P Fimbria–Specific B Cell Responses in Patients with Urinary Tract Infection

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Local immune response may be important in defense against urinary tract infection (UTI). P fimbria, an important virulence factor of *Escherichia coli*, is a noteworthy candidate for use in a vaccine against pyelonephritis (PN). Eleven patients with PN and 14 patients with lower urinary tract infection (LUTI) caused by *E. coli* were studied for mucosa-derived antibody-secreting cells (ASCs) and for urinary antibodies. In the 10 patients with P-fimbriated (P+) *E. coli*, an ASC response to P fimbria was found in 5 of 5 patients who had PN and 1 of 5 patients who had LUTI. The response to P fimbria was stronger among patients with P+ PN than among patients with PN caused by non–P-fimbriated *E. coli* (P <.001) or patients with P+ LUTI (P <.001). The response to P fimbria was also stronger than the response to outer-membrane protein A among all patients with PN. P fimbria–specific urinary immunoglobulin A antibody levels were higher among patients with P+ PN than those with P− PN. The results show a P fimbria–specific local immune response, which further encourages the use of P fimbria in locally administrable UTI vaccines.

The role of immunity in urinary tract infection (UTI) is somewhat unclear. Previous studies have shown an antibody response in the serum and in the urine of patients with UTI [1–5]. Antibodies against bacterial surface polysaccharide [1], outer-membrane proteins (Omps) of gram-negative bacteria [3], and fimbriae [4, 5] have been found. A local immune response in the urinary tract is indicated by the presence of locally produced urinary IgA [6, 7] and circulating pathogen-specific antibody-secreting cells (ASCs) [8]; the latter are thought to originate from the mucosa of the urinary tract and to be on their way back to those sites to provide local immune defense. It has become evident that the immune response depends on the clinical form of UTI: a significantly more vigorous immune response is seen in pyelonephritis (PN) than in lower urinary tract infection (LUTI) [8].

The presence of P fimbria, a cell-surface adhesin of *Escherichia coli*, is linked to PN [9–16]. It has been proposed that P fimbria contributes to the virulence of uropathogenic *E. coli* by several different mechanisms and at different stages of UTI. That P fimbria facilitates bacterial persistence in the intestinal flora has been suggested, because receptors for the fimbria are expressed in the large intestine [16]. P-fimbriated (P+) strains have been shown to establish bacteriuria more efficiently than strains without P fimbria [17]. It is presumed that P fimbria aids the adhesion of the bacteria to the endothelia of the ureters and thus facilitates the ascent of the bacteria to the kidney [18]. Use of a vaccine based on P fimbria and administered by the mucosal route has been a logical suggestion for prevention of this serious form of UTI. In animal experiments, P fimbria–based vaccines have proven to be protective [10–15].

To provide background data for a P fimbria–based approach to immunization in humans, we assessed the local immune response to P+ *E. coli*. We studied groups of patients with PN or LUTI caused by P+ or non–P-fimbriated (P−) *E. coli*. Responses were judged by the number of ASCs, presumed to derive from lymphocytes
stimulated on the local mucosa, and the level of antibodies in the urine. The antigens we used were, in addition to P fimbria, an Omp and an unrelated antigen, trinitrophenyl (TNP). TNP was used to test for polyclonal B cell activation. The responses were compared with responses to whole bacteria (the isolate from each individual episode of UTI) measured in a previous study [8].

METHODS

Study subjects. Patients were a subset of a group of 36 patients with UTI on whom we reported previously [8], selected because they had _E. coli_ isolated from a urine specimen (31/36 patients) and a sufficient number of peripheral blood mononuclear cells (PBMCs) available for assays of P fimbria–specific immune responses (25/31 patients). The present study included 11 patients with PN (6 women and 5 men; age range, 21–86 years) and 14 patients with LUTI (all women; age range, 20–75 years). For all of the patients with PN, it was reported that this was their first episode. Six of the 14 patients with LUTI had a history of ≥5 LUTIs, and 4 were experiencing their first or second LUTI. In the PN group, 5 patients had P⁺ _E. coli_ (all women), and 6 had P⁻ _E. coli_ (1 woman and 5 men); in the LUTI group (all women), 5 patients had P⁺ _E. coli_, and 9 had P⁻ _E. coli_, as determined by use of a commercial P fimbria latex test performed on _E. coli_ isolates (Orion Diagnostica). Of the 5 patients with P⁺ LUTI, 2 had no history of LUTI.

