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SPECIFIC AND NON-SPECIFIC OPSONISATION; ITS ROLE IN THE (NON-INFLAMMATORY) CLEARANCE OF TRANSLOCATED MICROORGANISMS*

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SUMMARY

Once bacteria invade the tissues phagocytosis is a crucial step in containing the microbes. Phagocytosis can only occur when bacteria are properly opsonised; loaded with antibody molecule and complement. Only when IgG and C3b and/or iC3b are present on the bacterial cell wall bacteria are recognised by phagocytic cells. Because phagocytic cells have receptors for the Fc fragment of the IgG molecule and for C3b it is possible that the phagocyte has also other receptors that could be involved in uptake of bacteria by the phagocytic cells, e.g.: mannose sensitive receptors, CD14 molecules, fibronectin binding receptors, etc.

However, the process of phagocytosis mediated by these receptors is much less efficient. Because the internal signalling pathway within the phagocyte is not known when bacteria are attacked via other receptors, efficiency of killing of bacteria via those alternative opsonins is unknown.

Many bacteria have developed strategies to prevent the binding of IgG and C3b. Capsules hinder binding and complement activation; many human pathogens have surface capsules e.g. *E. coli*, *H. influenza*, *S. aureus*. Many of the different inhibitors of opsonisation are discussed.

Finally, the role of opsonisation in the process of translocation is touched upon. However, data on the role of specific opsonins in eliminating translocating microbes are limited.

INTRODUCTION

The outcome of the interaction between certain microorganisms and PMN determines health or disease. Once the barriers of the skin and mucous membranes have been breached the host's health depends on PMN and other host resistance factors to combat invading microorganisms that can cause infection

*: This paper is an adapted version of the chapter " Neutrophil phagocytosis and killing: Normal function and microbial evasion" by Jan Verhoef and Maarten R. Visser. In: The natural immune system: The Neutrophil (Abramson, J.S., and Wheeler, J.G., Eds.). Oxford University Press, New York, 109-137 (1993).

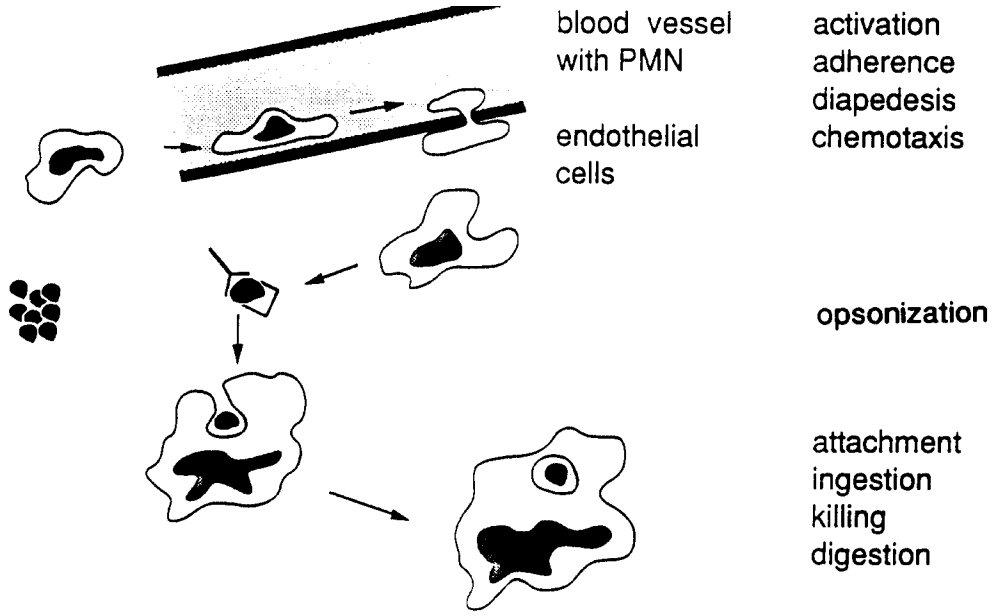

Figure 1: Processes involved in phagocytosis of bacteria by polymorphonuclear leukocytes.

(*Densen* and *Mandell*, 1990). PMN originate in the bone marrow and are continuously discharged in vast numbers into the bloodstream. They only live for a few days and each day about 10^{11} cells disappear from the body, even in the absence of infection.

For many infections the gastrointestinal tract can be a portal of entry for human pathogens. It is assumed that microorganisms can translocate from the gut into the regional lymph nodes (*Wells* et al., 1988). The mechanism of this process is still largely unknown. But there is some evidence that bacteria are taken up by phagocytes and then carried within the phagocytes to the mesenteric lymph nodes across the mucosal lung. Although it has also been shown that some bacteria invade certain mucosal cells and travel through these cells or between these cells (*Ménard* et al., 1994). It is now established *in vitro* that *Shigella* enters the epithelial barrier through M cells (*Phalipon* et al., 1995) that cove the dome of lymphoid follicles; subsequent invasion is primarily due to immigration of PMN which destroy the cohesion of the epithelial barrier.

