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P-fimbriae Studies on the Diagnosis and Prevention of Acute Pyelonephritis*

Summary: Theoretically there are several ways to prevent pyelonephritis and renal scarring caused by P-fimbriated *Escherichia coli*. Screening for individuals at risk, e.g. those carrying P-fimbriated pyelonephritogenic *E. coli* or those with high receptor density on their uroepithelial cells, could perhaps define a population where prophylaxis with a receptor analogue or vaccination with P-fimbriae may be relevant. Epidemiological measures in neonatal and maternity wards may prevent the nosocomial spread of virulent bacteria and reduce the number of colonized infants. However, no such methods have so far had any proven clinical relevance, and today, the important concern is still to try by conventional means – as we have always done – to get an early diagnosis and to treat the patient without delay.

Zusammenfassung: *Studien zur Bedeutung der P-Fimbrien für die Diagnose und Prävention der akuten Pyelonephritis.* Theoretisch gibt es verschiedene Möglichkeiten, die durch P-Fimbrien-tragende *Escherichia coli* verursachte Pyelonephritis und renale Narbenbildung zu verhüten. Ein Screening auf Risikopersonen, beispielsweise diejenigen, die mit P-Fimbrien-tragenden pyelonephritogenen *E. coli* besiedelt sind oder deren uroepitheliale Zellen eine hohe Rezeptordichte haben, könnte vielleicht eine Gruppe definieren, bei der eine Impfung mit einem Rezeptor-Analogon oder mit P-Fimbrien wichtig wäre. Epidemiologische Maßnahmen auf Neugeborenen- und Entbindungsstationen könnten die nosokomiale Verbreitung von virulenten Bakterien verhüten und die Anzahl besiedelter Säuglinge vermindern. Jedoch ist bisher für keine der genannten Methoden der Beweis der klinischen Effizienz erbracht, und derzeit ist es immer noch entscheidend, konventionelle Methoden einzusetzen, um, wie wir es immer getan haben, frühzeitig die Diagnose zu stellen und den Patienten ohne Verzug zu behandeln.

Introduction

The ultimate goal when treating patients with urinary tract infections (UTI) is to prevent renal damage. To do so we must try to identify the risk factors in this disease. There are individual parameters which have been identified as important for the development of renal scarring: the age and sex of the patient, the presence of gross anomalies of the urinary tract, the number of infections, the time until diagnosis and the efficiency of antimicrobial therapy. Small children suffering their first febrile infection who experience a delay in diagnosis and onset of therapy are at greatest risk (1).

It is also essential to try and identify bacterial factors important for the development of pyelonephritis and later renal scarring. *Escherichia coli* is the most common organism. Many factors, such as O and K-antigens, hemolysin production and resistance to antibacterial activity of serum have previously been connected with bacterial virulence. The identification of P-fimbriae as one important virulence factor in *E. coli* has given us new insight in the pathogenesis of the disease.

P-fimbriae are a special type of fimbriae which enable *E. coli* to bind specifically to uroepithelial cells of the human urinary tract (2, 3). This helps the bacteria to resist the flow of urine. P-fimbriae bind specifically to cells, such as erythrocytes or uroepithelial cells, from most humans, except those of the rare blood group phenotype, $\overline{p}$. The P blood group antigens correspond to specific glycosphingolipids. These glycosphingolipids are present on human erythocytes and also on uroepithelial cells and they all contain the carbohydrate structure α-D-Galp (1–4)-β-D-Galp, which is the minimal receptor structure that these bacteria bind to (4). We have characterized the exact surface of the molecule to which the P-fimbriae specifically bind. (5)

The PPA Test

Based on our knowledge of the receptor structure for P-fimbriae we have constructed a test, the PPA test, for the detection of P-fimbriated bacteria (6). The test consists of particles with receptors attached to the surface. Bacteria possessing P-fimbriae bind specifically to these receptors, thereby causing a rapid, visible particle agglutination. We have used the PPA test to study various clinical bacterial isolates. In children with acute, non-obstructive *E. coli* pyelonephritis, the majority of strains causing infection were P-fimbriated (2) (Table 1). In adults with acute pyelonephritis (7) and in urosepticaemia (8), there was also a high frequency of P-fimbriated *E. coli* strains. The faeces of healthy controls had a low incidence of *E. coli* strains expressing P-fimbriae (2, 7) (Table 1).

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* The present version – although unfortunately much delayed – incorporates latest findings on the subject as first oulined at Sils Maria.

Table 1: Isolation of P-fimbriated *Escherichia coli* from the urine of patients with UTI and from the faeces of healthy controls.*

Source	n	P-fimbriated *Escherichia coli* n	%
Urine from children with pyelonephritis	35	34	97%
Urine from adults with pyelonephritis	30	27	90%
Faeces from healthy controls	82	6	7%

* Modified from *Källenius* et al. (2) and *Jacobson* et al. (7).

