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Domains of group A streptococcal M protein that confer resistance to phagocytosis, opsonization and protection: implications for vaccine development.

Abstract

Streptococcus pyogenes (group A streptococcus) colonises skin and throat tissues resulting in a range of benign and serious human diseases. Opsonisation and phagocytosis are important defence mechanisms employed by the host to destroy group A streptococci. Antisera against the cell-surface M protein, of which over 150 different types have been identified, are opsonic and contribute to disease protection. In this issue of *Molecular Microbiology*, Sandin and colleagues have comprehensively analysed the regions of M5 protein that contribute to phagocytosis resistance and opsonisation. Human plasma proteins bound to M5 protein B- and C-repeats were shown to block opsonisation, an observation that needs to be carefully considered for the development of M protein-derived vaccines. Whilst safe and efficacious human group A streptococcal vaccines are not commercially available, candidate M protein-derived vaccines have shown promise in murine vaccine models and a recent phase 1 human clinical trial.

Keywords

Streptococcal, pyogenes, M protein, CMMB

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**Domains of group A streptococcal M protein that confer
resistance to phagocytosis, opsonisation and protection:
implications for vaccine development**

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Running title: M protein domains required for opsonisation

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Summary

Streptococcus pyogenes (group A streptococcus) colonises skin and throat tissues resulting in a range of benign and serious human diseases. Opsonisation and phagocytosis are important defence mechanisms employed by the host to destroy group A streptococci. Antisera against the cell-surface M protein, of which over 150 different types have been identified, are opsonic and contribute to disease protection. In this issue of *Molecular Microbiology*, Sandin and colleagues have comprehensively analysed the regions of M5 protein that contribute to phagocytosis resistance and opsonisation. Human plasma proteins bound to M5 protein B- and C-repeats were shown to block opsonisation, an observation that needs to be carefully considered for the development of M protein-derived vaccines. Whilst safe and efficacious human group A streptococcal vaccines are not commercially available, candidate M protein-derived vaccines have shown promise in murine vaccine models and a recent phase 1 human clinical trial.

Group A streptococcus (*Streptococcus pyogenes*) is the etiological agent of a range of human diseases including pharyngitis, impetigo, cellulitis, scarlet fever, bacteremia, toxic shock syndrome and necrotising fasciitis. Additionally, repeated *S. pyogenes* infections may lead to the development of the post-immune sequelae rheumatic fever, rheumatic heart disease and acute post-streptococcal glomerulonephritis. Although some experimental purified subunit vaccines against *S. pyogenes* are under development based on M protein, C5a peptidase, SpeB, group A carbohydrate and the fibronectin binding proteins Sfb1, SOF and FBP54 (Brandt *et al.*, 2000; Courtney *et al.*, 2003; Dale *et al.*, 1999; Guzman *et al.*, 1999; Ji *et al.*, 1997; Kapur *et al.*, 1994; Kawabata *et al.*, 2001; McArthur *et al.* 2005; Salvadori *et al.*, 1995), an effective and safe GAS vaccine is not available for human use.

M protein exists as a dimer consisting of two polypeptide chains complexed into an alpha-helical coiled-coil configuration that is anchored in the cell membrane. Each polypeptide may consist of up to four repeat regions (labelled A-D) that vary in size and amino acid composition. The C-terminal end of the molecule (including the C-repeat region) is highly conserved among GAS strains. The surface exposed N-terminal portion of M proteins usually consist of a hypervariable region (encompassing the A-repeat region) and a semi-variable B-repeat region (Figure 1). Antigenic differences in the hypervariable region of the protein formed the basis for the Lancefield serological classification of GAS and has since been expanded upon through the implementation of an *emm* typing system based on the sequencing of the N-terminal nucleotide residues of the *emm* gene (which encodes M-protein). There are currently more than 150 recognised *emm* genotypes (<http://www.cdc.gov/ncidod/biotech/strep/emmtypes.htm>). Additionally, other surface proteins of GAS that are structurally similar to M-protein have been characterised and are termed M-like proteins. These proteins differ in the types and numbers of repeats. Functional studies have demonstrated the ability of M-like proteins to interact with a variety human proteins including plasminogen (PAM) (Berge and Sjobring, 1993), IgG and IgA (Arp, protein H, Mrp, Sir, Enn) (Frithz *et al.*, 1989; Gomi *et al.*, 1990; Stenberg *et al.*, 1992; Stenberg *et al.*, 1994).

