Occurrence of Type-1C Fimbriae on Escherichia coli Strains Isolated from Human Extraintestinal Infections

By AULI PERE,1 MAIJA LEINONEN,2 VUOKKO VÄISÄNEN-RHEN,1 MIKAEL RHEN1 AND TIMO K. KORHONEN1

Department of General Microbiology, University of Helsinki1, and National Public Health Institute2, Mannerheimintie 172, SF-00280, Helsinki 28, Finland

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Two monoclonal antibodies specific for type-1C fimbriae of Escherichia coli were produced. In enzyme-linked immunosorbent assay and immunoblotting the antibodies, which were of the IgG1 isotype, reacted with type-1C, but not with P or type-1 fimbriae of E. coli strain KS71. Immunoblotting and immunoprecipitation of crude fimbrial extracts from 25 strains invariably gave an apparent molecular weight of 17000 for the type-1C fimbrillin. A total of 313 E. coli strains, isolated from patients with extraintestinal infection or from faeces of healthy children, were screened for the presence of type-1C fimbriae using both the monoclonal and polyclonal antibodies. Of these, 45 (14%) strains had type-1C fimbriae, with the highest frequency (27%) on strains isolated from patients with pyelonephritis. No faecal strain had type-1 C fimbriae, and the frequency on the other diagnostic groups ranged from 11 to 15%. Thus, no direct correlation between type-1C fimbriae and bacterial virulence in human extraintestinal infections was found. Type-1C fimbriae were detected on only a few E. coli serotypes, notably on all O6:K2:H1 and O22:K13:H1 strains tested.

INTRODUCTION

Enterobacterial fimbriae are filamentous surface proteins that serve as binding organelles (Duguid & Old, 1980; Korhonen et al., 1980). In Escherichia coli, specific adhesion to host epithelium, as in human pyelonephritis (Svanborg Edén et al., 1976) and diarrhoea (Gaastra & de Graaf, 1982), plays a significant role in bacterial pathogenicity.

The many types of fimbriae in E. coli are characterized by binding specificity, serological properties, serotype of the strains, and the associated clinical situation. Most E. coli strains possess mannose-binding type-1 fimbriae (Duguid & Old, 1982); no specific association has been demonstrated between type-1 fimbriae and bacterial virulence in humans. Most E. coli strains associated with human pyelonephritis carry P fimbriae (Väisänen-Rhen et al., 1984), which mediate bacterial adhesion to P-blood-group-specific glycosphingolipids on human uroepithelium (Korhonen et al., 1980, 1982; Leffler & Svanborg Edén, 1980; Källenius et al., 1981). Many E. coli strains causing meningitis or sepsis in neonates carry S fimbriae that bind to sialyl galactoside structures on human erythrocytes (Parkkinen et al., 1983; Korhonen et al., 1984, 1985) and a few pathogenic strains possess M fimbriae, which recognize the aminoterminal part of glycoporphin AM (Väisänen et al., 1982). E. coli strains causing diarrhoea in humans and animals carry fimbriae (such as K88, K99 and colonization factor antigens) which mediate bacterial adhesion to the host intestine (Gaastra & de Graaf, 1982). Pathogenic E. coli strains usually exhibit two or three fimbrial types (Väisänen-Rhen et al., 1984), which show fast phase variation and mostly occur on separate cells (Rhen et al., 1983d; Nowicki et al., 1984).

A fimbrial antigen termed type-1C (Ørskov et al., 1982) has been purified from three E. coli strains (Klemm et al., 1982; Rhen et al., 1983a, b). It is serologically distinct from other E. coli fimbriae but shares structural similarity with type-1 fimbriae. However, its binding properties have not been established, so its occurrence on E. coli strains and its biological function have
remained unknown. As a first step in determining the biological function of the type-1C fimbria, we prepared monoclonal antibodies against it and tested its presence in 313 *E. coli* strains isolated from patients with extraintestinal infections and from faeces of healthy children.