As soon as microbes invade the tissues, circulating PMN are activated, leave the bloodstream, adhere to activated endothelial cells, and move through the endothelial barrier to the site of the infection. This process of migration under guidance is called chemotaxis and is defined as directed cell movement in one direction in response to an agent which signals and induces the cell to move. While PMN migration occurs, the microbes are opsonised; that is, the microbial surface is coated with antibody and complement factors for recognition by PMN. PMN have receptors specifically designed to bind to the Fc fragment of the IgG molecule present on the surface of the opsonised bacteria and other receptors designed to bind to the activated complement factors. The complement factors and the antibody molecules are ligands that promote

attachment of the microbe to the cell enhancing an otherwise inefficient microbe-phagocyte interactions. After this receptor-mediated attachment, PMN engulf the microbes and ingestion takes place. Once a microbe is phagocytised by the PMN, it is usually rapidly killed and digested.

During the last decade, our understanding of the molecular basis of the different steps involved in the process of phagocytosis and killing (shown in Figure 1) has greatly increased. It is now known that the outcome of the interaction between PMN and microbes is determined not only by PMN but also by the microbes.

OPSONISATION

Opsonisation in the present of antibody and complement

Generally microorganisms are only phagocytised after they have been properly opsonised; that is, loaded with activated C3 and IgG. Opsonisation through activation of complement is primarily a function of C3b and iC3b. The PMN receptor that recognises C3b is CR1, while the receptor that recognises iC3b is CR-3 (CD11b/CD18) (*Metzger*, 1990). For most opsonised particles especially (including) encapsulated organisms, the iC3b-CR3 interaction enhances attachment of the particle to PMN but not its ingestion. Ingestion only occurs in the presence of antibodies (*Metzger*, 1990). Antibodies bind to specific antigens on the cell wall of bacteria; these antibody molecules also serve as ligands for the attachment of bacteria to PMN. Two receptors for IgG are present on the PMN cell membrane: FcRII and FcRIII. FcRII can bind IgG1 and IgG3 equally well and better than IgG2 and IgG4. FcRIII binds only to monomeric IgG (*Sawyer* et al., 1989).

Many bacteria have developed a defence against opsonophagocytosis and are thus able to escape phagocytosis by PMN. The most important anti-phagocytic defence of bacteria is an enveloping capsule. These capsules protect the microbes against PMN by interfering with opsonisation (*Finlay* and *Falkow*, 1989). For example, pathogens that cause pneumonia and meningitis, such as *H. influenzae, Neisseria meningitides, E. coli, Streptococcus pneumoniae, Klebsiella pneumoniae*, and group B streptococci, have polysaccharide capsules on their surface. Non-encapsulated derivatives of these organisms are less virulent. Although the chemical composition of these capsules can vary significantly between strains and species, most capsules are composed of polymers of repeated sugar residues. However, only a few types of capsules are commonly associated with disease. *H. influenzae* isolates can produce one of six different types of polysaccharide capsules, yet those organisms expressing type b capsules are predominantly isolated from serious infections. Capsules from bacterial pathogens prevent complement deposition on the bacterial surface, while capsules from non-virulent strains are less efficient at preventing this deposition. The capsules are only weakly immunogenic and mask the more immunogenic underlying bacterial surface structures that would directly activate complement. Thus, the capsule prevents opsonisation of the organism, conferring resistance to phagocytosis (*Finlay* and *Falkow*, 1989).

Some other cell wall components that help microbes evade the phagocytic defence of the host are peptidoglycans, protein A (*Staphylococcus aureus*), and protein M (group A streptococci). The

role of each of these cell wall components are discussed below in conjunction with the microbes on which they are found.

Opsonisation in the absence of antibody and complement

Some bacteria are able to adhere to PMN in the absence of antibodies and/or complement. *E. coli* adhesins, for example, are important components that may mediate adherence of *E. coli* directly to PMN without antibodies and complement. These adhesins can be divided into two groups: one where D-mannosides inhibit the adherence and one where they do not. The mannose-resistant (MR) phenotype is mediated by cell-bound adhesins or by specific protein fimbriae. Among the *E. coli* strains with MR haemagglutination, the P adhesins recognise the sequence α-D-Gal-(1-4)-β--D-Gal on the target cell receptors. P adhesin-expressing *E. coli* are frequently involved in human urinary tract infections.

