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Monoclonal Antibodies for Serotyping the P Fimbriae of Uropathogenic *Escherichia coli*

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Monoclonal antibodies (MAbs) against seven serologically different P fimbriae ($F7_1$, $F7_2$, F8, F9, F11, F12, and F13) of uropathogenic *Escherichia coli* were tested for their ability to detect the P fimbriae on wild-type strains. In a plate agglutination test the MAbs could detect the fimbriae on strains which expressed cloned fimbriae but not on wild-type strains. In a coagglutination test and in a whole-bacterium enzyme-linked immunosorbent assay the MAbs recognized the fimbriae on strains with cloned fimbriae and on wild-type strains. However, the coagglutination test has some disadvantages: only immunoglobulin G MAbs can be used, and the results cannot be read in an objective way. From these results, we concluded that the whole-bacterium enzyme-linked immunosorbent assay is the most convenient method for the determination of P fimbriae on wild-type *E. coli* strains. With this fast and easy method it is possible to do epidemiological studies on the distribution of P fimbriae among clinical isolates of uropathogenic *E. coli* and to extend the O:K:H serotype with the F serotype.

The ability of uropathogenic *Escherichia coli* to adhere to uroepithelial cells is an important virulence factor (15, 17, 18), enabling these bacteria to circumvent the flushing action of urine and to infect the urinary tract. Adhesion is mediated by fimbriae which bind to specific cell surface receptor molecules. The majority of these uropathogenic *E. coli* strains express P fimbriae (16), which recognize as a receptor structure the disaccharide α-D-Gal*p*-(1-4)-β-D-Gal*p* (10) on the uroepithelial cells. These P fimbriae differ in the molecular weights of their subunit proteins (7) and in serological properties (12, 13). Ørskov and Ørskov (12) could distinguish eight serologically different fimbriae on uropathogenic *E. coli* by using crossed-immunoelectrophoresis techniques. They suggested that these fimbriae be referred to as F antigens (F7 to F12). This typing scheme of P fimbriae was recently extended by the classification of pyelonephritis-associated pilus (Pap) fimbriae as F13 fimbriae (11). Parry et al. (13) were able to determine seven different antigenic determinants on fimbriae of uropathogenic *E. coli* by using a slide agglutination test and designated them a to g.

In both studies (12, 13) cross-absorbed polyclonal antisera were used. Recently, we described the production of monoclonal antibodies (MAbs) against the four different P fimbriae $F7_1$, $F7_2$, F9, and F11 (4). The main purpose of this study was to develop a rapid and easy method, with the aid of the MAbs, for the detection of serologically different P fimbriae on wild-type *E. coli* strains. Therefore, we compared three different methods: a plate agglutination test, a coagglutination test with *Staphylococcus aureus* as a carrier for the MAbs, and an enzyme-linked immunosorbent assay (ELISA) with whole bacteria as an antigen. A comparison of these three methods showed that the whole-bacterium ELISA was the best method for the screening of P fimbriae on uropathogenic *E. coli*.

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MATERIALS AND METHODS

Bacterial strains, plasmids, and growth media. The bacterial strains and plasmids used in this study are listed in Table 1. The C, SP, and J96 strains were kindly provided by I. Ørskov and F. Ørskov, Steve Parry, and Björn Lund, respectively. The SP strains were serotyped by P. A. M. Guinee (State Institute for Public Health, Bilthoven, The Netherlands). Plasmids pPIL110-70 and pPIL110-37 were kindly provided by Irma van Die, plasmid pRHU845 was provided by Staffan Normark, plasmid pDC1 was provided by Steven Clegg, and plasmid pANN921 was provided by Jörg Hacker. All the fimbriae encoded by these plasmids are P fimbriae. AM1727 is a *recA* derivative of JE2571, an *E. coli* K-12 strain deficient in the production of type 1 fimbriae (20). All wild-type strains were cultivated on blood agar plates (Oxoid Ltd.), and cloned strains were cultivated on brain heart infusion agar (Oxoid Ltd.). Selective pressure against the loss of plasmids was imposed by adding 50 μg of ampicillin, 100 μg of chloramphenicol, or 12.5 μg of tetracycline per ml, depending on the cloning vector used.

