J14; SpyCEP; S2; skin infection; bacteraemia; liposomes; URT (upper respiratory tract); intranasal

Vaccines are amongst the greatest medical achievements of modern civilisation. Today, over 70 vaccines have been licenced to prevent infection with approximately 30 different organisms.[1,2] Vaccine development has largely focused on the concepts of live attenuated, sub-unit and whole-cell vaccine designs. Of these one-third are sub-unit vaccines that contain highly immunogenic immunodominant antigens capable of producing antibodies to a single-strain of an organism.[2,3] However, for several infectious diseases this approach is ineffective or is associated with major disadvantages. The challenge is that many organisms are antigenically variable and due to their diversity, polyvalent vaccines have been developed. Examples include vaccines for *Streptococcus pneumoniae* and Human Papilloma Virus.[3]

Streptococcus pyogenes (group A streptococcus, GAS) is an important human pathogen for which vaccines are not yet available. For this organism, antigenic diversity is extensive and this challenges even a multivalent vaccine approach. An alternative approach is to use cryptic epitopes because these are poorly immunogenic in the native organism and they are thus not under immune selection pressure.[3] Although they may not be recognised as a result of natural infection,[4] they can be highly immunogenic when presented in isolation such as a peptide or a recombinant polypeptide fragment. Furthermore, because they are conserved they may be able to induce strain-transcending immunity. Cryptic epitopes can thus be exploited in vaccine development. Despite their recognised potential there is a paucity of literature on the description and utilisation of cryptic epitopes as vaccine candidates. Here, we provide an in-depth review on the development and potential use of two separate cryptic epitopes in a vaccine to prevent infection with GAS.

GAS is a Gram-positive organism that primarily infects the upper respiratory tract (URT) and the skin.[5,6] It is responsible for a wide array of infections ranging from superficial infections such as streptococcal pharyngitis and pyoderma to invasive necrotising fasciitis. The 'post-streptococcal' sequelae of rheumatic fever (RF)/rheumatic heart disease (RHD) and post-streptococcal glomerulonephritis are also of major concern. GAS infections and their sequelae are responsible for more than 500,000 deaths each year.[5] In 2015 there was an estimated 319 400 deaths due to RHD.[7]

Immunopathogenesis and obstacles in GAS vaccine development

Development of auto-reactive B and T-cells

Infection with GAS can lead to acute rheumatic fever (ARF), which predominantly affects people living in resource-poor settings. Subsequent streptococcal throat infections can cause recurrent ARF. Single or repeated episodes of ARF can result in RHD.[8] The genetic susceptibility to RF/RHD is associated with Class II MHC molecules (HLA-DR, DQ and DP) that present peptides from extracellular pathogens to $CD4^+$ T-cells.[9] These include HLA-DRB1, HLA-DRB4, HLA-DQA1 and HLA-DQB1.[10] The aetiology of the disease is not well understood but has been defined as an autoimmune illness (see below).[11] The streptococcal M-protein shares an alpha-helical coiled-coil structure and antigenic cross-reactivity with cardiac myosin. This

CONTACT; Manisha Pandey m.pandey@griffith.edu.au Institute for Glycomics, Griffith University, Glycomics 1 (G26), 1 Parklands Drive, Southport QLD 4215, Australia; Michael F. Good michael.good@griffith.edu.au Glycomics, Griffith University, Glycomics 1 (G26), 1 Parklands Drive, Southport QLD 4215, Australia.

*These authors contributed equally to this study.

© 2018 Griffith University. Published with license by Taylor & Francis
This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives License (http://creativecommons.org/licenses/by-nc-nd/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited, and is not altered, transformed, or built upon in any way.

phenomenon was first described by Kaplan,[12] and Zabriskie[13] and Meyeserian[12] as antibody cross-reactivity. This is a significant hindrance to GAS vaccine development where it is critical that a vaccine does not induce auto-reactive B- and T-cell responses. The evidence that an autoimmune pathogenic process might involve the M-protein was highlighted in an early study in 1969 where 21 children were vaccinated with type-3 streptococcal M-protein. Children received up to 33 injections of partially purified M-protein at doses of up to 1 mg per injection. Following vaccination, although these children developed 18 GAS infections (tonsillitis/pharyngitis), none were type-3 GAS infections. However, two of these infections were followed by RF and one by probable RF.[14] In other studies where subjects were immunized with three doses of M-protein there were no reported serious adverse events.[15,16] In 1979, the US Food and Drug Administration prohibited the development of a GAS vaccine after considering the findings of the independent advisory panel "Review of Bacterial Vaccines and Bacterial Antigens". The prohibition remained for nearly 30 years and was lifted in 2006 when subunit vaccines were being developed.[17]

The immunopathogenesis of group A streptococcal disease has been studied and the autoimmune potential of the M-protein has been identified in a number of previous studies reviewed extensively by Cunningham.[18,19] The B1B2/B2/B3A regions of the M-protein were found to contain myosin-cross reactive epitopes, with B2 peptide having 42% identity with cardiac myosin and B1A inducing myocardial lesions.[20] Therefore, the B-repeat region of the M-protein has been excluded in GAS vaccine development. Additionally, studies have also shown cross-reactivity between the C-repeat region and cardiac and skeletal myosin,[20-22] thus strengthening the case for the development of minimal subunit vaccines where host cross-reactive epitopes can be eliminated to reduce the risk of ARF and RHD.

M-protein sequence variation is associated with rheumatogenic GAS strains associated with the development of ARF. Examples include M-types 5 (M5) and 6 (M6). Immunization with M6 protein was shown to induce valvulitis and myocarditis in a Lewis rat model with both CD4+ and CD8+ T-cells detected in valvular lesions.[23] Additionally, immunization with human cardiac myosin generated T-cells that recognized the M5 protein.[24] Furthermore, passive transfer of M-protein A-repeat region-specific T-cells into naïve rats produced valvulitis providing further evidence that M-protein-specific T-cells may be key mediators in valvular heart disease.[25] M-types 1, 3, 5, 6, 14, 18, 19, 24, 27 and 29 have been previously associated with ARF.[26-31] The relationship between rheumatogenic GAS strains and acute pharyngitis was evaluated in an epidemiological study in the United States. A decrease in the prevalence of ARF was associated with a significant reduction in the proportion of cases of acute streptococcal pharyngitis in children caused by rheumatogenic GAS types.[30] In a more recent study, it was found that GAS strains belonging to *emm* pattern D (skin pattern) contributed to 49% of ARF-associated GAS strains, thus also suggesting a role of skin infection in the development of ARF.[32]

Antigenic strain variation with GAS and the need for repeat exposure to induce immune memory

A major hindrance to subunit vaccine development is the vast sequence diversity of the virulence factor, the M-protein. Strain-specific immunity is a result of the development of antibodies to the immunodominant amino-terminal epitopes on this protein.[27] The M-protein is encoded by the *emm* gene. There are over 200 distinct strains based on the serological M-types and more than 230 *emm* types have been identified using *emm* typing,[33,34] the gold standard molecular typing method that is based on the 5′-end 150 nucleotides of the *emm* gene.[35]

Early studies by Kuttner and Lenert[36] revealed the presence of type-specific antibodies in children recovering from streptococcal pharyngitis. A follow-up study found that type-specific antibodies from adults recovering from GAS infection in the URT were able to bind to homologous heat-killed streptococci but not strains of heterologous types.[37] In another study, type-specific antibodies were shown to reduce the risk of homologous pharyngeal infections.[38] Further studies by Lancefield reported that human antisera to types 3, 6 and 13 protected mice against homologous challenge with GAS to an extent roughly proportional to the antibody concentration detected in sera.[39] This supported the notion that M-protein-specific antibodies, post-pharyngeal infection with GAS, persist for extended periods of time, and confer homologous strain-specific immunity.

However, there is very little knowledge on the acquisition of immunity following GAS skin infection. We used a number of epidemiologically distinct GAS strains to model the development of acquired immunity to pyoderma and demonstrated that infection leads to antibody responses to the serotype-specific determinants on the M-protein and short-lived protective immunity to homologous strains. Memory B-cells do not develop after a single infection and immunity is rapidly lost.[4] Similarly, sequential infections with different strains resulted in short-lived immunity only to the last strain to which the mice had been exposed and not to any previous strains. However, two sequential infections with the same strain within a short time frame did induce enduring strain-specific immunity. Along with antigenic-diversity, if the requirement for multiple consecutive exposures to each serotype of GAS to induce a memory response also occurs in humans, then this represents a further serious impediment to the development of immunity to GAS. The need for multiple infections to induce immunological memory to a given strain begs the question of whether natural infection post-vaccination will be able to boost and maintain memory. This is a critical question for all vaccine candidates. Mice exposed to multiple strains, either sequentially or simultaneously, did not develop antibodies to a conserved M-protein vaccine peptide, J8, demonstrating that this epitope is cryptic to the immune system.[4] However, we have recently shown that skin infection can boost J8-induced immunity and furthermore that the infection serves to broaden the nature of immunity by engaging other antigens such as SpyCEP.[40]

GAS vaccine development

GAS vaccine development is divided into M-protein and non-M-protein-based approaches.[41] M-protein-based vaccines include fused recombinant peptides from the N-terminal region of the M-protein from multiple *emm* types of GAS (6-, 26- and 30-valent vaccines),[42-45] antigens from the conserved C-repeat region of the M-protein, StreptInCor (containing

selected T and B-cell epitopes),[46] SV1 (containing five 14-mer amino-acid sequences from differing C-repeat region)[47] and J8/J14, a cryptic epitope-based vaccine approach (containing a single B-cell epitope from the C3 repeat region).[48] Figure 1 represents a schematic of the M-protein with the location and targets of M-protein-based vaccines in development. The non-M-protein-based vaccines include virulence factors such as SpyCEP[49] and C5a peptidase,[50] and group carbohydrates.[51,52] A comprehensive discussion of M-protein and non-M-protein GAS vaccines is summarized in Table 1.

M-protein-based vaccines

To take advantage of the type-specific opsonic antibodies associated with the amino (N)-terminal region of the M-protein a multivalent M-protein vaccine was designed. The hexa-valent vaccine consisting of N-terminal subunits from 24, 5, 6, 19, 1 and 3 M-protein peptides was found to be immunogenic against all six M-protein peptides and no cross-reactivity between immune sera and human heart tissue was observed.[42,44] However, this vaccinate candidate was constrained by type-specific protection.[42,44] Therefore, the vaccine was advanced to a 26-valent N-terminal vaccine (StreptAvax), consisting of 26 N-terminal subunits from North American GAS isolates.[53] Although StreptAvax was shown to cross-opsonize non-vaccine M-types, it offered limited theoretical coverage against strains in many developing countries.[45,53] The 26 *emm* types present in the vaccine accounted for only 65% of all isolates in Africa, Asia, Middle-East and Pacific region, with the theoretical coverage of the vaccine in Africa being estimated to be 39% and in the Pacific region, 23.9%.[54] Regardless, this is the most advanced GAS vaccine candidate with the successful completion of a Phase II clinical trial.[55] The vaccine has since been refined to a 30-valent vaccine consisting of 30 N-terminal subunits from North America and Europe. The serotypes included in the vaccine account for 98% of all cases of pharyngitis in the United States and Canada, 90% of invasive disease cases in the United States and 78% of invasive disease cases in Europe.[56] The vaccine was shown to induce antibodies in rabbits against 24 of 40 non-vaccine serotypes.[43,57] Recently, these observations have led to the designing of M-protein-based vaccines utilizing an *emm* cluster-typing system in combination with computational structure-based peptide modelling. The preliminary data are promising, however, further investigations are required to confirm the feasibility of this approach.[58]

To elicit a broader range of protection, vaccine candidates targeting the conserved C-terminal region of the M-protein have been developed. StreptInCor, comprising 55 amino-acid residues from the C2 and C3 conserved regions of the M5 protein was shown to be protective in BALB/c[59], HLA class II transgenic mice[60] and SWISS mice.[61] Protective efficacy was demonstrated against M1, M5, M12, M22 and M87 GAS strains.[62] No autoimmune pathology was observed in heart or other organs[60] and an epidemiological study of Brazilian GAS isolates predicted the protective coverage to be 71%.[62] Another C-terminal vaccine candidate in development is SV1, consisting of five 14-mer amino-acid sequences ($J14_i$ variants) from differing C-repeat regions combined in a single recombinant construct. Unlike the J8-DT vaccine candidate, SV1 maintains alpha-helical structure without the need for additional flanking