E. coli possessing mannose-sensitive (MS) adhesins adhere avidly to urinary slime. In addition, a number of other adhesins have been detected (M, X, and S adhesins, type 1c and G fimbriae). MS fimbriae (type 1 adhesins) increase the susceptibility of *E. coli* to PMN phagocytosis in the absence of specific opsonins (*Sobel* and *Kaye*, 1990). MS adhesins recognise the mannose residues of three different membrane glycoproteins in the PMN: gp 150, gp 70-80, and gp 100. Gp 150 (CD11/CD18) is the major receptor for type 1 fimbriae (*Rodriguez-Ortega* et al., 1987). Adherence of *E. coli* to PMN via the interaction of type 1 fimbriae and mannose-containing receptors leads to phagocytosis and killing by PMN (*Sobel* and *Kaye*, 1990). In contrast, PMN lack receptors for P fimbriae, which block phagocytosis (*Svanborg* et al., 1984). S adhesins are widespread among *E. coli* isolates that cause sepsis or meningitis. These adhesins recognise a structure containing neuraminyl acid derivatives. In many strains these derivatives appear in the form of neuraminyl-α-(2-3)-galactoside.

A possible serum factor other than complement or antibody that functions as an opsonin is the lipopolysaccharide binding proteins (LBP). LBP is an acute-phase reactant that binds bacterial LPS. LBP can bind to the surface of Gram-negative bacilli and strongly enhances attachment of these particles to the CD14 molecule in the cell membrane of phagocytic cells. LBP bridges LPS-coated particles to PMN and macrophages by first binding to LPS and then to the CD14 receptor. This binding leads to enhanced phagocytosis (*Wright* et al., 1989).

Gonococci possess antigenically variable outer membrane proteins, termed PII proteins, that appear to mediate adherence to human PMN (*Farrell* and *Rest*, 1990). These PIIs in the membrane of bacteria (outer membrane proteins) bind to carbohydrate moieties of glycoconjugates in a lectin-like manner. Anti-PII monoclonal antibodies abrogate adherence of non-piliated gonococci to human neutrophils. The neutrophil receptors for PII^+ gonococci appear to be stored in a subcellular granule population (*Farrell* and *Rest*, 1990).

In plasma there is a high molecular weight glycoprotein, called fibronectin, that aids the reticuloendothelial system in clearing the cell of microorganisms and helps maintain vascular stability. Several bacteria, for example, *S. aureus* and groups A, B, C, and G streptococci, have receptors for fibronectin. Because PMN can also bind fibronectin, it is possible that this glycoprotein acts as a bridge between PMN and the bacteria and therefore facilitates phagocytosis in the absence of specific opsonins (*Proctor* et al., 1984). It is

also possible that fibronectin enhances the opsonic and protective activity of antibodies and complement (*Hill* et al., 1984).

MECHANISMS FOR AVOIDING OPSONISATION

Microbial adaptation to avoid opsonisation

Many microorganisms can escape from opsonisation by varying their surface antigenic structure. Some bacteria are master chameleons. For example, *N. gonorrhoeae* possess at least two mechanisms for altering surface antigens:

1. They can change PII proteins. Most *N. gonorrhoeae* express several different PII proteins at any given time, and a given strain can potentially express up to seven different PII proteins. The genetic control of each PII gene appears unrelated to other PII genes, which results in many different combinations. The regulation of PII gene expression depends on the repeating five nucleotide CTCtt, which is located within the PII leader sequence. Variations in the number of repeats of this pentamer will vary the reading frame of the downstream PII gene.
2. They can change pilins. There are usually many silent pili gene sequences. The gonococcus can undergo gene conversion by placing one of these incomplete sequences into the expression site, thus, synthesising a new antigenically distinct pilin molecule. Antigenic variation occurs in many other bacteria as well: e.g., Group B streptococci, *H. influenzae, P. aeruginosa, Salmonella, Borrelia*, etc.

Interference of antibiotics with opsonisation

Exposure of bacteria to antibiotics below the minimal inhibition concentration increases their susceptibility to the antimicrobial action of normal human PMNL. Low concentrations of antibiotics influence cell wall composition. Clindamycin, for example, has an inhibitory effect on the M protein of streptococci and protein A of *S. aureus* and thereby facilitates opsonisation and subsequent phagocytosis. Antibiotics may also interfere with K antigen synthesis and LPS assembly in *E. coli*. (During antimicrobial treatment K antigen synthesis may be inhibited). Therefore, bacteria are more readily opsonised and subsequently phagocytised. Thus, during infections antibiotics may act in different ways: they may either kill the microbe directly or change the cell wall composition in such a way that an increased number of receptors for IgG and C3b is produced, thereby enhancing opsonophagocytosis (*Gemmell* and *O'Dowd*, 1983; *Milatovic*, 1983; *Veringa* and *Verhoef*, 1987).

