FEMS Microbiology Letters 34 (1986) 329–334
Published by Elsevier

FEM 02426

Characterisation of a monoclonal antibody against the fimbrial F8 antigen of *Escherichia coli*

(Fimbriae; monoclonal antibody immunoblot; ELISA; mice)

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Received 27 December 1985
Revision received 31 January 1986
Accepted 2 February 1986

1. SUMMARY

A monoclonal IgG1 antibody against F8 fimbriae was obtained with the hybridoma technique using spleen cells from C3H/f mice immunised with a fimbrial preparation of *Escherichia coli* 2980 (O18ac : K5 : H$^-$: F1C, F8) and Sp 2/0 Ag8 myeloma cells. The hybrid cells were cloned twice by limiting dilution and grown in tissue culture. The monoclonal antibody was purified from culture supernatants on Protein A Sepharose. It reacted with F8 fimbriae in colony blot, enzyme-linked immunosorbent assay (ELISA) and immunoblot after electrotransfer from sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of fimbrial preparations. The antibody bound to and agglutinated F8-fimbriated bacteria.

2. INTRODUCTION

Fimbriae are filamentous protein structures extending from the bacterial surface, known to mediate the binding of bacteria to other cells [1–3]. *E. coli* fimbriae have been classified according to their receptor requirements into those which bind mannose-containing receptors in a mannose-sensitive (MS) way, and those which bind to other receptors in a mannose-resistant (MR) way [3]. MR fimbriae were found to be a functionally heterogenous group. It is known that some fimbriae recognise galactose-containing receptors belonging to the blood-group P system (P-fimbriae) [4,5] and others recognize parts of glycophorin A on human erythrocytes (M-fimbriae) or sialic acid-containing receptors (S-fimbriae) [6,7]. The serological classification of fimbriae as F antigens (F1–F13, Fy) [8,9] is independent of their receptor specificity. The F1 antigen corresponds to MS fimbriae and the other F antigens are MR fimbriae, whereby P-fimbriae are associated with serotypes F7–F13. In this study we describe a monoclonal antibody against the *E. coli* MR fimbrial antigen F8, belonging to the P-fimbriae [10].

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3. MATERIALS AND METHODS

3.1. Bacteria and fimbriae

The bacteria used are listed in Table 1. With the exception of 2131 and 21391, all strains were isolated from urine. Strain 2131 is a recombinant strain expressing F1A fimbriae on a genetic background of *E. coli* K-12. Strain 21391 is an *E. coli* K-12 clone harbouring the recombinant plasmid pANN921, which codes for the F8-antigen. pANN921 was derived from a cosmid which was isolated from a E. coli K-12 gene bank of strain 2980 (J. Hacker et al., manuscript in preparation). All strains were grown on Loeb agar at 37°C (strain 21391 in the presence of 20 μg/ml tetracycline). The isolation of the fimbriae was done as described previously [11,12]. Briefly, bacteria harvested from Loeb agar plates were suspended in 50 mM Tris buffer, pH 7.5, and treated with an Omnimixer (position 2; 3 × 3 min) at 4°C. The suspension was centrifuged at 10000 × *g* for 20 min and the supernatant was recentrifuged at 30000 × *g* for 20 min. After centrifugation of the second supernatant at 100000 × *g* for 2 h, the fimbriae were obtained as a pellet. They were resuspended in 50 mM Tris buffer, pH 7.5.

Table 1

E. coli strains used and their reactivity with the monoclonal antibody

The bacteria were grown on agar and, with the exception of strain 2131, did not express the F1A antigen.