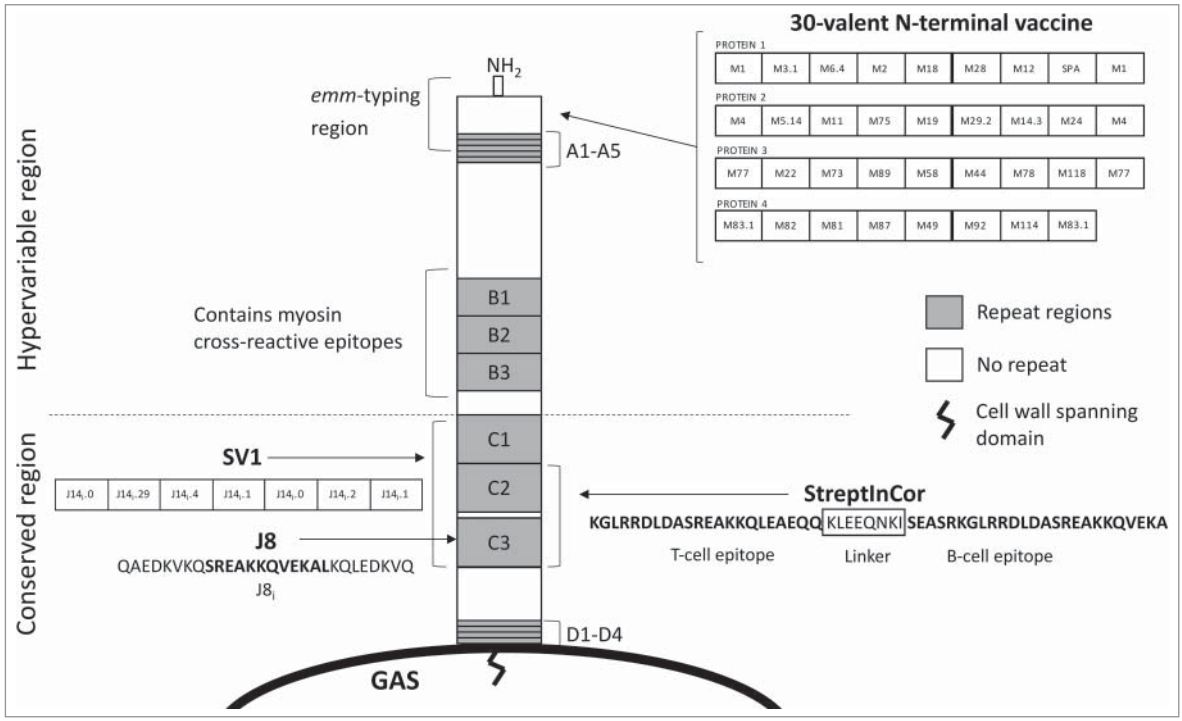

Figure 1. Idealized schematic illustrating M-protein based vaccine targets. The amino-terminal region: 30-valent N-terminal vaccine consisting of four different multivalent fusion proteins (containing eight or nine M-protein fragments)[42]; The B-repeat region: representing defined myosin cross-reactive epitopes[20]; The C1-C3 repeat regions: SV1 vaccine consisting of five 14-mer amino-acid sequences ($J14_i$ variants) combined in a single recombinant construct[46]; The C2-C3 repeat regions: StreptInCor vaccine containing immunodominant T (22 amino- acids) and B-cell (25 amino-acids) epitopes (**bold residues**) linked by eight amino-acid residues ([] boxed residues)[58]; The C3 repeat region: Minimal B-cell cryptic epitope within p145 defined as J8, **bold** residues are those contained within M-protein ($J8_i$), residues not in bold are from GCN4 protein (not from M-protein).[90]

Table 1. Status of M-protein and non-M-protein-based GAS vaccines.

Name	Composition	Advantages	Disadvantages	Status	Ref.
M-protein-based vaccines					
26-valent N-terminal (StreptAvax)	N-terminal subunits from 26 serotypes of GAS. Four different recombinant proteins (containing six or seven M-protein fragments linked in tandem) formulated with Alum	– Does not require carrier protein[53] – Ability to cross-opsonize M-types not included in vaccine[53] – No cross-reactivity between immune sera and human heart tissue[45,53]	– Constrained by type-specific protection – Theoretical coverage of vaccine in Africa 39% and Pacific region 23.9% [54]	Phase II clinical trial completed: Well tolerated and immunogenic in healthy adults[55]	45,53,55
30-valent N-terminal	N-terminal subunits from 30 serotypes of GAS. Four different multivalent fusion proteins (containing eight or nine M-protein fragments) formulated with Alum	– Serotypes account for 98% of all cases of pharyngitis in the U.S. and Canada and 90% of invasive diseases in the U.S. and 78% of invasive diseases in Europe[56] – Evoked bactericidal antibodies against all 30 vaccine serotypes of GAS (using serum from immunized rabbits)[43] – Contained significant levels of bactericidal antibodies against 24 of 40 non-vaccine serotypes of GAS tested[43]	– Heterologous protection not reported in animal models	Pre-clinical	43,56
StreptInCor	Based on the amino-acid sequences from the M5 protein conserved regions (C2 and C3 regions). Contains immunodominant T (22 amino- acids) and B-cell (25 amino-acids) epitopes linked by eight amino-acid residues	– Protective in BALB/c [59], HLA class II transgenic (mice containing human HLA II alleles)[60] and SWISS mice[61] – Antibodies able to neutralize/opsonize M1, M5, M12, M22 and M87 GAS[62] – No autoimmune pathological reactions were observed in heart or other organs[60] – Potential to elicit B and T-memory cells[129]	None identified yet	Pre-clinical	59-62,129
J8-DT/Alum (MJ8Vax)	Minimal B-cell epitope within p145 (C3-repeat region), conjugated to T-helper cell protein diphtheria toxoid (DT) and formulated with Alum	– Cryptic B-cell antigen capable of inducing protection against all GAS serotypes – Protection against streptococcal pyoderma, bacteraemia[65] and pharyngitis (J8-Lipo-DT)[96] – Pre-clinical data demonstrated no abnormal heart tissue pathology[91] – Minimal epitope size enhances safety profile	– Limited efficacy against hypervirulent *covR/S* mutants GAS strains[65,66]	Pilot Phase I trial completed: Well tolerated and immunogenic in healthy adults (manuscript in preparation)	65,66,92,96
J8-CRM+K4S2-CRM/Alum (MJ8CombiVax)	J8-CRM combined with a 20-mer B-cell epitope (K4S2) from SpyCEP and conjugated to CRM[66] * K4S2: A more soluble derivative of S2 (S2 with four Lysine residues) #CRM197 (CRM): Enzymatically inactive and non-toxic form of DT	– Act synergistically to opsonize GAS (with anti-J8 antibodies) and to block IL-8 degradation (with anti-SpyCEP antibodies) – Protect against hypervirulent *covR/S* mtant GAS[65-67]	None identified yet	In preparation for Phase I trial	65-67

SV1	Five 14-mer amino-acid sequences ($J14_i$ variants) from differing C-repeat regions combined in a single recombinant construct	– Maintains α-helical structure without the need for flanking sequence – Evidence of cross-recognition between $J14_i$ variants, may provide extended M-protein coverage – No autoimmune responses detected in studies with the Lewis Rat model for valvulitis – Present in 97% of M-proteins	None identified yet	Pre-clinical	[48,64]
Self-adjuvanting J14	J14 incorporated with fibril forming peptides Q11 and lipoamino-acids (C16) Vaccine constructs: (i) J14-spacer1-Q11-spacer2-C16-C16 (ii) J14-spacer2-C16-C16	– Capable of inducing significant dendritic cell uptake and J14-specific antibody responses	None identified yet	Pre-clinical	[130]
Non-M-protein-based vaccines					
GAS carbohydrate (GAS CHO)	GAS-CHO conjugated to tetanus toxoid (TT)	– GAS carbohydrate: conserved across all GAS strains[80] – Induction of phagocytosis promoting antibodies in rabbits[52] – Intranasal immunization (GAS-CHO/CTB): significant reduction in GAS throat colonisation[51]	– Cross-reactivity between GAS carbohydrate and human tissue[131-1332]	Pre-clinical	[51,52]
GAS carbohydrate (GAC) defective for GlcNAc sidechain	Gac1 mutant bacteria (GlcNAc sidechain deficient GAC) was used to extract GlcNAc-defective GAC (ΔGAC)	– GAS carbohydrate: conserved across all GAS strains – No cross-reactivity between human cardiac antigens due to removal of GlcNAc sidechain – Opsonisation and killing of diverse GAS serotypes – Passive protection in murine systemic infection model	None identified yet	Pre-clinical	[80]
GAS C5a peptidase (SCPA)	Recombinant, enzymatically inactive SCPA delivered intranasally (unadjuvanted)[71,134] or combined with MPL and formulated with Alum for subcutaneous immunization[50]	– Antigenically conserved among different serotypes, may induce protection across several GAS serotypes – Induction of high serum IgG and salivary IgA – Reduced the colonisation of M1, M2, M6, M11 and M49 GAS serotypes	None identified yet	Pre-clinical	[50,69-71]
Fibronectin binding protein (FBP54 or Sfb1)	Recombinant FBP54 (rFBP54) coupled with cholera toxin subunit B (CTB) and delivered orally and intranasally; Recombinant Sfb1 conjugated to CTB and delivered intranasally	– The *fbp54* gene: highly conserved among all test strains (>98%)[72] – FBP54/CTB oral/intranasal immunization: antigen-specific salivary IgA and serum IgG – Subcutaneous immunization with FBP54/CFA protected mice against intraperitoneal GAS infection[135]	–Intranasal immunization with Sfb1/CTB was ineffective against systemic bacterial growth and dissemination[136] – GAS pyoderma predisposes individuals to severe GAS infection, despite presence of anti-Sfb1IgG titres[137]	Pre-clinical	[72,73,135]

(*Continued on next page*)

Table 1. (*Continued*).

Name	Composition	Advantages	Disadvantages	Status	Ref.
		–Intranasal immunization with Sfb1/CTB protected mice against lethal GAS infection[73]			
Serine protease (SpyCEP)	Recombinant SpyCEP (rSpyCEP) administered parentally with CFA[49] or rSpyCEP combined with C-terminal peptide antigen (J8-DT) and administered subcutaneously with Alum[65]	– Reduced the dissemination of GAS from local to systemic infection [49] – Combination vaccination (J8-DT+rSpyCEP) resulted in profound protection against *covR/S* wild-type and mutant GAS skin challenges[65]	– Limited efficacy when administered alone[65,66]	Pre-clinical	[50,66]
Serum opacity factor (SOF2)	Purified recombinant SOF2 from SOF positive M-serotype 2 GAS	– Anti-rSOF2 serum able to opsonize SOF positive M2, M4 and M28 GAS types	– Anti-rSOF2 serum ineffective for SOF negative M5 GAS	Pre-clinical	[78]
Streptococcal pyrogenic exotoxin A (SPE A)	SPE A purified from *Staphylococcus aureus* (double, triple and hexa-amino acid mutants of SPE A)	– Protection from lethal GAS infection and no symptoms of streptococcal toxic shock syndrome (STSS)[75] – Immunization also led to survival and no signs of illness when challenged subcutaneously with wild-type SPE A[74]	None identified yet	Pre-clinical	[74,75]
Streptococcal pyrogenic exotoxin B (SPE B)	SPE B was purified from *Streptococcus pyogenes* A-20 (a protease producing clinical isolate)	– 100% survival in vaccinated mice following challenge with GAS A20[76] – Severity of skin lesions reduced in immunized mice[76]	– Passive immunization with anti-SPE B IgG conferred partial protection with 50% survival in the immunized cohorot in comparison to 28% survival in the control cohort [76]	Pre-clinical	[76]
Streptococcal pyrogenic exotoxin C (SPE C)	Double-site Y15A/N38D and the triple-site Y15A/H35A/N38D mutants constructed from 3D-structure of SPE C	– Non-mitogenic for rabbit splenocytes and human PBMCs[77] – Non-lethal in two rabbit models of STSS[77] – Highly immunogenic and vaccination protected rabbits from challenge with wild-type SpeC[77]	– None identified yet	Pre-clinical	[77]
Streptococcal pili (T-antigen)	Vaccine comprising of a combination of recombinant pilus proteins	– Protection against mucosal infection following immunization[79] – Cross-protection can be achieved between some T-types that share high homology, restricting number of variants required for broad coverage[56]	– Vast sequence diversity, protection is specific to strains that contain pilus like variants in vaccine [56]	Pre-clinical	[56,79]
Combo#5	Vaccine consisting of a combination of trigger factor (TF), inactivated versions of arginine deiminase (ADI), streptolysin O (SLO), Streptococcus pyogenes cell envelope proteinase (SpyCEP) and group A streptococcal C5a peptidase (SCPA) adjuvanted with Alum Vaccine consisting of Streptolysin O (SLO, aa 32–571), SPy0269 (aa 27–849), and	– Murine serum antibodies from BALB/c and humanized plasminogen mice were able to bind to live GAS – Anti-combo#5 sera from BALB/c and humanized plasminogen mice was opsonic against pM1.200 and 5448 GAS strains respectively – Mice were significantly protected following skin-challenge	– In a model for invasive disease, protection was compromised. M1 protein (positive control) vaccine was the only experimental vaccine that conferred protection (100% survival)	Pre-clinical	[112]