**METHODS**

*Bacteria.* *E. coli* strain KS71 and its fimbriae have been described previously (Korhonen et al., 1982; Rhen et al., 1983a, b, c, d; Nowicki et al., 1984; Rhen, 1985). Of the 313 test strains, 239 were isolated from urine of girls with acute pyelonephritis (*n* = 67), cystitis (*n* = 60) or asymptomatic bacteriuria (*n* = 62) and from faeces of healthy children (*n* = 50). The strains have been characterized in detail (Väisänen et al., 1981; Väisänen-Rhen et al., 1984). Seven strains were isolated from urine of boys with pyelonephritis. The other 67 strains were isolated from cases of neonatal sepsis or meningitis and have been described by us recently (Korhonen et al., 1985). The strains were stored in nutrient agar stabs at room temperature and were subcultured on nutrient agar plates as described previously (Korhonen et al., 1982).

**Fimbriae.** KS71ABC fimbriae, purified from agar-grown cells, were those described previously (Korhonen et al., 1982). KS71A, KS71B, KS71C and KS71D (type-1) fimbriae were purified from recombinant strains having only one of the fimbrial genes (Rhen et al., 1983c; M. Rhen, unpublished observations).

**Monoclonal antibodies.** BALB/C mice were immunized with purified KS71ABC fimbriae by four successive intraperitoneal injections of 50 μg over two weeks. A booster of 50 μg was given one month later. The method of cell fusion, which was done 3 d after the booster, was a slight modification of that described by Galfre et al. (1977). BALB/C myeloma cell line SP-2/O-Ag-14 (Flow Laboratories) was cultured in R-medium, which was RPMI 1640 medium buffered with sodium bicarbonate (Flow Laboratories) and supplemented with 17% (v/v) foetal calf serum (Flow Laboratories), 250 μg l(+)-glutamine ml⁻¹ (Fluka AG, Buchs, Switzerland), 100 IU G-Na-penicillin ml⁻¹ (Hoechst AG, Frankfurt am Main, FRG), 100 IU streptomycin sulphate ml⁻¹ (Sigma) and 25 IU nystatin or 30 μg amphotericin B ml⁻¹ (E. R. Squibb & Sons, Princeton, NJ, USA).

Spleen cells from two immunized mice and SP-2 cells of exponential growth phase were combined in a ratio of 10:1. After washing, cells were fused by adding, over a period of 1 min, 1 ml prewarmed (37°C) 50% (w/v) PEG 1500 (Fluka AG) in RPMI 1640 medium. Over the next 4 min, 10 ml prewarmed R-medium without foetal calf serum was added to the suspension. Cells were then sedimented by centrifugation (5 min, 400 g), resuspended in 40 ml R-medium, and distributed (100 μl per well) in 96-well, flat-bottomed microtest plates (Nunc, Roskilde, Denmark). After overnight incubation, 100 μl R-medium supplemented with hypoxanthine (100 μM), thymidine (16 μM) and aminopterin (0-4 μM) (HAT; Littlefield, 1964; Flow Laboratories) and containing spleen cells from an unimmunized BALB/C mouse was added to each well. Positive hybridomas were identified by testing samples of culture supernatants in enzyme-linked immunosorbent assay with purified KS71ABC fimbriae as antigens, and cloned by the limiting dilution in microtest plates containing BALB/C spleen cells as a feeder layer. Positive clones were expanded in R-medium, and culture supernatants were stored at -20°C. The hybrid cells were stored at -70°C or in liquid nitrogen in R-medium containing 7-5% (v/v) dimethylsulphoxide (Merck) at a cell density of about 5 × 10⁶ ml⁻¹.

**Enzyme-linked immunosorbent assay (ELISA).** Monoclonal antibodies in diluted culture supernatants were screened for specificity by ELISA (Engvall & Perlman, 1972) using purified fimbriae as antigens as previously described (Korhonen et al., 1982). Rabbit antiserum against purified type-1C fimbriae of strain KS71 was that previously described (Rhen et al., 1983a, b, d; Nowicki et al., 1984). Alkaline phosphatase-conjugated anti-rabbit and anti-mouse IgG were from Orion Diagnostica, Helsinki, Finland. Immunoglobulin isotypes of monoclonal antibodies were determined from diluted culture supernatants of the following class-specific rabbit anti-mouse immunoglobulins: anti-IgG1, anti-IgG2, anti-IgG2a, anti-IgG2b, anti-IgG3, anti-IgA and anti-IgM (Litton Bionetics, Charleston, USA). The level of antibody binding was detected with alkaline phosphatase-conjugated swine anti-rabbit IgG (Orion Diagnostica) and p-nitrophenyl phosphate (Sigma) as substrate.

