Current topics

P-fimbriae, bacterial adhesion, and pyelonephritis

JAN WINBERG

Department of Paediatrics, Karolinska Institute, Karolinska Hospital and National Bacteriologic Laboratory, Stockholm, Sweden

Bacterial tropism

Everyday observations usually seem self evident and are often not given the attention they merit. Examples are the fact that β haemolytic streptococci cause sore throat but not pneumonia; gonococci cause urethritis but not urinary tract infection (UTI) (which *Escherichia coli* do); and bacteria like *Neisseria gonorrhoea* and *Bordetella pertussis* that cause human disease are often harmless in animals. Bacterial adherence may play a role in this 'tropism'.

In nature bacteria often stick to surfaces like stones, leaves, and roots. Epithelial cell surfaces make no exception. Bacteria belonging to the 'normal' flora as well as pathogens may adhere by hydrophobic or electrostatic bonds, or both, of an 'unspecific' nature, or through a specific interaction between bacterial adhesins and epithelial cell receptors.¹⁻⁵ The ability of pathogens to adhere to mucous membranes is now recognised as a potential factor in virulence.

Specific bacterial adhesion

Adhesion is often mediated by fimbriae (pili),^{6,7} proteinaceous, hairlike extensions from the bacterial cell surface that may recognise specific receptor structures, often carbohydrates, on the epithelial cell membrane. These fimbriae have been studied mostly in *E coli* in relation to gastroenteritis and UTI, and in *N gonorrhoeae*.

Haemagglutination was the first observed manifestation of adhesion in enterobacteriaceae. The pioneering work was done in the 1950s by Duguid *et al.* but it took almost two decades before the full importance of this work was recognised.⁸

Adherence concept in UTI

The way bacteria reach the kidney in patients with acute pyelonephritis long remained an enigma.

Vesicoureteral reflux (VUR) provided a rationale for the ascent of bacteria from the bladder to the pelvis, and intrarenal reflux (IRR) for the spread into the renal parenchyma.⁹ Only a minority of patients, however, with acute, febrile pyelonephritis have gross VUR shown by micturition urethrocytography and still fewer have IRR.¹⁰ Furthermore, in many children with VUR the infection still remains limited to the bladder. The mechanistic concept of the initiation of urinary tract infection may thus need to be amended.

The host and the bacteria. In females with recurrent UTI, the outer urethral orifice and vaginal introitus are usually heavily colonised with Gram negative bacteria, even between infections.^{11,12} Urogenital epithelial cells from these patients bind *E coli* better than cells from healthy controls.¹³⁻¹⁵ This may be an expression of a more general biological abnormality, since buccal cells from women prone to UTI also show an increased adhesive capacity.¹⁶ In humans *E coli* strains isolated from patients with pyelonephritis bind better to uroepithelial cells than strains from patients with cystitis and asymptomatic bacteriuria.¹⁷

Thus, the initiation and progress of urinary tract infection may be governed by the interaction between host and bacteria. One main task in present research is to identify and characterise the two main actors: the adhesins of the bacteria and the receptors of the uroepithelial cells of the host.

Mannose sensitive and mannose resistant adhesion. Bacterial adhesion is divided into mannose sensitive (adhesion inhibited by mannose) and mannose resistant (adhesion not inhibited by mannose). Mannose sensitive adhesion is common among *E coli* pathogens as well as non-pathogens and is mediated by so called type 1 fimbriae,⁸ but since it has not so far been shown to play any role in the initiation of human pyelonephritis it is not mentioned further. Although the type of adhesion found

in most clinical isolates of *E coli* is mannose resistant,¹⁸ this is a very crude definition of the adhesin, telling us only that adhesion is not mediated by type 1 fimbriae.