3-antigen combo	S. pyogenes cell envelope protease (SpyCEP, aa 34–1613) tagless versions were cloned in the pET24b+ E. coli expression vector and purified	– Antigens conferred consistent cross-protection against a wide range of GAS strains in different mouse models of infection – Vaccine elicits antibodies capable of neutralizing two important virulence factors expressed by a large fraction of GAS isolates	– None identified yet	Pre-clinical	[138]
Spy 7	Vaccine consisting of seven recombinant antigens – C5a peptidase, oligopeptide-binding protein, putative pullulanase, nucleoside-binding protein, hypothetical membrane associated protein, cell surface protein and Spy AD	– Induction of anti-streptococcal antibodies and demonstrated protective efficacy against M1 and M3 GAS strains – Murine Spy7 antiserum demonstrated no discernible reactivity with human heart valve tissue using an ELISA based assay, suggesting an absence of cross reactive epitopes within any of the selected antigens	– Antigens have been tested in isolation as vaccine candidates, including six of those included in this study. Of these, C5a peptidase, cell surface protein, and SpyAD showed protective efficacy,[70,138,139] while oligopeptide-binding protein, putative pullulanase, and hypothetical membrane associated protein were not protective[138,140]	Pre-clinical	[141]
ADI and TF	Vaccine consisting of Arginine deiminase (ADI) and trigger factor (TF)	– These surface-exposed enzymes are expressed across multiple GAS serotypes exhibiting ≥99% amino acid sequence identity – Sera from human populations suffering repeated GAS infections and high levels of autoimmune complications do not recognize these enzymes – Protective efficacy demonstrated against intraperitoneal challenge with M1 GAS – Combination ADI and TF was observed to act synergistically, conferring significant protection against lethal subcutaneous M1 GAS challenge	– None identified yet	Pre-clinical	[142]

sequence.[47,63] Antibodies raised to SV1 were shown to bind to each of the 5 $J14_i$ variants which are present in 97% of M-proteins.[47,63] The studies with the Lewis Rat model for valvulitis suggested that the vaccine is safe and will elicit antibodies that recognize a broad range of GAS serotypes.[63] J14 has also been combined synthetically with 7 amino-acid peptides from different *emm* strains and induced protective antibodies in mice to strains both represented by and not represented by the amino-terminal sequences.[64]

An experimental vaccine J8-DT, targeting the conserved domain of M-protein and conjugated to diphtheria toxoid (focus of this review) has shown efficacy against multiple GAS strains. The efficacy of J8-DT was further improved to protect against *covR/S* mutant hypervirulent strains by incorporation of a SpyCEP epitope (S2) (see below). J8 conjugated to CRM 197 (enzymatically inactive non-toxic form of DT) in combination with K4S2-CRM is currently in preparation for a Phase I clinical trial.[65-67]

Non-M-protein-based vaccines

In recent years with the help of reverse vaccinology along with proteomics, whole genome sequencing, bio-informatics and microarray technology, a number of non-M-protein vaccine candidates have been identified[68] and are under pre-clinical development. Their highly-conserved nature across various serotypes and to date, no evidence of associated tissue cross-reactivity, makes them an attractive target for vaccine development.[56] Non-M-protein-based vaccine candidates that have been shown to play a role in immunity include C5a peptidase,[50,69-71] streptococcal fibronectin binding protein,[72,73] streptococcal pyrogenic exotoxins A,[74,75] B[76] and C,[77] *S. pyogenes* cell envelope protease,[49,65] serum opacity factor,[78] streptococcal pili,[79] and GAS carbohydrate.[51,52,80] However, a non-M-protein vaccine candidate has yet to progress into human clinical trials. It is believed that despite the role that non-M-protein antibodies play in GAS immunity, opsonic M-protein specific antibodies will be critical for clearing GAS infection.[81] A combination of M-protein and non-M-protein antigens could be exploited to improve protection which has been demonstrated with the MJ8CombiVax (J8-CRM+K4S2-CRM) vaccine.[67] A detailed analysis of each of these vaccine candidates is provided in Table 1.

Identifying a cryptic target for a GAS vaccine

Bessen and Fischetti[82] demonstrated the protective potential of the conserved region of the M-protein against GAS. Mice were immunized intranasally with synthetic peptides from the highly-conserved C-repeat region of the M-protein, which had been covalently linked to cholera toxin B subunit (CTB). These peptides corresponded to antigenic epitopes shared by many *emm* types. It was found that intranasal immunization with the cross-reactive epitopes coupled to CTB led to significant protection against pharyngeal colonisation by GAS. In parallel, Jones and Fischetti[83] showed that antibodies to the amino-terminal region of the M-protein, but not the conserved central region, were opsonic. Contrary to that, we demonstrated that a conserved region peptide, p145 (a 20-mer peptide from the 'C3-repeat' region), was able to induce opsonic antibodies in mice post-immunization.[84] The opsonization assay used stationary phase rather than log-phase organisms that are used in the 'classical' Lancefield assay. It was hypothesized that the diminished hyaluronic acid (HA) capsule associated with stationary phase GAS will allow better access of antibodies to the C-repeat region of the M-protein.[85] p145 peptide was identified by scanning the conserved C- region of the M-protein of GAS.[22,84] p145-specific affinity purified human antibodies collected from a highly endemic region of Australia, were also shown to be opsonic.[86] These findings suggested that p145 might be a suitable vaccine candidate. However, there were concerns regarding host tissue cross reactivity. Human studies suggested that while humoral responses may initiate RF/RHD, the key mediators of heart lesions are auto reactive T-cells. By molecular mimicry these T-cells also recognize heart tissue proteins. Heart infiltrating T-cell clones isolated from RHD patients have been shown to recognize GAS M5 protein and heart tissue proteins/peptides.[87,88] It was deemed prudent to define the minimal epitope within p145 that was immunogenic and able to induce opsonic antibodies.

The structure of the M-protein is a coiled-coil alpha helix and it was critical that the minimal epitope maintains helical folding in order to induce antibodies that recognize the native protein. To promote alpha-helical coiled-coil confirmation, small sequences (12 amino-acids in length) from p145 were flanked with a GCN4 peptide (from a DNA binding protein of yeast known to promote an alpha helical coiled-coil).[89] Chimeric peptides designated J1 to J9 were used to map the minimal epitope within p145 using age-stratified sera from Indigenous Australians living in a highly streptococcal endemic region[86] (Table 2). Sera from over 90% of individuals in the 20+ years age group recognized peptides J1, J2, J7, and J8 but the recognition of these peptides was much less in children (approximately 20%).[86] The epitopes were thus cryptic in that many years of exposure were required to induce an antibody response. Additional studies revealed that human antibodies to p145 could opsonize multiple serotypes of GAS including strains that exhibited slight differences in the p145 minimal epitope sequence.[90] Monoclonal antibodies from mice immunized with p145 recognized J7, J8 and J9.[91] These three peptides induced a significant antibody response to themselves (titre >12,800), although only J8 could induce an antibody response to p145. Having noted the potential of J7, J8 and J9, an additional chimeric peptide, termed J14, was synthesized from amino-acids 7–20 of p145 (amino-acids found within J7, J8 and J9).[92] p145 antisera bound to J14 and antisera from mice immunized with J14 recognized J7, J8, J9 and p145. J8 and J14 did not induce p145-specific T-cell responses in mice, which was seen as a bonus in terms of the safety profile of the vaccine.[91] Within p145, the T-cell epitopes were mapped to J2 and J3. This corresponds to residues 3–14 located at the amino-terminal region of p145.[91] Thus, a minimal cryptic B-cell epitope (J8) was defined, and this did not contain a potentially deleterious T-cell epitope from GAS, yet was able to stimulate antibodies that could opsonize GAS.[91] Although J8 did not contain a GAS-derived T-cell epitope recognized by mice, it does nevertheless contain one or more T-cell epitopes. J8 has 12 amino-acids copying the M-protein sequence, but also

Table 2. List of synthetic peptides of p145.

P145:									**L**	**R**	**R**	**D**	**L**	**D**	**A**	**S**	**R**	**E**	**A**	**K**	**K**	**Q**	**V**	**E**	**K**	**A**	**L**	**E**						
J1:	Q	L	E	D	K	V	K	Q	**L**	**R**	**R**	**D**	**L**	**D**	**A**	**S**	**R**	**E**	**A**	**K**	E	E	L	Q	D	K	V	K						
J2:		L	E	D	K	V	K	Q	A	**R**	**R**	**D**	**L**	**D**	**A**	**S**	**R**	**E**	**A**	**K**	**K**	E	L	Q	D	K	V	K	Q					
J3:			E	D	K	V	K	Q	A	E	**R**	**D**	**L**	**D**	**A**	**S**	**R**	**E**	**A**	**K**	**K**	**Q**	L	Q	D	K	V	K	Q	L				
J4:				D	K	V	K	Q	A	E	D	**D**	**L**	**D**	**A**	**S**	**R**	**E**	**A**	**K**	**K**	**Q**	**V**	Q	D	K	V	K	Q	L	E			
J5:					K	V	K	Q	A	E	D	K	**L**	**D**	**A**	**S**	**R**	**E**	**A**	**K**	**K**	**Q**	**V**	**E**	D	K	V	K	Q	L	E	D		
J6:						V	K	Q	A	E	D	K	V	**D**	**A**	**S**	**R**	**E**	**A**	**K**	**K**	**Q**	**V**	**E**	**K**	K	V	K	Q	L	E	D	K	
J7:							K	Q	A	E	D	K	V	K	**A**	**S**	**R**	**E**	**A**	**K**	**K**	**Q**	**V**	**E**	**K**	**A**	V	K	Q	L	E	D	K	V
J8:								Q	A	E	D	K	V	K	Q	**S**	**R**	**E**	**A**	**K**	**K**	**Q**	**V**	**E**	**K**	**A**	**L**	K	Q	L	E	D	K	V	Q
J9:									A	E	D	K	V	K	Q	L	**R**	**E**	**A**	**K**	**K**	**Q**	**V**	**E**	**K**	**A**	**L**	**E**	Q	L	E	D	K	V	Q	L
J14:							K	Q	A	E	D	K	V	K	**A**	**S**	**R**	**E**	**A**	**K**	**K**	**Q**	**V**	**E**	**K**	**A**	**L**	**E**	Q	L	E	D	K	V	K	

Bold residues are those contained within p145.
Underlined residues represent the T-cell epitope contained within p145.
Highlighted residues represent the B-cell epitope contained within p145.
*Adapted from Hayman *et al.* 1997. [91]

contains an additional 16 non-streptococcal amino-acids (GCN4 protein) that form part of the T-cell epitope of J8.[89,91]

The immunogenicity of the J8 peptide was determined using different adjuvants.[48] Quackenbush (outbred) and B10.BR mice were immunized with J8 peptide and lymph node cell proliferation to the peptide was determined for each mouse. For the Quackenbush mice, lymph node cells from only 2 of the 20 mice proliferated, whereas T-cells from 7 of the 8 immunized B10.BR mice responded to the J8 peptide.[48]

Development of a conjugate GAS vaccine

Immunological responsiveness to a vaccine is determined by T-cells being able to recognize processed fragments of an antigen (via the major histocompatibility molecule II [MHC II]). Failure of J8 to stimulate T-helper cells in an outbred population would limit its suitability as a vaccine. Therefore, J8 was conjugated to the carrier protein, diphtheria toxoid (DT), and the conjugate was used to immunize mice which were subsequently challenged via the skin or mucosal routes.[48]

J8-DT administered subcutaneously with Alum protected against streptococcal pyoderma and bacteraemia.[65] In this study, a scarification method was used to mimic superficial skin infection. Vaccinated mice had significantly reduced bacterial burden in the skin in comparison to non-vaccinated mice. In addition, vaccinated mice either did not develop a systemic infection or cleared infection significantly faster compared to the non-immunized cohort.[65] The vaccine was shown to induce a memory response using an adoptive transfer assay. J8-DT-immunized mice were rested for 10–12 weeks and splenocytes or purified B or T-cells were then transferred to naïve immunodeficient SCID mice. Adoptive transfer of splenocytes from immunized mice or B-cells from immunized mice along with T-cells from either immunized or naïve mice resulted in the recipients being immune and showing significantly reduced bacterial burden in the skin and blood following challenge infection. At the time of challenge, the reconstituted SCID mice did not have detectable J8-specific antibodies in their serum.[65] These data thus demonstrated that mice could be protected even if they did not have serum antibodies at the time of challenge, providing they had memory B-cells. Presumably the memory B-cells responded quickly to the infection, producing opsonizing antibodies.