**Polyacrylamide gel electrophoresis and immunoblotting.** Polyacrylamide gel electrophoresis in sodium dodecyl sulphate (SDS-PAGE) was done in 1-5 mm-thick slab gels by the system of Laemmli (1970). A low-molecular-weight electrophoresis calibration kit (Pharmacia) was used as a standard. Peptide bands were stained with Coomassie brilliant blue R250. Specificity of monoclonal antibodies was tested by immunoblotting. Fimbrial crude extracts (Korhonen et al., 1984) or purified fimbriae were subjected to SDS-PAGE, and subsequent electrophoretic transfer to nitrocellulose membranes and immunoblotting were essentially as described by Towbin et al. (1979). Peroxidase-conjugated rabbit anti-mouse immunoglobulins were from Dakopatts, Copenhagen, Denmark, and 4-chloro-l-naphthol, used in the colour reaction, was from Bio-Rad Laboratories.

**Immunoprecipitation.** Fimbrial crude extracts were obtained and precipitated with rabbit anti-type-1C fimbriae serum (Rhen et al., 1983a) as recently described (Korhonen et al., 1984).

**Agglutination tests.** Bacterial agglutination with antibodies was done as described by Rhen et al. (1983c). Rabbit antiserum was used in 10⁻², and monoclonal antibodies in 10⁻¹, dilutions. For negative control, agglutination tests were done in phosphate-buffered saline (PBS), pH 7.1, and in SP-2 culture supernatant.
RESULTS

Specificity of monoclonal antibodies

Mice were immunized with fimbriae purified from E. coli KS71 grown on agar plates. On agar strain KS71 forms three fimbriae: two types of P fimbriae (termed A and B) and type-1C (KS71C) fimbriae (Rhen et al., 1983a, b, c, d), hence the term KS71ABC fimbriae for the immunizing antigens. An SDS-PAGE analysis of KS71ABC fimbriae is shown in Fig. 1(a), lane A. We obtained four hybridoma clones producing antibodies specific for type-1C fimbriae. Two were chosen for further use; their reactions with strain KS71 fimbriae in ELISA are shown in Table 1. The hybridoma clones F4C1 and F4C2 were specific for type-1C fimbriae of strain KS71; no titres were observed with purified A, B or D (type-1) fimbriae of strain KS71. In contrast, the rabbit antiserum prepared against purified type-1C fimbriae showed titres against KS71A, KS71B and KS71D fimbriae, which were less than 1% of the reaction with KS71C fimbriae.

Specificity of the monoclonal antibodies was also tested by immunoblotting. Fig. 1(b) shows immunoblotting of KS71ABC fimbriae (lane A) and of purified KS71C fimbriae (lane B) with F4C1; identical results were obtained with F4C2. Both monoclonal antibodies reacted strongly with KS71C, but not with KS71A or KS71B, fimbriae. It was concluded that the clones F4C1 and F4C2 were specific for the KS71C fimbriae.

Isotype analysis by ELISA showed that both F4C1 and F4C2 were of the IgG1 isotype.

Cross-reaction of $F4C1$ with heterologous strains

We used immunoblotting of crude fimbrial extracts to assay cross-reactions of the monoclonal antibody F4C1 with type-1C fimbriae of strains other than KS71. Fig. 1(a) shows an SDS-PAGE analysis of fimbrial extracts from eight E. coli strains that were agglutinated by rabbit anti-KS71C serum and by F4C1 and F4C2 (lanes C to J), and from two strains not agglutinated by the antibodies (lanes K and L). The positive strains represented major serotypes of type-1C-fimbriated strains (see below). Most strains possessed more than one fimbrial antigen, as shown by multiple peptide bands with apparent molecular weights of 17000 to 22000. In each case, only a peptide with an apparent molecular weight of 17000 reacted with F4C1 (Fig. 1b).

