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# Mechanisms of evasion of complement by *Porphyromonas gingivalis*

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## 1. ABSTRACT

The complement system is an important host response to invading bacteria. Activation leads to deposition on the bacterial surface of C3b and its' inactivation products and phagocytosis of the opsonised bacteria by host cells. Alternatively the entire complement pathway including terminal components C5b-9 may be activated on the cell surface which gives rise to generation and insertion of the membrane attack complex into the bacterial membrane and cell lysis. Bacterial resistance to complement may be by enzyme digestion of complement components or by the generation or acquisition from the host of cell surface molecules which allow the organism to adopt host complement control proteins. The involvement of surface polysaccharides can be deduced from the very strong association of resistance with the presence of capsule and extended or modified LPS O-antigens in several species. However, in many cases the mechanism is unclear. The proteases of *Porphyromonas gingivalis* breakdown C3 and C5 and prevent the deposition of C3b on the bacterial cell surface. Greater deposition of both C3b and C5b-9 occurs in protease deficient mutants but mutants do not show loss of resistance to complement mediated lysis. Instead, complement resistance in *P.gingivalis* is associated with the presence on the cell surface of an anionic branched mannan and appears independent of capsule serotype.

## 2. INTRODUCTION

The complement system is an important part of the immune response to invading organisms, mediating attraction of host phagocytic cells to an area of infection and opsonisation of the invading micro-organisms thereby increasing their uptake. In some circumstances, particularly with Gram negative bacteria, the complement system is able to bring about lysis of the invading organism. This may be accomplished without prior exposure to allow the development of acquired immunity and is therefore thought to be important early in life and to have been established early in evolution. Bacteria have adapted to survive in this environment by destroying components of the complement system, by synthesising protective surface layers, by adopting or synthesising carbohydrates with similar patterns to those of the host, or by adopting regulatory molecules from the host. The most successful in this subversion are also some of the most highly pathogenic bacteria.

It is well recognised that defence of the periodontal tissues from subgingival bacteria and their extracellular products is mediated in part via a plasma exudate, gingival crevicular fluid, derived from the extensive capillary network in these tissues and neutrophil-dominated phagocytic cell recruitment to this region. Hence, the twin functions of complement in opsonisation and direct bacterial killing are

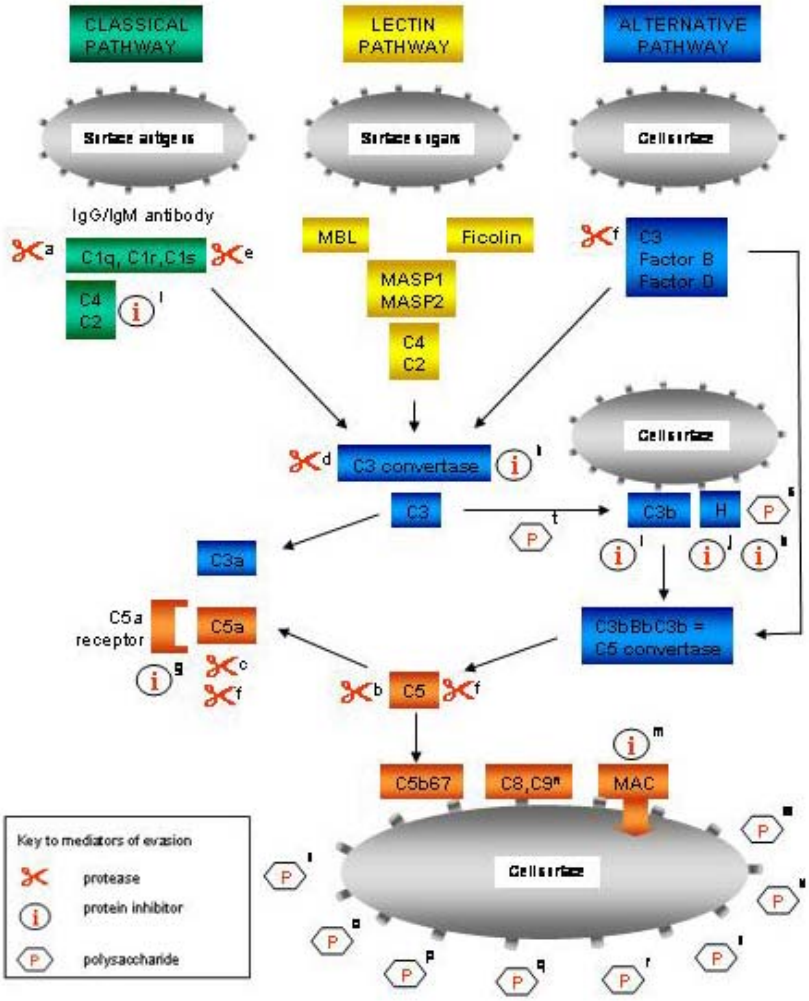


**Figure 1.** Activation routes of the complement system and means of evasion by human pathogens.

likely to be important elements of the host defence against periodontal bacteria. The survival of a bacterial species in an inflamed gingival pocket will therefore be dependent upon an ability to subvert or deregulate the complement system. Surprisingly few studies have examined the phenomenon of mechanisms of complement evasion by periodontal bacteria. In this review we examine some of the more well characterised strategies applied by other human pathogenic organisms and compare these to our understanding of how the anaerobic gram negative rod, *Porphyromonas gingivalis*, has successfully solved the problem of maintaining itself in a complement rich environment.

## 3. THE COMPLEMENT SYSTEM

The complement system is comprised of a series of plasma proteins, activated in a cascade. The central molecule of this system is complement C3 which is present in normal human serum at 0.75-1.75 g/l. Cleavage of this molecule can occur through three pathways but all give rise to two active molecules, C3a and C3b. C3a is a soluble factor which acts as an anaphylotoxin while C3b is deposited onto the cell surface and acts as an opsonin. C3b forms part of the C5 convertase, bringing about the next step in the activation cascade, and can also feed back to activate other molecules of C3, giving amplification of activity. Activation of C5 similarly gives rise to C5a, an anaphylotoxin, and C5b which interacts with C6 and C7 to give a complex which briefly has the capacity to bind to the lipid membrane. When C8 binds to this cell associated complex, one or more molecules of C9 may also become associated to form the terminal complement complex which forms a trans-membrane channel, the membrane attack complex. The membrane attack complex has been shown to be particularly important in killing of Gram-negative bacteria (1). Resistance to serum killing has been identified as a virulence trait in pathogenic bacteria and reviewed recently by Rooijakkers and Strijp (2).

