actively expressed in circulating neutrophils are those which are either subject to rapid turnover (i.e. those of the contractile machinery and membrane receptors) or those whose rate of biosynthesis needs to be increased during inflammation. It does not appear that mature cells actively synthesize, or up-regulate during 'priming', those components normally present in high concentrations and/or not subject to turnover in non-activated cells (i.e. granule enzymes or the components of the NADPH oxidase). These up-regulated proteins appear to play an important role in the function of these cells during an acute inflammatory response and further work is clearly necessary to characterize these proteins and identify their biological functions.

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Bacterial capsules and interactions with complement and phagocytosis

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There is now considerable evidence that the capsular polysaccharides on the surface of both Gram-negative and Gram-positive bacteria play a key role in virulence by conferring serum resistance and inhibiting phagocytosis by polymorphonuclear leucocytes (PMNL) (Peterson et al., 1978; Horwitz & Silverstein, 1980; Kim et al., 1986; Allen et al., 1987). In this regard, the best studied examples are the Gram-positive bacteria *Staphylococcus aureus* and *Streptococcus pneumoniae*. Studies on *S. aureus* have shown that encapsulated strains have increased virulence compared with their unencapsulated variants (Melly et al., 1974). Encapsulated strains are more resistant to serum killing and serum-mediated opsonization and phagocytosis by PMNL (Peterson et al., 1978). This is an important initial defence mechanism in the non-immune host. The resistance of *S. aureus* to complement-mediated opsonization is not due to capsule-mediated inhibition of the alternative pathway of complement activation (Verbrugh et al., 1979). Rather, encapsulated *S. aureus* do bind opsonically active C3b, but underneath the capsule; thus bound C3b is masked from C3b receptors on the PMNL cell surface (Wilkinson et al., 1979). As a result there is only a poor correlation between the total amount of C3b deposited and the effective opsonization of *S. aureus*. Recent studies (Verbrugh et al., 1982; Karakawa et al., 1988) have shown that specific capsular antibodies play a crucial role in the phagocytosis of *S. aureus*. Indeed it is likely that phagocytosis of encapsulated *S. aureus* occurs by opsonization by capsule-specific antibodies and uptake by PMNL via Fc receptors. The presence of specific capsular antibodies will cause activation of the classical pathway complement in the capsule, thereby increasing opsonization and allowing C3b-mediated phagocytosis.

A primary virulence determinant of *S. pneumoniae* is the capsule. Virulent encapsulated *S. pneumoniae* are resistant to the bacteriocidal action of complement and in the absence of specific antibodies are poorly phagocytosed and killed by PMNL (Winkelstein et al., 1976). When encapsulated *S. pneumoniae* activate complement by the alternative pathway, the capsule plays no role in this activation (Winkelstein et al., 1976). As for *S. aureus*, C3b molecules generated by the alternative pathway are deposited on the pneumococcal cell wall beneath the capsule (Winkelstein et al., 1980), resulting in reduced opsonic potency and a reduction in C3b-mediated phagocytosis by PMNL. The presence of specific capsular antibodies causes C3b deposition on the pneumococcal capsule by classical pathway activation of complement, maximizing phagocytosis of the pneumococcus via both Fc and C3b receptors on the PMNL. The inaccessibility of C3b deposited on the pneumococcal cell wall can be demonstrated by immune adherence. Human erythrocytes possessing a C3b receptor on their surface can be agglutinated by exposed C3b. Pneumococci opsonized in non-immune serum fail to agglutinate such cells, while pneumococci opsonized in the presence of specific antibodies cause immune adherence (Brown et al., 1982). Clearly in both *S. aureus* and *S. pneumoniae*, the capsule plays a key role in non-immune serum in masking the cell-wall-bound C3b from the appropriate C3b receptor on the PMNL.

The presence of a capsule has been implicated in the virulence of many Gram-negative bacteria (Makela et al., 1980; Robbins et al., 1980). In contrast to the cell surface of Gram-positive bacteria, the complex nature of the cell envelope of Gram-negative bacteria, including the presence of lipopolysaccharide (LPS) and outer membrane and surface proteins, in addition to capsular polysaccharide, make the unequivocal assignment of particular roles for the capsule more difficult. Possibly the best studied capsules of Gram-negative bacteria are the capsular polysaccharides (or K-antigens) of *Escherichia coli*. In part this is a reflection of our well-developed understanding of the genetics of *E. coli* and the ease of the application of the techniques of molecular biology. More than 50 K-antigens have been described for *E. coli* (Orskov et al., 1977). Virulent, extra-intestinal isolates of *E. coli* are encapsulated with particular K-antigens being associated with *E. coli* isolates from certain infections (Robbins et al., 1974; Kajser et al., 1977). Encapsulated *E. coli* have

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Abbreviations used: PMNL, polymorphonuclear leucocyte; LPS, lipopolysaccharide; Mab, monoclonal antibody.