MAbs against the P fimbriae $F7_1$, $F7_2$, F8, F9, F11, F12, and F13. The production of MAbs against the P fimbriae $F7_1$, $F7_2$, F9, and F11 was recently described (4). Briefly, spleen cells of immunized BALB/c mice were fused with the myeloma cell line SP2/0, and the resulting hybridomas were grown for 3 weeks. Antibody production against fimbriae was tested in an ELISA, and positive clones were subcloned by a limiting dilution step. After additional growth, the cells were used for antibody production in ascitic fluid. MAbs were partially purified from ascitic fluid as described previously (4). For this study we used various MAbs against different P fimbriae, selected on the basis of specificity and immunoglobulin subclass. The characteristics of the MAbs used are shown in Table 2. The production of MAbs against purified F8, F12, and F13 (Pap) fimbriae will be described elsewhere (J. M. de Ree and J. F. van den Bosch, submitted for publication). F8 fimbriae were purified from the cloned strain AM1727 (pANN921), F12 fimbriae were purified from the wild-type strain C1979, and F13 fimbriae were purified

TABLE 1. Bacterial strains used in this study

Bacterial strain	Serotype	Fimbriae or antigenic determinants	Reference
Wild type			
C1212 (AD110)	O6:K2:H1	$F7_1$, $F7_2$, and 1C	12
C1254	O75:K⁻:H5	F8	12
C1018	O2:K5:H4	F9	12
C1976	O1:K1:H7	F11	12
C1979	O16:K1:H⁻	F12	12
C134	O4:K12:H5	Fy, F12, F13, and 1C	12
SP133	O14:?	c and e	13
SP7	O18:K5	c, d, and f	13
SP57	O157:K⁻	a and b	13
SP201	Nontypable	g and e	13
SP144	O6:K13	f and g	13
SP88	O157:K⁻	a and b	13
SP101	O1:K1	b, c, and e	13
J96	O4:K6:H5	F13 (Pap)	6
Cloned			
AM1727(pPIL110-70)		$F7_1$	19
AM1727(pPIL110-37)		$F7_2$	20
AM1727(pANN921)		F8	Hacker, in preparation
AM1727(pPIL288-10)		F9	2
AM1727(pPIL291-15)		F11	3
AM1727(pRHU845)		F13 (Pap)	6
AM1727(pDC1)		Clegg	1

from the cloned strain AM1727 (pRHU845). The immunoglobulin subclasses of the MAbs were determined with an ELISA as described previously (4).

Plate agglutination test. Bacterial agglutination assays were performed essentially as described for O-antigen typing by Guinee et al. (5). Bacteria were grown for 16 h and suspended in phosphate-buffered saline (PBS) with 0.25% Formalin to an optical density at 660 nm of 0.7. Bacteria were stained by adding a 0.05% gentian violet solution in PBS with 0.25% Formalin. Serial dilutions of the MAbs were made in 100 μl of PBS with 0.25% Formalin in microtiter plates (Thovadec). To each well, 100 μl of stained bacteria was added. Agglutination was read after overnight incubation at 4°C.

Coagglutination. *S. aureus* Cowan 1 NCTC 8530 was kindly provided by Loek van Alphen. These bacteria were grown for 16 h on Columbia agar plates. After three washings with PBS, the bacteria were suspended in PBS with 0.5% Formalin and incubated for 3 h at room temperature. Subsequently, the bacteria were washed four times with PBS, incubated for 10 min at 80°C, and washed two times with PBS. Finally, a 10% (vol/vol) suspension was made in PBS with 0.01% sodium azide. This suspension was stored at 4°C.

Immunoglobulin G (IgG) MAbs were coated to protein A of the *S. aureus* suspension as described by Kromwall (9). Briefly, 50 μl of undiluted MAb was incubated with 0.5 ml of the *S. aureus* suspension for 10 min at room temperature. Agglutination assays were performed on glass slides by mixing 1 drop of a bacterial suspension in PBS and 1 drop of an MAb-coated *S. aureus* suspension. Agglutination was scored within 1 min.

Whole-bacterium ELISA. Bacteria cultivated overnight were suspended in 50 mM bicarbonate coating buffer (pH 9.6) to an optical density of 1.0 at 660 nm and diluted eight times in coating buffer. Microtiter plates (activated polyvinyl chloride; Flow Laboratories, Inc.) were coated with 100 μl of the diluted bacterial suspension per well and dried overnight at 37°C. On the next day, the microtiter plates were washed four times with PBS containing 0.05% Tween 20 (PBST). After 1 h of incubation at room temperature with 15% newborn calf serum (GIBCO Laboratories) in PBST (NBST), the microtiter plates were washed four times with PBST and then incubated with 100 μl of 1:100-diluted MAb in NBST for 2 h at room temperature. Subsequently, the wells were washed four times with PBST and incubated for 3 h at room temperature with 100 μl of goat anti-mouse IgG (heavy and light chains) conjugated to peroxidase (Nordic Laboratories); the conjugate was diluted 1:1,000 in NBST. Antifimbria antibody activity was detected colorimetrically by adding 0.05% *o*-phenylenediamine in citrate-phosphate buffer (pH 4.5) containing 0.005% H_2O_2. The reaction was developed in the dark for 15 min at room temperature and terminated with 2 M H_2SO_4. The A_{492} was measured in a Titertek Multiskan (Flow Laboratories, Inc.). Wells filled with H_2O were used as blanks.