Activation of C3 is achieved through one of three pathways, the classical, alternative and lectin pathways (Figure 1). The alternative pathway is initiated by cleavage of C3 which occurs spontaneously at a low rate. The cleavage product C3a is released and C3b is covalently bound to $-NH_2$ or –OH groups on the surface of bacteria. Factor B binds to C3b and is cleaved by Factor D, a serine protease, liberating Ba and leaving C3bBb, a C3 convertase, on the cell surface. C3bBb may be protected

**Table 1.** Evasion of complement activity by bacteria

| Fig key | Bacteria | Effector | Target | Reference |
|---|---|---|---|---|
| | | **Enzymes** | | |
| a | *Pseudomonas aeruginosa* | elastase, alkaline protease | C1q, C3 | 12 |
| b | *Staphylococcus epidermidis* | extracellular protease | C5 | 13 |
| c | *Streptococcus pyogenes* | endopeptidase | C5a | 14, 15 |
| d | *Streptococcus pyogenes* | Streptococcal pyrogenic exotoxin B (SPE B) | Properdin | 16 |
| e | *E.coli* 0157:H7 | metalloprotease, StcE | C1 esterase inhibitor | 34,35,36 |
| f | *Porphyromonas gingivalis* | RgpA, RgpB | C3, C5, C5aR | 17,18, 25,26 |
| | | **Other exoproteins** | | |
| g | *Staphylococcus aureus* | Chemotaxis inhibitory protein (CHIPS) | Blocks receptors for C5a and formylated peptide | 31,32 |
| h | *Staphylococcus aureus* | Staphylococcal complement inhibitor (SCIN) | surface bound C3 convertases | 33 |
| | | **Outer membrane proteins** | | |
| i | *Bordetella pertussis* | BrkA protein | Inhibits deposition of C4 | 38,39 |
| j | *Yersinia enterocolitica* | YadA protein | binds Factor H | 41,42 |
| k | *Streptococcus pyogenes* | M protein | binds C4BP and Factor H with reduced activation of AP on cell surface | 43,44 |
| l | *Streptococcus pneumoniae* | Pneumococcal surface protein A | blocks C3b deposition | 45,46 |
| m | *E. coli* | TraT protein | binds CD59, prevents insertion of MAC | 47,48 |
| | | **Cell surface polysaccharide** | | |
| n | *Bordetella bronchiseptica* | High molecular weight O side chain (rough strain) | resists bactericidal effect of serum | 49 |
| o | *Vibrio cholerae* | capsular material | resists bactericidal effect of serum | 55 |
| p | *Staphylococcus aureus* | capsular material | resists bactericidal effect of serum | 56 |
| q | *Burkholderia pseudomallei* | capsular material | resists bactericidal effect of serum | 57 |
| r | *Haemophilus influenzae* | endogenous sialylation of lipopolysaccharide (LPS) | | 60 |
| s | *Neisseria gonorrhoeae* | sialylation of LPS by host CMP-N-acetylneuraminic acid | binds C4BP and Factor H with X7 reduction in C3b | 58, 61-63 |
| t | *Neisseria meningitidis* | endogenous sialylation of lipooligolysaccharide | resists absorption of C3 and killing by immune serum and complement, | 59 |
| u | *Neisseria meningitidis* | strain specific capsule | prevents insertion of MAC | 62 |
| v | *Salmonella enterica serovar Typhimurium* | LPS O antigen (length) | resists bactericidal effect of serum | 50 |
| w | *Porphyromonas gingivalis* | anionic branched mannan | resists bactericidal effect of serum | 24 |

from dissociation by binding of properdin. Association of another C3b molecule to the C3 convertase gives the C5 convertase.

The classical pathway is activated following antibody binding to an epitope on the invading organism and a resultant conformational change of the immunoglobulin molecule. Activation of the pathway is initiated by binding of C1q to antigen-antibody complexes and subsequent binding of the serine proteases C1r and C1s to C1q. C1s cleaves C4 and C2 which together form the C3 convertase, C4b2a, on the cell surface of micro-organisms.

The lectin pathway gains specificity through the lectins mannose binding lectin and ficolin. These are pattern recognition molecules which recognise pathogen-associated molecular patterns shared by a broad class of microorganisms. MBL binds to a range of sugars with 3- and 4- hydroxyl groups in the pyranose ring including mannose, N-acetyl mannosamine, N-acetyl glucosamine, fucose and glucose (3). Three members of the ficolin family have been described in humans of which two are thought to be serum lectins. L-ficolin binds N-acetylglucosamine while H-ficolin binds N-acetyl glucosamine and N-acetyl galactosamine. D-galactose and sialic acid, which are more frequently found in mammalian glycoproteins, are not bound by these lectins. Both ficolins consist of a lectin domain and a collagen-like domain and occur in oligomers (4). When binding of MBL or ficolin takes place the lectin pathway is activated through MBL-associated serine proteases of which three (MASP 1-3) have been described. The exact role of each remains to be clarified but it has been reported that MASP-1 cleaves C3 and C2 while MASP-2 cleaves C4 and C2 (5).