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increased resistance to complement-mediated killing and to phagocytosis and killing by PMNL (Gemska et al., 1980; Kim et al., 1986; Allen et al., 1987). In addition, certain K-antigens, such as the K1-antigen, a homopolymer of 2-8-linked N-acetylenuraminic acid, are poorly immunogenic, being chemically related to carbohydrate moieties found on the mammalian cell surface (Schauer, 1981; Finne, 1982). This molecular mimicry effectively means that as well as conferring a level of resistance to the non-specific arms of the immune system, the presence of certain K-antigens may lead to the failure of the host to generate an adequate humoral response (Cross et al., 1983).

Encapsulated E. coli are in general resistant to complement-mediated killing and, in the absence of specific antibodies, to complement-mediated opsonophagocytosis. Exposing encapsulated E. coli isolates to human serum absorbed with isogenic unencapsulated E. coli, followed by exposure to PMNL, results in little or no phagocytosis and killing (Horwitz & Silverstein, 1980). However, addition of specific capsule antibodies to pre-absorbed serum results in classical pathway activation of complement on the capsule surface of E. coli and phagocytosis via Fc and C3b receptors on the PMNL surface. This indicates that the capsule is having a strong effect on complement activation by the mammalian cell surface and masking structures such as LPS known to be capable of activating complement. However, the situation is likely to be much more complex than this, for instance it has been shown that antibodies to the somatic O-antigen of certain encapsulated E. coli are protective (Kajiser & Olling, 1973). In addition, opsonization of a serum-resistant K12 strain with an anti-04 monoclonal antibody (Mab) is sufficient to induce phagocytosis and killing by PMNL (Abe et al., 1988). However, the extent of phagocytosis and killing is enhanced when the bacteria are opsonized with an anti-K12 Mab or when both Mabs are used in conjunction (Abe et al., 1988). Clearly, therefore, in this case sufficient LPS is exposed to allow specific opsonization and classical activation of complement followed by phagocytosis via Fc and C3b receptors. This may reflect the low level of encapsulation of this strain (Abe et al., 1988). Therefore it is likely that the anti-phagocytic role of any particular K antigen may be dependent on the chemical structure and molecular arrangement of the polysaccharide and the overall topography of the particular bacterial cell surface.

The role of the capsule on the virulence of the E. coli K1-antigen to the virulence of E. coli has come from studies using the cloned genes encoding for synthesis (Boulnois & Roberts, 1989) of the K1 (Allen et al., 1987) and other antigens (F. K. Saunders, I. S. Roberts & G. J. Boulnois, unpublished work). The expression of the genes for the production of the K1-antigen in well-characterized laboratory strains of E. coli provides an opportunity to investigate the role that this capsule plays in resistance to complement killing and in the phagocytosis by PMNL. Laboratory strains of E. coli are rapidly killed by the bacteriocidal action of complement and are quickly phagocytosed and degraded within the PMNL. The presence of the cloned K1-genes in the laboratory strain conferred only a slight protection to the bacteriocidal activity of complement compared with the clinical K1 strain, indicating that a combination of cell surface structures in addition to the capsule may be important in this respect (Allen et al., 1987). However, the presence of the K1 capsule on the surface of the laboratory strain did inhibit the rate of uptake of this strain by PMNL. The fact that these experiments were performed in the presence of heat-inacti- vated serum makes precise interpretation of the antiphagocytic role of the K1-antigen difficult in this case. It is possible that the negative charge imparted by the capsule may be exerting an effect. The presence of the K1-antigen on the surface of the laboratory strain of E. coli had no effect on the rate of killing and intraphagocytic degradation, in contrast to the clinical K1 isolate which appeared highly resistant. Clearly these sorts of experiments are useful in attempting to dissect out a particular role for the capsule. However, the fact that many laboratory strains are deep, rough mutants lacking O-antigen side-chains and part of the core of LPS, which in certain clinical strains have been implicated in resistance to phagocytic uptake (Rozenberg-Arksa et al., 1985), may well lead to confusing results. Moreover, the correct assembly and maintenance of different components of the Gram-negative envelope may be mutually dependent, such that in laboratory strains expression of the cloned capsule functions results in an incomplete or aberrant capsule on the cell surface (Allen et al., 1987). Indeed, we have recently established that different recombinant plasmids encoding the same K-antigen confer different levels of serum resistance. This may reflect expression of differing amounts of the capsular polysaccharide in question. Previous studies have indicated in the case of E. coli K1 that the extent of polysaccharide production dramatically influences complement activation, opsonization and virulence (Bortolussi et al., 1979).