NORMAL OPSONISATION AND EVASION OF OPSONISATION BY SPECIFIC MICROBES

Staphylococci

The major cell wall components of *S. aureus* are peptidoglycan, teichoic acids, and protein A. Peptidoglycan is a polysaccharidic polymer composed of B-linked (*Densen* and *Mandell*, 1990; *Sawyer* et al., 1989; *Berridge* and *Irvine*, 1984) chains containing alternat-

Table 1: Opsonins for *Stapylococcus aureus*

Unencapsulated strains:
- Antibodies against peptidoglycan
- C_3b and iC_3b generated by antigen-antibody reaction via the classical complement pathway
- C_3b and iC_3b generated by the classical and alternative pathway interaction with peptidoglycan
Encapsulated strains:
- Antibodies against polysaccharide capsule
- C_3b and iC_3b generated by the interaction of anticapsular antibodies with capsule

ing subunits of N-acetylmuramic acid and N-acetylglucosamine. Penta-peptide chains are linked to the muramic acid residue and are cross-linked by a pentaglycine bridge attached to L-Lysine on one chain and D-alanine on the other (*Davis* et al., 1990). Teichoic acids are simple glycerol or ribitol phosphates in repeating units, while protein A is a 42 kD protein with the capacity to bind human IgG subclasses (except IgG3) via their Fc terminals. Antibodies against peptidoglycan are opsonic (*Verbrugh* et al., 1980). When antibodies against peptidoglycan were isolated from serum and incubated with staphylococci, these bacteria were readily phagocytised. Peptidoglycan was also able to directly activate the complement system leading to deposition of C3b on the surface of the bacteria. However, more than 50% of *S. aureus* isolates obtained from blood cultures of patients are encapsulated. These capsular polysaccharides may interfere with the effective opsonisation by anti-peptidoglycan antibodies and hinder the interaction of complement with peptidoglycan (*Wilkinson* et al., 1979; *Verbrugh* et al., 1982; *Karakawa* et al., 1988). Anticapsular antibodies are needed for the efficient phagocytosis of these encapsulated bacteria.

Four mechanisms of opsonisation of unencapsulated *S. aureus* strains are described: the interaction of PMN with *S. aureus* through antibodies against peptidoglycan; the interaction through antibodies and C3b; the interaction through C3b generated by direct interaction of peptidoglycan with complement (classical pathway); and the interaction through direct activation via the alternative pathway (*Verbrugh* et al., 1980; *Wilkinson* et al., 1979; *Verbrugh* et al., 1982; *Karakawa* et al., 1988; *Nelles* et al., 1985). Antibodies against the 0-acetyl group of capsular polysaccharide are most efficient in opsonisation. While anti-peptidoglycan antibodies promote phagocytosis *in vitro*, their opsonic capacity *in vivo* is unclear, as most strains grown under *in vivo* condition contain a capsule that shields the peptidoglycan from specific antibodies (Table 1).

It would be of interest to test the protective capacity of both antibodies against peptidoglycan and capsules in an animal model.

During *S. aureus* infection of the host antibodies against teichoic acid are also produced. Their role in opsonisation is questionable and is probably indirect via activation of the complement cascade. In contrast, protein A probably does play a triple anti-phagocytic role in the bacteria-cell recognition process by virtue of its binding to the Fc portion of IgG: 1) extracellular soluble protein A

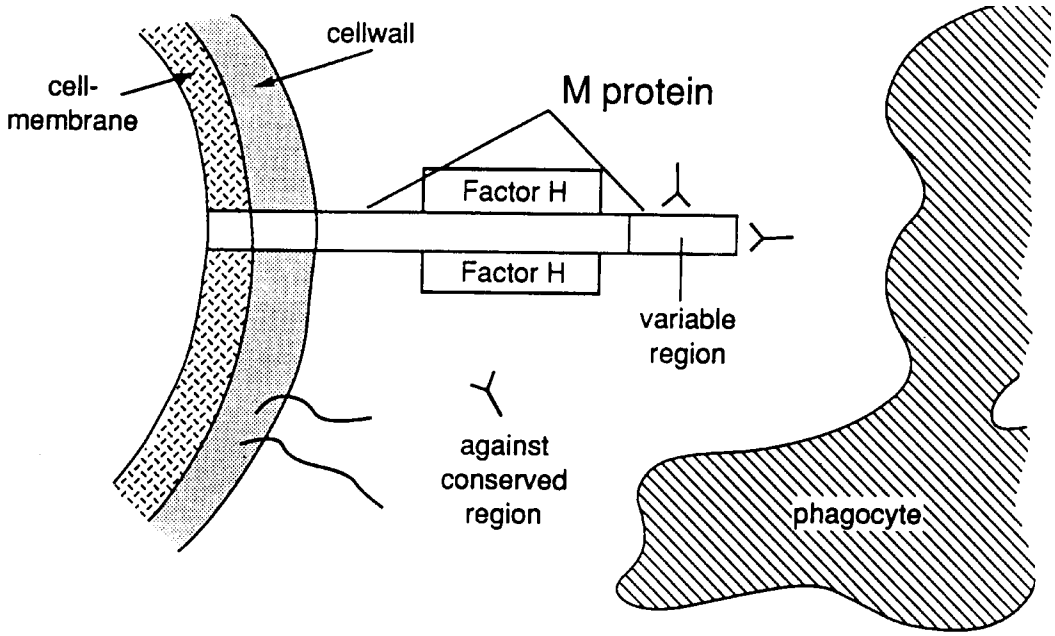

Figure 2: Interaction of M-proteins of streptococci with opsonic antibodies and factor H of the complement system.

can react with the Fc terminal of IgG molecules of human serum, thereby producing immune aggregates that consume complement. 2) extracellular protein A can bind to the Fc portion of specific anti-staphylococcal antibodies coating the microorganism with their Fab fragment, thereby preventing further interaction of the complex with the Fc receptor of phagocyte, and 3) cell-bound protein A binds to the Fc fragment of any IgG molecule in its neighbourhood, thereby eliminating non-specific and specific antibodies.