Control of the complement system is vital for the host as bystander damage can occur to host cells if the process is poorly regulated. This is partly achieved by the highly labile nature of some of the complement components eg Factor B and C2 and of the complexes formed on activation. In addition there are regulatory proteins for the complement convertases which all contain a series of short consensus repeating units (SCRs). Factor H and FHL-1 are the main fluid phase regulators of the alternative pathway, acting as co-factors for the serine protease factor I in cleaving C3bBb (6). Factor H is a 150kDa plasma protein composed of 20 individually folded SCRs of approximately 60 amino acids based on a framework of four invariant cysteine residues and highly conserved acidic, proline, asparagine, glycine, tyrosine, phenylalanine and tryptophan residues (7). FHL-1 is a 42kDa protein which is identical with the first seven SCRs of Factor H and includes an extension of four hydrophobic amino acid residues at its C-terminus (8). This SCR is also found in the functionally related proteins, C4binding protein (C4BP) and decay accelerating factor (DAF, CD55) and in complement receptors CR1 (CD35) and CR2 (CD21). C4BP plays a similar role as co-factor for factor I in the classical pathway (9) It is a 500-kDa plasma glycoprotein, composed of seven identical alpha chains and

a unique beta chain linked together by a central core (10). C-terminal parts of both chains contain two cysteine residues each and an amphipathic alpha helix region, which are both required for intracellular polymerization of the molecule. The alpha chains contain eight SCRs and the beta chain contains three (11). DAF accelerates decay of the C3 convertases of both the classical and alternative pathways.

Other inhibitors include C1 inhibitor, a member of the serpin family of protease inhibitors, which inhibits the activity of C1r and C1s. Inhibition of the terminal complement complex is brought about by CD59 which binds to C8, C9 components of the MAC and inhibits formation of the lytic pore.

## 4. MECHANISMS OF EVASION OF THE COMPLEMENT SYSTEM BY BACTERIA

The complexity of the complement system allows the opportunity for subversion by pathogenic bacteria in several ways (Table 1). To date there is little information pertaining to evasion of this system by periodontal bacteria with the notable exception of *P.gingivalis*. However it is likely that similar strategies will be employed to those described for other organisms whose survival in the host is dependent upon subversion of this important arm of the innate host defence. The following section describes some of these well recognised themes.

### 4.1. Proteases

Enzymatic cleavage of host defence proteins has been demonstrated in a number of bacteria. *Pseudomonas aeruginosa* is able to cleave C1q and C3 by the activity of elastase and alkaline protease enzymes (12). An extracellular proteinase from an oral strain of *Staphylococcus epidermidis* can degrade complement protein C5 (13). An endopeptidase of *Streptococcus pyogenes* cleaves C5a and similar enzymes can be demonstrated in other streptococcal strains of human origin suggesting that inactivation of chemotactic signals is a universal virulence mechanism of streptococci (14, 15). Properdin, a complement regulator which promotes activity by binding and stabilising the C3bBb convertase, is cleaved by a cysteine protease of *S .pyogenes,* SPE B (16).

These studies demonstrate that complement activity is blocked by bacterial degradation of one or more of the components of the complement cascade. However in some bacteria protease activity can truly be described as subversive since the enzymatic cleavage of the complement components generates biologically active fragments. In *P.gingivalis* degradation of human serum proteins, including complement factors C3 and C5, was first demonstrated by immunological methods and suggested as a means to explain the high pathogenic potential of the organism by the group of Sundqvist (17). The same group demonstrated *P.gingivalis* can bring about the generation and degradation of complement C5a (18). Prior to the classification of the Arg- and Lys-gingipains (19) Schenkein suggested that degradation of complement may be dependent on the trypsin-like protease activity of *P. gingivalis* but that the proteases are not likely to destroy fluid phase complement components at the concentrations present in gingival crevicular fluid (20, 21). He went on to demonstrate that *P.gingivalis* W83 failed to accumulate $^{125}$I-C3 on the cell surface following opsonization with serum due to cell associated proteolytic activity and deposition was increased following treatment with a cysteine protease inhibitor TLCK (22). The group suggested that *P.gingivalis* protease activity mimicked that of complement factor D, enabling it to activate C3 in serum without significant C3 accumulation on the cell surface (23). This group confirmed that when protease activity is inhibited greater C3b deposition occurs and in addition deposition of C5b-9 is increased. A similar effect is seen in protease deficient mutants (24). However, although our recent studies confirmed these findings, we were unable to demonstrate any loss of resistance to complement mediated lysis in the presence of protease inhibitors or in mutants deficient in gingipains suggesting that there is an additional mechanism of evasion of serum killing which is independent of protease activity.

Wingrove *et al* reported that the degradation of C3 and C5 by *P.gingivalis* might lead to generation of active fragments and were able to demonstrate the generation of a C5a-like fragment which is biologically active for neutrophil activation (25). Discipio *et al* demonstrated that C5 treated with Rgp, one of the Arg-X specific proteases of *P. gingivalis*, elicited eosinophil polarisation responses which were greater if the C5 had first been oxidised. Polarisation of eosinophils also occurred when oxidised C5 was treated with Kgp, the lysine specifc member of the gingipain protease family, although unoxidised C5 was not affected by this enzyme (26). The results of Schenkein and Discipio suggest that *P.gingivalis* subverts the complement response rather than simply inactivating it.

In addition to cleavage and activation of components of the complement cascade the proteases of *P.gingivalis* are also able to bring about cleavage of the human C5a receptor and so prevent neutrophil activation (27). Initially this would appear to counteract any advantage gained by the generation of the C5a-like fragment described by Wingove *et al* (25). However a second receptor for C5a has subsequently been described, C5L2, (28, 29) and the effect of proteases of *P.gingivalis* on this receptor has not yet been investigated. Unlike C5a receptor, C5L2 is not coupled to G protein intracellular signalling pathways and an anti-inflammatory function for this receptor has been hypothesised (30). Other bacteria achieve inactivation of the C5a receptor by non-enzymatic means. Recently *S. aureus* has been shown to produce two exoproteins which are able to block activity. Chemotaxis inhibitory protein (CHIPS) blocks receptors for C5a and formylated peptide on neutrophils with high affinity binding (31, 32). Staphylococcal complement inhibitor (SCIN) binds to C3bBb and C4b2a and so inactivates C3 convertases (33).