All of the 11 patients with PN received treatment in a hospital (the Central Hospital of Central Finland, Jyväskylä, or the University Central Hospital, Tampere, Finland). PN was diagnosed by the following criteria: typical clinical symptoms, axillary temperature of >38°C, C-reactive protein level of >40 mg/L, leukocyturia, and isolation of a pathogen from a urine specimen (at least 10⁵ cfu/mL). For 5 of 11 patients, the pathogen was also isolated from a blood sample. The 14 patients with LUTI attended the local Health Center of Kylö in Jyväskylä. The diagnosis of LUTI was based on urinary urgency, urinary frequency, and/or dysuria, together with leukocyturia and bacteriuria at a level >10⁵ cfu/mL, in the absence of the signs of PN listed above. Four healthy volunteers (all women; age range, 23–38 years) from among the laboratory personnel served as control subjects.

Informed consent was obtained from all patients. The human experimentation guidelines of the US Department of Health and Human Services and those of the authors’ institutions were followed in conducting the clinical research.

Collection of specimens. Samples of blood (from patients with PN and patients with LUTI) and of urine (only from patients with PN) were obtained during the acute phase and the convalescent phase of the disease. It was difficult to obtain acute-phase samples from different patients at a comparable stage of the disease, because onset of symptoms varies and patients seek medical care after a varying length of time; this problem was solved by collection of 2 acute-phase samples. To catch the peak (or near-peak) of the ASC response, which, according to our studies of oral vaccines [19, 20], is expected 7 days after antigenic exposure, sample collection was performed as follows: The first acute-phase specimens were obtained on the day of (urine sample) and the day after (blood sample) the patient’s first visit to the health center (for patients with LUTI) or admission to the hospital (for patients with PN). In both groups, the second acute-phase samples were obtained 7–8 days after the onset of the symptoms, and the last (convalescent-phase) samples were obtained 3–7 weeks after the onset of the disease. Only 1 acute-phase sample was obtained from patients who did not seek medical care until 1 week after the onset of symptoms. The term “acute-phase sample” is used in this article to describe the acute-phase sample that yielded the highest number of ASCs.

Samples of blood were obtained from 4 healthy laboratory personnel for use as controls. All blood samples were assayed using ELISPOT immediately after the sample was drawn. Urine samples were frozen at −70°C and stored for 6–12 months before ELISAs were performed.

Antigens (P fimbria, OmpA, and TNP). The P fimbria isolate used in the ASC assay was obtained from Dr. Auli Pere (Department of General Microbiology, University of Helsinki, Helsinki). It had been isolated from strain IH 11086. This strain is of serotype O4:K12:H1, and it was originally isolated from a patient with PN [21]. The method of isolation of the P fimbria [22] and a detailed description of the IH 11086 strain [21] have been published elsewhere. The OmpA preparation was received as a gift from Dr. Eveliina Tarkka (National Public Health Institute, Helsinki). It had been isolated from _E. coli_ strain P2. The TNP preparation contained TNP coupled to bovine serum albumin (BSA) and was received as a gift from Dr. Olli Mäkelä (Department of Serobacteriology, University of Helsinki).

Isolation of mononuclear cells. PBMCs containing mainly lymphocytes were obtained by Ficoll-Paque (Pharmacia) centri-fugation of heparinized venous blood. The isolated cells were washed 3 times with Hanks’ buffered salt solution (Flow Laboratories) and suspended in culture medium to a concentration of 2 × 10⁶ cells/mL, as described elsewhere [23].