In recent years *S. epidermidis*, and other coagulase negative staphylococci has become major pathogens in hospitalised patients. Because of its ability to adhere to plastics, these organisms are formidable pathogens in the presence of foreign bodies. Principal adhesins that are responsible for the binding of *S. epidermidis* to catheters are a capsular polysaccharide and a protease-sensitive surface constituent from the slime-producing strains of *S. epidermidis*. In addition to promoting adherence to foreign bodies, these adhesins may also protect coagulase-negative staphylococci against phagocytosis. Antibodies to these adhesins may neutralise this shield and provide opsonisation of the bacteria. Monoclonal antibodies against *S. epidermidis* adhesins facilitate phagocytosis of homologous and heterologous *S. epidermidis* strains (*Kojima* et al., 1990; *Timmerman* et al., 1991). The major PMN receptor for *S. epidermidis* opsonins is the FcRIII receptor (*Schutze* et al., 1991). However, for strains that have a hydrophobic surface structure, antibodies by themselves are not sufficient for opsonisation. These strains also need C3b or iC3b (*Pascual* et al., 1988).

Streptococci

A major component of the *S. pyogenes* cell wall is M protein. M proteins interfere with opsonisation and therefore

can be regarded as important virulence factors. They form hair-like projections on the surface of group A streptococci. About 80% of M protein consists of blocks of repeating amino acid sequences. The amino acids near the C terminus bind M protein to the bacterial cytoplasmic membrane and, together with a region rich in prolines and glycines, they anchor M protein in the cell wall. This part of M protein is highly conserved and is also present in other cell wall proteins of Gram-positive bacteria (e.g. staphylococcal protein A; streptococcal protein G). The N-terminal end is the variable part of M protein and has an excess of negatively charged amino acids. PMN also exhibit a net negative charge on their surface. The negative charge of M proteins may thus have evolved to hinder contact between streptococci and phagocytic cells.

To add to this anti-phagocytic effect, M protein-positive streptococci do not bind C3b efficiently and thus evade opsonisation (Figure 2). This appears to be due to complement factor H binding of to the M protein, which prevents the deposition of C3b on the surface of *S. pyogenes*. In a sense, the M protein-bearing streptococcus cleverly disguises itself as a normal human cell to evade the complement system. During infection antibodies to the conserved and variable parts of the M proteins are made. Antibodies against the conserved part cannot bind when factor H is present and therefore do not have an opsonic capacity. Antibodies against the variable region are opsonic and also neutralise the region's negative charge and thereby further assist phagocytosis. However, they are only type-specific. Different streptococci with serologically different M proteins need different antibodies for efficient phagocytosis. One can thus be infected repeatedly with different group A streptococci.

Group B streptococci have cell wall components designed to evade the premature host defences of the neonate. These streptococci have polysaccharides and proteins in their cell walls which allow the strains to be differentiated into serotypes (Ia, Ib, Ic, II and III). The capsular polysaccharide is type-specific, while the C polysaccharide is group-specific and common to all strains of group B streptococci. Surface proteins are additional antigenic markers (*Baker* et al., 1982).

Most late-onset infections (onset of infections 6 days to 3 months after birth) are caused by group B streptococci belonging to serotype III, while early onset of infection can be caused by serotypes I, II, or III. Infections with meningitis are almost always caused by serotype III strains. The classical complement pathway and heat-stable opsonins are required for maximal opsonic activity by human sera for type I, II, and III strains (*Baker* et al., 1986). For clinical isolates of type Ia group B streptococci, opsonisation and phagocytosis may proceed via the classical complement pathway in an antibody-independent fashion, and C1 activation may be initiated by interactions with surface-bound capsular polysaccharides in these strains (*Baker* et al., 1982; *Levy* and *Kasper*, 1986; *Edwards* et al., 1982). Deficient opsonic activity of neonatal sera for clinical isolates of this serotype correlates with low levels of the classic pathway components C1q and C4. Since complement proteins in the neonate are not maternally derived and since levels of components in both the classical and alternative pathways are only 30-50 % of those in maternal or adult control sera at term, physiologically low levels of complement components or their receptors on phagocytes may provide a partial explanation for age-related susceptibility to group B streptococcal disease.