Some bacteria are able to subvert host complement control using enzyme mediated methods. For example, enterohemorrhagic *E.coli* 0157:H7 strains secrete a metalloprotease, StcE, which is able to cleave the C1 esterase inhibitor and localise it on the cell membrane (34, 35, 36).

### 4.2. Outer membrane proteins

Serum resistance of *Bordetella pertussis* has been attributed to an outer membrane protein BrkA (37). BrkA has a predicted molecular mass of 103 kDa and has two RGD motifs, an outer membrane localization signal, and a common proteolytic cleavage site. BrkA inhibits deposition of C4 on the cell surface (38). In other work Berggard *et al* suggest that complement resistance is dependent on C4BP binding to a site similar to C4b although sequence homology has not been identified (39). In this study binding was shown to be dependent on two sites on the bacterial surface of which one was contributed by filamentous haemagglutinin. A study by Pishko *et al* (40) investigated the variation in complement sensitivity in other studies and reported that *B.pertussis* grown in broth culture is sensitive to killing by serum complement in non-immune serum but that resistance is acquired *in vivo*.

Resistance of *Yersinia enterocolitica* to complement killing was described by China *et al* who showed this was mediated by the outer membrane protein YadA and suggested this was brought about through the binding of Factor H (41). More recently resistance has been shown to be dependent on outer membrane proteins rather than LPS by Biedzka-Sarek *et al*. Resistance to complement killing by *Y. enterocolitica* was shown to be dependent in the long term on YadA and influenced in the short term by another outer membrane protein Ail (42).

The nature of the cell wall of Gram positive bacteria confers resistance to complement killing but in addition some Gram positive bacteria possess outer membrane proteins which resist deposition of C3b and its' cleavage products conferring resistance to opsonisation and phagocytosis. Several strains of Streptococci have this ability. The M protein of *Streptococcus pyogenes* binds C4BP and Factor H resulting in reduced activation of AP on cell surface (43, 44). Similarly *Streptococcus pneumoniae* has Factor H binding proteins encoded by the *pspC* locus Pneumococcal surface protein C (PspC) and the factor H-binding inhibitor of complement (Hic) (45). Pneumococcal surface protein A (PspA) is a choline binding protein with sequence homology to PspC which confers reduced C3 activation *in vivo* and in vitro and inhibits C3 deposition (46).

Many Gram-negative bacteria are susceptible to complement lysis and it is possible to demonstrate strain variation of resistance and hence link resistance to specific attributes. The *E. coli* TraT protein binds the host complement regulator CD59 thus preventing insertion of MAC through the lipid bilayer (47, 48).

### 4.3. Cell surface polysaccharide

Cell surface polysaccharides of bacteria were originally distinguished serologically and their classification as K or O antigens reflects this. Different O and K serotypes result from variation in sugar composition and linkage specificity in the polysaccharides, as well as their substitution with non-carbohydrate residues. Bacterial lipopolysaccharides (LPS) typically consist of a hydrophobic domain known as lipid A (or endotoxin) which is embedded in the outer membrane, a nonrepeating core oligosaccharide, and a distal polysaccharide or O-antigen. The length of this molecule has been associated with resistance to complement lysis in several bacteria. In a study by Byrd *et al* (49) a strain of *Bordetella parapertussis* and a smooth strain of *Bordetella bronchoseptica* possessed high molecular weight O-side chain and were resistant to complement killing whereas a rough strain of *B.bronchiseptica* lacking this side chain was sensitive. *Salmonella enterica*, serovar typhimurium can decorate its cell surface with long or very long LPS molecules depending on the number of polymers of O sugar repeat-units incorporated (50). The degree of complement activation and susceptibility to phagocytosis in Salmonella strains show a direct correlation with the length of these repeat-units. In addition the length and density of the repeat units is important for resistance to the bactericidal activity of complement.

K antigen was originally described as being associated with capsule, and more than 80 distinct K serotypes in *E.coli* result from structural diversity in these polymers (51). To date 6 K-serotypes have been described in *P.gingivalis* which contribute significantly to the virulence of the organism (52, 53) and as K negative strains show variation (54) more classifications may yet be made. .

In outbreaks of *Vibrio cholerae* in Bangladesh, strains isolated in 2002 were more susceptible to the bactericidal activity of serum compared to strains from 1993 to 1995 and the susceptible strains had less capsular material than the earlier resistant isolates (55). Similarly different capsular serotypes of *Staphylococcus aureus* show difference in virulence in a mouse model(56). Mice challenged with a serotype $5^+$ (CP5) strain showed a significantly higher level of bacteremia than those challenged with serotype $8^+$ (CP8) strain. CP5 strains were also more resistant to *in vitro* killing by whole mouse blood. The degree of O-acetylation was similar in purified capsular polysaccharides CP5 and CP8 but CP5 showed greater N-acetylation. Contribution of the capsule to the virulence of *Burkholderia pseudomallei* was studied by Reckseidler-Zenteno *et al* (57). The group constructed a capsule deletion mutant by in-frame deletion of the glycosyltransferase gene *wcbB* which was serum sensitive and in a mouse model it showed less ability to persist in the blood. To define the role of the capsule a double mutant lacking both capsule and O-polysaccharide was constructed and *in vitro* serum killing assays carried out with the addition of purified capsular polysaccharide and O-polysaccharide. Some resistance was obtained by the addition of *B. pseudomallei* capsular polysaccharide to the assay but not by the addition of O-polysaccharide. Furthermore preincubation of the capsular polysaccharide with serum before the addition of bacteria increased the survival of the double mutant in serum to the same level as in heat inactivated serum.

However, in *P.gingivalis* serum resistance appears not to be associated with capsule (24). Mutagenesis at the capsule encoding locus in strain W50 at PG0106-PG0120, leads to the loss of capsule expression on the basis of negative staining and serological analysis. However, the resultant capsule minus mutants are not significantly more

sensistive to killing than the parent W50. Moreover, naturally capsule minus strains such as 381 are also resistant to the lytic effect of complement.

