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Invited review for *Vaccine*

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

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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 litterature 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.

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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.

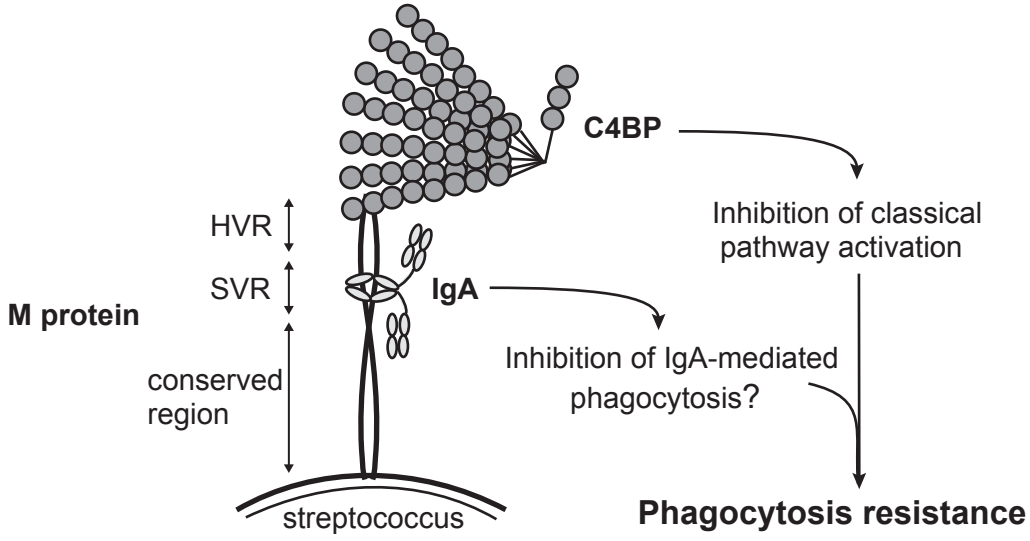
C4BP
Inhibition of classical
pathway activation
HVR
SVR
M protein
IgA
conserved
region
Inhibition of IgA-mediated
phagocytosis?
streptococcus
Phagocytosis resistance

Figure 1

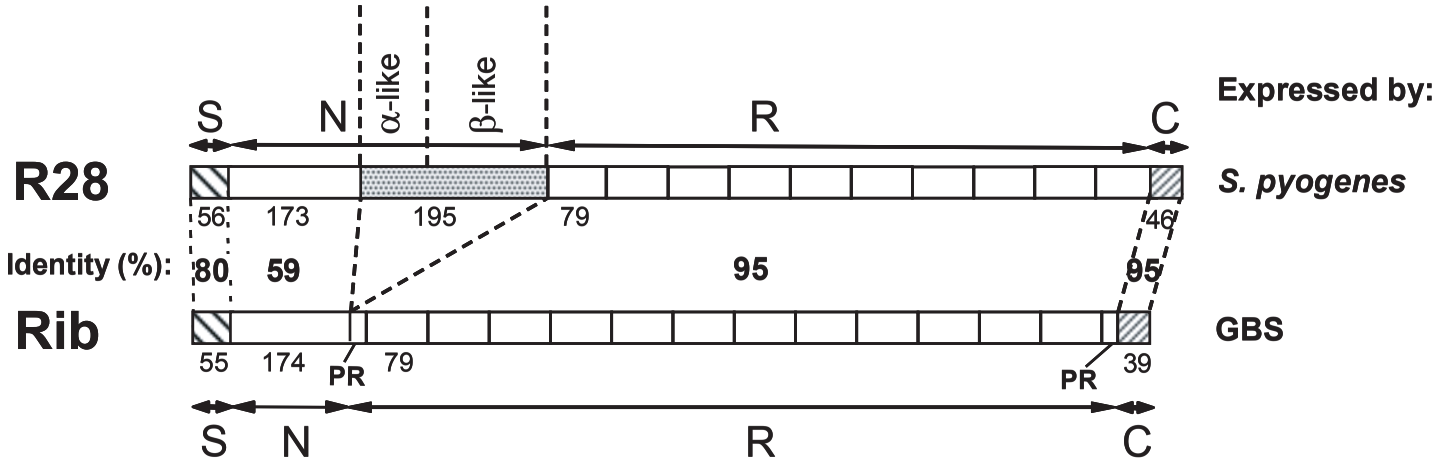
Expressed by:
S
N
α-like
β-like
R
C
R28
S. pyogenes
56
173
195
79
46
Identity (%):
80
59
95
95
Rib
GBS
55
174
PR
79
PR
39
S
N
R
C

Figure 2

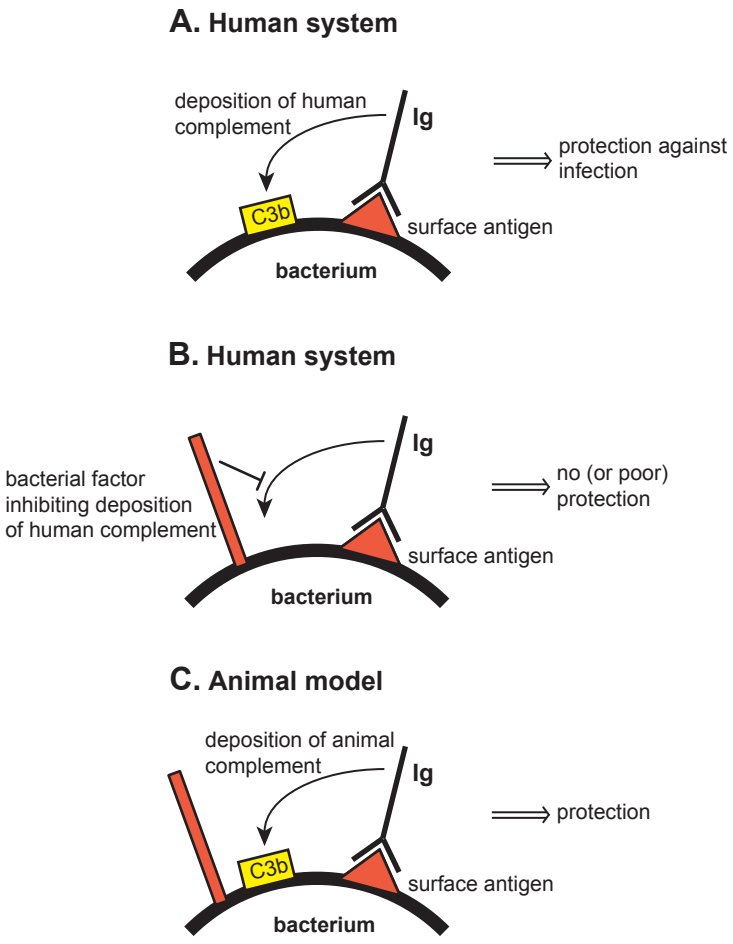
A. Human system
deposition of human complement
Ig
C3b
surface antigen
bacterium
protection against infection
B. Human system
bacterial factor inhibiting deposition of human complement
Ig
surface antigen
bacterium
no (or poor) protection
C. Animal model
deposition of animal complement
Ig
C3b
surface antigen
bacterium
protection

Figure 3