

# Molecular and serological characterization of p fimbriae from uropathogenic escherichia coli

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## GENERAL AND SUMMARIZING DISCUSSION

The ability of pathogenic organisms to colonize a mucous membrane is a prerequisite for most infections. To colonize a mucous membrane bacteria belonging to the normal flora as well as pathogens have to adhere to epithelial cells to persist in their habitat, and not to be removed by clearing mechanisms like peristalsis, mucous secretions or other fluids, e.g. urine. Most bacteria have evolved adhesins to overcome the clearing mechanisms. With the adhesins the bacteria can recognize specific receptors, usually carbohydrates on the epithelial cells. The surfaces of the epithelial cells are covered with a great variety of carbohydrates, where they can act as receptors. The molecular basis of the receptor structures are under investigation, and some of these specific receptor structures are elucidated (1-4).

For many bacteria the adhesins have been characterized as proteinaceous appendages called fimbriae. Among uropathogenic Escherichia coli strains especially the P fimbriae have been associated with pyelonephritis. This thesis describes the molecular and especially the serological characterization of the various P fimbriae of uropathogenic E. coli. The main goal of this study was to develop a set of specific antibodies against the serologically different P fimbriae. With these specific antibodies the presence of P fimbriae on uropathogenic E. coli strains and the role of P fimbriae in the pathogenesis of pyelonephritis could be studied.

At the onset of this study we planned to reach the ultimate goal by purifying the P fimbriae from uropathogenic E. coli strains and to raise polyclonal antisera against these purified fimbriae. Subsequently, these antisera could be made fimbriae specific by absorption techniques. However, each wild-type E. coli strain often expresses various serologically different P fimbriae together with type 1 fimbriae, which makes it extremely difficult to purify one specific type of P fimbriae. Furthermore, purification of fimbriae

from wild-type strains in general yielded low amounts of fimbriae. For these reasons we decided to clone the genes encoding for the various types of P fimbriae. Subsequently, a gene cluster encoding for specific P fimbriae was transformed to an E. coli K12 strain which does not produce any kind of fimbriae (Chapter 2 and 3). This molecular cloning of the genes encoding for a certain type of P fimbriae made it possible to purify all serologically different P fimbriae separately. Furthermore, the cloning of the genes clusters, encoding for the P fimbriae, on high copy plasmids increased the production of the fimbriae, which made it possible to purify large amounts of these fimbriae.

Polyclonal rabbit antisera raised against these purified cloned P fimbriae still showed many cross reactions against heterologous P fimbriae (Chapter 4). For this reason we started to produce monoclonal antibodies (MABs) against the various purified P fimbriae (Chapter 4-6). Most of these MABs recognized only an epitope on the homologous P fimbriae. From these specific MABs we selected a set of MABs for serotyping the P fimbriae of clinical E. coli isolates (Chapter 7). With these MABs we were able to identify approximately 70% of the P fimbriated urinary E. coli isolates (Chapter 8). The most common serotypes of the P fimbriated urinary tract infections (UTI) strains were the F11, F7<sub>1</sub>, and F8 fimbriae. However, it is still difficult to draw definite conclusions about which of the P fimbriae are the most important in the pathogenesis of UTI, due to the relatively small collections that were screened so far.

The sera of four patients with pyelonephritis were analyzed for the presence of specific antibodies against the P fimbriae (Chapter 9). In all cases antifimbriae antibodies were found, which strongly suggests that these P fimbriae are expressed in vivo and are not merely a laboratory phenomenon. However, the antibodies did not inhibit adherence, indicating that these antibodies were directed against the fimbriae proteins rather than to the "minor components" responsible for adherence.

Recently, several gene clusters encoding for P fimbriae were analyzed (5-9). Surprisingly, mutations in the fimbriae subunit gene did not abolish the adhesion to uroepithelial cells, although such mutants are unable to produce fimbriae. In contrast, mutants with lesions in two downstream genes were able to synthesize fimbriae but completely lost the ability to adhere to uroepithelial cells (10). This strongly suggests that these two genes encode for minor components involved in adhesion, and that the fimbriae itself are

not the actual adhesins.

In Chapter 6 some MAbs are described which recognize an epitope on the minor components which are involved in adhesion. These MAbs were able to inhibit adhesion, whereas the fimbriae specific MAbs did not inhibit adhesion. Most anti-fimbriae MAbs described in this study reacted only with homologous fimbriae whereas the MAbs which recognize the minor components involved in adhesion showed a cross reaction with three different fimbriae. This suggests that the minor components may have a more conservative structure compared with the P fimbriae which are more susceptible to antigenic variation.