In *Neisseria gonorrhoeae* sialylation of lipopolysaccharide (LPS) by host CMP-N-acetylneuraminic acid (CMP-NANA), catalyzed by bacterial sialyltransferase, renders gonococci resistant to absorption of complement component C3, to killing by immune serum and complement, to killing by phagocytes and to entry into epithelial cell lines (58). In strains of *N. meningitides,* sialic acid incorporation into LPS is endogenous and this organism also possesses strain specific polysaccharide capsule which prevents insertion of the complement membrane attack complex (59). Similarly endogenous sialylation of lipopolysaccharide in *Haemophilus influenzae* has been shown to contribute to serum resistance when wild-type strains were compared with non-sialylated mutants (60). Ram *et al* have suggested that the complement control protein factor H acts as a lectin for sialic acid and other polyanions such as heparin and most sulphated glycosaminoglycans (61). Factor H acts as a co-factor for factor I mediated cleavage of C3b so that the enhanced interaction of Factor H with the sialic acid of the cell surface could promote the local inactivation of the complement cascade. Complement resistance brought about by binding of soluble host derived complement control proteins factor H and C4 binding protein onto cell surfaces has been confirmed in several strains, including *N. gonorrhoeae* (62, 63) and *Streptococcus pyogenes* (44).

Recent studies from our laboratory have now determined the identity of a cell surface polysaccharide responsible for serum resistance. The study of this polymer was initiated during our investigation of the glycosylation of the Arg-gingipains where it became apparent that the glycans attached to these enzymes were immunologically related to a repeating unit cell surface polymer cross reactive with a monoclonal antibody (MAb 1B5) originally raised against one isoform of RgpA (64). Glycosylation of the Arg-gingipains appears to be important for both enzyme stabilisation (65) and for immune recognition (66) of these proteases by the serum IgG response of periodontal patients. Hence characterisation of the post-translational modifications to the Rgps may offer insights into the mechanism of persistence of this organism in the periodontal tissues. We therefore investigated the identity of the MAb 1B5 reactive cell surface polymer. The polysaccharide (APS: anionic polysaccharide) was purified using a combination of gel-filtration and ion-exchange chromatography and the structure determined by 1 D and 2D NMR and GC-MS analysis. APS consists of a branched mannan structure built up of $\alpha$ -1$\rightarrow$6 linked Man backbone with $\alpha$-1$\rightarrow$2 linked Man side-chains with two residues of Man-1-phosphate in phospho-diester linkage at $\rightarrow$2)Man in two terminal Man residues in the repeating unit. The polymeric structure of APS was retained following dephosphorylation implying that the phospho-diester is in the side-chains and not in the backbone. Given that MAb 1B5 reacts with all *P. gingivalis* laboratory strains and isolates examined to date, it appears that APS expression is highly conserved in this species.

Synthesis of APS is dependent upon the expression of genes at the *porR* locus in the *P. gingivalis* genome (*pg1135-pg1142*): deletions at this locus or insertional inactivation of *porR* (*pg1138*) lead to loss of reactivity with MAb 1B5, a more fragile cell phenotype and loss of electron dense staining at the cell surface as revealed by electron microscopy. However the composition of the O-antigen of LPS and capsule expression is unaffected by these mutations. We recently reported that loss of APS expression is associated with an exquisite sensitivity to complement mediated lysis in *P. gingivalis* W50. Whether complement resistance is conferred by the anionic nature of the mannan due to its phophorylation status or whether the presence of the mannan at the cell surface confers a steric hindrance effect on complement deposition at the outer membrane is yet to be established.

Hence it appears that in the case of *P. gingivalis*, two distinct mechanisms of complement evasion are in operation. The Arg-gingipains are required to mediate complement degradation, particularly C3 activation, in the fluid phase and this in turn leads to reduced deposition of complement components on the cell surface and presumably reduced opsonophagocytosis. When complement deposition does occur, the presence of the branched mannan at the cell surface protects the cell from complement mediated lysis.

## 5. PERSPECTIVE

Bacteria have developed a multiplicity of strategies to avoid the chemotactic, opsonic and lytic effects of the complement system and the same organism may have several different strategies to obtain complement resistance. Studies in the literature show there is a long history of examination of enzyme activity against components of the convertases of the classical and alternative pathways and against C5. *P.gingivalis* Arg- and Lys- gingipains have been shown to be effective mediators of complement subversion. However much less is known about bacterial evasion of the lectin pathway, possibly because that pathway was more recently discovered. There are also several studies described above which demonstrate the adoption of host complement inhibitors to the surface of bacteria mediated by outer membrane proteins as in *E.coli* or by polysaccharide as in Neisseria spp. and *H. influenzae.* There is no evidence to date that any similar binding of control proteins occurs in *P.gingivalis* but this possibility has not been fully investigated.

There are multiple examples in the literature of complement evasion mediated by bacterial polysaccharides. However, the mechanisms by which cell surface polysaccharides afford this protection has not always been established. The role of the phosphorylated branched mannan of *P. gingivalis* in the evasion of complement and the broader issue of the interaction of this surface polysaccharide with the innate defences of the colonised host are still under investigation in these laboratories.

## 6. REFERENCES


1. P.W.Taylor: Bactericidal and bacteriolytic activity of serum against gram-negative bacteria. *Microbiol Rev*, 47 (1), 46-83 (1983)

2. S.H.Rooijakkers and J.A.Van Strijp: Bacterial complement evasion. *Mol Immunol*, 44 (1-3), 23-32 (2007)

3. M.W.Turner: The role of mannose-binding lectin in health and disease. *Mol Immunol*, 40 (7), 423-429 (2003)

4. T.Fujita, M.Matsushita and Y.Endo: The lectin-complement pathway-its role in innate immunity and evolution. *Immunol Rev*, 198, 185-202 (2004)

5. M.Matsushita, Y.Endo and T.Fujita: Cutting edge: complement-activating complex of ficolin and mannose-binding lectin-associated serine protease. *J Immunol*, 164 (5), 2281-2284 (2000)

