



LUND UNIVERSITY

Host-pathogen interactions in *Streptococcus pyogenes* infections, with special reference to puerperal fever and a comment on vaccine development.

Areschoug, Thomas; Carlsson, Fredric; Stålhammar-Carlemalm, Margaretha; Lindahl, Gunnar

Published in:
Vaccine

DOI:
[10.1016/j.vaccine.2004.08.010](https://doi.org/10.1016/j.vaccine.2004.08.010)

2004

[Link to publication](#)

Citation for published version (APA):

Areschoug, T., Carlsson, F., Stålhammar-Carlemalm, M., & Lindahl, G. (2004). Host-pathogen interactions in *Streptococcus pyogenes* infections, with special reference to puerperal fever and a comment on vaccine development. *Vaccine*, 22 Suppl 1(Suppl 1), S9-S14. <https://doi.org/10.1016/j.vaccine.2004.08.010>

Total number of authors:
4

General rights

Unless other specific re-use rights are stated the following general rights apply:
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: <https://creativecommons.org/licenses/>

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

Invited review for *Vaccine*

**Host-pathogen interactions in *Streptococcus pyogenes* infections,
with special reference to puerperal fever and a comment on vaccine
development**

Thomas Areschoug, Fredric Carlsson, Margaretha Stålhammar-Carlemalm and

Gunnar Lindahl*

Department of Medical Microbiology, Dermatology and Infection;

Lund University; Sölvegatan 23; SE-223 62 Lund; Sweden

*Corresponding author. Tel: +46-46-173244; Fax: +46-46-189117;

e-mail: gunnar.lindahl@mmb.lu.se

Abstract

Streptococcus pyogenes (group A streptococcus) causes a variety of diseases, including acute pharyngitis, impetigo, rheumatic fever and the streptococcal toxic shock syndrome. Moreover, *S. pyogenes* was responsible for the classical example of a nosocomial infection, the epidemics of puerperal fever (childbed fever) that caused the death of numerous women in earlier centuries. The most extensively studied virulence factor of *S. pyogenes* is the surface M protein, which inhibits phagocytosis and shows antigenic variation. Recent data indicate that many M proteins confer phagocytosis resistance because the variable N-terminal region has non-overlapping sites that specifically bind two components of the human immune system, the complement inhibitor C4b-binding protein (C4BP) and IgA-Fc. Concerning puerperal fever, molecular and epidemiological analysis suggests that the *S. pyogenes* surface protein R28 may have played a pathogenetic role in these epidemics. This article summarizes the properties of M protein and the R28 protein and considers a potential problem encountered in connection with the use of animal models for vaccine development.

Keywords: Group A streptococcus / puerperal fever / vaccines / C4b-binding protein / IgA-Fc

Abbreviated title: *Streptococcus pyogenes* infections

1. Introduction

Streptococcus pyogenes (group A streptococcus) is a Gram-positive bacterium that causes some of the most common infections in humans [1]. With regard to nosocomial infections, the subject of this issue of *Vaccine*, it is of particular interest that *S. pyogenes* caused the classical example of such infections, the epidemics of puerperal fever (childbed fever) that resulted in the death of numerous women during the 18th and 19th centuries. Although puerperal fever is now rare, it remains an important example of an infectious disease that emerged because of changes in society, in this case the construction of large maternity hospitals where the disease was acquired.

In addition to its importance as a pathogen, *S. pyogenes* is an attractive model system for analysis of some key problems in microbial pathogenesis. For example, *S. pyogenes* has unique advantages for studies of antigenic variation, a phenomenon of major importance in vaccine development. Moreover, *S. pyogenes* is an interesting system for analysis of phagocytosis resistance, a common and important microbial virulence mechanism. This short review focuses on the properties of two *S. pyogenes* surface proteins, the M protein, which confers resistance to phagocytosis, and the R28 protein, which has been implicated in the pathogenesis of puerperal fever. In addition, we will comment on a general problem that may complicate attempts to develop vaccines against a human pathogen such as *S. pyogenes*.

2. *Streptococcus pyogenes*

The two most common infections caused by *S. pyogenes* are acute tonsillitis ('strep throat') and the skin infection impetigo. During the last two decades, *S. pyogenes* has also emerged as the cause of a streptococcal toxic shock syndrome (STSS) with high mortality [1]. Importantly, infection with *S. pyogenes* is occasionally followed by one of two non-suppurative sequelae that may be due to autoimmunity, acute rheumatic

fever (ARF) and acute post-streptococcal glomerulonephritis (APSGN). ARF is a major cause of acquired heart disease in developing countries and unexpected outbreaks occurred in the US in the 1980s, but like APSGN this disease is presently uncommon in the Western world [1].