P-fimbriae and corresponding epithelial receptor (D-galactose - $\frac{1-4}{\alpha}$ - D-galactose)

One research frontier is the definition and chemical characterisation of specific epithelial receptors for different kinds of adhesins within the heterogeneous mannose resistant group; another the purification and characterisation of the corresponding fimbriae. Two research groups have approached the first problem along two different avenues and have located a receptor structure for pyelonephritogenic *E coli* in the same kind of substances—glycosphingolipids associated with the P-blood group system. Källénus *et al*, who based their work upon Duguid's original observation that enterobacteria have specific haemagglutination abilities, showed that pyelonephritogenic *E coli* specifically agglutinated human erythrocytes in a mannose resistant manner and that the agglutination capacity was proportional to the ability to adhere to uroepithelial cells. Systematic work, showed that the P-blood group antigens acted as receptors for pyelonephritogenic *E coli*.¹⁹⁻²⁴ The fimbriae mediating the receptor binding were therefore named P-fimbriae.²² The P-blood group antigens consist of different oligosaccharide chains bound to ceramide, a sphingo lipid.

About 70 people in the world are known to lack P-antigens—they are of the phenotype $\bar{p}$. A key observation was that pyelonephritogenic *E coli* did not adhere to red cells or epithelial cells from these subjects.¹⁹

Continued work has shown that a disaccharide, a D-galactose - $\frac{1-4}{\alpha}$ - D-galactose, that is part of the oligosaccharide chains of the P-blood group antigens is the minimal receptor active element.²⁰ The evidence comes from various studies:

(1) Failure of P-fimbriated *E coli* to agglutinate $\bar{p}$ erythrocytes; low adhesion to uroepithelial cells from subjects with phenotype $\bar{p}$; isolated P-fimbriae show the same binding specificity as whole bacteria.²⁵

(2) Inhibition of agglutination and adhesion by the D-galactose - $\frac{1-4}{\alpha}$ - D-galactose structure, which has been synthesised.

(3) Non-agglutinable erythrocytes are made agglutinable after passive coating with a synthesised glycolipid containing the D-galactose - $\frac{1-4}{\alpha}$ - D-galactose disaccharide.

(4) By coating uroepithelial cells with the same

synthetic glycolipid their binding capacity for P-fimbriated bacteria increases.

(5) In vivo, a soluble receptor analogue interferes with the infectious process.

Svanborg-Edén who has made major contributions in the field of bacterial adhesion in UTI was early in the search for a receptor active compound. Together with Leffler²⁶ she independently reported that P-blood group antigen related glycosphingolipids were functional as receptors for one pyelonephritogenic *E coli* strain.

Clinical studies

E coli expressing P-fimbriae on their surface were found in the urine of more than 90% of children with a first, acute, febrile non-obstructed pyelonephritis, but less often in distal infections (Table 1). P-fimbriae most likely help the bacteria to resist the flow of urine by attaching to the uroepithelium (Figure), and to the kidney cells where the receptor active glycosphingolipids are present.¹⁰

The P-fimbriated *E coli* isolated from children with pyelonephritis also seems to dominate the periurethral and faecal flora in that child (Table 2). This suggests that faecal colonisation with P-fimbriated *E coli* may be the first step in the series of events leading to overt UTI. The classic antithesis of the 'special pathogenicity theory' and the 'prevalence theory' of initiation of pyelonephritis is not an 'either/or', but an 'as well as': specially pathogenic bacteria dominate the faecal flora. Thus, factors promoting or preventing intestinal colonisation may be of importance in the pathogenesis of this disease.

Table 1 Isolation of P-fimbriated *E coli* from urine of patients with UTI and from faeces of healthy controls

	No	(%)
Patients		
Febrile symptomatic (clinical pyelonephritis)	35	(94)
Afebrile, symptomatic (cystitis)	26	(19)
Afebrile, asymptomatic	36	(14)
Controls		
Faeces	82	(7)

Table 2 Occurrence of P-fimbriated *E coli* in periurethral area and in faeces of pyelonephritis patients and healthy controls

Group	Periurethral occurrence (No)	Faecal occurrence No (%)	Dominating faecal strain No (%)
Patients (n=10)	10	10 (100)	9 (90)
Controls (n=52)	ND	5 (10)	1 (2)

ND = not done.