In summary, the precise role the capsule plays in the virulence of Gram-negative organisms has been difficult to establish. It is important in conferring resistance to opsonophagocytosis, but the role of other cell surface factors play in this process and the extent to which they might modulate capsule function awaits elucidation.

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Consequences of bacterial attachment in the urinary tract


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Introduction

Escherichia coli bacteria attach to the epithelial lining of the urinary tract [1]. The attachment is mediated by bacterial surface structures, adhesins, which bind to receptors in the epithelial cell membrane [2]. The adhesins are mostly associated with pili or fimbriae [3]. They are lectins, i.e. proteins which recognize carbohydrate sequences [2]. The receptor specificity is, however, not provided by the fimbrial protein, but by additional proteins bound at the tip and/or alongside the fimbrial rod [4-6]. The receptor specificity is afforded by oligosaccharide sequences in epithelial cell glycoconjugates, glycolipids and glycoproteins [2].

An individual E. coli strain may express adhesins with several different receptor specificities. For uropathogenic E. coli these include the Galα1→4Galβ sequence [7, 8] or GalNAc-terminal of globo-series of glycolipids [9, 10], mannose-containing structures, e.g. in secretory IgA [11], sialic acid residues in glycoproteins and gangliosides, etc. [12]. The final binding of bacteria to the epithelial cells will thus depend both on the expression of adhesins by the bacterial cell and on the available receptors on the target cell.

The globo-series of glycolipid receptors for uropathogenic E. coli have received special attention. They were the first group of receptor structures to be identified, and the functional consequences of binding to these receptors have been extensively analysed.

Adherence enhances bacterial persistence

When the adherence concept was first formulated, it was said that bacteria needed to attach to components of epithelial surfaces in order to resist elimination by the flow of secretions [13]. Bacterial persistence at mucosal surfaces has indeed been shown to be influenced by attachment to Galα1→4Galβ-containing receptors. This is relevant at two sites: the large intestine and the urinary tract itself.

The large intestine serves as the ecological niche for Enterobacteriaeae. Furthermore, it constitutes the reservoir for E. coli causing extraintestinal infections [14]. The likelihood of causing such infections is related to the time of persistence. Strains which are resident in the large intestine have an increased chance to produce urinary tract infection [15, 16].

Determinants of persistence in the large intestine are likely to be numerous and of complex nature. We have investigated the presence in colonic mucosa of receptors for attaching E. coli [17]. Colonic epithelial cells were obtained from two origins: colonic specimens from patients undergoing colorectal surgery and a colonic carcinoma cell line HT29. Bacteria with Galα1→4Galβ specificity bound to cells of both origins.

The role of attachment in persistence in the large intestine was analysed by correlating bacterial receptor specificity with the time of persistence as deduced from sequential faecal cultures from children with urinary tract infection. The strains with Galα1→4Galβ specific adhesins persisted significantly longer than strains lacking such adhesins (A. Wold, G. Larsson & C. Svanborg Eden, unpublished work).

The role of attachment for bacterial persistence in the urinary tract has been studied in an experimental model for urinary tract infection [18, 19]. E. coli strains were genetically manipulated to differ in the expression of Galα1→4Galβ specific adhesins, but to retain other virulence factors. This was achieved in two ways: a deletion mutation in a fully virulent pyelonephritis strain eliminated the expression of adhesins. A non-virulent and non-adhering strain was transformed with the chromosomal DNA fragments encoding adherence factors. The ability of the virulent and transformant to persist in the urinary tract was compared with the adherent parent and the non-adhering recipient strain, respectively. By both procedures, it could be demonstrated that attachment enhanced bacterial persistence both in kidneys and bladders.

Role of adherence for induction of inflammation

The injection of adherent E. coli strains into the mouse urinary tract results in a mucosal and systemic inflammatory response, which can be measured as the production of interleukins in urine and serum and as the recruitment of inflammatory cells to that site [20, 21]. This process was first shown to be dependent on the lipid A moiety of endotoxin [22]. A high degree of inflammation did not occur in lipopolysaccharide (LPS) non-responder mice which are genetically incapable of reacting to lipid A. The adhesins, however, are required to anchor the bacteria on to the epithelial cells to present the toxin to the tissues. Adhesins synergistically increased the toxicity of lipid A, and blocking of adherence reduced the inflammation caused by whole bacteria [22].

The ability of the adhesin-receptor interaction to activate inflammation independent of lipid A was analysed by intravesical installation of isolated fimbriae with and without the adhesin. A significant response to the fimbriae occurred both in C3H/HeN (LPS responder) and C3H/HeJ (LPS non-responder mice) (Table 1). These results represent the first evidence in vivo that the binding of a bacterial adhesin to a glycoconjugate receptor initiates a signal in the target cell.

The inflammatory process has two components: an afferent phase during which interleukin 6 (IL-6) and other media-