Remarkably, resistance to penicillin has never been described for *S. pyogenes*, a fact that simplifies treatment of infections and limits spread of the pathogen. Nevertheless, development of a vaccine against this common pathogen is highly desirable, because *S. pyogenes* infections cause great costs to society and because a vaccine would be expected to reduce the incidence of ARF.

3. M protein and its role in phagocytosis resistance

The most extensively studied virulence factor of *S. pyogenes* is the surface M protein, identified already in the 1920s through the classical work of Rebecca Lancefield [2]. The M protein inhibits phagocytosis by human neutrophils and allows *S. pyogenes* to grow rapidly in whole human blood [3]. In addition, M protein may have other functions, such as promoting adhesion to human epithelial cells [4].

Several lines of evidence indicate that M protein plays a major role in human infections [3]. For example, recent studies in a primate model of *S. pyogenes* infection demonstrated that M protein promotes pharyngeal colonization, a feature that may be explained by its antiphagocytic property and/or by its ability to promote adhesion to epithelial cells [5]. Importantly, all clinical isolates of *S. pyogenes* express M protein, which occurs in >120 different types [6]. The M protein is stable within a single strain, but exhibits extensive sequence variability between strains, in particular in the N-terminal so-called hypervariable region (HVR), which determines the M type of the strain. Antibodies directed against the HVR are protective and the immunity is type-

specific [3, 7]. In other words, M protein exhibits antigenic variation. This antigenic variation has so far precluded the development of a vaccine based on M protein.

The mechanism by which M protein inhibits phagocytosis has remained unclear, and the extensive sequence variability in the HVR has made it difficult to understand how this region could have the specific function to prevent phagocytosis. A clue to this problem was obtained through the finding that many HVRs specifically bind the same ligand, in spite of the extreme sequence variability between different HVRs. The bound ligand is human C4b-binding protein (C4BP) [8, 9], a ~570 kDa plasma protein that inhibits activation of the classical pathway of complement activation [10, 11]. More than 50% of all *S. pyogenes* strains bind C4BP [12], and all available evidence indicates that C4BP binds to the HVR of the M protein expressed by these strains [8, 9]. It is important to note, however, that many M proteins do not bind C4BP, and the exact molecular function of the HVR in these M proteins remains unknown.

We have used the M22 protein as a model system to study M protein function (Fig. 1). The antiphagocytic property of this M protein may be explained as cooperation between two variable ligand-binding regions [13]. The HVR binds human C4BP and an adjacent semi-variable region (SVR) binds human IgA-Fc. The HVR makes an important contribution to phagocytosis resistance because bacteria-bound C4BP retains its ability to inhibit complement activation, thereby preventing opsonization due to complement deposition [13, 14]. Surprisingly, *S. pyogenes* lacking M protein activates the classical complement pathway even in normal human serum. This may explain why *S. pyogenes* exploits human C4BP for protection against complement activation [13]. The SVR, which binds human IgA-Fc [15], also makes an important contribution to phagocytosis resistance [13], but the mechanism by which this region contributes to virulence is not clear. Possibly, the IgA-binding region acts by inhibiting the binding of

IgA-Fc to the human IgA-receptor CD89 on phagocytes, thereby interfering with IgA effector functions [16].

Importantly, the model shown in Fig. 1 can explain the existence of “protective” and “non-protective” epitopes in M protein. Such epitopes were first described by Jones and Fischetti [17], who showed that a mAb directed against the HVR of the M6 protein was protective (i.e. it blocked the antiphagocytic function of this M protein), while a mAb directed against the conserved C-repeat region was non-protective. We have confirmed this finding using polyclonal antibodies directed against different parts of the M22 protein, and find that protective antibodies block binding of the two ligands C4BP and IgA, while non-protective antibodies do not have this property [13]. Thus, the key property of a protective antibody may be its ability to inhibit binding of ligand(s) to the N-terminal variable region of an M protein. Antibodies raised against the HVR of M22 block binding of both C4BP and IgA, most likely because such antibodies sterically interfere with binding of both ligands. Similarly, antibodies to the SVR block binding of both ligands and are protective [13].

Studies of antigenic variation in M protein are important for analysis of the mechanisms by which *S. pyogenes* evades host immunity. In addition, M protein has several advantages as a model system for analysis of antigenic variation, a phenomenon of major importance in biology. For example, the stability of M protein within a single *S. pyogenes* strain facilitates studies of M protein function. Moreover, M proteins can readily be studied in soluble form and different ligand-binding regions can be studied as synthetic peptides [9, 18]. The evolution of extreme sequence divergence in the N-terminal region of M proteins is of considerable interest, because the variability appears to be at least as great as that observed in HIV-1 and influenza virus [9].