Figure Transmission electron micrograph showing a P-fimbriated *E coli* adhering to a periurethral cell. B is a bacterial cell; C is cytoplasm of periurethral cell. The arrow points to a fimbriae-like structure anchoring bacterium to periurethral cell surface. (Reproduced by courtesy of Infection and Immunity.²⁷

Svenson *et al* have developed a P-specific particle agglutination test (PPA-test) by means of which P-fimbriated bacteria can be identified by a simple slide agglutination.²⁸ This opens new possibilities for studying the occurrence of P-fimbriated *E coli*.

Experimental infections

Experimental *E coli* pyelonephritis is difficult to induce in animals with a normal urinary tract,²⁹ possibly because of the absence of relevant receptor structures for the inoculated bacteria. An exception is the monkey, in several species of which glycosphingolipids corresponding to the antigens of the human-P-blood group system are present in kidney tissue and on blood cells. Consequently, P-fimbriated *E coli* adhere well to uroepithelial and red blood cells and, when inoculated into the

urinary tract, cause persistent bacteriuria and acute and chronic pyelonephritis. Non-adhering, non-P-fimbriated *E coli* are less infectious.^{30 31} In vivo experiments in the monkey have shown that infection can be prevented by the simultaneous administration of P-fimbriated *E coli* and a synthetic soluble receptor analogue. This is evidence for in vivo relevance of the P-fimbriae.²⁴ Svanborg-Edén *et al*,³² recently reported that globotetraose (a P-blood group specific oligosaccharide) interfered with bacterial adhesion and blocked experimental infection in the mouse.

Vaccination with highly purified P-fimbriae²⁵ can also modify experimental infection and protect against severe renal damage. These findings suggest that antifimbrial antibodies may be protective (unpublished data). In vitro they prevent adherence.^{25 33}

Future development and speculations

It is still unclear why some patients, mainly females, have a large number of infections, while others are never infected. Direct measurement of P-fimbriae receptor density or availability, or both, on epithelial cells suggest that women prone to infection have more of these receptors than others.³⁴

The finding of faecal dominance of P-fimbriated *E coli* in infected patients may have implications for treatment. For example, persistence of P-fimbriated *E coli* in the faecal flora may increase the risk for recurrence.

The crucial question whether colonisation of the gut with P-fimbriated bacteria predisposes to UTI has been examined. By means of the PPA test nosocomial spread of P-fimbriated *E coli* among staff and patients has been shown in a hospital ward. A retrospective study suggested that infants cared for earlier in this ward had an increased incidence of pyelonephritis.

The significance of small bacterial counts in the urine in infants with pyuria and clinical symptoms compatible with pyelonephritis is obscure. P-fimbriation—shown by means of the PPA test—of the few isolated *E coli* may in these instances support the idea that pyelonephritis can be associated with low bacterial numbers.³⁵

The protective effect of vaccination of monkeys with a highly purified P-fimbriae preparation suggests that immunity plays a role in the defence against infection. The very first infection in a small infant will hit a host unprotected by anti-fimbrial immunity and may therefore be especially dangerous. This may provide another rationale for early diagnosis and treatment of infants with pyelonephritis.

Adhesins and receptors other than the P-antigen related ones will probably soon become characterised. Their role in the pathogenesis of UTI, as well as the role of other factors such as the mucous gel covering epithelial cells awaits further elucidation.

In summary, acute and recurrent UTI are complex biological events. Recent advances in the understanding of the host/parasite interactions on a molecular level will help us to understand the delicate complexity of the pathogenesis of pyelonephritis and should lead to better management and prevention of renal damage.

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Correspondence to Professor J Winberg, Department of Paediatrics, Karolinska Hospital, S-104 01, Stockholm, Sweden.