6. S.Kuhn and P.F.Zipfel: Mapping of the domains required for decay acceleration activity of the human factor H-like protein 1 and factor H. *Eur J Immunol*, 26 (10), 2383-2387 (1996)

7. J.Ripoche, A.J.Day, T.J.Harris and R.B.Sim: The complete amino acid sequence of human complement factor H. *Biochem J*, 249 (2), 593-602 (1988)

8. P.Kraiczy and R.Wurzner: Complement escape of human pathogenic bacteria by acquisition of complement regulators. *Mol Immunol*, 43 (1-2), 31-44 (2006)

9. Y.Hardig, A.Hillarp and B.Dahlback: The amino-terminal module of the C4b-binding protein alpha-chain is crucial for C4b binding and factor I-cofactor function. Biochem J, 323 (2), 469-475 (1997)

10. A.Hillarp and B.Dahlback: Cloning of cDNA coding for the beta chain of human complement component C4b-binding protein: sequence homology with the alpha chain. *Proc Natl Acad Sci* U S A, 87 (3), 1183-1187 (1990)

11. A.M.Blom: Structural and functional studies of complement inhibitor C4b-binding protein. *Biochem Soc Trans*, 30 (Pt 6), 978-982 (2002)

12. Y.Q.Hong and B.Ghebrehiwet: Effect of *Pseudomonas aeruginosa* elastase and alkaline protease on serum complement and isolated components C1q and C3. *Clin Immunol Immunopathol,* 62 (2), 133-138 (1992)

13. J.L.Moon, A.Banbula, A.Oleksy, J.A.Mayo and J.Travis: Isolation and characterization of a highly specific serine endopeptidase from an oral strain of *Staphylococcus epidermidis*. *Biol Chem*, 382 (7), 1095-1099 (2001)

14. P.P.Cleary, U.Prahbu, J.B.Dale, D.E.Wexler and J.Handley: Streptococcal C5a peptidase is a highly specific endopeptidase. *Infect Immun*, 60 (12), 5219-5223 (1992)

15. P.P.Cleary, J.Handley, A.N.Suvorov, A.Podbielski and P.Ferrieri: Similarity between the group B and A streptococcal C5a peptidase genes. *Infect Immun*, 60 (10), 4239-4244 (1992)

16. N.Tsao, W.H.Tsai, Y.S.Lin, W.J.Chuang, C.H.Wang and C.F.Kuo: Streptococcal pyrogenic exotoxin B cleaves properdin and inhibits complement-mediated opsonophagocytosis. *Biochem Biophys Res Commun*, 339 (3), 779-784 (2006)

17. G.Sundqvist, J.Carlsson, B.Herrmann and A.Tarnvik: Degradation of human immunoglobulins G and M and complement factors C3 and C5 by black-pigmented Bacteroides. *J Med Microbiol*, 19 (1), 85-94 (1985)

18. G.Sundqvist, A.Bengtson and J.Carlsson: Generation and degradation of the complement fragment C5a in human serum by *Bacteroides gingivalis*. *Oral Microbiol Immunol*, 3 (3), 103-107 (1988)

19. M.A.Curtis, H.K.Kuramitsu, M.Lantz, F.L.Macrina, K.Nakayama, J.Potempa, E.C.Reynolds and J.Aduse-Opoku: Molecular genetics and nomenclature of proteases of *Porphyromonas gingivalis*. *J Periodontal Res*, 34 (8), 464-472 (1999)

20. H.A.Schenkein: The effect of periodontal proteolytic Bacteroides species on proteins of the human complement system. *J Periodontal Res*, 23 (3), 187-192 (1988)

21. H.A.Schenkein and C.R.Berry: Production of chemotactic factors for neutrophils follo*wing the interaction of Bacteroides gingivalis with purified C5.* J Periodontal Res, 23 (5), 308-312 (1988)

22. H.A.Schenkein: Failure of *Bacteroides gingivalis* W83 to accumulate bound C3 following opsonization with serum. *J Periodontal Res*, 24 (1), 20-27 (1989)

23. H.A.Schenkein: Complement factor D-like activity of *Porphyromonas gingivalis* W83. *Oral Microbiol Immunol*, 6 (4), 216-220 (1991)

24. J.M.Slaney, A.Gallagher, J.Aduse-Opoku, K.Pell and M.A.Curtis: Mechanisms of resistance of *Porphyromonas gingivalis* to killing by serum complement. *Infect Immun*, 74 (9), 5352-5361 (2006)

25. J.A.Wingrove, R.G.DiScipio, Z.Chen, J.Potempa, J.Travis and T.E.Hugli: Activation of complement components C3 and C5 by a cysteine proteinase (gingipain-1) from *Porphyromonas* (*Bacteroides*) *gingivalis*. *J Biol Chem*, 267 (26), 18902-18907 (1992)

26. R.G.Discipio, P.J.Daffern, M.Kawahara, R.Pike, J.Travis, T.E.Hugli and J.Potempa: Cleavage of human complement component C5 by cysteine proteinases from *Porphyromonas* (*Bacteroides*) *gingivalis*. Prior oxidation of C5 augments proteinase digestion of C5. *Immunology*, 87 (4), 660-667 (1996)

27. M.A.Jagels, J.A.Ember, J.Travis, J.Potempa, R.Pike and T.E.Hugli: Cleavage of the human C5A receptor by proteinases derived from *Porphyromonas gingivalis*: cleavage of leukocyte C5a receptor. *Adv Exp Med Biol*, 389, 155-164 (1996)

28. M.Ohno, T.Hirata, M.Enomoto, T.Araki, H.Ishimaru and T.A.Takahashi: A putative chemoattractant receptor, C5L2, is expressed in granulocyte and immature dendritic cells, but not in mature dendritic cells. *Mol Immunol*, 37 (8), 407-412 (2000)

29. S.A.Cain and P.N.Monk: The orphan receptor C5L2 has high affinity binding sites for complement fragments C5a and C5a des-Arg(74) *J Biol Chem*, 277 (9), 7165-7169 (2002)