Pre-clinical data on immunogenicity and safety of J8-DT demonstrated no abnormal heart tissue pathology in a Lewis rat model for cardiac valvulitis.[92] In addition, a dose escalating toxicology assessment of J8-DT in rabbits demonstrated no treatment-related or toxicologically significant effects.[92] The vaccine has been tested in a pilot Phase I clinical trial and was shown to be immunogenic with no serious adverse events reported in the study (manuscript submitted).

J8-DT-mediated systemic protection required J8-specific IgG to mediate GAS clearance from the site of infection.[65,66,93] However, protection against URT infection may require an IgA response.[94,95] We observed that intramuscular immunization with J8-DT/Alum resulted in high serum J8-specific IgG titres but no salivary J8-specific IgA titres. Following intranasal challenge there was minimal protection as demonstrated from estimating bacterial burden in nasal secretions, throats and Nasal Associated Lymphoid Tissue (NALT; a murine homolog to human tonsils).[96]

We explored different approaches to induce mucosal immunity. Immunization of mice with J8-DT/CTB (cholera toxin B, CTB) (and J14-DT/CTB) led to protection following challenge via the URT route.[97] However, CTB is not a suitable adjuvant for human studies. We therefore explored other potential approaches to induce mucosal immunity. Immunization with J14 formulated with bacterial outer membrane proteins (J14/proteosomes) and administered intranasally to outbred mice resulted in J14-specific IgA in saliva and a decreased colonisation in mice post-challenge with GAS.[94] In a further study, J14 was incorporated into a lipopeptide construct to which a universal T-cell epitope and a self-adjuvanting lipid moiety, Pam (2)Cys, were attached.[98] This vaccine formulation (P25-P2C-J14) induced salivary J14-specific antibodies, which coincided with reduced throat colonisation post-intranasal GAS challenge.[99] More recently we have explored the use of liposomes composed of neutral lipids encapsulating DT and displaying lipidated J8 on their surface (J8-Lipo-DT). This liposome construct induced peptide-specific IgA and protected against intranasal GAS challenge.[96]

Anti-J8 antibodies are not observed following a GAS infection of mice. Additionally, there is a lack of anti-J8 antibody secreting cells (ASCs) in the spleen and long lived plasma cells (LLPCs) in the bone marrow.[4] In contrast, following immunization with J8-DT, significant numbers of J8-specific ASCs

were observed in the spleens of mice. Furthermore, following sequential infections of J8-vaccinated mice with different strains of GAS, the numbers of J8-specific ASCs increased significantly and the degree of protective immunity similarly increased. Thus, while J8 is cryptic following infection of naïve mice, J8-specific B-cells (induced by vaccination with a J8 conjugate vaccine) can nevertheless be boosted by infection[4,41,94]

J8, being highly conserved and cryptic, overcomes the barrier of antigenic variability found within circulating GAS strains. In a recent study by Sanderson-Smith et al., 2014,[99] J8 was found to have high sequence homology among differing *emm* types; 173 of the 175 *emm* types, collected globally, contained either the J8 or J8.1 allele.[99] These two J8 allelic sequences are immunologically cross-reactive. Antisera raised to both allelic sequences recognize the parent peptide (p145) equally (unpublished data). Further supporting these data is a study from Cambodia where 28% and 69% of the isolates carried the J8 or J8.1 allele respectively, thus, predicting the theoretical coverage of the vaccine to be 97%.[100] Likewise, in another study carried out in Lao, where among 124 GAS isolates, 34 *emm* types were observed: 15% and 82% of the isolates predicted to contain the J8 or J8.1 allele respectively and the theoretical coverage of the J8 vaccine was predicted to be 97%.[101] These studies provide encouraging data supporting the potential of cryptic epitope J8 in combating one of the major impediments to GAS vaccine development – antigenic strain variation. This is further strengthened by extensive animal studies where immunization with vaccines based on cryptic epitopes (J8-DT or J8-DT+K4S2-DT) provided protection against GAS strains from multiple *emm* types belonging to different clades and *emm* clusters.[65-67]

Pathogenesis of *covR/S* mutant GAS strains

While J8-DT is a highly efficacious vaccine that protects against multiple GAS strains of various *emm* types, its efficacy against hyper-virulent *covR/S* mutant strains is compromised. The *covR/S* system plays an important role in regulating ~15% of the genome of which a majority includes virulence gene expression (mostly virulence factors responsible for invasiveness of an isolate during infection).[102] Several virulence factor genes are upregulated as a result of *covR/S* mutation including *S.pyogenes* cell envelope proteinase (SpyCEP, *cepA*), streptodornase of serotype 1 (Sda1, *sda1*), streptolysin O (SLO, *slo*), streptococcal inhibitor of complement (SIC, *sic*) and the hyaluronic acid capsule synthesis operon (HA, *hasABC*).[103] SpyCEP, a CXC chemokine protease is a cell wall anchored serine protease that can also be released as a soluble enzyme.[104] SpyCEP can cleave human interleukin-8 (IL-8) and KC and MIP-2 in mice, thereby disrupting neutrophil chemotaxis to the site of infection and assisting GAS to become systemic.[104] Invasive blood isolates have been shown to have increased SpyCEP activity compared to non-invasive isolates.[105] The role of neutrophils in SpyCEP mediated pathogenesis of GAS was demonstrated utilising human microvascular endothelial cells where infection with GAS Δ*cepA* mutant (gene encoding SpyCEP, *cepA*, deleted) led to significantly higher neutrophil chemotaxis in comparison to a *covR/S* mutant GAS strain. In addition, it was demonstrated that *covR/S* mutant GAS survived neutrophil killing significantly more than Δ*cepA* mutant bacteria.[106] Furthermore, following subcutaneous skin-infection *covR/S* mutant GAS demonstrated increased lesion size which correlated with histopathological analysis where an impaired neutrophil recruitment to the site of infection was noted.[106]

Hypervirulent *covR/S* mutant GAS have been associated with reduced colonisation capacity.[103] However, *covR/S* mutant GAS displayed enhanced ability to establish URT infection in a mouse model when compared to a Δ*cepA* mutant.[105] On the contrary, in the same study the observations were reversed when the contribution of SpyCEP to GAS adherence and invasion was examined using HEp-2 human epithelial cells. The Δ*cepA* mutant was found to be ~3 fold more adherent and ~2 fold more invasive than the *covR/S* mutant parent strain.[105] These data are supported by another study where *covR/S* mutant GAS had significantly decreased adherence to HEp-2 cells and HaCaT keratinocytes in comparison to wild-type GAS.[103] *covR/S* mutant GAS were found to have significantly more hyaluronic acid capsule than wild-type GAS. Hypercapsulation was associated with impaired adherence through the masking of GAS adhesins and extracellular binding proteins.[103]

SpyCEP is highly conserved between GAS isolates.[104,107] Initial studies by Rodriguez-Ortega et al., 2006,[68] using a whole genome proteomic bioinformatic approach identified SpyCEP (Spy0416) as a potential vaccine candidate that led to partial protection following intranasal infection with M23 GAS. In another study, SpyCEP immunization led to reduced dissemination of GAS to the blood and spleen following challenge.[49] Similarly, intranasal immunization with rSpyCEP significantly reduced *covR/S* mutant GAS dissemination from URT to blood liver or spleen.[49] Furthermore, SpyCEP vaccination has been shown to reduce the intensity of intranasal infection with bioluminescent GAS (*covR/S* wild-type).[108] However, bacterial counts in nasal tissues on day-4 post-infection were not significantly different between vaccinated and control groups, indicating that SpyCEP alone was unlikely to be a viable vaccine candidate.[108]

Development of a combination vaccine to broaden the scope of J8-DT

The data on the mechanism of J8-DT-mediated protection highlighted a critical role of neutrophils.[65] Following skin challenge with *covR/S* wild-type GAS, vaccinated neutrophil-depleted mice suffered significantly higher bacterial burdens in skin and blood when compared to vaccinated neutrophil-sufficient mice.[65] These data suggested that J8-DT may have compromised efficacy against strains of GAS that have a mutation in the *covR/S* regulon, preventing neutrophil ingress to the site of infection and hampering phagocytosis. This was supported by histological examination that demonstrated a lack of neutrophils at the site of infection.[65] To protect neutrophil-attracting CXC chemokines from degradation, antibodies were generated using a truncated recombinant SpyCEP fragment (rSpyCEP: amino-acid residues 35–587)[49] combined with J8-DT. Vaccination with this combination vaccine (J8-DT+ rSpyCEP) led to significant protection against pyoderma and bacteraemia.[65] *In-vitro* studies showed that anti-SpyCEP antibodies protected IL-8 from degradation mediated by supernatants from *covR/S*

mutant GAS strains.[106] These data demonstrated that J8-DT and rSpyCEP act synergistically to opsonize GAS (with anti-J8 antibodies) and to block IL-8 degradation (with anti-SpyCEP antibodies). The combination vaccine resulted in profound protection against *covR/S* wild-type and mutant GAS skin challenges.

The combination J8-DT+rSpyCEP is promising; however, rSpyCEP is a large protein, which may have the ability to induce an unwanted autoimmune response. Although rSpyCEP has been previously used as a vaccine candidate with no known side effects[49]; to eliminate any potential risks that may impede future vaccine progress, epitope mapping of rSpyCEP was undertaken. Peptide S2 (AA 205–224) was recognized by antisera from rSpyCEP-immunized mice. Antibodies generated to S2 could completely protect IL-8 from SpyCEP-mediated proteolysis.[66] We also demonstrated that human plasma samples with a confirmed antibody response to GAS could only partially protect IL-8 from degradation, suggesting that native SpyCEP may be cryptic or subdominant conferring a survival advantage to the organism.[67] Like J8, S2 is highly conserved with 95% homology found between the vaccine candidate S2 and S2.1 (Table 3), further suggesting that it is not under immune pressure. Both rSpyCEP- and S2-antisera also protected the related mouse chemokine, MIP-2, against degradation. Subsequently, mice vaccinated with the combination vaccine (J8-DT+S2-DT) and challenged via the skin route with stationary or log phase *covR/S* mutant organisms had significantly reduced bacterial burden in skin and blood when compared to PBS controls.[66] Furthermore, histological examination revealed that immunized mice had a large influx of neutrophils to the site of infection. Mucosal immunity was also assessed in the context of J8 and S2 mediated protection. J8 and S2 expressed on the surface of liposomes (J8/S2-Lipo-DT) and administered to mice intranasally elicited J8- and S2-specific IgA titres that were comparable to the titres induced by the individual vaccine constructs (J8-Lipo-DT and S2-Lipo-DT respectively).[96] Following intranasal-challenge with 5448AP GAS (a *covR/S* mutant), immunized mice had significantly reduced bacterial colonisation in comparison to PBS controls in throat swabs and NALT.[96] Recently a more soluble derivative of S2 (S2 with four Lysine residues; K4S2) in combination with J8-DT has demonstrated comparable efficacy.[67] A comprehensive summary of cryptic/B-cell epitopes utilized in vaccines designed by our group is presented in Table 4.

Animal models in GAS vaccine development

GAS is a human-specific pathogen; consequently, use of an animal model to study vaccine efficacy and immunopathogenesis of the organism poses several challenges. GAS isolated from humans rarely show natural virulence for mice and serial passaging is required to increase the virulence of the organism. Additionally, lack of responsiveness to GAS superantigens further limits the utility of animal models to assess vaccine efficacy in the context of humans; colonization is often difficult to achieve and true pharyngitis does not occur.[109] A potential way forward would be to develop a human GAS pharyngeal challenge model and efforts to implement this strategy are currently underway.[110]

Despite these limitations, mouse models provide a complex multi-factorial immune system that cannot be recapitulated in an *in-vitro* environment. The recent emergence of humanized mice is a pivotal step in the advancement of translational vaccine research. Humanized mice expressing human MHC recognize GAS superantigens[111] and therefore can be utilized to assess vaccine efficacy against clinical GAS isolates that rarely show natural virulence in mice. Humanized plasminogen mice can be used to model GAS invasive disease in humans. Since GAS streptokinase has a higher affinity for human plasminogen than mouse plasminogen, these mice can mimic the activation of human plasminogen by streptokinase which is vital for systemic dissemination.[112] Another alternative would be to use non-human primate (NHP) models that are biologically closer to humans. Streptococcal pharyngitis has been previously assessed in NHPs.[113,114] In addition, different experimental vaccine candidates inducing significantly different level of protection in two different mouse models;[112] suggests that progression to human clinical trials requires standardisation of animal models for the advancement of GAS vaccine development.[112] Overall, a combination of various readouts (*in-vivo* protection studies in mouse and *in-vitro* opsonophagocytic assays) may provide valuable insight into the mechanistic aspects as well as protective efficacy of vaccines in humans.