In summary, our studies of the M22 protein indicate that the antiphagocytic property of this protein may be fully explained through its ability to bind two

components of the humoral immune system, C4BP and IgA [13, 14]. This finding provides the first molecular explanation for the ability of M protein to confer phagocytosis resistance. It may appear surprising that plasma proteins play an essential role in infections caused by *S. pyogenes*, a pathogen that usually causes infections on mucosal surfaces. However, a plasma exudative response contributes to a first-line defense system [19] and neutrophils are recruited to the site of an infection, so it seems likely that the most important interactions between *S. pyogenes* and the human immune system occur on mucosal surfaces. An important subject for future studies will be to analyze how phagocytosis resistance is conferred by M proteins that do not bind C4BP and/or IgA.

4. Puerperal fever and the R28 protein

In the 18th and 19th centuries, numerous women died from puerperal fever (childbed fever), the disease that was the subject of the classical studies of Semmelweis and caused one of the major controversies in medical history [20, 21]. It is now generally accepted that puerperal fever was due to transfer of bacteria to women in labour by attending hospital physicians, who had previously performed autopsies or examined other patients. The epidemics of puerperal fever were due to *S. pyogenes* infections [22] and the clinical picture was probably similar to that of the recently identified streptococcal toxic shock syndrome (STSS), including shock and multiorgan failure [1].

Although puerperal fever is now rare, studies of this disease are of interest because it represents the classical example of a nosocomial infection and of an emerging infectious disease caused by changes in society. Virtually nothing is known about molecular mechanisms in puerperal fever, but an epidemiological study in Britain showed that the surface protein R28 was common among *S. pyogenes* strains isolated from recent outbreaks of puerperal fever, but was found in only a minority of

pharyngitis strains [23]. This over-representation of R28-expressing strains among puerperal fever isolates led us to hypothesize that the R28 protein played an important role in the epidemics of puerperal fever [24]. For obvious reasons, *S. pyogenes* isolates from the 18th and 19th century epidemics of puerperal fever were not available for characterization, but analysis of cases of puerperal fever reported in the literature in which the *S. pyogenes* strains were serotyped supported the hypothesis that R28-expressing strains were overrepresented also among puerperal fever isolates recovered earlier in the 20th century [24]. Although many *S. pyogenes* strains isolated from 20th century cases of puerperal fever probably did not express R28, this finding does not contradict the hypothesis that R28 may favor the emergence of epidemics, because endemic isolates, that cause sporadic cases or small outbreaks, would be expected to be non-clonal, while only epidemic strains would be expected to be clonal [25].

Molecular characterization of the R28 protein [24] showed that it is a member of a family of extremely repetitive surface proteins first identified in the group B streptococcus (GBS; *Streptococcus agalactiae*) [26], which is often found in the normal vaginal flora and causes serious invasive disease in newborns [27]. The protein family that includes R28 was first defined by the GBS proteins Rib and α [26, 28, 29] and among these GBS proteins R28 is most closely related to Rib (Fig. 2). Indeed, R28 can be viewed as a chimera derived from Rib and two other GBS surface proteins [24]. These data indicated that the gene encoding R28 may have originated in GBS, a hypothesis supported by the finding that some strains of GBS express a protein virtually identical to R28 [30, 31]

The similarity between R28 of *S. pyogenes* and Rib of GBS suggests that these two streptococcal surface proteins may have similar function. Interestingly, the R28 protein promotes binding of *S. pyogenes* to a human epithelial cell line of cervical origin [24], and one can speculate that the Rib protein of GBS has similar function,

allowing GBS to colonize the human genital tract. However, attempts to demonstrate that Rib is an adhesin have been unsuccessful so far.

To summarize, the R28 protein of *S. pyogenes* is epidemiologically associated with cases of puerperal fever and promotes binding to a human cervical cell line derived from the cervix. Interestingly, a bacterial mutant lacking R28 did not bind to these epithelial cells, a finding that led us to hypothesize that the R28 protein confers tissue tropism, specifically promoting adhesion of *S. pyogenes* to cells in the female genital tract [24]. According to this hypothesis, exposure to an R28-expressing *S. pyogenes* strain in connection with child delivery would increase the risk for a serious infection (puerperal fever), because the R28-expressing bacteria can rapidly establish an infection in the wounded tissue. Importantly, strains of *S. pyogenes* produce potent exotoxins, which probably are required for the development of puerperal fever and other invasive *S. pyogenes* infections [32]. In contrast, GBS lacks the toxins of *S. pyogenes* and may therefore not be able to cause puerperal fever, although it colonizes the female genital tract. Thus, a working model for the molecular pathogenesis of puerperal fever is now emerging.