30. N.P.Gerard, B.Lu, P.Liu, S.Craig, Y.Fujiwara, S.Okinaga and C.Gerard: An anti-inflammatory function for the complement anaphylatoxin C5a-binding protein, C5L2. *J Biol Chem*, 280 (48), 39677-39680 (2005)

31. B.Postma, M.J.Poppelier, J.C.van Galen, E.R.Prossnitz, J.A.Van Strijp, C.J.de Haas and K.P.Van Kessel: Chemotaxis inhibitory protein of *Staphylococcus aureus* binds specifically to the C5a and formylated peptide receptor. *J Immunol*, 172 (11), 6994-7001 (2004)

32. B.Postma, W.Kleibeuker, M.J.Poppelier, M.Boonstra, K.P.Van Kessel, J.A.Van Strijp and C.J.de Haas: Residues 10-18 within the C5a receptor N terminus compose a binding domain for chemotaxis inhibitory protein of *Staphylococcus aureus*. *J Biol Chem*, 280 (3), 2020-2027 (2005)

33. W.J.van Wamel, S.H.Rooijakkers, M.Ruyken, K.P.Van Kessel and J.A.Van Strijp: The innate immune modulators staphylococcal complement inhibitor and chemotaxis inhibitory protein of *Staphylococcus aureus* are located on beta-hemolysin-converting bacteriophages. *J Bacteriol*, 188 (4), 1310-1315 (2006)

34. W.W.Lathem, T.E.Grys, S.E.Witowski, A.G.Torres, J.B.Kaper, P.I.Tarr and R.A.Welch: StcE, a metalloprotease secreted by *Escherichia coli* O157:H7, specifically cleaves C1 esterase inhibitor. *Mol Microbiol*, 45 (2), 277-288 (2002)

35. W.W.Lathem, T.Bergsbaken and R.A.Welch: Potentiation of C1 esterase inhibitor by StcE, a metalloprotease secreted by *Escherichia coli* O157:H7. *J Exp Med*, 199 (8), 1077-1087 (2004)

36. T.E.Grys, L.L.Walters and R.A.Welch: Characterization of the StcE protease activity of *Escherichia coli* O157:H7. *J Bacteriol*, 188 (13), 4646-4653 (2006)

37. R.C.Fernandez and A.A.Weiss: Cloning and sequencing of a *Bordetella pertussis* serum resistance locus. *Infect Immun*, 62 (11), 4727-4738 (1994)

38. M.G.Barnes and A.A.Weiss: BrkA protein of *Bordetella pertussis* inhibits the classical pathway of complement after C1 deposition. *Infect Immun*, 69 (5), 3067-3072 (2001)

39. K.Berggard, G.Lindahl, B.Dahlback and A.M.Blom: *Bordetella pertussis* binds to human C4b-binding protein (C4BP) at a site similar to that used by the natural ligand C4b. *Eur J Immunol*, 31 (9), 2771-2780 (2001)

40. E.J.Pishko, D.J.Betting, C.S.Hutter and E.T.Harvill: *Bordetella pertussis* acquires resistance to complement-mediated killing *in vivo*. *Infect Immun*, 71 (9), 4936-4942 (2003)

41. B.China, M.P.Sory, B.T.N'Guyen, M.De Bruyere and G.R.Cornelis: Role of the YadA protein in prevention of opsonization of *Yersinia enterocolitica* by C3b molecules. *Infect Immun*, 61 (8), 3129-3136 (1993)

42. M.Biedzka-Sarek, R.Venho and M.Skurnik: Role of YadA, Ail, and Lipopolysaccharide in Serum Resistance of *Yersinia enterocolitica* Serotype O:3. *Infect Immun*, 73 (4), 2232-2244 (2005)

43. R.D.Horstmann, H.J.Sievertsen, J.Knobloch and V.A.Fischetti: Antiphagocytic activity of streptococcal M protein: selective binding of complement control protein factor H. *Proc Natl Acad Sci* U S A, 85 (5), 1657-1661 (1988)

44. D.Perez-Caballero, I.Garcia-Laorden, G.Cortes, M.R.Wessels, S.R.de Cordoba and S.Alberti: Interaction between complement regulators and *Streptococcus pyogenes*: binding of C4b-binding protein and factor H/factor H-like protein 1 to M18 strains involves two different cell surface molecules. *J Immunol*, 173 (11), 6899-6904 (2004)

45. C.Neeleman, S.P.Geelen, P.C.Aerts, M.R.Daha, T.E.Mollnes, J.J.Roord, G.Posthuma, H.van Dijk and A.Fleer: Resistance to both complement activation and phagocytosis in type 3 pneumococci is mediated by the binding of complement regulatory protein factor H. *Infect Immun*, 67 (9), 4517-4524 (1999)

46. B.Ren, M.A.McCrory, C.Pass, D.C.Bullard, C.M.Ballantyne, Y.Xu, D.E.Briles and A.J.Szalai: The virulence function of *Streptococcus pneumoniae* surface protein A involves inhibition of complement activation and impairment of complement receptor-mediated protection. *J Immunol*, 173 (12), 7506-7512 (2004)

47. M.M.Binns, J.Mayden and R.P.Levine: Further characterization of complement resistance conferred on *Escherichia coli* by the plasmid genes *traT* of R100 and *iss* of ColV,I-K94. *Infect Immun*, 35 (2), 654-659 (1982)

48. R.Rautemaa, G.A.Jarvis, P.Marnila and S.Meri: Acquired resistance of *Escherichia coli* to complement lysis by binding of Glycophosphoinositol-Anchored Protectin (CD59) *Infect Immun*, 66 (5), 1928-1933 (1998)

49. D.W.Byrd, R.M.Roop, H.P.Veit and G.G.Schurig: Serum sensitivity and lipopolysaccharide characteristics in

*Bordetella bronchiseptica*, *B. pertussis* and *B. parapertussis*. *J Med Microbiol*, 34 (3), 159-165 (1991)