Many pre-clinical studies in GAS vaccine development rely on hypothesis-driven research in mice. Recently, the translation of mouse data into humans has been questioned. A recent study claimed that genomic responses in mouse models correlate poorly with the human condition.[115] A subsequent report reevaluated the same gene expression dataset in a more rigorous and less biased manner and reported the exact opposite findings.[116] To combat the caveats associated with *in-vivo* research, rigorous standards need to be implemented when undertaking mouse studies. Proper use of controls, sufficient statistical power to determine cohort sizes and attention to data interpretation will improve the translational impact of these experiments.[117] Additionally, discounting the practicality and the utility of mouse-based research may compromise future scientific discoveries.[118] Thus, an ongoing discussion on mouse models in all disease states is necessary to advance translational research in a more efficient and effective way.

Table 3. Multiple sequence alignment of S2 variants.

S2:	N	S	D	N	I	K	E	N	Q	F	**E**	D	F	D	E	D	W	E	N	F
S2.1:	N	S	D	N	I	K	E	N	Q	F	**G**	D	F	D	E	D	W	E	N	F
	*	*	*	*	*	*	*	*	*	*		*	*	*	*	*	*	*	*	*

S2 sequence alignment performed using bioinformatics program (Clustal Omega; http://www.ebi.ac.uk/Tools/msa/clustalo/). Data representing 95% homology between S2 and S2.1. An * (asterix) indicates positions which have single, fully conserved residues. Single amino-acid polymorphism represented in **bold**. A total of 96 BLAST hits returned with 62 hits containing 100% homology with S2 and 34 hits containing 100% homology with S2.1.

Table 4. J8-based vaccine modifications.

Peptide	Sequence	Adjuvant/ delivery system	Application/ outcomes	Ref.
p145	LRRDLDASREAKKQVEKALE	CFA *not suitable for human use	**p145/CFA**: p145-spcific antibodies from mice immunized with p145/CFA were able to opsonize several serotypes of GAS	84
J8	Highly conserved peptide from p145: QAEDKVKQ**SREAKKQVEKAL**KQLEDKVQ	Alum	**J8-DT/Alum**: J8 conjugated to T-helper cell carrier protein, DT, and administered subcutaneously to mice with Alum protected against streptococcal pyoderma and bacteraemia	65
	12-mer peptide ftom p145 in **bold**, total 28-mer	CTB *not suitable for human use	**J8-DT/CTB**: Mice immunized intranasally with J8-DT/CTB had significantly increased survival following intranasal challenge with GAS and salivary IgA correlated with protection	97
		Liposomes	**J8-Lipo-DT**: Mice immunized intranasally with J8-Lipo-DT induced high J8-specific salivary IgA and mice were protected following URT-challenge	96
		LCP	**LCP-J8**: Mice immunized subcutaneously with LCP-J8 and LCP-J8 in CFA induced high J8-specific serum IgG and antisera from these mice was able to opsonize multiple GAS strains. Antibodies did not cross react with human heart tissue proteins.	143,144
			Tetraepitopic LCP (LCP system incorporating 4 different non-host cross-reactive peptide epitopes of the GAS M-protein): Parenteral immunisation induced high antigen-specific serum IgG responses and the antisera was able to opsonize multiple GAS strains. immunized mice were also protected following systemic challenge.	145
			LCP-8830-J8 (LCP system incorporating 2 different peptide epitopes of the GAS M-protein, 8830 and J8): Mucosal immunization induced an antigen-specific systemic IgG. Antisera was able to opsonize a homologous and heterologous GAS strain. immunized mice were protected following systemic and mucosal challenge.	146
J14	Highly conserved peptide from p145: KQAEDKVK**ASREAKKQVEKALE**QLEDKVK	CTB *not suitable for human use	**J14-DT/CTB**: Mice immunized intranasally with J14-DT/CTB had significantly increased survival following intranasal challenge with GAS and detection of salivary IgA was coincided with protection	97
	14-mer peptide from p145 in **bold**, total 29-mer	Proteosomes	**J14/Proteosomes**: Intranasal immunization of mice induced J14-specific salivary IgA that led to decreased URT colonisation in mice post-intranasal challenge	94
		Pam(2)Cys	**P25-P2C-J14**: Mice immunized intranasally with P25-P2C-J14 induced J14-specific salivary IgA that coincided with a significant reduction in throat colonisation	98
rSpyCEP	Encompassing amino-acid residues 35–587 (GenBank No. DQ413032)	CFA *not suitable for human use	**rSpyCEP/CFA**: Mice immunized with rSpyCEP/CFA had reduced bacterial dissemination from local intramuscular and intranasal sites of GAS infections and anti-rSpyCEP antibodies were able to inhibit SpyCEP cleaving of IL-8	49,104
		Alum		

			J8-DT+rSpyCEP/Alum: Combination vaccination resulted in profound protection against *covR/S* wild-type and mutant GAS skin challenges	[65]
S2	Highly conserved peptide from rSpyCEP (AA 205–224): NSDNIKENQFEDFDEDWENF	Alum	**J8-DT+S2-DT/Alum**: Combination vaccination resulted in protection against pyoderma and bacteraemia following skin-infection with *covR/S* mutant GAS and antibodies generated to S2 could completely protect IL-8 from SpyCEP mediated proteolysis	[66]
		Liposomes	**J8/S2-Lipo-DT**: Combination intranasal vaccination induced J8 and S2-specific salivary IgA that coincided with reduced bacterial colonisation of the URT following intranasal infection with *covR/S* mutant GAS	[96]
K4S2 (S2 with 4 lysine residues)	A more soluble derivative of S2	Alum	**J8-CRM+K4S2-CRM/Alum (MJ8CombiVax)**: Parenteral immunization of mice with MJ8CombiVax was immunogenic and protective against *covR/S* mutant skin challenge. Immunogenicity and protective efficacy was comparable to J8-DT+S2-DT vaccination.	[67]

*CRM197 (CRM): Enzymatically inactive and non-toxic form of DT

CFA: Complete Freud's adjuvant; Alum: Aluminum hydroxide; CTB: Cholera toxin B subunit; Liposomes: Liposomes composed of neutral lipids encapsulating DT and displaying lipidated peptide on surface; LCP: Lipid core peptide; Proteosomes: Bacterial outer membrane proteins; Pam(2)Cys: Lipopeptide construct containing a universal T-cell epitope and a self-adjuvanting lipid moiety.

Other cryptic vaccines in preclinical development

The implementation of cryptic epitopes as vaccine candidates is not unique to GAS vaccine development and has been employed in other fields as well. *Plasmodium* spp. parasites evade immunity through switching antigen expression and/or by expressing antigens that exist in multiple allelic forms. However, some important antigens/epitopes are cryptic and such as not under immune pressure. The circumsporozoite protein (CSP) protein is found on the surface of sporozoites (introduced into the blood stream following a mosquito bite). The amino-terminal region of the CSP is responsible for liver invasion by sporozoites.[119] A cryptic, 21 amino-acid epitope, from the amino-terminal region of the CSP protein, was identified that induced antibodies capable of blocking liver cell invasion.[120] However, in the native state the epitope was not immunogenic, protecting the parasite's ability to invade hepatocytes.[120]

Bacillus anthracis is the causative agent of anthrax in animals and humans. Anthrax toxin is composed of a protective antigen (PA), a cell binding protein, and two enzyme components. PA-based vaccination has shown protective efficacy following anthrax challenge.[121-123] The licensed Bioanthrax/AVA vaccine, composed predominantly of PA, requires multiple injections and yearly boosts to maintain immunity. It has also demonstrated a high degree of reactogenicity.[124-126] PA-specific neutralising antibody repertoire has been shown to be limited to a few dominant specificities thus leaving the vaccine vulnerable to *B.anthracis* strains resistant to PA-specific humoral immunity.[127,128] A protective cryptic antigen within PA was identified that could elicit humoral immunity and potent neutralisation of lethal toxin *in-vitro*.[128] Immunization with full-length PA did not induce antibodies specific for the epitope.[128]

Conclusion

Vaccine development strategies have primarily focused on dominant epitopes; however, immunodominance can be a hindrance to the progression of a vaccine due to its common association with antigenic polymorphism. Therefore, a focus should be placed on defining cryptic epitopes that induce protective immune responses to a vast array of antigenically variable organisms. While cryptic epitopes are not recognized, or recognized poorly, as a result of natural infection,[4] they can induce antibodies that may recognize the organism and induce strain-transcending immunity.[65,66]

The cryptic epitope, J8, is minimal and this enhances its safety profile, and S2 contains only 20 amino-acids. They work synergistically to induce strain-transcending immunity that prevents infection with virulent streptococci. This strategy of identifying non-dominant/cryptic epitopes has been successfully applied to a few organisms that readily evade immunity and enable the design of highly immunogenic and effective vaccines.

Acknowledgments

We thank Emma Langshaw for critically reviewing the manuscript and Ainslie Calcutt for her assistance with analysis of the S2 data sequence.

Funding

This work was supported by grants from the National Heart Foundation of Australia (APP1044023), a National Health and Medical Research Council (NHMRC) (Australia Program grant (APP1037304) and a NHMRC project grant (APP1083548). We also acknowledge funding from the National Foundation of Medical Research and Innovation (NFMRI, Australia), and the Australian Tropical Medicine Commercialisation grant. An APA and a GLYPRS Scholarship awarded to VO and a NHMRC Fellowship grant to MFG.