The antigenic heterogeneity displayed by various M-proteins has enabled it to evolve a number of different functions. It has long been recognised that GAS can avoid phagocytosis-mediated killing by interfering with the complement pathway. M proteins may play a role in this resistance by binding regulatory components of the complement control system. The hypervariable region of M-protein can bind both Factor H and Factor H-like protein (Johnsson *et al.*, 1998), while the C-repeat region can also bind Factor H (Kotarsky *et al.*, 2001). The C-repeat regions of a number of different M-proteins also bind human serum albumin (Akesson *et al.*, 1994; Retnoningrum and Cleary, 1994). The hypervariable region of M-protein may bind C4bp (Johnsson *et al.*,

1996), which is an important regulator in the classical complement pathway, and contributes to phagocytosis resistance (Berggard *et al.*, 2001). Numerous M-protein types can bind fibrinogen via the B-repeat region and, again, this property contributes to phagocytosis resistance (Ringdahl *et al.*, 2000).

Although research towards an effective vaccine for the prevention of GAS infections has been pursued for over 70 years, a commercial vaccine is still not available. M-protein is a major protective antigen of GAS and has been the focus for much of the vaccine research. Efforts to develop an M protein-derived vaccine were hindered in the late 1960's by the observation of an increased prevalence of rheumatic fever in vaccinated versus control children in vaccine trials using a crude M protein preparation (Massell *et al.*, 1969). M-protein shares epitopes with a number of host proteins including, cardiac and skeletal myosin, tropomyosin, laminin and keratin (Cunningham, 2003). More recently, protective regions within the M-protein have been and separate from the regions containing the cross-reactive epitopes responsible for the induction of the autoimmune driven post-streptococcal sequelae. Two of the most promising approaches include multivalent, type specific vaccines (Kotloff *et al.*, 2004; McNeil *et al.*, 2005) and peptide based vaccines based on the C-repeat region of M-protein (Batzloff *et al.*, 2003).

In this issue of *Molecular Microbiology*, Sandin *et al.* have examined domains of the M5 protein for their capacity to promote resistance to phagocytosis under nonimmune conditions. These authors have unequivocally demonstrated that the B-repeats, but neither the C-repeats nor the amino-terminal hypervariable region, are required for phagocytosis resistance. However, in the presence of human plasma proteins, only antibodies raised against the amino-terminal hypervariable domain were able to opsonise M5 group A streptococci. The presence of human fibrinogen inhibited the binding of specific antisera to the M5 B-repeats. Human fibrinogen was found to bind the B-repeats in previous studies (Carlsson *et al.*, 2005; Ringdahl *et al.*, 2000).

Similarly, antibodies raised against the M5 C-repeats failed to bind in the presence of human serum albumin, which is capable of binding to the C-repeats (Akesson *et al.*, 1994; Retnoningrum and Cleary, 1994). The significance of these data rests in the fact that there are more than 150 different M types and, thus, antisera specific to the hypervariable region of one M type will not opsonise other M types. The direction of opsonising antibodies to the hypervariable region of M protein and the protection of more highly conserved repeat regions of M protein through binding to human serum proteins represents a highly evolved mechanism of immune resistance for group A streptococci with important implications for the development of M protein-based vaccines.

The M-protein family is extensive in group A streptococcus and exhibits a diverse range of antigenic and functional properties. The study of Sandin *et al.* on M5 protein should be extended to other members of the M-protein family that interact with other human proteins. Such studies might also be broadly useful in conjunction with the examination of the vaccine potential of adhesins and other microbial proteins able to bind human target molecules. Sandin *et al.* observe that the phagocytosis assay used by them and others might only represent the situation encountered during septicaemic infection. Whilst human plasma proteins might be present at inflamed mucosal and skin infection sites, it remains to be determined whether specific antibodies against the B- and C-repeats of M protein are inhibited from binding under these conditions. *In vivo* confirmation and further investigation of this mechanism of immune evasion might require the development of humanised transgenic mice expressing human serum albumin and human fibrinogen. A transgenic mouse line expressing human plasminogen has already been constructed to examine the role of the plasminogen activation system in invasive disease (Sun *et al.*, 2004; Walker *et al.*, 2005).

Following a long hiatus, several groups are beginning human trials of prototype M protein-based vaccines. The definition of the relationship between binding of human serum proteins and opsonisation capacity is an important consideration for such group A streptococcal vaccine studies. Such work also has broader implications for selection and analysis of potential protective antigens against other bacterial pathogens, in particular those able to bind human proteins.

Figure legend

Figure 1. Structural and functional characteristics of M and M-like proteins. The molecule is anchored in the group A streptococcal cell wall via the C terminus (Pro/Gly rich and hydrophobic domains) and exists as a coiled coil structure (left). M protein consists of A-, B-, C- and D-repeats with capacity to bind several human plasma proteins as indicated (right). A- or D-repeats may not be present in some M or M-like proteins. Different M proteins have the capacity to interact with only a subset of the human plasma proteins indicated. Adapted from Fischetti (2000).

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Figure 1