50. G.L.Murray, S.R.Attridge and R.Morona: Altering the length of the lipopolysaccharide O antigen has an impact on the interaction of *Salmonella enterica* serovar Typhimurium with macrophages and complement. *J Bacteriol*, 188 (7), 2735-2739 (2006)

51. C.Whitfield and I.S.Roberts: Structure, assembly and regulation of expression of capsules in *Escherichia coli*. *Mol Microbiol,* 31 (5), 1307-1319 (1999)

52. M.L.Laine, B.J.Appelmelk and A.J.Van Winkelhoff: Novel polysaccharide capsular serotypes in *Porphyromonas gingivalis*. *J Periodontal Res*, 31 (4), 278-284 (1996)

53. M.L.Laine, B.J.Appelmelk and A.J.Van Winkelhoff: Prevalence and distribution of six capsular serotypes of Porphyromonas gingivalis in periodontitis patients. J Dent Res, 76 (12), 1840-1844 (1997)

54. J.Aduse-Opoku, J.M.Slaney, A.Hashim, A.Gallagher, R.P.Gallagher, M.Rangarajan, K.Boutaga, M.L.Laine, A.J.Van Winkelhoff and M.A.Curtis: Identification and characterization of the capsular polysaccharide (K-antigen) locus of Porphyromonas gingivalis. Infect Immun, 74 (1), 449-460 (2006)

55. F.Qadri, A.M.Svennerholm, S.Shamsuzzaman, T.R.Bhuiyan, J.B.Harris, A.N.Ghosh, G.B.Nair, A.Weintraub, S.M.Faruque, E.T.Ryan, D.A.Sack and S.B.Calderwood: Reduction in capsular content and enhanced bacterial susceptibility to serum killing of Vibrio cholerae O139 associated with the 2002 cholera epidemic in Bangladesh. Infect Immun, 73 (10), 6577-6583 (2005)

56. A.Watts, D.Ke, Q.Wang, A.Pillay, A.Nicholson-Weller and J.C.Lee: Staphylococcus aureus strains that express serotype 5 or serotype 8 capsular polysaccharides differ in virulence. Infect Immun, 73 (6), 3502-3511 (2005)

57. S.L.Reckseidler-Zenteno, R.DeVinney and D.E.Woods: The capsular polysaccharide of Burkholderia pseudomallei contributes to survival in serum by reducing domplement factor C3b deposition. Infect Immun, 73 (2), 1106-1115 (2005)

58. M.J.Gill, D.P.McQuillen, J.P.van Putten, L.M.Wetzler, J.Bramley, H.Crooke, N.J.Parsons, J.A.Cole and H.Smith: Functional characterization of a sialyltransferase-deficient mutant of Neisseria gonorrhoeae. Infect Immun, 64 (8), 3374-3378 (1996)

59. M.M.Estabrook, J.M.Griffiss and G.A.Jarvis: Sialylation of Neisseria meningitidis lipooligosaccharide inhibits serum bactericidal activity by masking lacto-N-neotetraose. Infect Immun, 65 (11), 4436-4444 (1997)

60. D.W.Hood, K.Makepeace, M.E.Deadman, R.F.Rest, P.Thibault, A.Martin, J.C.Richards and E.R.Moxon: Sialic acid in the lipopolysaccharide of Haemophilus influenzae: strain distribution, influence on serum resistance and structural characterization. Mol Microbiol, 33 (4), 679-692 (1999)

61. S.Ram, A.K.Sharma, S.D.Simpson, S.Gulati, D.P.McQuillen, M.K.Pangburn and P.A.Rice: A novel sialic acid binding site on factor H mediates serum resistance of sialylated Neisseria gonorrhoeae. J Exp Med, 187 (5), 743-752 (1998)

62. S.Ram, F.G.Mackinnon, S.Gulati, D.P.McQuillen, U.Vogel, M.Frosch, C.Elkins, H.K.Guttormsen, L.M.Wetzler, M.Oppermann, M.K.Pangburn and P.A.Rice: The contrasting mechanisms of serum resistance of Neisseria gonorrhoeae and group B Neisseria meningitidis. Mol Immunol, 36 (13-14), 915-928 (1999)

63. S.Ram, M.Cullinane, A.M.Blom, S.Gulati, D.P.McQuillen, B.G.Monks, C.O'Connell, R.Boden, C.Elkins, M.K.Pangburn, B.Dahlback and P.A.Rice: Binding of C4b-binding protein to porin: a molecular mechanism of serum resistance of Neisseria gonorrhoeae. J Exp Med, 193 (3), 281-295 (2001)

64. M.A.Curtis, A.Thickett, J.M.Slaney, M.Rangarajan, J.Aduse-Opoku, P.Shepherd, N.Paramonov and E.F.Hounsell: Variable carbohydrate modifications to the catalytic chains of the RgpA and RgpB proteases of *Porphyromonas gingivalis* W50. *Infect Immun*, 67 (8), 3816-3823 (1999)

65. N.Paramonov, M.Rangarajan, A.Hashim, A.Gallagher, J.Aduse-Opoku, J.M.Slaney, E.Hounsell and M.A.Curtis: Structural analysis of a novel anionic polysaccharide from *Porphyromonas gingivalis* strain W50 related to Arg-gingipain glycans. *Molecular Microbiology*, 58 (3), 847-863 (2005)

66. J.M.Slaney, M.Rangarajan, J.Aduse-Opoku, S.Fawell, I.Darby, D.Kinane and M.A.Curtis: Recognition of the carbohydrate modifications to the RgpA protease of *Porphyromonas gingivalis* by periodontal patient serum IgG. *J Periodontal Res*, 37 (3), 215-222 (2002)

**Abbreviations:** Mannose binding lectin MBL, MBL-associated serine protease MASP, Short consensus repeating unit SCR, C4 binding protein C4BP, Decay accelerating factor DAF, Chemotaxis inhibitory protein of *S.aureus* CHIPS, Staphylococcal complement inhibitor SCIN, Lipopolysaccharide LPS

**Key words:** Innate immunity, complement, complement evasion, complement resistance, *Porphyromonas gingivalis*.

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