5. Vaccine development: a problem with animal models

No vaccine is available against *S. pyogenes* infections. A priori, the M protein would appear to be a promising vaccine component, because it is expressed by all strains and elicits protective immunity, but the antigenic variation between M proteins expressed by different strains has so far precluded the development of a vaccine. Moreover, possible cross-reactions between M protein and human tissues have caused major concerns [3]. However, work is in progress to develop a multivalent vaccine based on a combination of many different HVRs, which may not elicit unwanted cross-reactivities [33]. Possibly, a vaccine could also be based on the conserved parts of M protein [34,

35] although antibodies directed against this part might be expected to have poor activity because they do not block the antiphagocytic function of the HVR. Another interesting vaccine candidate expressed by all strains of *S. pyogenes* is ScpA, a surface localized C5a-peptidase that shows a high degree of conservation between strains [36]. However, little is yet known about the ability of ScpA to elicit protective immunity in a human system. Several other surface proteins are potentially interesting as vaccine components, but have the drawback that they may not be expressed by all strains of *S. pyogenes* [24, 37-40]

A major problem in vaccine development is the lack of a good animal model for infections caused by *S. pyogenes*, a specific human pathogen. In this regard, *S. pyogenes* is similar to many other human pathogens. However, a vaccine must first be tested in an animal model, even if the model is poor. This is acceptable, as long as the results are interpreted with caution, but a problem that is sometimes overlooked is that a virulence factor with a key role in human infections may be of little or no importance in an animal model, making results obtained in the animal model of uncertain significance. This problem is well demonstrated by the properties of the *S. pyogenes* M22 protein (Fig. 1), which binds human C4BP and IgA-Fc, but does not bind C4BP or IgA of mouse origin [18, 41], excluding the use of mice as a model to study M22 and similar M proteins. This type of problem should be kept in mind in evaluating data obtained in the mouse, which is the only animal model commonly used to study the pathogenesis of *S. pyogenes* infections [42, 43].

The use of animal models becomes particularly complicated if a pathogen actively interferes with human defenses, as in the M22 system, in which human C4BP bound to the M protein interferes with complement deposition (Fig. 1). This problem is presented in a schematic way in Fig. 3. In the human system, an antibody binding to a bacterial surface antigen may cause complement activation, resulting in opsonization of

the bacterium by immunoglobulin (Ig) and complement, resulting in protection of the host against infection (Fig. 3A). Importantly, efficient opsonization commonly requires both Ig and complement [44], implying that a microorganism may evade opsonization by interfering with deposition of either Ig or complement. This situation is shown in Fig. 3B, where the bacterium expresses a surface structure that specifically inhibits deposition of human complement. One bacterial surface molecule with these properties is the *S. pyogenes* M22 protein. In this situation, the human host is poorly protected and infection may ensue. However, if the same bacterium infects an animal host, the bacterial surface protein that interferes with complement deposition will not function, due to its specificity for human complement. Therefore, the bacteria will be opsonized by both Ig and complement and the animal host may be protected, although the bacterium expresses a virulence factor that would allow infection in the human system. In the case of the *S. pyogenes* M22 protein, this situation would correspond to the inability of M22 to bind mouse C4BP.

With regard to vaccine development, it is of particular relevance to compare Figs 3B and 3C. An antibody directed against a surface antigen may be protective in an animal model, suggesting that this antigen is a suitable vaccine candidate, but in the human situation this antibody would not be protective. For example, an *S. pyogenes* surface antigen that elicits protective immunity in an animal model may not be protective in humans, because M protein actively blocks opsonization. Thus, it is essential to find ways to evaluate whether a vaccine candidate, that elicits protective immunity in an animal model, would also be protective in humans.

6. Concluding remarks

S. pyogenes remains a major cause of human disease. In addition to common infections such as acute pharyngitis and impetigo, the emergence of the streptococcal toxic shock syndrome and the persistent problem of rheumatic fever [1] demonstrate the importance of this pathogen. Although puerperal fever is now rare, this disease remains interesting as the classical example of a nosocomial infection and as a model of emerging infectious diseases. As a model system, *S. pyogenes* has several advantages for analysis of interactions with the human immune system, in particular for studies of antigenic variation. Different approaches to the development of an *S. pyogenes* vaccine are being tried, and the results will be of considerable general interest, because it has not yet been possible to develop a good vaccine against any pathogen exhibiting antigenic variation.