The important question now is how to use our knowledge of P-fimbriae and their role in the pathogenesis of pyelonephritis in the prevention of pyelonephritis and eventually of renal scarring. Hypothetically, there are several potential ways of doing this. Firstly, one could screen for individuals at risk. Such individuals might be those who are colonized with P-fimbriated pyelonephritogenic *E. coli* in the faeces, or individuals with a high P-fimbriae receptor density on uroepithelial cells. Secondly, if we found such a risk population, we could speculate about preventing infection either by receptor blockage with a receptor analogue or by vaccination with P-fimbriae or perhaps by colonizing the individuals with an avirulent *E. coli* strain.

Carriers of P-fimbriated Escherichia coli

Pyelonephritis is usually an ascending infection originating in the faecal flora. Children with pyelonephritis caused by P-fimbriated *E. coli* also have these strains as the dominating strains in their stool (2). In adults, too, the urinary strain is often found as the dominating strain of the patients' faecal flora (7).

Patients with renal scarring due to pyelonephritis are at an increased risk of recurrent UTI and acute pyelonephritis. We studied faecal and urine colonization in these patients with the aim of seeing whether it was possible to predict the development of acute pyelonephritis by examining the patients' faecal flora for P-fimbriated *E. coli*. One of five episodes of pyelonephritis was caused by the same P-fimbriated *E. coli* strain as was found in the patients' faecal flora six months earlier (unpublished data). Since small children are at the highest risk of attracting renal scarring, we also chose to investigate the faecal flora of a group of about 700 neonates for a time period of more than two years, studying their faecal colonization and the incidence of acute pyelonephritis. We studied an area in Stockholm where all children were referred to one particular hospital, Danderyd Hospital. About 15% of the children born in this hospital are referred to the neonatal ward of the hospital.

We found that in recent years, many more than the expected number of children with pyelonephritis had previously been cared for in this ward (9). In 1981/1982, more than half of the children with diagnosed pyelonephritis had previously (up to 17 months earlier) been cared for in this ward (Table 2).

Table 2: Children from the referral area of Danderyd Hospital who developed pyelonephritis before the age of two years.*

Year of birth	Acute pyelonephritis n	Care in the neonatal ward n	%
1979	22	4	18%**
1980	18	6	33%
1981	27	15	55% ($p<0.001$)
1982	10	7	70% ($p<0.001$)

* Modified from *Tullus* et al. (9);
** Expected incidence was 15%.

We were able to collect ten urinary strains from children with acute pyelonephritis who had attended the neonatal ward. All ten strains were of a particular serotype – O6K5 – and were P-fimbriated. This supported our theory that these infections were caused by a nosocomial spread of a particular pyelonephritogenic *E. coli* strain.

We also found a high frequency of this strain in the faeces of staff and among some of the children leaving the ward. This fact also supported the theory that the outbreak originated in the neonatal ward and was caused by a particular pyelonephritogenic *E. coli* strain. Further investigations have also shown outbreaks of *E. coli* infections in 1975 and 1976 probably originating from nosocomially spread bacteria.

We also found that nine of 13 cases of *E. coli* septicemia during the study period seemed to be correlated to the outbreaks of pyelonephritis and were thus possibly caused by the nosocomial spread of virulent *E. coli* strains in the ward (unpublished data).

This study is interesting in several respects. One important question is: what makes this particular *E. coli* strain so virulent or "pyelonephritogenic"? Secondly, although many children in the ward were probably colonized with this particular strain for certain periods of time, most children did *not* acquire UTI. What made some of the children particularly vulnerable to this particular strain?

Pyelonephritogenic Escherichia coli Clones

The expression of P-fimbriae seems to be one important virulence factor for uropathogenic *E. coli* strains. We have also known for a long time that strains causing pyelonephritis are often of specific O and K serotypes in particular combinations. Other factors, such as hemolysin production, are also frequent among pyelonephritis *E. coli* strains. *E. coli* serotypes O2:K1:H4, O4:K12:H5 and

Infection 13 (1985) Nr. 3

O6:K2ac:H1 were shown to be associated with acute pyelonephritis in non-pregnant women in a disproportionately high frequency (10).
In a Finnish study, about 100 strains causing acute pyelonephritis in children were investigated (11). 43% of these strains could be grouped into seven groups or clones. These clones were defined by a number of parameters: P-fimbration, type 1 fimbration, O and K serotype, α hemolysis, outer membrane protein (OMP) pattern, lipopolysaccharide (LPS) pattern, plasmid content and biochemical markers. All strains belonging to these groups were P-fimbriated. The hypothesis is that there is a limited number of pyelonephritogenic *E. coli* clones causing most cases of pyelonephritis in individuals without other underlying diseases.
The P-fimbriated O6:K5 *E. coli* strain which caused the above-mentioned outbreak appears to belong to yet another such virulent *E. coli* clone (unpublished data).

P-fimbriae Receptor Density

Not all individuals acquire pyelonephritis when their faeces is colonized with virulent bacteria. One reason for this could be a difference between the ability of uroepithelial cells from different individuals to bind bacteria. We have already found that there is a difference in the binding capacity of cells from infection-prone and non-infection-prone individuals. This was investigated using an old technique (12) whereby we mixed bacteria with cells, washed away the bacteria, filtered them down and counted the number of bacteria per cells with the naked eye. This was a time-consuming method and only a few cells could be counted.
We have now developed a new technique which enables us to count a large number of cells in a very short time (13). We stain the bacteria with fluorescein and then use the FACS technique, which can sort cells according to fluorescence or cell size. This method has proven to be versatile for the rapid and specific analysis of P-fimbriae receptor densities with a large number of cells.