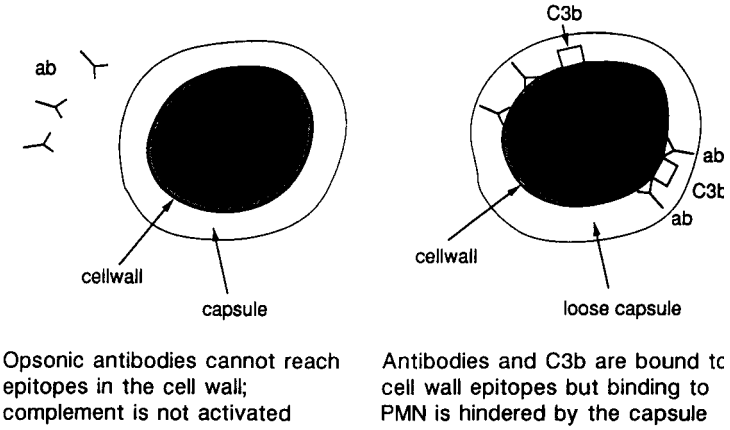

Figure 3: Ways capsules can interfere with phagocytosis.

Direct activation of C_1 may paradoxically be an extra virulence factor of the capsular polysaccharides. During infection large amounts of capsular material may be liberated from the bacteria. These fluid-phase macromolecules may then deplete complement by activating of C_1. Complement components necessary for opsonisation may then be absent.

Monoclonal antibodies (IgM and IgG) against type-specific antigens have been shown to be opsonic and to protect mice against lethal challenge with group B streptococci (*Egan* et al., 1983; *Hill* et al., 1984). Interestingly, IgA monoclonal antibodies were also shown to be opsonic (*Bohnsack* et al., 1989).

Opsonisation is an important factor in host defence against *S. pneumoniae*. Again, a polysaccharide capsule is the important virulence factor that hampers opsonophagocytosis. Of the 83 different serotypes of *S. pneumoniae* 23 cause nearly 90% of the pneumococcal infections. Like most bacteria, pneumococci are opsonised in the presence of complement through C3b and iC3b. Both activated complement factors are recognised by their specific receptors in the membrane of the PMN (CR1 and CR3). CR1 can specifically recognise C3b, while CR3 recognises iC3b. It is possible to differentiate between the C3b-CR1- and iC3b-CR3-specific interactions with monoclonal antibodies (*Gordon* et al., 1986). For example, the monoclonal antibody OKM10 that is able to block CR3 mediated phagocytosis of type 6A and 14 strains by 50-80%. These strains bear almost exclusively iC3b. Blockade of the CR1 receptor had no effect. For serotype III strains that bear C3b, iC3b, and C3d on the capsule, CR3- mediated phagocytosis accounted for only 20% of the uptake. Again, there was no evidence for CR1-mediated phagocytosis. The iC3b ligand elicits more release of superoxide, myeloperoxidase (MPO), and lactoferrin than C3b. The iC3b-CR3 interaction is thus the primary trigger for phagocytosis of iC3b-bearing pneumococci and for stimulation of intracellular bactericidal processes (*Hofstetter*, 1986). For comparison, it was shown that C3b, iC3b, and C3 make up 17%, 64% and 19% respectively, on *S. aureus* and 53%, 44%, and 2%, respectively, on *E. coli* (*Gordon* et al., 1988). Even among capsulated pneumococci a diversity exists in opsonisation. C3b and iC3b can be bound to the cell wall via a covalently linked thiolester-reactive

binding or via an amide linkage. Interestingly, the C3b molecules are bound almost exclusively to the capsule via the thiolester-reactive site, while the amide linkage is used for unencapsulated surfaces. The amide-linked molecules are far more potent activators of phagocytosis than the thiolester binding ones. This explains the ready phagocytosis of unencapsulated pneumococci and provides the capsule with another virulence mechanism.

Capsular polysaccharides may thus be regarded as virulence factors because they interfere with phagocytosis. This interference may be due to a variety of mechanisms preventing complement consumption (Figure 3). For example, binding of inefficient opsonins such as C3b, or thiolester-active binding of the C3b or iC3b molecule which makes the opsonisation less active by shielding or binding specific antibodies to cell wall antigens.

Haemophilus influenzae: Studies with *H. influenzae* have shown the crucial role for antibodies and complement in host defence. *H. influenzae* is the cause of respiratory tract infections in children and adults. In young children this microorganism can also cause bacteraemia and meningitis. Although unencapsulated *H. influenzae* strains can cause serious infections, most invasive infections are caused by the encapsulated strains. The capsule of *H. influenzae* type b is a polyribosyl-ribitol phosphate (PRP). Not only are antibodies against PRP necessary for opsonisation and protection of the host against recurrent infections (*Cates* et al., 1985), but classical complement components are also important for adequate opsonisation. This was shown in experiments with C1q-deficient serum, which demonstrated that opsonisation of *H. influenzae* type b may proceed through activation of the alternative pathway of complement, but that opsonisation via the classical pathway is much more efficient (*Roord* et al., 1983). The addition of C1q to C1q-deficient serum greatly enhanced opsonic activity. Also, there is an increased risk for *H. influenzae* infections in patients with a deficiency of other early components of the classical pathway of complement. In contrast, serum from patients with factor D (alternative pathway component) deficiency shows no impairment in opsonic activity for *H. influenzae*. This again underlines the predominant role of the classical pathway of complement in opsonisation of *H. influenzae* type b.