Assay of specific ASCs. Specific ASCs were enumerated using ELISPOT [8, 23, 24]. In this assay, isolated lymphocytes are allowed to secrete antibodies in microtiter plate wells previously coated with the antigen of interest. The secreted antibodies react with the antigen in the immediate vicinity of the secreting cell. These antibodies are visualized with enzyme-conjugated antisera, followed by substrate overlay in agarose; the latter immobilizes the decaying substrate and turns the area of antibody into a colored spot.
The plates were coated with the preparation of P fimbria at a concentration of 5 μg/mL, OmpA at 0.2 μg/mL, or TNP-BSA at 5 μg/mL of PBS (pH 7.4) and incubated for 2 h at 37°C or overnight at 20°C. The next steps have been described in detail elsewhere [8, 23, 24]. In brief, the nonspecific binding sites were blocked with 1% BSA (30 min at 37°C), and 10^5 cells in culture medium were incubated in each well (2–3 h at 37°C). The antibodies secreted during this time were detected with alkaline phosphatase–conjugated antisera (Orion Diagnostica; diluted 1:100 in 1% BSA-PBS), followed by the substrate in agarose at 50°C. A response was defined as the presence of ≥2 specific ASCs/10^6 PBMCs.

**Detection of antibodies in urine specimens.** Antibodies to P fimbria in urine specimens from patients with PN were quantitated using ELISA as follows: Coating and blocking of non-specific binding sites was done as described for the ELISPOT assay. Urine specimens (undiluted and diluted 1:2 in PBS) were incubated in the wells for 30 min at 37°C. The enzyme-conjugated antisera (as in ELISPOT) were incubated in the wells for 1 h at 37°C. Finally, the substrate (p-nitrophenyl phosphate [Sigma]; 1 mg/mL of a 1-mol/L solution of diethylamine buffer) was added and allowed to react for 1.5 h. The absorbances were measured with a Tittertek Multiskan spectrophotometer (Lab systems Oy) at 405 nm. Net absorbances were calculated by subtracting the value for a PBS-coated blank well from that of the sample well.

**Statistical analysis.** The significance of differences in the ASC responses between the 2 groups was analyzed using Student’s t test with log-transformed data. P < .05 was considered to be statistically significant.

**RESULTS**

Eleven patients with PN (5 with P + PN and 6 with P – PN) and 14 patients with LUTI (5 with P + LUTI and 9 with P – LUTI) caused by E. coli were investigated for circulating ASCs specific to P fimbria, OmpA, or TNP. No difference was found in the numbers of positive blood cultures between the P + PN (2/5 patients) and P – PN (3/6 patients) groups. Urinary antibodies to P fimbria were assessed only in patients with PN.

**ASCs specific to P fimbria.** No P fimbria–specific ASCs were found in the blood of the 4 healthy control subjects. Among the patients with PN, P fimbria–specific ASCs were found in all 5 of the patients who had P + E. coli (geometric mean ASC count, 294 ASCs/10^6 PBMCs; tables 1 and 2). In all cases, a response was found in all 3 isotypes, and IgA was the predominant isotype (figure 1). The weakest response was seen in isotype IgG in 2 cases and in IgM in 3. The kinetics of the response (figure 2) was similar to that observed in our earlier study, in which whole bacteria were used as antigen [8]: the high numbers of ASCs found in the acute-phase samples were followed by a decrease in the convalescent samples. The vigor of the response to P fimbria was significantly weaker than that of the response to whole bacteria (P < .01) and stronger than that of the response to OmpA (P < .01) in the same patients (figure 1 and table 2).

Of the 6 patients with P – PN, 1 had a clear-cut ASC response (85 ASCs/10^6 cells), and 2 others had weak responses (3 and 4 ASCs/10^6 cells) (tables 1 and 2). The only female patient with P – PN had no P fimbria–specific ASCs.