E. coli strains		Reaction with the monoclonal antibody in colony blot and ELISA
Freiburg collection number	Seroformula	
2980 [a]	O18ac:K5:H$^-$:F1C, F8	+
21472	O18ac:K5:H$^-$:F1C, F8	+
21473	O18ac:K5:H$^-$:F1C, F8	+
21474	O18ac:K5:H$^-$:F1C, F8	+
21475	O18ac:K5:H$^-$:F1C, F8	+
20043	O83:K5:H$^-$:F1C, Fy	−
21084	O6:K2:H1:F1C, F7	−
21085	O75:K$^-$:H5:F8	+
21478	O75:K$^+$:H5:F8	+
21479	O75:K$^+$:H5:F8	+
21480	O75:K$^+$:H5:F8	+
21086	O2:K5:H4:F9	−
21087	O7:K1:H1:F10	−
21088	O1:K1:H7:F11	−
21089	O16:K1:H$^-$:F12	−
2131	K-12:F1A (recombinant)	−
21391	K-12:F8 (cloned)	+

[a] Fimbriae of this strain were used as immunogen.

3.2. Monoclonal antibodies

Female C3H/f mice, 6–8 weeks old, were injected subcutaneously and intraperitoneally with the fimbrial preparation from *E. coli* 2980 (20 μg protein in Freund's complete adjuvant) twice in a 4-week interval. Three weeks after the second injection the mice were given 20 μg protein in PBS intravenously, and 3 days later they were killed with ether. Spleen cells (10^8) of the immunised mice were fused with Sp2/0-Ag8 myeloma cells (10^7) [13] using polyethylene glycol 1540 as fusing agent. Hybrid clones were screened for homologous anti-F, anti-O and K specificity by the ELISA technique, and positive clones were subcloned by limiting dilution.

Supernatants of the clones were purified by affinity chromatography on protein A-Sepharose 4B (Pharmacia, Uppsala, Sweden) at pH 8.6, and after washing the column with 50 mM Tris-HCl 150 mM NaCl pH 8.6, elution was done with 50 mM glycin-HCl/150 mM NaCl at pH 2.3. Antibody-containing fractions were adjusted to pH 7.5 and dialysed against PBS. The purified antibodies were stored at −80°C at a concentration of 2.5 mg/ml.

3.3. Serological techniques

For ELISA tests [14] microtitre plates were coated at 37°C with antigen diluted in 50 mM carbonate buffer, pH 9.6. The antigen concentration was 20 μg/ml with protein and 100 μg/ml with LPS or capsular (K) polysaccharide. When fimbriated bacteria were used as antigens, 10^8 cells per ml were used after fixation with glutaraldehyde [15]. The unspecific sites were blocked with 4% (w/v) BSA in PBS/0.05% (v/v) Tween-20, pH 7.5. Peroxidase-conjugated goat anti-mouse immunoglobulin (Medac, Hamburg) was used and the peroxidase substrate was 2,2'-azino(3-ethylbenzenthiazoline sulphonic acid) in phosphate buffer at pH 6. The extinction was measured at 405 nm.

For immunoblots [16] fimbrial subunits were electrotransferred (90 mA, 16 h, 4°C) from SDS-polyacrylamide gels to nitrocellulose sheets in 25 mM Tris/190 mM glycin, pH 7.5, containing 20% methanol. The nitrocellulose was washed with phosphate-buffered saline (PBS), incubated with 4% bovine serum albumin (BSA) in PBS/0.05% Tween-20 for 1 h, washed again and incubated with the purified monoclonal antibody in a concentration of about 10 μg/ml in PBS-Tween overnight at 4°C. After washing at least 4 times with 0.05% Tween-20 in PBS, the sheets were incubated for 1 h at room temperature with peroxidase-conjugated goat anti-mouse IgG (Miles). After several washings they were incubated for 1 h at room temperature with substrate/H_2O_2 and then washed with water. Reaction of protein bands with the antibodies was indicated by a dark blue-grey colour. The reagent was 4-chloro-1-naphthol (30 mg in 10 ml methanol, to which were added 50 ml of 10 mM Tris-HCl, pH 7.5, containing 200 mM NaCl and 20 μl H_2O_2).

For colony blots, single colonies were blotted from Loeb agar plates to nitrocellulose paper sheets, air dried, and washed with PBS-Tween/4% BSA containing 0.02% NaN_3. The procedure was continued as described for the immunoblot.