Acknowledgements

Work in our laboratory is supported by the Swedish Research Council, Lund University, the Royal Physiographic Society in Lund, the Swedish Society for Medical Research, and the Trusts of Golje, Kock and Österlund.

References

- [1] Stevens DL, Kaplan EL, editors. Streptococcal infections. Clinical aspects, microbiology and molecular pathogenesis. Oxford University Press, New York, 2000.
- [2] Lancefield RC. The antigenic complex of *Streptococcus haemolyticus*. I. Demonstration of a type-specific substance in extracts of *Streptococcus haemolyticus*. J Exp Med 1928;47(1):91-103.
- [3] Fischetti VA. Streptococcal M protein: molecular design and biological behavior. Clin Microbiol Rev 1989;2(3):285-314.
- [4] Courtney HS, Hasty DL, Dale JB. Molecular mechanisms of adhesion, colonization, and invasion of group A streptococci. Ann Med 2002;34(2):77-87.
- [5] Ashbaugh CD, Moser TJ, Shearer MH, White GL, Kennedy RC, Wessels MR. Bacterial determinants of persistent throat colonization and the associated immune response in a primate model of human group A streptococcal pharyngeal infection. Cell Microbiol 2000;2(4):283-92.
- [6] Facklam RF, Martin DR, Lovgren M, Johnson DR, Efstratiou A, Thompson TA, Gowan S, Kriz P, Tyrrell GJ, Kaplan E, Beall B. Extension of the Lancefield classification for group A streptococci by addition of 22 new M protein gene sequence types from clinical isolates: *emm103* to *emm124*. Clin Infect Dis 2002;34(1):28-38.
- [7] Lancefield RC. Persistence of type-specific antibodies in man following infection with group A streptococci. J Exp Med 1959;110(2):271-92.
- [8] Johnsson E, Thern A, Dahlbäck B, Hedén LO, Wikström M, Lindahl G. A highly variable region in members of the streptococcal M protein family binds the human complement regulator C4BP. J Immunol 1996;157(7):3021-9.

- [9] Morfeldt E, Berggård K, Persson J, Drakenberg T, Johnsson E, Lindahl E, Linse S, Lindahl G. Isolated hypervariable regions derived from streptococcal m proteins specifically bind human C4b-binding protein: implications for antigenic variation. *J Immunol* 2001;167(7):3870-7.
- [10] Scharfstein J, Ferreira A, Gigli I, Nussenzweig V. Human C4-binding protein. I. Isolation and characterization. *J Exp Med* 1978;148(1):207-22.
- [11] Fujita T, Gigli I, Nussenzweig V. Human C4-binding protein. II. Role in proteolysis of C4b by C3b-inactivator. *J Exp Med* 1978;148(4):1044-51.
- [12] Thern A, Stenberg L, Dahlbäck B, Lindahl G. Ig-binding surface proteins of *Streptococcus pyogenes* also bind human C4b-binding protein (C4BP), a regulatory component of the complement system. *J Immunol* 1995;154(1):375-86.
- [13] Carlsson F, Berggård K, Stålhammar-Carlemalm M, Lindahl G. Evasion of phagocytosis through cooperation between two ligand-binding regions in *Streptococcus pyogenes* M protein. *J Exp Med* 2003;198(7):1057-68.
- [14] Berggård K, Johnsson E, Morfeldt E, Persson J, Stålhammar-Carlemalm M, Lindahl G. Binding of human C4BP to the hypervariable region of M protein: a molecular mechanism of phagocytosis resistance in *Streptococcus pyogenes*. *Mol Microbiol* 2001;42(2):539-51.
- [15] Johnsson E, Areschoug T, Mestecky J, Lindahl G. An IgA-binding peptide derived from a streptococcal surface protein. *J Biol Chem* 1999;274(21):14521-4.
- [16] Pleass RJ, Areschoug T, Lindahl G, Woof JM. Streptococcal IgA-binding proteins bind in the C α 2-C α 3 interdomain region and inhibit binding of IgA to human CD89. *J Biol Chem* 2001;276(11):8197-204.