In the LUTI group, 1 of the 5 patients with P + E. coli had an IgA-dominated ASC response to P fimbria (62 ASCs/10^6 cells), which is close to the range of P fimbria–specific ASCs among the patients with P + PN (70–741 ASCs/10^6 cells). All 4 of the other patients with P + LUTI had no ASC response. The mean response (± SEM) in the P + LUTI group (2 ± 12 ASCs/10^6 cells) was significantly lower than that in the P + PN group (294 ± 116 ASCs/10^6 cells) (P < .001). None of the patients with P – LUTI had ASC responses to P fimbria.

The disease history of the patients with LUTI did not appear to influence ASC responses. Moreover, the difference between patients with P – PN and those with P – LUTI was not influenced by sex, because all of the patients in these groups were female.

**ASCs specific to OmpA, TNP, or whole bacteria.** No ASCs specific to OmpA, TNP, or whole bacteria were found in the blood of the 4 healthy control subjects. ASCs specific to the OmpA antigen were found in 10 patients with UTIs, more commonly in the PN group (9/11 patients) than in the LUTI group (1/14 patients) (table 1). The responses in both groups were, in general, weak (range, 4–47 ASCs/10^6 cells in the PN group and

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**Table 1. Numbers of patients with pyelonephritis (PN) or lower urinary tract infection (LUTI) who had specific antibody-secreting cell (ASC) responses to P fimbria, outer-membrane protein A (OmpA), trinitrophenyl, or whole bacteria.**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>P + PN (n = 5)</th>
<th>P – PN (n = 6)</th>
<th>P + LUTI (n = 5)</th>
<th>P – LUTI (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P fimbria</td>
<td>5</td>
<td>3^a</td>
<td>1^b</td>
<td>0</td>
</tr>
<tr>
<td>OmpA</td>
<td>5</td>
<td>4</td>
<td>0</td>
<td>1^c</td>
</tr>
<tr>
<td>Trinitrophenyl</td>
<td>3</td>
<td>4</td>
<td>1^d</td>
<td>0</td>
</tr>
<tr>
<td>Whole bacteria^e</td>
<td>5</td>
<td>6</td>
<td>3</td>
<td>7</td>
</tr>
</tbody>
</table>

NOTE. A response was defined as the presence of ≥2 pathogen-specific ASCs/10^6 PBMCs. PBMCs, peripheral blood mononuclear cells; P+: P-fimbriated *Escherichia coli*; P –, non–P-fimbriated *E. coli*.

a Two of the 3 patients in the P + PN group who had positive responses to P fimbria had only a weak response, of 3 and 4 specific ASCs/10^6 PBMCs, whereas, in the P + PN group, the responses ranged from 69 to 740 ASCs/10^6 PBMCs.

b The 1 patient with a positive response to P fimbria in the P + LUTI group had 62 ASCs/10^6 PBMCs.

c The 1 patient with a positive response to OmpA in the P + LUTI group had a weak response, of 3 ASCs/10^6 PBMCs.

d The 1 patient with a positive response to trinitrophenyl in the P + LUTI group had a weak response, of 3 ASCs/10^6 PBMCs.

^e Data are from [8].
Table 2. Geometric mean (GM) antibody-secreting cell (ASC) responses (IgA + IgG + IgM) to P fimbria, outer-membrane protein A (OmpA), trinitrophenyl, and whole bacteria in the acute phase of the disease in 11 patients with pyelonephritis and 14 patients with lower urinary tract infection.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Pyelonephritis</th>
<th>Lower urinary tract infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GM ASC count ± SEM, ASCs/10^6 PBMCs</td>
<td>No. of patients</td>
</tr>
<tr>
<td>P fimbria P^-</td>
<td>NR</td>
<td>6</td>
</tr>
<tr>
<td>P+</td>
<td>294 ± 116</td>
<td>5</td>
</tr>
<tr>
<td>OmpA</td>
<td>9 ± 5</td>
<td>11</td>
</tr>
<tr>
<td>Trinitrophenyl</td>
<td>13 ± 13</td>
<td>11</td>
</tr>
<tr>
<td>Whole bacteria b</td>
<td>1643 ± 3272</td>
<td>11</td>
</tr>
</tbody>
</table>

NOTE. To allow the calculation of the GM, an ASC count of 1 ASC/10^6 PBMCs was assigned to the numerous patients with ASC responses of 0 specific ASCs/10^6 PBMCs. We compensated for the influence of this by setting the limit for NR for GM at <4 ASCs/10^6 PBMCs. NR, no response; NS, not significant; PBMCs, peripheral blood mononuclear cells; P+, P-fimbriated Escherichia coli; P^- non-P-fimbriated E. coli.