Agglutinations were performed with bacterial suspensions in 0.9% NaCl ($A_{600} = 1$). The antibodies were also diluted in 0.9% NaCl. After incubation (1 h at 37°C and then 16 h at 4°C), agglutination was estimated microscopically.

3.4. SDS-PAGE

The disintegration of fimbriae and their electrophoretic separation on polyacrylamide gels (running gel 13%, separating gel 5%) has been described [12,16].

3.5. Radioiodination of antibodies

The purified antibodies were iodinated with the chloramine-T method [17]. Free iodine was removed by chromatography on Sephadex G-25 with PBS as eluent.

3.6. Absorption of ^{125}I-antibody to fimbriated E. coli

E. coli bacteria, grown on agar at 37°C for 40 h, were suspended in PBS to a density of 10^9 cells/ml. Of each suspension, 1 ml was centrifuged at 8000 × *g* for 15 min and the respective pellets were gently resuspended in PBS containing 5 μg of the monoclonal anti-F8 antibody. After shaking gently for 60 min at 37°C, the mixtures were centrifuged and washed 5 times with PBS, by centrifuging as above. The final pellet was suspended in 1 ml PBS and 50-μl portions were counted in a γ-counter.

4. RESULTS

4.1. Bacteria and fimbriae

E. coli 2980 (O18ac : K5 : H^-) used for the production of monoclonal anti-fimbrial antibodies, expresses two types of fimbriae, defined as F1C and F8, by crossed immunoelectrophoresis [9,10]. For comparison, F test strains and strains in which the F8 antigen had been detected by crossed immune electrophoresis, as well as the *E. coli* K-12 strain expressing the cloned F8-antigen (Hacker, manuscript in preparation) were included. The

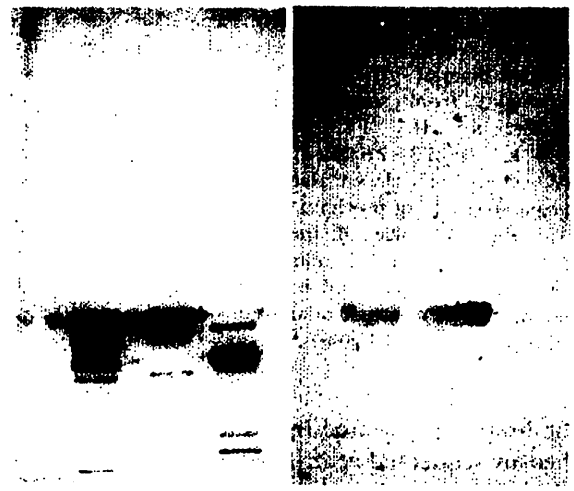

Fig. 1. (A) SDS-PAGE of fimbrial preparations from *E. coli* 2980 (F8, F1C), 21085 (F8) and 20043 (F1C, Fy) (from left to right). Of each preparation 15 μg protein were applied; the gel was stained with Coomassie blue. (B) Immunoblot of the same fimbrial preparations as in 1A, after electro-transfer to nitrocellulose. The immunostaining is described in the text. The monoclonal antibody was used in a concentration of about 10 μg/ml and the conjugate of anti-antibody and peroxidase was used in a dilution of 1 : 1000.