Occurrence of type-1C fimbriae on E. coli strains

We then screened for the presence of type-1C fimbriae in 313 E. coli strains isolated from patients with extraintestinal infection or from faeces of healthy children. The assay consisted of bacterial agglutination by F4C1, F4C2 and polyclonal rabbit antiserum against KS71C fimbriae. A strain was considered type-1C-positive if it was agglutinated by rabbit anti-KS71C serum and/or the monoclonal antibodies but not in PBS or by SP-2 culture supernatant. In all, 45 strains were considered type-1C-positive. Of these, 44 were agglutinated by polyclonal serum and by the monoclonal antibodies. One strain was agglutinated by polyclonal serum only. Bacterial agglutinations were confirmed by SDS-PAGE analyses of immunoprecipitates obtained from fimbrial extracts with rabbit antiserum against KS71C fimbriae. This was done on 25 type-1C-positive and 46 type-1C-negative strains, including all strains showing a strong autoagglutination in PBS or SP-2 culture supernatant. Analysis of immunoprecipitates from positive strains showed a 17000 molecular weight peptide band, which was lacking in precipitates from strains considered to be type-1C-negative (not shown). The two methods thus gave identical results.

A total of 45 (14%) of the strains possessed type-1C fimbriae (Table 2); 20 (27%) of the strains from pyelonephritis, but none of the faecal strains, were type-1C-fimbriated. In the other diagnostic groups the frequency of type-1C fimbriae ranged from 11 to 15%. Thus, no specific association was found between possession of type-1C fimbriae and bacterial virulence.

Serotypes of the type-1C-fimbriated strains

Serotypes of the strains used in this study have been described previously (Väisänen-Rhen et al., 1984; Korhonen et al., 1985). Type-1C-fimbriated strains belonged to seven O groups only: O6 (23 strains), O4 (five strains), O18 (five strains), O22 (five strains), O75 (two strains), O25
Fig. 1. (a) SDS-PAGE analysis of purified KS71ABC (lane A) and KS71C fimbriae (lane B) and of fimbrial extracts (lanes C to L). Lanes C to J are extracts from strains agglutinated by F4C1: lanes K and L are from strains that were not agglutinated. Serotypes of the strains are: O4:K12 (lanes A and B), O4 (lane C), O6:K2:H1 (lanes D and E), O6:K5:H+ (lane F), O6:K5:H1 (lane G), O18:K5:H- (lane H), O18 (lane I), O22 (lane J), O6 (lane K), and O18:K5:H7 (lane L). The migration distances of KS71A, KS71B and KS71C fimbribins are indicated on the left, and the positions of standard proteins (kDal) on the right. (b) Immunoblotting of duplicate gels with F4C1 monoclonal antibody.

DISCUSSION

Type-1C fimbriae resemble other, non-type-I fimbriae of *E. coli* in that they occur in only a few *E. coli* serotypes. All of the type-1C-fimbriated strains studied belonged to seven O groups (O2, O4, O6, O18, O22, O25, O75), and two of the strains were rough (Table 3). The most frequent serotypes were O6:K2:H1 (25% of type-1C-fimbriated strains) and O22:K13:H1 (7%); in fact, all strains with these two serotypes in our material possessed type-1C fimbriae. Strains with serotype O6:K2:H1 are among the virulent clonal groups identified in *E. coli* strains from pyelonephritis (Väisänen-Rhen et al., 1984) and bacteremic sepsis (Korhonen et al., 1985); however, it is noteworthy that none of the other clones associated with virulence had type-1C fimbriae. Our results are in accordance with those of Ørskov et al. (1982), who found type-1C fimbriae on urinary isolates with serotypes O4, O6, O18, O23, O75, O78 and O83.
Table 1. *Serological reactions of the monoclonal antibodies with fimbriae from E. coli KS71*