References

1. The Australian immunisation handbook. Canberra: Australian Department of Health; 2015 update.
2. Landry S, Heilman C. Future directions in vaccines: The payoffs of basic research. Health Aff (Millwood). 2005;24:758–69. doi:10.1377/hlthaff.24.3.758. PMID:15886171.
3. Good MF, Yanow SK. Cryptic epitope for antibodies should not be forgotten in vaccine design. Expert Rev Vaccines. 2016;15:675–6. doi:10.1586/14760584.2016.1154791. PMID:26881480.
4. Pandey M, Ozberk V, Calcutt A, Langshaw E, Powell J, Rivera-Hernandez T, Ho MF, Philips Z, Batzloff MR, Good MF. Streptococcal immunity is constrained by lack of immunological memory following a single episode of pyoderma. PLoS Pathog. 2016;12:e1006122. doi:10.1371/journal.ppat.1006122. PMID:28027314.
5. Carapetis JR, Steer AC, Mulholland EK, Weber M. The global burden of group A streptococcal diseases. Lancet Infect Dis. 2005;5:685–94. doi:10.1016/S1473-3099(05)70267-X. PMID:16253886.
6. Jackson SJ, Steer AC, Campbell H. Systematic Review: Estimation of global burden of non-suppurative sequelae of upper respiratory tract infection: Rheumatic fever and post-streptococcal glomerulonephritis. Trop Med Int Health. 2011;16:2–11. doi:10.1111/j.1365-3156.2010.02670.x. PMID:21371205.
7. Watkins DA, Johnson CO, Colquhoun SM, Karthikeyan G, Beaton A, Bukhman G, Forouzanfar MH, Longenecker CT, Mayosi BM, Mensah GA, et al. Global, regional, and national burden of rheumatic heart disease, 1990–2015. N Engl J Med. 2017;377:713–22. doi:10.1056/NEJMoa1603693. PMID:28834488.
8. Sika-Paotonu D, Beaton A, Raghu A, Steer A, Carapetis J. Acute rheumatic fever and rheumatic heart disease. In: Ferretti JJ, Stevens DL, Fischetti VA, editors. Streptococcus pyogenes: Basic Biology to Clinical Manifestations. Oklahoma City (OK): University of Oklahoma Health Sciences Center (c) The University of Oklahoma Health Sciences Center; 2016.
9. Guilherme L, Kohler KF, Postol E, Kalil J. Genes, autoimmunity and pathogenesis of rheumatic heart disease. Ann Pediatr Cardiol. 2011;4:13–21. doi:10.4103/0974-2069.79617. PMID:21677799.
10. Klingenberg R. The heart in rheumatic, autoimmune and inflammatory diseases. Eur Heart J. 2017;38:2985. doi:10.1093/eurheartj/ehx556. PMID:29088444.
11. Guilherme L, Kalil J. Rheumatic fever and rheumatic heart disease: Cellular mechanisms leading autoimmune reactivity and disease. J Clin Immunol. 2010;30:17–23. doi:10.1007/s10875-009-9332-6. PMID:19802690.
12. Kaplan MH, Meyeserian M. An immunological cross-reaction between group-A streptococcal cells and human heart tissue. Lancet (London, England). 1962;1:706–10. doi:10.1016/S0140-6736(62)91653-7. PMID:14453769.
13. Zabriskie JB. Mimetic relationships between group A streptococci and mammalian tissues. Adv Immunol. 1967;7:147–88. doi:10.1016/S0065-2776(08)60128-5. PMID:4868522.
14. Massell BF, Honikman LH, Amezcua J. Rheumatic fever following streptococcal vaccination. Report of three cases. JAMA. 1969;207:1115–9. doi:10.1001/jama.1969.03150190037007. PMID:5818242.
15. D'Alessandri R, Plotkin G, Kluge RM, Wittner MK, Fox EN, Dorfman A, Waldman RH. 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. 1978;138:712–8. doi:10.1093/infdis/138.6.712. PMID:368261.
16. 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. 1973;52:1885–92. doi:10.1172/JCI107372. PMID:4719668.
17. Revocation of status of specific products; Group A streptococcus. Direct final rule. Fed Regist. 2005;70:72197–9. PMID:16323338.
18. Cunningham MW. Rheumatic fever, autoimmunity, and molecular mimicry: The streptococcal connection. Int Rev Immunol. 2014;33:314–29. doi:10.3109/08830185.2014.917411. PMID:24892819.
19. Cunningham MW. Post-streptococcal autoimmune sequelae: Rheumatic fever and beyond. In: Ferretti JJ, Stevens DL, Fischetti VA, editors. Streptococcus pyogenes: Basic Biology to Clinical Manifestations. Oklahoma City (OK): University of Oklahoma Health Sciences Center (c) The University of Oklahoma Health Sciences Center; 2016.
20. 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;65:3913–23. PMID:9284171.
21. Bessen D, Jones KF, Fischetti VA. Evidence for two distinct classes of streptococcal M protein and their relationship to rheumatic fever. J Exp Med. 1989;169:269–83. doi:10.1084/jem.169.1.269. PMID:2642529.
22. Pruksakorn S, Currie B, Brandt E, Phornphutkul C, Hunsakunachai S, Manmontri A, Robinson JH, Kehoe MA, Galbraith A, Good MF. Identification of T cell autoepitopes that cross-react with the C-terminal segment of the M protein of group A streptococci. Int Immunol. 1994;6:1235–44. doi:10.1093/intimm/6.8.1235. PMID:7981150.
23. Quinn A, Kosanke S, Fischetti VA, Factor SM, Cunningham MW. Induction of autoimmune valvular heart disease by recombinant streptococcal m protein. Infect Immun. 2001;69:4072–8. doi:10.1128/IAI.69.6.4072-4078.2001. PMID:11349078.
24. Galvin JE, Hemric ME, Kosanke SD, Factor SM, Quinn A, Cunningham MW. Induction of myocarditis and valvulitis in lewis rats by different epitopes of cardiac myosin and its implications in rheumatic carditis. Am J Pathol. 2002;160:297–306. doi:10.1016/S0002-9440(10)64373-8. PMID:11786423.
25. Kirvan CA, Galvin JE, Hilt S, Kosanke S, Cunningham MW. Identification of streptococcal m-protein cardiopathogenic epitopes in experimental autoimmune valvulitis. J Cardiovasc Transl Res. 2014;7:172–81. doi:10.1007/s12265-013-9526-4. PMID:24346820.
26. Bisno AL. Group A streptococcal infections and acute rheumatic fever. N Engl J Med. 1991;325:783–93. doi:10.1056/NEJM199109123251106. PMID:1870652.
27. Fischetti VA. Streptococcal M protein: Molecular design and biological behavior. Clin Microbiol Rev. 1989;2:285–314. doi:10.1128/CMR.2.3.285. PMID:2670192.
28. Johnson DR, Stevens DL, Kaplan EL. Epidemiologic analysis of group A streptococcal serotypes associated with severe systemic infections, rheumatic fever, or uncomplicated pharyngitis. J Infect Dis. 1992;166:374–82. doi:10.1093/infdis/166.2.374. PMID:1634809.
29. Lee GM, Wessels MR. Changing epidemiology of acute rheumatic fever in the United States. Clin Infect Dis. 2006;42:448–50. doi:10.1086/499817. PMID:16421786.
30. 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. 2006;42:441–7. doi:10.1086/499812. PMID:16421785.
31. Wolfe RR. Incidence of acute rheumatic fever: A persistent dilemma. Pediatrics. 2000;105:1375. doi:10.1542/peds.105.6.1375a. PMID:10877673.
32. Williamson DA, Smeesters PR, Steer AC, Steemson JD, Ng AC, Proft T, et al. M-protein analysis of streptococcus pyogenes isolates associated with acute rheumatic fever in New Zealand. J Clin Microbiol. 2015;53:3618–20. doi:10.1128/JCM.02129-15. PMID:26292296.
33. Beall B, Facklam R, Thompson T. Sequencing emm-specific PCR products for routine and accurate typing of group A streptococci. J Clin Microbiol. 1996;34:953–8. PMID:8815115.
34. Smeesters PR, McMillan DJ, Sriprakash KS. The streptococcal M protein: A highly versatile molecule. Trends Microbiol. 2010;18:275–82. doi:10.1016/j.tim.2010.02.007. PMID:20347595.
35. Smeesters PR, Mardulyn P, Vergison A, Leplae R, Van Melderen L. Genetic diversity of group A streptococcus m protein: Implications for typing and vaccine development. Vaccine. 2008;26:5835–42. doi:10.1016/j.vaccine.2008.08.037. PMID:18789365.
36. Kuttner AG, Lenert TF. The occurrence of bacteriostatic properties in the blood of patients after recovery from streptococcal pharyngitis. J Clin Invest. 1944;23:151–61. doi:10.1172/JCI101478. PMID:16695089.
37. Rothbard S. Bacteriostatic effect of human sera on group a streptococci: Type-specific antibodies in sera of patients convalescing from group a streptococcal pharyngitis. J Exp Med. 1945;82:93–106. doi:10.1084/jem.82.2.93. PMID:19871491.
38. Wannamaker LW, Denny FW, Perry WD, Siegel AC, Rammelkamp CH, Jr. Studies on immunity to streptococcal infections in man. Am J Dis Child. 1953;86:347–8.
39. Lancefield RC. Persistence of type-specific antibodies in man following infection with group A streptococci. J Exp Med. 1959;110:271–92. doi:10.1084/jem.110.2.271. PMID:13673139.
40. Pandey M, Ozberk V, Langshaw EL, Calcutt A, Powell J, Batzloff RM, Rivera-Hernandez T, Good MF. Skin infection boosts memory B-cells specific for a cryptic vaccine epitope of group A streptococcus and broadens the immune response to enhance vaccine efficacy npj Vaccines. Vaccine. 2018;3.
41. Steer AC, Dale JB, Carapetis JR. Progress toward a global group a streptococcal vaccine. Pediatr Infect Dis J. 2013;32:180–2. doi:10.1097/INF.0b013e318281da11. PMID:23328823.
42. Dale JB. Multivalent group A streptococcal vaccine designed to optimize the immunogenicity of six tandem M protein fragments. Vaccine. 1999;17:193–200. doi:10.1016/S0264-410X(98)00150-9. PMID:9987154.
43. Dale JB, Penfound TA, Chiang EY, Walton WJ. New 30-valent M protein-based vaccine evokes cross-opsonic antibodies against non-vaccine serotypes of group A streptococci. Vaccine. 2011;29:8175–8. doi:10.1016/j.vaccine.2011.09.005. PMID:21920403.
44. Kotloff KL, Corretti M, Palmer K, Campbell JD, Reddish MA, Hu MC, et al. Safety and immunogenicity of a recombinant multivalent group a streptococcal vaccine in healthy adults: Phase 1 trial. JAMA. 2004;292:709–15. doi:10.1001/jama.292.6.709. PMID:15304468.
45. McNeil SA, Halperin SA, Langley JM, Smith B, Warren A, Sharratt GP, Baxendale DM, Reddish MA, Hu MC, Stroop SD, et al. Safety and immunogenicity of 26-valent group a streptococcus vaccine in healthy adult volunteers. Clin Infect Dis. 2005;41:1114–22. doi:10.1086/444458. PMID:16163629.
46. Guilherme L, Fae KC, Higa F, Chaves L, Oshiro SE, Freschi de Barros S, Puschel C, Juliano MA, Tanaka AC, Spina G, et al. Towards a vaccine against rheumatic fever. Clin Dev Immunol. 2006;13:125–32. doi:10.1080/17402520600877026. PMID:17162355.
47. McMillan DJ, Dreze PA, Vu T, Bessen DE, Guglielmini J, Steer AC, Carapetis JR, Van Melderen L, Sriprakash KS, Smeesters PR. Updated model of group A Streptococcus M proteins based on a comprehensive worldwide study. Clin Microbiol Infect. 2013;19:E222–9. doi:10.1111/1469-0691.12134. PMID:23464795.
48. Batzloff MR, Hayman WA, Davies MR, Zeng M, Pruksakorn S, Brandt ER, 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. doi:10.1086/374800. PMID:12721940.
49. Turner CE, Kurupati P, Wiles S, Edwards RJ, Sriskandan S. Impact of immunization against SpyCEP during invasive disease with two streptococcal species: Streptococcus pyogenes and streptococcus equi. Vaccine. 2009;27:4923–9. doi:10.1016/j.vaccine.2009.06.042. PMID:19563892.
50. Cleary PP, Matsuka YV, Huynh T, Lam H, Olmsted SB. Immunization with C5a peptidase from either group A or B streptococci