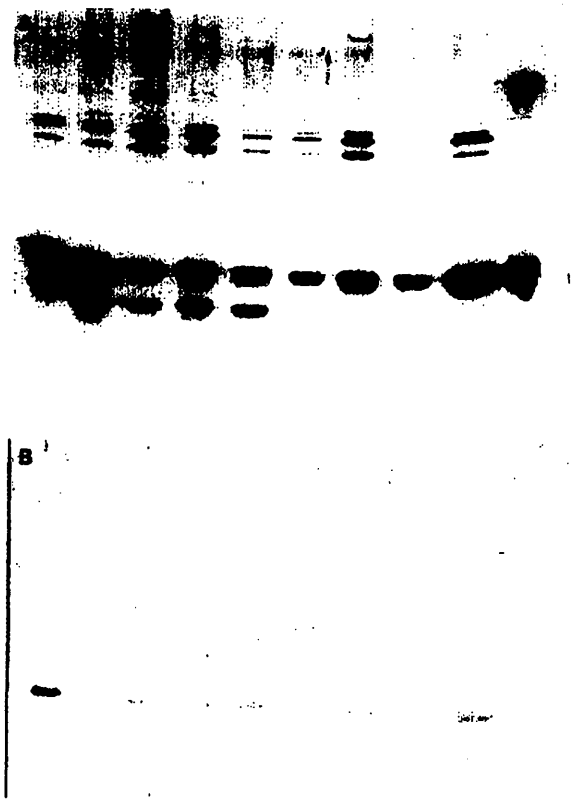

Fig. 2. (A) SDS-PAGE of fimbrial preparations from *E. coli* strains expressing fimbrial F8 antigen alone or in combination with the fimbrial F1C antigen. The preparations are (from left to right) from strains 2980, 21472-21475 (*E. coli* O18ac : K5 : H$^-$: F8, Fy), 21478-21480 (*E. coli* O75 : K$^+$: H5 : F8), 21085 (*E. coli* O75 : K$^-$: H5 : F8), 21391 (*E. coli* K-12 : F8, cloned; Hacker, in preparation). From each preparation, 15 μg protein was applied; the gel was stained with Coomassie blue. (B) Immunoblot of the same fimbrial preparations, after electro-transfer to nitrocellulose. The monoclonal antibody was used in a concentration of about 10 μg/ml and the conjugate of anti-antibody and peroxidase was used at a dilution of 1 : 1000.

fimbriae were sheared from the bacteria and purified by sequential centrifugation.

4.2. Production and characterisation of monoclonal antibodies

Female C3H/f mice, 6–8 weeks old, were immunised with isolated fimbriae of *E. coli* 2980 and their spleen cells were fused with Sp2/0-Ag8 myeloma cells [13]. The supernatants of the hybrid clones were screened by ELISA for antibody

Table 2

Adsorption of ^{125}I-labelled monoclonal anti-F8 antibody to *E. coli* cells

5 μg Antibody protein (9.5×10^6 cpm) was added to 10^9 *E. coli* cells in a total volume of 1 ml.

Bacterial strain	Fimbrial antigens expressed	Monoclonal anti-F8 antibody adsorbed	
		cpm ($\times 10^5$)	μg protein adsorbed
2980	F1C, F8	9.38	0.5
21085 [a]	F8	0.98	0.05
20043	F1C, Fy	0.06	0.017

[a] Electron microscopy revealed that strain 21085 was less fimbriated than strain 2980.

specificity with isolated fimbriae, LPS and capsular polysaccharide of the immunizing strain. One clone exhibiting only anti-fimbrial specificity was subcloned twice by limiting dilution and propagated in tissue culture. The supernatant which showed anti-fimbrial activity was purified by affinity chromatography on protein-A Sepharose 4B. Agar gel double diffusion test with anti-mouse Ig antisera showed that the monoclonal antibody was IgG1, κ.

4.3. Anti-fimbrial specificity

The antibody was analysed by ELISA and colony blot for cross-reactions with fimbriae of *E. coli* strains expressing F8, F1C or other *E. coli* F antigens including those of F7–F12 test strains. It reacted only with strain 2980 and F8 test strain 21805, and not with strains which do not express the F8 antigen. To ensure F8 specificity, the antibody was used in colony blots and ELISA with *E. coli* strains expressing the F8 antigen, including the *E. coli* K-12 strain carrying the recombinant DNA for F8-fimbriae (Hacker et al., manuscript in preparation). All these strains reacted in both assays, ELISA titre being in the range of 10^{-5}–10^{-6} (Table 1).