Antibody titre, as tested by ELISA, is given as the logarithm of the reciprocal of the highest dilution of hybridoma culture supernatant or antiserum giving an absorbance (at 405 nm) four times higher than that given by SP-2 culture supernatant. Rabbit preimmune serum gave titres of <2 for all fimbriae. The results are means of duplicate determinations with variation of ±0.2 or less. For the monoclonal antibodies, titres of supernatants of different days varied by ±0.3.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>KS71ABC</th>
<th>KS71A</th>
<th>KS71B</th>
<th>KS71C</th>
<th>KS71D</th>
</tr>
</thead>
<tbody>
<tr>
<td>F4C1</td>
<td>4.0</td>
<td>&lt;2.0</td>
<td>&lt;2.0</td>
<td>5.2</td>
<td>&lt;2.0</td>
</tr>
<tr>
<td>F4C2</td>
<td>3.8</td>
<td>&lt;2.0</td>
<td>&lt;2.0</td>
<td>4.9</td>
<td>&lt;2.0</td>
</tr>
<tr>
<td>Polyclonal anti-KS71C serum</td>
<td>3.9</td>
<td>2.3</td>
<td>2.0</td>
<td>4.9</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Table 2. *Occurrence of type-IC fimbriae on E. coli strains*

<table>
<thead>
<tr>
<th>Diagnostic group</th>
<th>No. of strains tested</th>
<th>No. of type-IC-fimbriated strains*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyelonephritis</td>
<td>74</td>
<td>20 (27)</td>
</tr>
<tr>
<td>Cystitis</td>
<td>60</td>
<td>8 (13)</td>
</tr>
<tr>
<td>Asymptomatic bacteriuria</td>
<td>62</td>
<td>7 (11)</td>
</tr>
<tr>
<td>Bacteremic sepsis and meningitis</td>
<td>67</td>
<td>10 (15)</td>
</tr>
<tr>
<td>Faecal</td>
<td>50</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>313</td>
<td>45 (14)</td>
</tr>
</tbody>
</table>

* Percentage given in parentheses.

Table 3. *Serotypes of the type-IC-fimbriated strains of E. coli*

<table>
<thead>
<tr>
<th>Serotype</th>
<th>No. of type-IC-fimbriated strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>06:K2:H1</td>
<td>11</td>
</tr>
<tr>
<td>06:K5:H1</td>
<td>5</td>
</tr>
<tr>
<td>06:K13:H1</td>
<td>1</td>
</tr>
<tr>
<td>06:K5:H-</td>
<td>1</td>
</tr>
<tr>
<td>Other O6</td>
<td>5</td>
</tr>
<tr>
<td>O4:K12:H5</td>
<td>2</td>
</tr>
<tr>
<td>Other O4</td>
<td>3</td>
</tr>
<tr>
<td>O18:K5:H-</td>
<td>2</td>
</tr>
<tr>
<td>O18:K12:H-</td>
<td>1</td>
</tr>
<tr>
<td>Other O18</td>
<td>2</td>
</tr>
<tr>
<td>O22:K13:H1</td>
<td>3</td>
</tr>
<tr>
<td>Other O22</td>
<td>2</td>
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<tr>
<td>O75:K5:H5</td>
<td>1</td>
</tr>
<tr>
<td>O75:K95:H-</td>
<td>1</td>
</tr>
<tr>
<td>O25:K5:H1</td>
<td>2</td>
</tr>
<tr>
<td>R:K5:H1</td>
<td>1</td>
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<tr>
<td>R:K1:H-</td>
<td>1</td>
</tr>
<tr>
<td>O2:K2</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
</tr>
</tbody>
</table>