The third complement component C3 assumes a central role in the complement system. It participates in both the classical and the alternative pathways as well as in the amplification loop and is one of the major opsonins. Patients with C3 deficiency suffer from recurrent and often severe respiratory tract and systemic infections, that are frequently due to *H. influenzae* type b (*Roord* et al., 1983).

Enterobacteriaceae

Enterobacteriaceae are able to evade host defence. This capacity is mainly determined by properties of the bacterial cell wall. As shown in Figure 4, the Gram-negative bacterial cell wall consists of an inner cytoplasmic membrane, an intermediate murein or peptidoglycan layer, and an outer phospholipid-LPS bilayer in which proteins are inserted. LPS is anchored by the lipid region (lipid A) in the outer leaflet of the outer membrane with a covalently bound core-oligosaccharide structure directed outward. In addition, the outer part of the LPS of most strains, that are present in nature (wild-type strains), contains a polysaccharide chain (O antigen) bound to the distal terminal of the core-oligosaccharide. Some strains (e.g., many types of *E. coli* and *Klebsiella*) contain a surrounding capsular polysaccharide

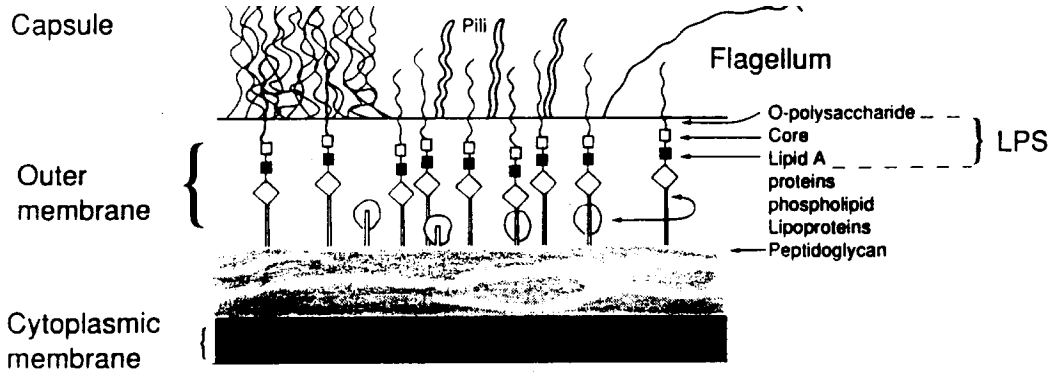

Figure 4: The Gram-negative bacterial cell wall.*

(K antigen). In addition, extruding protein structures, flagellae, pili, or fimbriae, may be present. These pili and fimbriae mediate the capacity of strains to adhere to and colonise mucosal surfaces.

Certain types of O an K antigens confer resistance to the bactericidal action of serum and phagocytosis by granulocytes. It has been suggested that the presence of large amounts of these polysaccharide structures may physically hinder the access of complement and/or antibodies to target structures on the bacterial surface, thereby either preventing activation of the complement pathways or yielding formation of the membrane attack complex at a site too distant from the bacterial cytoplasmic membrane (*Vermeulen* et al., 1988). Also, the anti-phagocytic effects of these structures may be related to interference with the hydrophobic nature of the bacterial surface.

Indeed, rough Gram-negative bacillary strains, defined by their deficiency of the polysaccharide side chain (O antigen), are sensitive to the bactericidal activity of the complement system and are readily ingested by granulocytes. Lipid A can bind the first complement factor (C1) directly, leading to an antibody-independent activation of the classical pathway. The polysaccharide region of LPS can activate the alternative pathway by a lipid A-independent, antibody-independent mechanism and has a modulating effect on the expression of lipid A binding and C1 activation. In addition to complement, specific antibodies to the surface structures are required for killing encapsulated (K+) and smooth (O+) bacilli in serum and for phagocytosis of such bacilli by PMN (*Vermeulen*, et al., 1988). Thus, Gram-negative bacilli that contain O and K antigens have an increased capacity of surviving in the bloodstream. Indeed, most episodes of Gram-negative bacteraemia are caused by smooth and/or encapsulated strains. Nevertheless, rough and unencapsulated strains of Gram-negative bacilli may also cause bacteraemia and septic shock. This is, however, rare and mostly seen in patients with severely diminished host defence. Once the imbalance between host defence and bacterial virulence has al-

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lowed the microorganisms to invade and survive in the bloodstream, the ensuing cascade of events and ultimately the patient's outcome are dependent on the Gram-negative bacillary strain. All Gram-negative bacillary strains possess the capacity, upon invasion into the bloodstream, to cause septic shock syndrome.