Fig. 1A shows that the fimbrial preparation of *E. coli* 2980 exhibited two major peptide bands in SDS-PAGE (16.6 and 19.7 kDa). Previous immunoblot analysis [21] had shown that the smaller peptide (16.6-kDa) corresponded to the fimbrial F1C subunit. Immunoblot with the monoclonal

antibody revealed reactivity with the slower-moving 19.7-kDa band (Fig. 1B). Fimbrial preparations of all strains exhibiting the F8 antigen were analyzed in immunoblots with the monoclonal antibody. As shown in Fig. 2, they expressed in SDS-PAGE either only one (19.7-kDa) band or two (16.6-kDa and 19.7-kDa) bands, and only the 19.7-kDa band was reactive with the antibody.

4.4. Reaction of the monoclonal anti-F8 antibody with whole E. coli *cells*

The monoclonal antibody (2.5 mg/ml) agglutinated strain 2980 to a dilution of 1 : 4000 (in saline). Binding of the antibody to the bacteria was also monitored with *E. coli* 2980 and with F8 test strain 21085 using ^{125}I-labelled antibody. As shown in Table 2, strain 2980 bound 10 times more antibody protein (per 10^9 cells) than test strain 21085. Electron microscopic inspection (not shown) revealed that strain 21085 was less fimbriated than strain 2980. Binding of the monoclonal antibody to strain 20043, which was fimbriated but did not express F8 fimbriae, was 3 log numbers lower, the values corresponding practically to blank values.

5. DISCUSSION

In the course of our immunochemical studies of *E. coli* surface antigens we prepared a monoclonal antibody against the fimbriae of uropathogenic *E. coli* 2980 (O18ac : K5), which had been described previously [12]. Crossed immunoelectrophoresis had previously shown that this strain expresses the fimbrial F1C and F8 antigens. The monoclonal antibody, obtained by hybridisation [13] of spleen cells from C3H/f mice immunised with the fimbrial preparation with non-producer Sp2/0-Ag8 hybridoma cells, reacted with the homologous fimbrial preparation but not with the O18ac-LPS or the K5-polysaccharide. It was therefore taken to be fimbriae-specific. Only *E. coli* strains containing the F8 antigen reacted with the monoclonal antibody in colony blot and ELISA. The F8 antigen is associated mainly with 2 O : K : H combinations (O18ac : K5 : H^- and O75 : K : H5) probably reflecting two clones ([9], I. Ørskov, unpublished observation). Several such strains were therefore analysed by immunoblotting. Fimbrial preparations from strains 2980 and 21472-21475 (*E. coli* O18ac : K5 : H^-) exhibited two bands, 19.7 and 16.6 kDa, in SDS-PAGE. The monoclonal antibody reacted only with the 19.7-kDa band, which emphasised that this band represents the fimbrial F8 subunit. The 16.6-kDa band has previous been identified as fimbrial F1C subunit [21]. Strains 21478-21480 (*E. coli* O75 : K^+ : H5) and K mutant 21085 exhibited only the 19.7-kDa band, which reacted with the monoclonal antibody. The genetic determinant coding for F8-fimbriae was recently cloned from the chromosome of the O18 : K5 strain 2980 (Hacker et al., manuscript in preparation). Fimbriae from this *E. coli* K-12 clone carrying the recombinant F8-DNA (21391) also reacted with the monoclonal antibody.

Reactivity of the monoclonal antibody in ELISA as well as in and colony blot and immunoblot indicated that the F8-specific antibody recognises a surface-expressed sequential rather than a conformational epitope. This was also shown by absorbing ^{125}I-labelled antibody to whole fimbriated *E. coli* 2980 and F8 test strain 21085. In this test *E. coli* 2980 reacted 10-fold better than 21085, which was probably due to a lesser fimbriation of the latter, as shown by electron microscopy. Surface accessibility of the F8 antigen to the monoclonal antibody was also demonstrated by the agglutination of *E. coli* 2980, although this was observed only after 24 h.

The results described here allow a complete fimbrial characterization of uropathogenic *E. coli* 2980. Together with other results on monoclonal anti-fimbrial antibodies [18–20], they expand the spectrum of fimbriae-specific probes for *E. coli* pathogens.

ACKNOWLEDGEMENT

These studies were supported by a grant from the Deutsche Forschungsgemeinschaft.

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