enhances clearance of group A streptococci from intranasally infected mice. Vaccine. 2004;22:4332–41. doi:10.1016/j.vaccine.2004.04.030. PMID:15474726.
51. Sabharwal H, Michon F, Nelson D, Dong W, Fuchs K, Manjarrez RC, Sarkar A, Uitz C, Viteri-Jackson A, Suarez RS, et al. Group A streptococcus (GAS) carbohydrate as an immunogen for protection against GAS infection. J Infect Dis. 2006;193:129–35. doi:10.1086/498618. PMID:16323141.
52. 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. doi:10.1093/infdis/171.3.593. PMID:7876606.
53. 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. doi:10.1128/IAI.70.4.2171-2177.2002. PMID:11895984.
54. Steer AC, Law I, Matatolu L, Beall BW, Carapetis JR. Global emm type distribution of group A streptococci: Systematic review and implications for vaccine development. Lancet Infect Dis. 2009;9:611–6. doi:10.1016/S1473-3099(09)70178-1. PMID:19778763.
55. McNeil SA HS, Langley JM, Smith B, Warren A, Baxendale DM, Sharratt GP, et al. A double-blind, randomized phase II trial of the safety and immunogenicity of 26-valent group A streptococcus vaccine in healthy adults. Int Cong Ser. 2006;1289:303–6. doi:10.1016/j.ics.2005.12.002.
56. Dale JB, Batzloff MR, Cleary PP, Courtney HS, Good MF, Grandi G, Halperin S, Margarit IY, McNeil S, Pandey M et al. Current approaches to group A streptococcal vaccine development. In: Ferretti JJ, Stevens DL, Fischetti VA, editors. Streptococcus pyogenes: Basic Biology to Clinical Manifestations. Oklahoma City (OK): University of Oklahoma Health Sciences Center (c) The University of Oklahoma Health Sciences Center; 2016.
57. Dale JB, Penfound TA, Tamboura B, Sow SO, Nataro JP, Tapia M, Kotloff KL. Potential coverage of a multivalent M protein-based group A streptococcal vaccine. Vaccine. 2013;31:1576–81. doi:10.1016/j.vaccine.2013.01.019. PMID:23375817.
58. Dale JB, Smeesters PR, Courtney HS, Penfound TA, Hohn CM, Smith JC, Baudry JY. Structure-based design of broadly protective group a streptococcal M protein-based vaccines. Vaccine. 2017;35:19–26. doi:10.1016/j.vaccine.2016.11.065. PMID:27890396.
59. Guilherme L, Postol E, Freschi de Barros S, Higa F, Alencar R, Lastre M, Zayas C, Puschel CR, Silva WR, Sa-Rocha LC, et al. A vaccine against S. pyogenes: Design and experimental immune response. Methods. 2009;49:316–21. doi:10.1016/j.ymeth.2009.03.024. PMID:19409999.
60. Guerino MT, Postol E, Demarchi LM, Martins CO, Mundel LR, Kalil J, Guilherme L. HLA class II transgenic mice develop a safe and long lasting immune response against StreptInCor, an anti-group A streptococcus vaccine candidate. Vaccine. 2011;29:8250–6. doi:10.1016/j.vaccine.2011.08.113. PMID:21907752.
61. Postol E, Alencar R, Higa FT, Freschi de Barros S, Demarchi LM, Kalil J, Guilherme L. StreptInCor: A candidate vaccine epitope against S. pyogenes infections induces protection in outbred mice. PLoS One. 2013;8:e60969. doi:10.1371/journal.pone.0060969. PMID:23593359.
62. De Amicis KM, Freschi de Barros S, Alencar RE, Postol E, Martins Cde O, Arcuri HA, Goulart C, Kalil J, Guilherme L. Analysis of the coverage capacity of the StreptInCor candidate vaccine against Streptococcus pyogenes. Vaccine. 2014;32:4104–10. doi:10.1016/j.vaccine.2013.08.043. PMID:23994376.
63. McNeilly C, Cosh S, Vu T, Nichols J, Henningham A, Hofmann A, Fane A, Smeesters PR, Rush CM, Hafner LM, et al. Predicted Coverage and Immuno-Safety of a Recombinant C-Repeat Region Based Streptococcus pyogenes Vaccine Candidate. PLoS One. 2016;11:e0156639. doi:10.1371/journal.pone.0156639. PMID:27310707.
64. Brandt ER, Sriprakash KS, Hobb RI, Hayman WA, Zeng W, Batzloff MR, Jackson DC, Good MF. New multi-determinant strategy for a group A streptococcal vaccine designed for the Australian Aboriginal population. Nat Med. 2000;6:455–9. doi:10.1038/74719. PMID:10742155.
65. Pandey M, Langshaw E, Hartas J, Lam A, Batzloff MR, Good MF. A synthetic M protein peptide synergizes with a CXC chemokine protease to induce vaccine-mediated protection against virulent streptococcal pyoderma and bacteremia. J Immunol (Baltimore, Md: 1950). 2015;194:5915–25. doi:10.4049/jimmunol.1500157.
66. Pandey M, Mortensen R, Calcutt A, Powell J, Batzloff MR, Dietrich J, Good MF. Combinatorial Synthetic Peptide Vaccine Strategy Protects against Hypervirulent CovR/S Mutant Streptococci. J Immunol (Baltimore, Md: 1950). 2016;196:3364–74. doi:10.4049/jimmunol.1501994.
67. Pandey M, Powell J, Calcutt A, Zaman M, Phillips ZN, Ho MF, Batzloff MR, Good MF. Physicochemical characterisation, immunogenicity and protective efficacy of a lead streptococcal vaccine: Progress towards Phase I trial. Sci Rep. 2017;7:13786. doi:10.1038/s41598-017-14157-7. PMID:29062085.
68. Rodriguez-Ortega MJ, Norais N, Bensi G, Liberatori S, Capo S, Mora M, Scarselli M, Doro F, Ferrari G, Garaguso I, et al. Characterization and identification of vaccine candidate proteins through analysis of the group A Streptococcus surface proteome. Nat Biotechnol. 2006;24:191–7. doi:10.1038/nbt1179. PMID:16415855.
69. Cheng Q, Stafslien D, Purushothaman SS, Cleary P. The group B streptococcal C5a peptidase is both a specific protease and an invasin. Infect Immun. 2002;70:2408–13. doi:10.1128/IAI.70.5.2408-2413.2002. PMID:11953377.
70. 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. PMID:9169735.
71. Ji Y, McLandsborough L, Kondagunta A, Cleary PP. C5a peptidase alters clearance and trafficking of group A streptococci by infected mice. Infect Immun. 1996;64:503–10. PMID:8550199.
72. Bronze MS, Beachey EH, Dale JB. Protective and heart-crossreactive epitopes located within the NH2 terminus of type 19 streptococcal M protein. J Exp Med. 1988;167:1849–59. doi:10.1084/jem.167.6.1849. PMID:2455015.
73. 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. 1999;179:901–6. doi:10.1086/314655. PMID:10068585.
74. Roggiani M, Stoehr JA, Olmsted SB, Matsuka YV, Pillai S, Ohlendorf DH, Schlievert PM. Toxoids of streptococcal pyrogenic exotoxin A are protective in rabbit models of streptococcal toxic shock syndrome. Infect Immun. 2000;68:5011–7. doi:10.1128/IAI.68.9.5011-5017.2000. PMID:10948118.
75. Schlievert PM, Assimacopoulos AP, Cleary PP. Severe invasive group A streptococcal disease: Clinical description and mechanisms of pathogenesis. J Lab Clin Med. 1996;127:13–22. doi:10.1016/S0022-2143(96)90161-4. PMID:8592092.
76. Kuo CF, Wu JJ, Lin KY, Tsai PJ, Lee SC, Jin YT, Lei HY, Lin YS. Role of streptococcal pyrogenic exotoxin B in the mouse model of group A streptococcal infection. Infect Immun. 1998;66:3931–5. PMID:9673282.
77. McCormick JK, Tripp TJ, Olmsted SB, Matsuka YV, Gahr PJ, Ohlendorf DH, Schlievert PM. Development of streptococcal pyrogenic exotoxin C vaccine toxoids that are protective in the rabbit model of toxic shock syndrome. J Immunol (Baltimore, Md: 1950). 2000;165:2306–12. doi:10.4049/jimmunol.165.4.2306.
78. Courtney HS, Hasty DL, Dale JB. Serum opacity factor (SOF) of Streptococcus pyogenes evokes antibodies that opsonize homologous and heterologous SOF-positive serotypes of group A streptococci. Infect Immun. 2003;71:5097–103. doi:10.1128/IAI.71.9.5097-5103.2003. PMID:12933852.
79. Mora M, Bensi G, Capo S, Falugi F, Zingaretti C, Manetti AG, Maggi T, Taddei AR, Grandi G, Telford JL. Group A Streptococcus produce pilus-like structures containing protective antigens and Lancefield T antigens. PNAS. 2005;102:15641–6. doi:10.1073/pnas.0507808102. PMID:16223875.

80. van Sorge NM, Cole JN, Kuipers K, Henningham A, Aziz RK, Kasirer-Friede A, Lin L, Berends ETM, Davies MR, Dougan G, et al. The classical lancefield antigen of group a Streptococcus is a virulence determinant with implications for vaccine design. Cell Host Microbe. 2014;15:729–40. doi:10.1016/j.chom.2014.05.009. PMID: 24922575.
81. Cunningham MW. Pathogenesis of group A streptococcal infections. Clin Microbiol Rev. 2000;13:470–511. doi:10.1128/CMR.13.3.470-511.2000. PMID:10885988.
82. 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. PMID:2458320.
83. 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;167:1114–23. doi:10.1084/jem.167.3.1114. PMID:2450950.
84. Pruksakorn S, Currie B, Brandt E, Martin D, Galbraith A, Phornphutkul C, Hunsakunachai S, Manmontri A, Good MF. Towards a vaccine for rheumatic fever: Identification of a conserved target epitope on M protein of group A streptococci. Lancet (London, England). 1994;344:639–42. doi:10.1016/S0140-6736(94)92083-4. PMID:7520963.
85. Good MF, Pandey M, Batzloff MR, Tyrrell GJ. Strategic development of the conserved region of the M protein and other candidates as vaccines to prevent infection with group A streptococci. Expert Rev Vaccines. 2015;14:1459–70. doi:10.1586/14760584.2015.1081817. PMID:26485214.
86. Brandt ER, Hayman WA, Currie B, Carapetis J, Wood Y, Jackson DC, Cooper J, Melrose WD, Saul AJ, Good MF. 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. doi:10.1046/j.1365-2567.1996.d01-754.x. PMID:8958044.
87. Fae KC, da Silva DD, Oshiro SE, Tanaka AC, Pomerantzeff PM, Douay C, Charron D, Toubert A, Cunningham MW, Kalil J, et al. Mimicry in recognition of cardiac myosin peptides by heart-intralesional T cell clones from rheumatic heart disease. J Immunol (Baltimore, Md: 1950). 2006;176:5662–70. doi:10.4049/jimmunol.176.9.5662.
88. Guilherme L, Fae KC, Oshiro SE, Tanaka AC, Pomerantzeff PM, Kalil J. Rheumatic fever: How S. pyogenes-primed peripheral T cells trigger heart valve lesions. Ann N Y Acad Sci. 2005;1051:132–40. doi:10.1196/annals.1361.054. PMID:16126952.
89. Relf WA, Cooper J, Brandt ER, Hayman WA, Anders RF, Pruksakorn S, Currie B, Saul A, Good MF. Mapping a conserved conformational epitope from the M protein of group A streptococci. Pept Res. 1996;9:12–20. PMID:8727479.
90. Brandt ER, Hayman WA, Currie B, Pruksakorn S, Good MF. Human antibodies to the conserved region of the M protein: Opsonization of heterologous strains of group A streptococci. Vaccine. 1997;15:1805–12. doi:10.1016/S0264-410X(97)00178-3. PMID:9364687.
91. 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;9:1723–33. doi:10.1093/intimm/9.11.1723. PMID:9418133.
92. Batzloff MR, Fane A, Gorton D, Pandey M, Rivera-Hernandez T, Calcutt A, Yeung G, Hartas J, Johnson L, Rush CM, et al. Preclinical immunogenicity and safety of a Group A streptococcal M protein-based vaccine candidate. Hum Vaccin Immunother. 2016;12:3089–96. doi:10.1080/21645515.2016.1222999. PMID:27541593.
93. Pandey M, Wykes MN, Hartas J, Good MF, Batzloff MR. Long-term antibody memory induced by synthetic peptide vaccination is protective against Streptococcus pyogenes infection and is independent of memory T cell help. J Immunol (Baltimore, Md: 1950). 2013;190:2692–701. doi:10.4049/jimmunol.1202333.
94. Batzloff MR, Yan H, Davies MR, Hartas J, Lowell GH, White G, Burt DS, Leanderson T, Good MF. Toward the development of an antidisease, transmission-blocking intranasal vaccine for group a streptococcus. J Infect Dis. 2005;192:1450–5. doi:10.1086/466528. PMID:16170764.
95. Bessen D, Fischetti VA. Passive acquired mucosal immunity to group A streptococci by secretory immunoglobulin A. J Exp Med. 1988;167:1945–50. doi:10.1084/jem.167.6.1945. PMID:3290383.
96. Zaman M, Ozberk V, Langshaw EL, McPhun V, Powell JL, Phillips ZN, Ho MF, Calcutt A, Batzloff MR, Toth I, et al. Novel platform technology for modular mucosal vaccine that protects against streptococcus. Sci Rep. 2016;6:39274. doi:10.1038/srep39274. PMID:27976706.
97. 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. doi:10.1016/S0264-410X(02)00205-0. PMID:12034109.
98. Batzloff MR, Hartas J, Zeng W, Jackson DC, Good MF. Intranasal vaccination with a lipopeptide containing a conformationally constrained conserved minimal peptide, a universal T cell epitope, and a self-adjuvanting lipid protects mice from group A streptococcus challenge and reduces throat colonization. J Infect Dis. 2006;194:325–30. doi:10.1086/505146. PMID:16826480.
99. Sanderson-Smith M, De Oliveira DM, Guglielmini J, McMillan DJ, Vu T, Holien JK, Henningham A, Steer AC, Bessen DE, Dale JB, et al. A systematic and functional classification of Streptococcus pyogenes that serves as a new tool for molecular typing and vaccine development. J Infect Dis. 2014;210:1325–38. doi:10.1093/infdis/jiu260. PMID:24799598.
100. Turner P, Ngeth P, Turner C, Sao S, Day NP, Baker C, Steer AC, Smeesters PR. Molecular Epidemiology of Group A Streptococcus Infections in Cambodian Children, 2007–2012. Pediatr Infect Dis J. 2015;34:1414–5. doi:10.1097/INF.0000000000000878. PMID:26780027.
101. Rattanavong S, Dance DA, Davong V, Baker C, Frost H, Phetsouvanh R, Vongsouvath M, Newton PN, Steer AC, Smeesters PR. Group A streptococcal strains isolated in Lao People's Democratic Republic from 2004 to 2013. Epidemiol Infect. 2016;144:1770–3. doi:10.1017/S0950268815002927. PMID:26645633.
102. Churchward G. The two faces of Janus: Virulence gene regulation by CovR/S in group A streptococci. Mol Microbiol. 2007;64:34–41. doi:10.1111/j.1365-2958.2007.05649.x. PMID:17376070.
103. Hollands A, Pence MA, Timmer AM, Osvath SR, Turnbull L, Whitchurch CB, Walker MJ, Nizet V. Genetic switch to hypervirulence reduces colonization phenotypes of the globally disseminated group A streptococcus M1T1 clone. J Infect Dis. 2010;202:11–9. doi:10.1086/653124. PMID:20507231.
104. Turner CE, Kurupati P, Jones MD, Edwards RJ, Sriskandan S. Emerging role of the interleukin-8 cleaving enzyme SpyCEP in clinical Streptococcus pyogenes infection. J Infect Dis. 2009;200:555–63. doi:10.1086/603541. PMID:19591574.
105. Edwards RJ, Taylor GW, Ferguson M, Murray S, Rendell N, Wrigley A, Bai Z, Boyle J, Finney SJ, Jones A, et al. Specific C-terminal cleavage and inactivation of interleukin-8 by invasive disease isolates of Streptococcus pyogenes. J Infect Dis. 2005;192:783–90. doi:10.1086/432485. PMID:16088827.
106. Zinkernagel AS, Timmer AM, Pence MA, Locke JB, Buchanan JT, Turner CE, Mishalian I, Sriskandan S, Hanski E, Nizet V. The IL-8 protease SpyCEP/ScpC of group A Streptococcus promotes resistance to neutrophil killing. Cell Host & Microbe. 2008;4:170–8. doi:10.1016/j.chom.2008.07.002.
107. Sumby P, Zhang S, Whitney AR, Falugi F, Grandi G, Graviss EA, Deleo FR, Musser JM. A chemokine-degrading extracellular protease made by group A Streptococcus alters pathogenesis by enhancing evasion of the innate immune response. Infect Immun. 2008;76:978–85. doi:10.1128/IAI.01354-07. PMID:18174342.
108. Alam FM, Bateman C, Turner CE, Wiles S, Sriskandan S. Non-invasive monitoring of Streptococcus pyogenes vaccine efficacy using biophotonic imaging. PLoS One. 2013;8:e82123. doi:10.1371/journal.pone.0082123. PMID:24278474.