All the assays used in this study for the detection of P fimbriae on uropathogenic E. coli were phenotypic assays. The disadvantage of phenotypic assays is that they do not demonstrate whether or not the genetic information for the expression of fimbriae is present in the bacteria. For E. coli strains expressing type 1 fimbriae it has been described that these bacteria can switch from a fimbriated phenotype to a non-fimbriated phenotype, a process called phase variation (11). It has also been described that a strain with a specific P fimbriae serotype was able to switch to a type 1C fimbriae serotype (12), and O'Hanley et al. (13) showed that expression of P fimbriae required four or more passages in vitro in 40% of the strains tested. Thus, phase variations in the fimbriae phenotype may result in an underestimation of the percentage of P fimbriae expressing strains among strain collections when only phenotypic assays are used. This can be circumvented by using phenotypic assays in combination with a genotypic assay. With a DNA probe encoding for the P fimbriae gene cluster it is possible to demonstrate whether the strains expressing a non-fimbriate phenotype, do have the genetic information for expression of P fimbriae on the chromosome or not.

Preliminary experiments have shown that with a DNA probe it is indeed possible to demonstrate that the information for P fimbriae is present in a wild-type urinary isolate of E. coli (unpublished results). As a DNA probe we used a part of the F7<sub>1</sub> gene cluster which encoded for a conserved region. With this probe we were able to demonstrate in a Southern blot (14) that the reference strain for F12 fimbriae, C1979, possesses the information for P fimbriae on the chromosome. Other studies have also shown that it is possible to construct probes from the conserved regions which hybridize with all the different P fimbriae genes, for use in identifying potentially P fimbriated strains (13, 15).

As described above the developed MAb's against the P fimbriae can have, in combination with a genotypic assay, great value for diagnostic and epidemiological studies. With the set of P fimbriae specific MAb's the O:K:H serotyping for E. coli can easily be extended with the F serotype.

Recently, much interest has been focussed on P fimbriae as a candidate for a vaccine against pyelonephritis. In vitro studies have demonstrated that antibodies which bind to the fimbriae (16, Chapter 4) or to the minor components involved in adhesion (Chapter 6) inhibit the association between the adhesins and the receptors. In vivo, anti-fimbriae antibodies have been shown to inhibit or delay experimental infection in monkeys and mice (16-18). Based on these in vivo and in vitro studies it was suggested that the P fimbriae might be used for vaccination against pyelonephritis. However, several problems have to be considered with regard to development of a vaccine, e.g. antigenic variation of the P fimbriae; the difference between fimbriae and actual adhesins; the target population; and the route of vaccination.

As shown in Chapter 8, we were able to identify approximately 75% of the P fimbriae on urinary isolates with 9 different MAb's. Thus, at least 10 serologically different P fimbriae may be involved in the pathogenesis of pyelonephritis. Although polyclonal antisera showed many cross reactions with different F types of P fimbriae (Chapters 4 and 9), it was shown in Chapter 9 that in vivo developed anti-P fimbriae antibodies will not always be anti-adhesive for the infecting E. coli strains. Therefore, it seems logical that a possible vaccine has to be based on the minor components which are responsible for adherence, rather than on the fimbriae itself. An advantage might also be that the minor components have less antigenic variation than the fimbriae, since the anti-adhesive MAb's described in Chapter 6 showed cross reaction with minor components of three different P fimbriae.

With regard to the target population for vaccination one has to be aware of the finding that P fimbriae are mainly of importance in the pathogenesis of pyelonephritis in the non-compromised host. Although most studies agree on the association between P fimbriae and acute pyelonephritis in young children (19, 20), less agreement exists on the association with lower UTI (19, 20) or with UTI in elderly patients (21, Chapter 8). Furthermore, Lomberg et al. (21) have shown that the incidence of P-fimbriated E. coli strains in recurrent pyelonephritis patients with vesicoureteric reflux is only 25% compared with 50% in such patients without reflux. Therefore, vaccination may only be effective in patients without urological disorders.

With regard to the route of vaccination, recently a new approach was proposed by Uehling et al. (22). In contrast to other studies using parenteral vaccination, Uehling et al. administered a vaccine into the vagina of monkeys. They suggested that this local vaccine application will result into a local mucosal sIgA response which might prevent colonisation by pathogenic P-fimbriated E. coli of the periurethral zone and the bladder.

In conclusion, not only the pathogenesis of E. coli pyelonephritis is multi-factoral (23), but also the role of adherence in the pathogenesis is very complicated. Obviously, more research is needed to reach the ultimate goal, the proper management and prevention of UTI.