P Blood Group Phenotype in Relation to Renal Scarring

Renal scarring is often caused by acute pyelonephritis in children. It has been proposed that individuals of the P_1 blood group phenotype have an increased P receptor density on uroepithelial cells and that this explains the finding that children with recurrent UTI and pyelonephritis have a significantly higher proportion of the P_1 blood group phenotype (14). We determined the P blood group phenotype in 56 adult female patients with verified renal scarring and a history of febrile UTI. However, we found no increase of the P_1 blood group phenotype among our patients (15). The P receptor density on uroepithelial cells from patients with renal scarring is now being studied with the FACS technique, and preliminary data indicate an increased density compared to healthy controls.

Animal Models

If we are to study the pathogenesis of the disease and the possible preventive measures, there is a need for a relevant animal model. This animal must possess relevant cell receptors, should have a similar immunological repertoire and should be anatomically similar to man. In cooperation with *Roberts* et al. from Tulane University, U.S.A., we studied the existence of P-fimbriae receptors in different species using the FACS technique and by analysing the glycolipids from various organs. We found that only man, monkeys, some pigs but no other animal species have P-fimbriae-specific receptors (16). We have also used monkeys in studies on pyelonephritis.
The administration of P-fimbriated *E. coli* to the bladder in monkeys will cause pyelonephritis. A non-P-fimbriated strain will not cause this disease. A receptor analogue added to the model in addition to the bacteria inhibited the infection. *Roberts'* group was also able to prevent infection by vaccination with purified P-fimbriae (17).

Early Diagnosis

As previously stated, the ultimate goal of treating patients with UTI is to prevent renal damage. The rapid identification and treatment of acute pyelonephritis have been shown to reduce the risk of progressive renal damage (18). It is therefore important to detect pyelonephritis as early as possible. *Winberg* has used the term "delay nephropathy", which means that the delay in initiating therapy is a cause of renal damage. A delay in treatment of five days or more leads to a higher risk of renal scarring (18) than that following early treatment.
The primary diagnostic criterion of a UTI is the identification of bacteria in a bladder aspirate or significant numbers of organismus ($> 10^5$ bacteria/ml) in preferably repeated specimens of voided bladder urine. However, during a period of a few months, six children at the Sacchs Children's Hospital presented with symptoms and signs of acute pyelonephritis but not "significant" numbers of bacteria in their urine (19). Small numbers ($\leq 10^4$) of P-fimbriated *E. coli* were found in all urine samples. Three children were initially treated inadequately or they were not treated at all and later developed "significant" bacteriuria with P-fimbriated *E. coli.* Thus, the finding of P-fimbriated *E. coli* in small numbers in the urine of children with other clinical signs of pyelonephritis can be of diagnostic value.
Another preventive measure during the course of infection would be to interfere with the inflammatory reaction, which probably contributes to renal damage. Hypothetically, there are several ways of doing this in the early stages of the disease. In animal models, the depletion of complement in an attempt to decrease chemotaxis and opsonization and thus decrease phagocytosis was shown to decrease acute renal damage (20, 21).

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Book Review

H. Friemel (Hrsg.):
Immunologische Arbeitsmethoden
3. Auflage. 522 Seiten, 146 Abbildungen, 45 Tabellen
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Aus den Bereichen der spezifischen und unspezifischen Immunsysteme werden die geläufigsten und auch selteneren immunologischen Testmethoden abgehandelt. Der Schwerpunkt liegt eindeutig – und völlig zu Recht – bei den wissenschaftlichen Labormethoden und nicht bei den alteingeführten Routineuntersuchungen.

In größeren Übersichtskapiteln (Antikörper; Antigen-Antikörper; Immunzelle; Immunchemie) werden die Labortechniken kochbuchartig, d. h. nachmachbar einfach beschrieben.

Dabei folgt die Methodenbeschreibung einer sehr sinnvollen Systematik: Beschreibung des Testprinzips, der Testdurchführung mit erforderlichen Geräten und Materialien, Diskussion der Methode und Literaturhinweise.

Von Auflage zu Auflage wurde Ballast abgeworfen und neu entwickelte Techniken aufgenommen. Insgesamt bringt die Auswahl der Arbeitsmethoden ein repräsentatives aktuelles Spektrum. Hinweise auf Kapitel, die in der nächsten Auflage hinzukommen sollten, werden den Autoren nicht fehlen (Ko-Kulturen, Immunglobulinsynthese *in vitro,* Chemilumineszenz von Granulozyten u. a.).

Der Aufbau des Buches würde sich für eine Herstellung im Loseblattsystem vorzüglich eignen. Laboranden und Wissenschaftler würden sich leichter zum Kauf entscheiden, als innerhalb acht Jahren die dritte Auflage anschaffen zu müssen.

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Infection 13 (1985) Nr. 3