109. Henningham A, Gillen CM, Walker MJ. Group a streptococcal vaccine candidates: Potential for the development of a human vaccine. Curr Top Microbiol Immunol. 2013;368:207–42. PMID:23250780.
110. Perepared for WHO PD-VAC. Status of vaccine research and development of vaccines for streptococcus pyogenes.
111. Nooh MM, El-Gengehi N, Kansal R, David CS, Kotb M. HLA transgenic mice provide evidence for a direct and dominant role of HLA class II variation in modulating the severity of streptococcal sepsis. J Immunol (Baltimore, Md: 1950). 2007;178:3076–83. doi:10.4049/jimmunol.178.5.3076.
112. Rivera-Hernandez T, Pandey M, Henningham A, Cole J, Choudhury B, Cork AJ, Gillen CM, Ghaffar KA, West NP, Silvestri G, et al. Differing efficacies of lead group A streptococcal vaccine candidates and full-length M protein in cutaneous and invasive disease models. mBio. 2016;7.
113. Skinner JM, Caro-Aguilar IC, Payne AM, Indrawati L, Fontenot J, Heinrichs JH. Comparison of rhesus and cynomolgus macaques in a Streptococcus pyogenes infection model for vaccine evaluation. Microb Pathog. 2011;50:39–47. doi:10.1016/j.micpath.2010.10.004. PMID:21035535.
114. Virtaneva K, Porcella SF, Graham MR, Ireland RM, Johnson CA, Ricklefs SM, Babar I, Parkins LD, Romero RA, Corn GJ, et al. Longitudinal analysis of the group A Streptococcus transcriptome in experimental pharyngitis in cynomolgus macaques. PNAS. 2005;102:9014–9. doi:10.1073/pnas.0503671102. PMID:15956184.
115. Seok J, Warren HS, Cuenca AG, Mindrinos MN, Baker HV, Xu W, et al. Genomic responses in mouse models poorly mimic human inflammatory diseases. PNAS. 2013;110:3507–12. doi:10.1073/pnas.1222878110. PMID:23401516.
116. Takao K, Miyakawa T. Genomic responses in mouse models greatly mimic human inflammatory diseases. PNAS. 2015;112:1167–72. doi:10.1073/pnas.1401965111. PMID:25092317.
117. Justice MJ, Dhillon P. Using the mouse to model human disease: Increasing validity and reproducibility. Dis Models Mech. 2016;9:101–3. doi:10.1242/dmm.024547.
118. Osuchowski MF, Remick DG, Lederer JA, Lang CH, Aasen AO, Aibiki M, Azevedo LC, Bahrami S, Boros M, Cooney R, et al. Abandon the mouse research ship? Not just yet! Shock (Augusta, Ga). 2014;41:463–75. doi:10.1097/SHK.0000000000000153. PMID:24569509.
119. Rathore D, Sacci JB, de la Vega P, McCutchan TF. Binding and invasion of liver cells by Plasmodium falciparum sporozoites. Essential involvement of the amino terminus of circumsporozoite protein. J Biol Chem. 2002;277:7092–8. doi:10.1074/jbc.M106862200. PMID:11751898.
120. Rathore D, Nagarkatti R, Jani D, Chattopadhyay R, de la Vega P, Kumar S, McCutchan TF. An immunologically cryptic epitope of Plasmodium falciparum circumsporozoite protein facilitates liver cell recognition and induces protective antibodies that block liver cell invasion. J Biol Chem. 2005;280:20524–9. doi:10.1074/jbc.M414254200. PMID:15781464.
121. Little SF, Ivins BE, Fellows PF, Friedlander AM. Passive protection by polyclonal antibodies against Bacillus anthracis infection in guinea pigs. Infect Immun. 1997;65:5171–5. PMID:9393812.
122. Pezard C, Weber M, Sirard JC, Berche P, Mock M. Protective immunity induced by Bacillus anthracis toxin-deficient strains. Infect Immun. 1995;63:1369–72. PMID:7890396.
123. Turnbull PC, Leppla SH, Broster MG, Quinn CP, Melling J. Antibodies to anthrax toxin in humans and guinea pigs and their relevance to protective immunity. Med Microbiol Immunol (Berl). 1988;177:293–303. doi:10.1007/BF00189414.
124. Demicheli V, Rivetti D, Deeks JJ, Jefferson T, Pratt M. The effectiveness and safety of vaccines against human anthrax: A systematic review. Vaccine. 1998;16:880–4. doi:10.1016/S0264-410X(98)00023-1. PMID:9682332.
125. Institute of Medicine Committee to Assess the S. Efficacy of the Anthrax V. In: Joellenbeck LM, Zwanziger LL, Durch JS, Strom BL, editors. The Anthrax Vaccine: Is It Safe? Does It Work? Washington, (DC): National Academies Press (US) Copyright 2002 by the National Academy of Sciences. All rights reserved; 2002.
126. Pittman PR, Kim-Ahn G, Pifat DY, Coonan K, Gibbs P, Little S, Pace-Templeton JG, Myers R, Parker GW, Friedlander AM. Anthrax vaccine: Immunogenicity and safety of a dose-reduction, route-change comparison study in humans. Vaccine. 2002;20:1412–20. doi:10.1016/S0264-410X(01)00462-5. PMID:11818160.
127. Albrecht MT, Li H, Williamson ED, LeButt CS, Flick-Smith HC, Quinn CP, Westra H, Galloway D, Mateczun A, Goldman S, et al. Human monoclonal antibodies against anthrax lethal factor and protective antigen act independently to protect against Bacillus anthracis infection and enhance endogenous immunity to anthrax. Infect Immun. 2007;75:5425–33. doi:10.1128/IAI.00261-07. PMID:17646360.
128. Oscherwitz J, Yu F, Jacobs JL, Liu TH, Johnson PR, Cease KB. Synthetic peptide vaccine targeting a cryptic neutralizing epitope in domain 2 of Bacillus anthracis protective antigen. Infect Immun. 2009;77:3380–8. doi:10.1128/IAI.00358-09. PMID:19487468.
129. Guilherme L, Ferreira FM, Kohler KF, Postol E, Kalil J. A vaccine against Streptococcus pyogenes: The potential to prevent rheumatic fever and rheumatic heart disease. Am J Cardiovasc Drugs. 2013;13:1–4. doi:10.1007/s40256-013-0005-8. PMID:23355360.
130. Azmi F, Ahmad Fuaad AA, Giddam AK, Batzloff MR, Good MF, Skwarczynski M, Toth I, et al. Self-adjuvanting vaccine against group A streptococcus: Application of fibrillized peptide and immunostimulatory lipid as adjuvant. Bioorg Med Chem. 2014;22:6401–8. doi:10.1016/j.bmc.2014.09.042. PMID:25438764.
131. Kirvan CA, Swedo SE, Heuser JS, Cunningham MW. Mimicry and autoantibody-mediated neuronal cell signaling in Sydenham chorea. Nat Med. 2003;9:914–20. doi:10.1038/nm892. PMID:12819778.
132. Shikhman AR, Cunningham MW. Immunological mimicry between N-acetyl-beta-D-glucosamine and cytokeratin peptides. Evidence for a microbially driven anti-keratin antibody response. J Immunol (Baltimore, Md: 1950). 1994;152:4375–87.
133. Shikhman AR, Greenspan NS, Cunningham MW. Cytokeratin peptide SFGSGFGGGY mimics N-acetyl-beta-D-glucosamine in reaction with antibodies and lectins, and induces in vivo anti-carbohydrate antibody response. J Immunol (Baltimore, Md: 1950). 1994;153:5593–606.
134. Simpson WJ, Musser JM, Cleary PP. Evidence consistent with horizontal transfer of the gene (emm12) encoding serotype M12 protein between group A and group G pathogenic streptococci. Infect Immun. 1992;60:1890–3. PMID:1563779.
135. Kawabata S, Kunitomo E, Terao Y, Nakagawa I, Kikuchi K, Totsuka K, Hamada, S. 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. doi:10.1128/IAI.69.2.924-930.2001. PMID:11159987.
136. McArthur J, Medina E, Mueller A, Chin J, Currie BJ, Sriprakash KS, Talay SR, Chhatwal GS, Walker MJ. Intranasal vaccination with streptococcal fibronectin binding protein Sfb1 fails to prevent growth and dissemination of Streptococcus pyogenes in a murine skin infection model. Infect Immun. 2004;72:7342–5. doi:10.1128/IAI.72.12.7342-7345.2004. PMID:15557665.
137. Goodfellow AM, Hibble M, Talay SR, Kreikemeyer B, Currie BJ, Sriprakash KS, Chhatwal GS. Distribution and antigenicity of fibronectin binding proteins (SfbI and SfbII) of Streptococcus pyogenes clinical isolates from the northern territory, Australia. J Clin Microbiol. 2000;38:389–92. PMID:10618121.
138. Bensi G, Mora M, Tuscano G, Biagini M, Chiarot E, Bombaci M, Capo S, Falugi F, Manetti AG, Donato P, et al. Multi high-throughput approach for highly selective identification of vaccine candidates: The Group A Streptococcus case. Mol Cell Proteomics. 2012;11:M111. 015693. doi:10.1074/mcp.M111.015693. PMID:22286755.
139. Reid SD, Green NM, Sylva GL, Voyich JM, Stenseth ET, DeLeo FR, Palzkill T, Low DE, Hill HR, Musser JM. 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. doi:10.1128/JB.184.22.6316-6324.2002. PMID:12399501.

140. Fritzer A, Senn BM, Minh DB, Hanner M, Gelbmann D, Noiges B, Henics T, Schulze K, Guzman CA, Goodacre J, et al. Novel conserved group A streptococcal proteins identified by the antigenome technology as vaccine candidates for a non-M protein-based vaccine. Infect Immun. 2010;78:4051–67. doi:10.1128/IAI.00295-10. PMID:20624906.
141. Reglinski M, Lynskey NN, Choi YJ, Edwards RJ, Sriskandan S. Development of a multicomponent vaccine for Streptococcus pyogenes based on the antigenic targets of IVIG. J Infect. 2016;72:450–9. doi:10.1016/j.jinf.2016.02.002. PMID:26880087.
142. Henningham A, Chiarot E, Gillen CM, Cole JN, Rohde M, Fulde M, Ramachandran V, Cork AJ, Hartas J, Magor G, et al. Conserved anchorless surface proteins as group A streptococcal vaccine candidates. J Mol Med. 2012;90:1197–207. doi:10.1007/s00109-012-0897-9. PMID:22527883.
143. Olive C, Batzloff M, Horvath A, Clair T, Yarwood P, Toth I, Good MF. Group A streptococcal vaccine delivery by immunization with a self-adjuvanting M protein-based lipid core peptide construct. Indian J Med Res. 2004;119(Suppl):88–94. PMID:15232170.
144. Olive C, Batzloff MR, Horvath A, Wong A, Clair T, Yarwood P, Toth I, Good MF. 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. doi:10.1128/IAI.70.5.2734-2738.2002. PMID:11953422.
145. Olive C, Ho MF, Dyer J, Lincoln D, Barozzi N, Toth I, Good MF. Immunization with a tetraepitopic lipid core peptide vaccine construct induces broadly protective immune responses against group A streptococcus. J Infect Dis. 2006;193:1666–76. doi:10.1086/504266. PMID:16703510.
146. Olive C, Sun HK, Ho MF, Dyer J, Horvath A, Toth I, Good MF. Intranasal administration is an effective mucosal vaccine delivery route for self-adjuvanting lipid core peptides targeting the group A streptococcal M protein. J Infect Dis. 2006;194:316–24. doi:10.1086/505580. PMID:16826479.