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## SAMENVATTING

De bacterie Escherichia coli is een van de belangrijkste verwekkers van urineweginfecties. De uropathogene E. coli bacteriën hebben een aantal virulentie eigenschappen ontwikkeld, waardoor ze in staat zijn om vanuit de faeces via de urethra de urinewegen te infecteren. Een van deze virulentie factoren is het aanhechtend vermogen van de E. coli bacteriën aan het urine- wegepitheel. De aanhechting vindt plaats met behulp van P fimbriae, waardoor de bacteriën zich specifiek aan receptoren op het urinewegepitheel kunnen hechten. Tot nu toe zijn er + 10 serologisch verschillende soorten P fimbriae beschreven.

Het doel van het onderzoek, beschreven in dit proefschrift, was een set van antisera te ontwikkelen gericht tegen de verschillende P fimbriae. Met behulp van deze set van antisera kunnen de P fimbriae aanwezig op de uropathogene E. coli geserotypeerd worden en kan de rol van P fimbriae in de pathogenese van pyelonephritis bestudeerd worden.

De meeste uropathogene E. coli bacteriën zijn in staat om verschillende serotypen van deze P fimbriae tot expressie te brengen, waardoor de zuivering van één type P fimbriae bemoeilijkt wordt. Bovendien is het aantal tot expressie gebrachte P fimbriae op de uropathogene E. coli gering, waardoor de opbrengst aan zuivere P fimbriae na isolatie laag is.

Om deze redenen werd besloten om de genen coderend voor één type P fimbriae te cloneren en te transformeren naar een E. coli K12 stam die geen fimbriae kan produceren (Hoofdstuk 2 en 3). Hierdoor werd het mogelijk om alle serologisch verschillende P fimbriae afzonderlijk te zuiveren. De genen clusters coderend voor de P fimbriae werden gecloneerd in high-copy plasmiden, waardoor relatief grote hoeveelheden P fimbriae konden worden gezuiverd.

Polyclonale antisera opgewekt tegen de gezuiverde P fimbriae reageerden

met de homologe, maar ook met de meeste heterologe P fimbriae (Hoofdstuk 4). Daarom werd gestart met de produktie van monoclonale antilichamen (MAbs) gericht tegen de verschillende P fimbriae (Hoofdstuk 4-6). De meeste van deze MAbs reageerden alleen met de homologe P fimbriae. Uit deze fimbriae specifieke MAbs werd na selectie een set van MAbs samengesteld voor serotypering van P fimbriae van uropathogene E. coli isolaten. Voor deze serotypering werd een eenvoudige methode ontwikkeld waarin hele bacteriën in een ELISA werden gebruikt (Hoofdstuk 7). Vervolgens werden de P fimbriae geserotypeerd van enkele oude en nieuwe collecties van uropathogene E. coli stammen (Hoofdstuk 8). Met behulp van de set van 9 verschillende specifieke MAbs bleek het mogelijk om meer dan 70% van de P fimbriae van uropathogene E. coli stammen te serotyperen. De meest voorkomende serotypen in deze collecties waren de F11, F7<sub>1</sub> en F8 fimbriae. In veel gevallen bleek een bepaald F type geassocieerd te zijn met bepaalde O:K serotypen.

De sera van vier patiënten met pyelonefritis werden geanalyseerd op de aanwezigheid van specifieke antilichamen gericht tegen P fimbriae (Hoofdstuk 9). In alle sera werden deze anti-P fimbriae antilichamen gevonden. Dit suggereert dat de P fimbriae zowel in vitro als in vivo gevormd worden. De antilichamen in deze sera waren niet in staat om de aanhechting van de uropathogene E. coli aan urinewegepitheelcellen te remmen. Deze antilichamen zijn dus waarschijnlijk gericht tegen de P fimbriae en niet tegen de componenten in de P fimbriae die verantwoordelijk zijn voor de aanhechting. Alle P fimbriae specifieke MAbs bleken niet in staat de aanhechting van homologe clones te remmen.

In Hoofdstuk 6 werden echter ook een aantal MAbs beschreven die de aanhechting wél bleken te remmen en dus een epitoolp herkennen op de eiwitten die betrokken zijn bij de aanhechting aan het urinewegepitheel. In tegenstelling tot de P fimbriae specifieke MAbs reageerden deze MAbs niet alleen met de homologe P fimbriae maar ook met een aantal heterologe P fimbriae. Dit suggereert dat de structuur van de componenten, die verantwoordelijk zijn voor de aanhechting, meer geconserveerd is dan de structuur van de P fimbriae.