Similar associations between fimbriation and *E. coli* serotypes have been reported for *P* fimbriae (Väisänen-Rhen et al., 1984), *S* fimbriae (Korhonen et al., 1984) and for fimbrial antigens associated with enterotoxigenic strains (Gaastra & de Graaf, 1982). The reason for these associations is not known, but they probably result from a common clonal evolution of strains with certain antigenic characters (Achtman et al., 1983).
Type-1C fimbriae have been purified from three *E. coli* strains: C1023 (an O83:K24:H31 strain; Klemm *et al.*, 1982), KS71 and ER2 (O4:K12 strains; Rhen *et al.*, 1983a, b). The three fimbriins have marked chemical similarities: an apparent molecular weight of 17000 and almost identical amino acid compositions and amino-terminal amino acid sequences. Interestingly, their chemical properties are similar to those of mannos-binding type-1 fimbriae (Salit & Gotschlich, 1977; Hermodson *et al.*, 1978). The apparent molecular weight of 17000 that was found for all type-1C fimbriins by immunoblotting with monoclonal antibodies (Fig. 1) or by immunoprecipitation with a polyclonal serum (not shown) suggests that type-1C fimbriae on different *E. coli* strains have a rather conserved chemical structure. In this respect they resemble *S* fimbriae of *E. coli* (Korhonen *et al.*, 1984) but differ from *P* fimbriae, which show marked heterogeneity in the apparent molecular weight of their subunits (Korhonen *et al.*, 1982; Rhen *et al.*, 1983b, d; A. Pere, V. Väisänen-Rhen, M. Rhen, J. Tenhunen & T. K. Korhonen, unpublished results). Moreover, many *E. coli* strains possess two variants of *P* fimbriae (Rhen *et al.*, 1983d; A. Pere, V. Väisänen-Rhen, M. Rhen, J. Tenhunen & T. K. Korhonen, unpublished results); this was not observed for type-1C fimbriae (Fig. 1b).

The two monoclonal IgG1 antibodies were specific for type-1C fimbriae of *E. coli* KS71, as assayed by ELISA (Table 1) and immunoblotting (Fig. 1). The fact that these monoclonal antibodies agglutinated all but one of the strains agglutinated by polyclonal anti-KS71C serum points to a strong conserved immunodeterminant on this fimbrial protein. However, type-1C fimbriae from different strains show variations in serological cross-reactivity with other *E. coli* fimbriae. Type-1C fimbriae of strain ER2 cross-react with *P* fimbriae of the same strain both in ELISA and in immunoprecipitation (Rhen *et al.*, 1983b); the latter indicates a strong degree of cross-reactivity. Polyclonal anti-KS71C serum shows a weak reaction with KS71A, KS71B and KS71D fimbriae and with type-1 fimbriae of strain 2131 (Table 1; Rhen *et al.*, 1983b); these cross-reactions do not result in immunoprecipitation (Rhen *et al.*, 1983a, b, d; Rhen, 1985). In the present study, a *P*-fimbrial protein with an apparent molecular weight of 19500 was coprecipitated with type-1C fimbriae from a few O6 and O18 strains by the polyclonal anti-KS71C serum (not shown). It thus seems that, in addition to conserved antigenic determinants, type-1C fimbriae have weaker determinants occurring on certain *P* fimbriae. The properties of these *P* fimbriae will be described in a separate report (A. Pere, V. Väisänen-Rhen, M. Rhen, J. Tenhunen & T. K. Korhonen, unpublished results). These examples illustrate the serological complexity of *E. coli* fimbriae.

Type-1C fimbriae did not seem to be associated with virulence in human extraintestinal infections caused by *E. coli*. They were most frequent among strains from pyelonephritis and absent from faecal ones (Table 2). However, the low percentages indicate that type-1C fimbriae are not directly involved in bacterial virulence in these infections; their frequency on *E. coli* strains that cause intestinal infections is unknown. Most of the type-1C-fimbriated strains described here have factors known to contribute to bacterial virulence in pyelonephritis: 78% of them are haemolytic and 67% *P*-fimbriated (Väisänen-Rhen *et al.*, 1984; Korhonen *et al.*, 1985). The association of type-1C fimbriae with these virulence factors may merely reflect common clonal evolution (Achtman *et al.*, 1983), or type-1C fimbriae may enhance virulence via mechanisms such as phase variation (Rhen *et al.*, 1983d; Nowicki *et al.*, 1984) which is thought to help bacteria avoid host immune responses. Fimbria-specific monoclonal antibodies should be useful tools for studying fimbrial phase variation.

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