- [17] 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(3):1114-23.
- [18] Sandin C, Linse S, Areschoug T, Woof JM, Reinholdt J, Lindahl G. Isolation and detection of human IgA using a streptococcal IgA-binding peptide. *J Immunol* 2002;169(3):1357-64.
- [19] Persson CG, Erjefält JS, Greiff L, Erjefält I, Korsgren M, Lindén M, Sundler F, Andersson M, Svensson C. Contribution of plasma-derived molecules to mucosal immune defence, disease and repair in the airways. *Scand J Immunol* 1998;47(4):302-13.
- [20] Semmelweis I. Die Aetiologie, der Begriff und die Prophylaxis des Kindbettfiebers (Pest: CA Hartleben), 1861. English translation by Carter KC: The etiology, concept and prophylaxis of chilbed fever, University of Wisconsin Press, 1983.
- [21] Gilbert GL. Group A streptococci and puerperal fever. Then and now. *Med J Aust* 1985;143(7):271-2.
- [22] Lancefield RC, Hare R. The serological differentiation of pathogenic and non-pathogenic strains of hemolytic streptococci from parturient women. *J Exp Med* 1935;61(2):335-49.
- [23] Colman G, Tanna A, Efstratiou A, Gaworzewska ET. The serotypes of *Streptococcus pyogenes* present in Britain during 1980-1990 and their association with disease. *J Med Microbiol* 1993;39(3):165-78.
- [24] Stålhammar-Carlemalm M, Areschoug T, Larsson C, Lindahl G. The R28 protein of *Streptococcus pyogenes* is related to several group B streptococcal surface proteins, confers protective immunity and promotes binding to human epithelial cells. *Mol Microbiol* 1999;33(1):208-19.

- [25] Achtman M. Epidemic spread and antigenic variability of *Neisseria meningitidis*. Trends Microbiol 1995;3(5):186-92.
- [26] Wästfelt M, Stålhammar-Carlemalm M, Delisse AM, Cabezon T, Lindahl G. Identification of a family of streptococcal surface proteins with extremely repetitive structure. J Biol Chem 1996;271(31):18892-7.
- [27] Schuchat A. Group B streptococcus. Lancet 1999;353(9146):51-6.
- [28] Stålhammar-Carlemalm M, Stenberg L, Lindahl G. Protein Rib: a novel group B streptococcal cell surface protein that confers protective immunity and is expressed by most strains causing invasive infections. J Exp Med 1993;177(6):1593-603.
- [29] Michel JL, Madoff LC, Olson K, Kling DE, Kasper DL, Ausubel FM. Large, identical, tandem repeating units in the C protein alpha antigen gene, *bca*, of group B streptococci. Proc Natl Acad Sci USA 1992;89(21):10060-4.
- [30] Areschoug T, Stålhammar-Carlemalm M, Larsson C, Lindahl G. Group B streptococcal surface proteins as targets for protective antibodies: identification of two novel proteins in strains of serotype V. Infect Immun 1999;67(12):6350-7.
- [31] Lachenauer CS, Creti R, Michel JL, Madoff LC. Mosaicism in the alpha-like protein genes of group B streptococci. Proc Natl Acad Sci USA 2000;97(17):9630-5.
- [32] Kotb M, Norrby-Teglund A, McGeer A, El-Sherbini H, Dorak MT, Khurshid A, Green K, Peeples J, Wade J, Thomson G, Schwartz B, Low DE. An immunogenetic and molecular basis for differences in outcomes of invasive group A streptococcal infections. Nat Med 2002;8(12):1398-404.

- [33] 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(4):2171-7.
- [34] Batzloff MR, Hayman WA, Davies MR, Zeng M, Pruksakorn S, Brandt ER, Good MF. 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(10):1598-608.
- [35] Bessen D, Fischetti VA. Synthetic peptide vaccine against mucosal colonization by group A streptococci. I. Protection against a heterologous M serotype with shared C repeat region epitopes. *J Immunol* 1990;145(4):1251-6.
- [36] 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(6):2080-7.
- [37] Dale JB, Chiang EY, Liu S, Courtney HS, Hasty DL. New protective antigen of group A streptococci. *J Clin Invest* 1999;103(9):1261-8.
- [38] 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(4):901-6.
- [39] 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(9):5097-103.
- [40] 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(2):924-30.