* By Student's t test.

b Data are from [8].

only 3 ASCs/10^6 cells for the patient with a specific ASC response in the LUTI group; P < .001; figure 1 and table 2). In most cases, the responses were predominantly IgA responses.

ASCs specific to the unrelated antigen, TNP, were found in 8 patients with UTI, more frequently in those with PN (7/11 patients) than in those with LUTI (1/14 patients) (table 1). The responses were, in general, weak (in the PN group, the range of responses to TNP was 0–137 ASCs/10^6 cells, vs. responses to whole bacteria of 10–34,565 ASCs/10^6 cells among the same patients), and IgA was predominant in all cases. All patients who had an ASC response to TNP also had a response to OmpA. All 11 patients with PN and 10 of the 14 patients with LUTI had ASC responses to whole bacteria (figure 1 and tables 1 and 2). In most cases in both patient groups, the response was dominated by IgA ASCs. Among the patients with PN, no differences were found between patients with P+ E. coli and those with P^- pathogens.

Urinary antibodies to P fimbria. Urinary antibodies to P fimbria were measured only in samples from patients with PN. The net IgA absorbances were significantly higher among patients with P+ PN than among those with P^- PN (P < .01; figure 3).

DISCUSSION

One of the most promising approaches for immunization against UTI is local immunization with P fimbria, an important virulence factor of the most common uropathogen, E. coli. The present study provides background data for a P fimbria–based immunization approach in humans. In particular, the present study was aimed at characterization of mucosal immune response to P fimbria in patients with UTIs, because local immune response appears to be beneficial in the immune defense against UTI [7, 25, 26].

Serologic variability exists among P fimbriae [21, 27], an aspect that needs to be considered both in P fimbria–based immunization studies and in serologic studies. Moreover, a bacterial strain may have >1 variant of P fimbriae with differing chemical and serologic properties [21]. On the other hand, immunologic cross-reactivity between P fimbria variants in serologic assays [21, 28], as well as cross-protective capacity between 2 different P fimbria–based vaccines [14], has been demonstrated in previous studies. To get broad coverage for our assay, we chose P fimbria isolated from strain IH 11086, because this has been shown to be precipitated by numerous different antisera raised to fimbriae of different strains [21]. It appears...
Figure 2. Kinetics of the antibody-secreting cell (ASC) response (IgA + IgG + IgM) to P fimbria in blood samples from 5 patients with pyelonephritis caused by P-fimbriated Escherichia coli.

Figure 3. Levels of IgA, IgG, and IgM antibodies specific to P fimbria in the urine of patients with pyelonephritis caused by P-fimbriated (P+; 5 patients) or non–P-fimbriated (P−; 6 patients) Escherichia coli. Data are net absorbance, as measured by ELISA (the value for a PBS-coated blank well subtracted from the value for the sample well). Geometric means are indicated by dark horizontal lines. **, by Student’s t test. P < .01.

that this choice was successful, because all of the patients with P+ PN in the present study had responses in our assay.