Pseudomonas aeruginosa

Studies on the opsonic requirements of *P. aeruginosa* have shown that antibodies against mucoid exopolysaccharide (MEP; the primary constituent of the slime coat of mucoid strains) are important opsonins. These mucoid strains are the primary pathogens in cystic fibrosis patients. MEP promotes the adherence of *P. aeruginosa* strains to tracheal cells and respiratory mucins.

The heteropolymeric nature of MEP is explained by the presence of both common and type-specific epitopes; the common epitopes are divided into those that bind opsonising and those that bind non-opsonising antibodies. Most naturally occurring antibodies to MEP function poorly in in-vitro opsonophagocytosis assays with complement and are unable to protect host animals. In contrast, antibodies that are highly opsonic protect host animals against infection. and have been found in older CF patients who are not colonised with *P. aeruginosa*. Opsonising antibodies to MEP are usually not found in younger non-colonised or chronically colonised CF patients. These findings suggest a protective effect for the opsonising antibodies. MEP, therefore, has become a promising vaccine candidate for the prevention of *P. aeruginosa* infection in CF patients. Unfortunately, in humans MEP appears to be poorly immunogenic in inducing opsonic antibodies (*Schreiber* et al., 1991; *Garner* et al., 1990).

Neisseria

Specific antibodies and the complement system play key roles in host defence against *N. meningitides* and *N. gonorrhoea*. They can lyse bacteria, enhance phagocytosis and neutralise the effects mediated by endotoxin (*Jarvis* and *Vedros*, 1987; *Ross* et al., 1987). In *N. meningitides* the presence of a bactericidal antibody, however, is of utmost importance. Anti-capsular polysaccharide antibodies and anti-outer membrane protein antibodies in general facilitate phagocytosis and killing. Antibodies against meningococcal LPS also appear to contribute to opsonophagocytosis. However, for optimal phagocytosis complement should also be present.

Although the alternative pathway is not able to halt meningococcal dissemination in susceptible infants (indicating the importance of antibodies and the classical complement pathway), the relative importance of the alternative pathway is shown in families with a sex-linked properdin (alternative pathway) deficiency. Individuals belonging to these families experience multiple, sometimes fatal, episodes of fulminant group B, C, and Y meningococcal infections (*Jarvis* and *Vedros*, 1987). In contrast, individuals with a deficiency in early classical complement components show an unexpected lack of meningococcal disease. Some unknown compensatory mechanisms must be involved in the resistance against meningococci in these patients. Perhaps antibodies and the successful use of the alternative pathway may be responsible for this resistance mechanism.

Other microorganisms

The opsonic requirements of many other microorganisms (bacteria, fungi, parasites) have been studied. The general conclusion is that cell wall antigens

may determine whether the microorganisms is readily phagocytised. In some microorganisms the capsule is the determining factor, while in others proteins or lipopolysaccharides may be responsible for the resistance to opsonisation. For example, the expression of plasmid-encoded proteins is associated with resistance to complement-mediated opsonisation and neutrophil phagocytosis in the cell wall of *Yersinia enterocolitica*. PMN also play a role in eliminating virus particles (*van Strijp* et al., 1989, *Turner*, 1990). For example, interaction of herpes virions through PMN-complement CR1 and CR3 results solely in binding to PMN, but not in internalisation (*van Strijp* et al., 1989). For internalisation, interaction with FcR is mandatory. Recently, it has been shown that some parasites (e.g., *Trypanosoma cruzei*, the causative agent of Chagas' disease) produce a glycoprotein (gp160) that restricts complement activation by inhibiting C3 convertase formation. This glycoprotein is similar to a human complement regulatory protein, the decay-acceleration factor (*Joiner* et al., 1988).

CONCLUSION

Opsonins are of crucial importance for host defence against invading microorganisms. Many microbes have developed strategies to combat the interaction of bacteria with opsonins and thus evade recognition by phagocytic cells. Patients with low levels of opsonins, such as patients with hypogammmaglobulinaemia with complement deficiencies suffer from recurrent infection.

How important opsonins are in the containment of translocated bacteria from the gut into the mesenteric lymph nodes is not known. It is possible that at the site of the regional lymph node alternative opsonins and receptors are important, such as CD14 in the membrane of phagocytic cells, fibronectin binding receptors, binding via mannose-sensitive receptors or α-D-Gal(1-4)-ß-D-Gal.

It is also possible that in some tissues bacteria are fixed to cells and that phagocyte had to eliminate these fixed bacteria. This process appears not to involve opsonins and is mainly driven by surface charges (*Vandenbroucke*, 1988; *Pascual*, 1989). However, there is evidence that the phagocytic cell is damaged in the process of eating bacteria that are fixed to other cells (*Vandenbroucke*, 1988). This process does not appear to be very efficient.

In conclusion, opsonins are important; in some events phagocytosis can occur in the absence of opsonins. But it is likely that phagocytosis in the absence of opsonins is less efficient.

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