- [41] Accardo P, Sánchez-Corral P, Criado O, García E, Rodríguez de Córdoba S. Binding of human complement component C4b-binding protein (C4BP) to *Streptococcus pyogenes* involves the C4b-binding site. *J Immunol* 1996;157(11):4935-9.
- [42] 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(2):503-10.
- [43] Ashbaugh CD, Warren HB, Carey VJ, Wessels MR. Molecular analysis of the role of the group A streptococcal cysteine protease, hyaluronic acid capsule, and M protein in a murine model of human invasive soft-tissue infection. *J Clin Invest* 1998;102(3):550-60.
- [44] Brown EJ. Cooperation between IgG Fc receptors and complement receptors in host defence. In: van de Winkel JGJ, Hogarth PM, editors. *The immunoglobulin receptors and their physiological roles in immunity*, New York, Kluwer Academic Publishers, 1998:141-49.
- [45] Hedén LO, Frithz E, Lindahl G. Molecular characterization of an IgA receptor from group B streptococci: sequence of the gene, identification of a proline-rich region with unique structure and isolation of N-terminal fragments with IgA-binding capacity. *Eur J Immunol* 1991;21(6):1481-90.
- [46] Jerlström PG, Chhatwal GS, Timmis KN. The IgA-binding β antigen of the c protein complex of Group B streptococci: sequence determination of its gene and detection of two binding regions. *Mol Microbiol* 1991;5(4):843-9.

Figure legends

Fig. 1. Model for the role of human C4BP and IgA in resistance to phagocytosis conferred by M22 and related M proteins [13]. The two ligands bind to separate sites in the N-terminal part of M22, which is a dimeric coiled coil. According to the model, bacteria-bound C4BP protects against complement-mediated opsonization, which occurs via the classical pathway even under non-immune conditions. The ability to bind IgA-Fc may allow the bacteria to interfere with phagocytosis promoted by “natural” IgA [13, 16]. HVR, hypervariable region; SVR, semivariable region. The conserved part of the M22 protein contains the C-repeat region. Reproduced from *The Journal of Experimental Medicine* (2003) 198, 1057-1068, by copyright permission of The Rockefeller University Press.

Fig. 2. Structural features of the R28 protein of *Streptococcus pyogenes* and comparison with the Rib protein of group B streptococcus (GBS). The alignment of R28 and Rib is based on the sequences of R28 in *S. pyogenes* strain AL368 [24] and Rib in GBS strain BM110 [26]. S, signal peptide; N, non-repeated N-terminal region; R, repeat region; PR, partial repeat; C, C-terminal region. The number of amino acids in each region and percentage residue identity (bold figures) between corresponding regions are indicated. Each protein includes 10-12 identical repeats with a length of 79 amino acids, as indicated. The repeats are identical within one protein, but not between proteins. The processed forms of R28 and Rib have lengths of 1204 and 1176 amino acids, respectively. The non-repeated N-terminal region of R28 includes a region (shaded), which does not fit into the alignment with Rib. This region can be divided into two subregions, one with homology to the repeat region of the GBS α protein, which

belongs to the same family as Rib and R28 [24], and one with homology to the GBS β protein, which is unrelated to the Rib and α proteins [45, 46]. Thus, the R28 protein can be viewed as a chimera derived from three different GBS proteins [24]. Reproduced from *The Journal of Infectious Diseases* (2000) 182, 142-149 by copyright permission of The University of Chicago Press.

Fig. 3. Illustration of a potential problem with animal models in vaccine development.

(A) When a bacterium infects a human host, an antibody binding to a bacterial surface antigen may cause complement activation, resulting in opsonization of the bacterium by both immunoglobulin (Ig) and complement, and protection of the host against infection. Importantly, efficient opsonization commonly requires deposition of both Ig and complement, implying that a microorganism may evade opsonization by interfering with deposition of either Ig or complement. (B) If the pathogenic bacterium expresses a surface structure that specifically inhibits deposition of human complement, the inefficient opsonization by Ig alone may not protect the human host against disease. (C) If the same bacterium infects an animal host, the bacterial factor that interferes with human complement deposition will not function, resulting in opsonization by both Ig and complement and protection of the animal host. Thus, antibodies to the surface antigen would be protective in the animal model but not in humans.