Measurement of local immune response in humans can be achieved by measuring antibodies in secretions [29] or by examining circulating ASCs [20, 30, 31]. The latter approach is based on studies in animals showing that lymphocytes, after contact with antigen in the mucosa, migrate to regional lymph nodes and later return via the thoracic duct and blood to the mucosa [29]. Numerous studies have confirmed the existence of this lymphocyte cycle in humans, too; specific ASCs can be found transiently in the circulation after mucosal antigen contact (e.g., after oral vaccination) [19, 30–32]. In current studies, the cells are assayed using the ELISPOT method, in which cells isolated from peripheral blood are allowed to secrete antibodies in microtiter plate wells that have been coated with the particular antigen. Imprints of these cells can be enumerated in the wells after immunoenzymatic reactions. Using this method, we have found circulating pathogen-specific ASCs in patients with diarrhea [24] and UTI [8]. In the present study, a local immune response to a particular antigen, P fimbria, was mounted in patients with UTI, as evidenced by the presence of both circulating ASCs and urinary antibodies. Both of these responses were dominated by IgA, the mucosal immunoglobulin isotype [29]. In fact, the ASC response to P fimbria was quite vigorous. It was significantly stronger than the response to another E. coli structure, OmpA (P < .001).

Some investigators have called into question whether P fimbria really is expressed in vivo [33, 34]. However, evidence for its in vivo expression has emerged from several studies [4, 17, 35]. Antibodies specific to P fimbria have been found in serum from patients with PN [4]. The present study, in which a marked immune response was apparent both as circulating ASCs and as urinary antibodies, provides further confirmation of the results of these studies. The in vivo expression of P fimbria is also supported by studies in animals, in which P fimbria–based vaccines inducing P fimbria–specific antibodies have proven to be protective against UTI [10–12, 14, 15].

P+ E. coli have been reported to be the pathogenic agent in 91% of children having their first episode of acute PN [36] and in 100% of a group of 25 hospitalized children and adults with acute PN [37]. P fimbriae are progressively less common in E. coli strains isolated from patients with cystitis or asymptomatic bacteriuria or from feces [16, 38, 39]. In the present study, an ASC response was mounted to P fimbria in all patients with P+ PN, whereas only 1 of 5 patients with P+ LUTI had a response. Consistent with this, in previous studies, anti-fimbrial antibodies have been found to be absent in urine from patients with LUTI [5]; in the present study, urine specimens from patients with LUTI were not available, but anti-fimbrial antibodies were found in urine from patients with P+ PN. On the other hand, some evidence exists that P fimbria of E. coli is a virulence factor even in LUTI [40].

In 1 of the patients with PN, a clear ASC response (85 ASCs/10^6 cells) was mounted even though the infecting strain was not found to be P+. This could be the result of a failure to detect P fimbria with the latex test. It is known that E. coli are subject to fimbrial phase variation in vivo in the urinary tract [35, 41]: some strains have shown a predominantly P fimbria–positive phase (e.g., 95%) in urine specimens but poor expression of P fimbria (e.g., 1%) after growth on agar plates [35, 41]. Unfortunately, the bacterial strains in the present study could not be tested for pap, the operon for P fimbria, because the strains were no longer available. With regard to the patient
discussed above, the possibility exists that another, undetected P* E. coli strain would simultaneously have been present in the urinary tract. A third explanation is that nonspecific stimulation of the immune system occurred, as is suggested by the exceptionally high numbers of immunoglobulin-secreting cells (ISCs) found in this patient (235,000 ISCs/10^6 PBMCs [8]), which far exceeded the high numbers of pathogen-specific ASCs seen in the same patient (35,000 ASCs/10^6 PBMCs [8]). A similarly excessive increase in ISCs was seen in our earlier study of patients with diarrhea, in which it was suggested that polyclonal stimulation might explain the phenomenon [24]. The hypothesis that some degree of polyclonal stimulation also occurs in patients with PN is supported by the fact that a considerable ASC response to TNP, an unrelated antigen, was found in several patients, including the patient discussed above, who had the highest TNP-ASC numbers of all.

In conclusion, the present study shows that a local immune response to P fimbria is mounted in patients with P+ UTI. It also confirms the earlier finding of a significantly stronger ASC response in patients with PN, compared with patients with LUTI. In PN, the responses to P fimbria were quite strong, which confirms that these structures are expressed in vivo and further encourages the use of P fimbria both in immunodiagnosis and as an antigen in locally administered vaccines.

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References