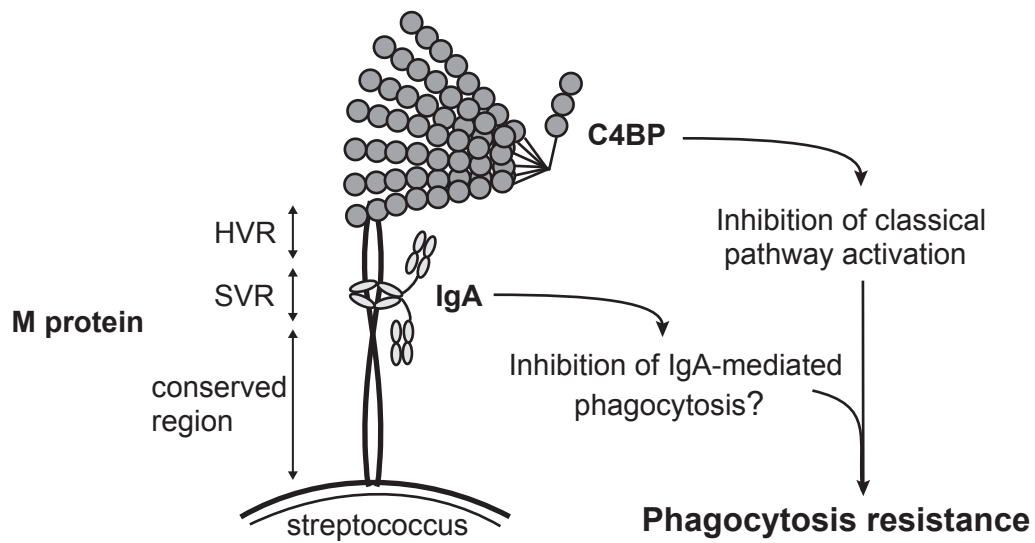


Figure 1

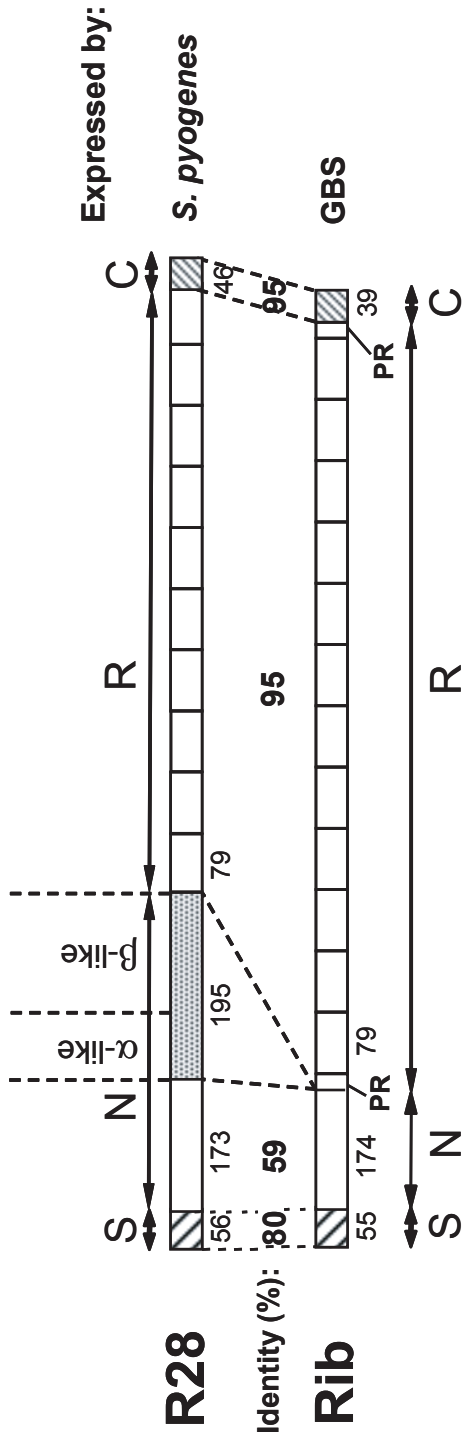
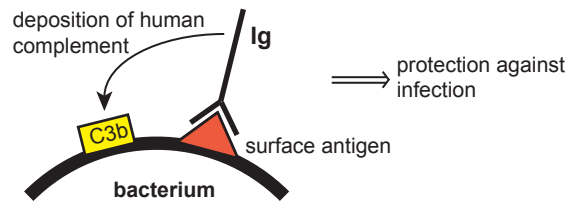
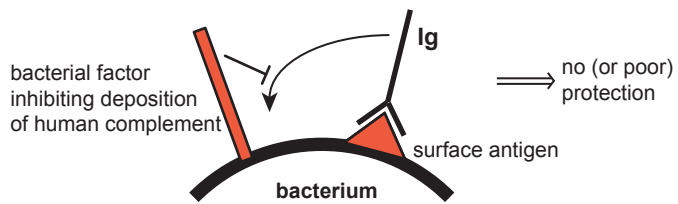


Figure 2

A. Human system



B. Human system



C. Animal model

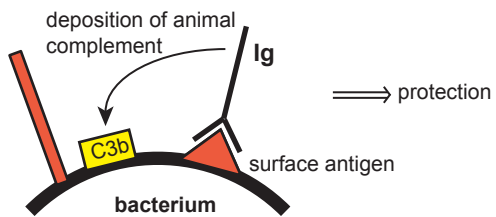


Figure 3