RESULTS

The reaction of the MAbs raised against the P fimbriae $F7_1$, $F7_2$, F9, and F11 was tested in the plate agglutination assay with strains expressing homologous fimbriae. All MAbs reacted with the fimbriae on the homologous *E. coli* AM1727 strains expressing cloned fimbriae (Table 3). Strain AM1727 (pPIL288-10) showed autoagglutination with this method. Only MAb M6-8 reacted with the fimbriae on the wild-type strain C1212. None of the other MAbs recognized the fimbriae on the homologous wild-type strains with this plate agglutination assay.

The MAbs were also tested in a slide agglutination test (data not shown), and none of them reacted with the homologous wild-type strains. Subsequently, we tried to improve this agglutination by coating the MAbs to protein A of an *S. aureus* suspension as described above. With this coagglutination test only the IgG MAbs raised against the $F7_1$, $F7_2$, and F11 fimbriae were tested. In this assay all the MAbs recognized the fimbriae on the homologous cloned strains (Table 4). MAb M7-6 also reacted with the fimbriae on the heterologous cloned strain, AM1727 (pDC1). MAbs M6-3 and M2-1 also agglutinated the wild-type strain C1212, which expresses homologous fimbriae. MAb M7-6 did not agglutinate the homologous wild-type strain C1976. However, this

TABLE 2. MAbs used in this study

MAb	Immunoglobulin subclass	Fimbriae that MAb was raised against	Fimbriae recognized by MAb in a fimbria ELISA
M6-3	IgG2a	$F7_1$	$F7_1$[a]
M6-8	IgM	$F7_1$	$F7_1$[a]
M2-1	IgG2b	$F7_2$	$F7_2$ and F9[a]
M17-9	IgM	F8	F8[b]
M4-7	IgM	F9	F9[a]
M7-6	IgG1	F11	F11 and Clegg[a]
M14-5	IgM	F12	F12[b]
M8-16	IgM	F13 (Pap)	F13 (Pap)[b]

[a] See reference 4.
[b] de Ree and van den Bosch, submitted.

TABLE 3. Titers of MAbs in a plate agglutination test against homologous wild-type and cloned *E. coli* strains

Bacterial strain	Fimbriae	Titer[a] of			
		$F7_1$ MAb M6-8	$F7_2$ MAb M2-1	F9 MAb M4-7	F11 MAb M7-6
C1212	$F7_1$, $F7_2$, and 1C	40	0		
C1018	F9			0	
C1976	F11				0
AM1727(pPIL110-70)	$F7_1$	5,120			
AM1727(pPIL110-37)	$F7_2$		10,240		
AM1727(pPIL288-10)	F9			AA[b]	
AM1727(pPIL291-15)	F11				>40,000

[a] Expressed as the dilution of MAb which still showed agglutination.
[b] AA, Autoagglutination.

MAb did react with the fimbriae on the wild-type strains SP133 and SP101.

All the MAbs described in Table 2 were tested in an ELISA with whole bacteria as an antigen. In this ELISA all the MAbs reacted with the fimbriae on the homologous cloned strains (Table 5). The F11 MAb M7-6 also reacted with the fimbriae on the heterologous cloned strain AM1727 (pDC1). All the MAbs reacted with the fimbriae on the homologous wild-type strains, except for the F11 MAb M7-6, which did not react with the F11 fimbriae on the wild-type strain C1976. However, this F11 MAb did react with the fimbriae on the wild-type strains SP133 and SP101. MAb M14-5, raised against purified F12 fimbriae from the wild-type strain C1979, also reacted with the fimbriae on the wild-type strain C1979. In contrast, the F12 fimbriae on strain C134 were not recognized by MAb M14-5.

MAb M17-9, raised against purified F8 fimbriae from the cloned strain AM1727 (pANN921), reacted with the fimbriae on the wild-type strains C1254 and SP7.

DISCUSSION

In this paper we tested three different methods for the determination of serologically different P fimbriae on clinical isolates of *E. coli*. The classification of these P fimbriae was recently described by Ørskov and Ørskov (12). They used polyclonal antisera raised against whole cell cultures which had been made more fimbria specific by absorption with the homologous nonfimbriated strain grown at 20°C. The disadvantage of polyclonal antisera against P fimbriae is that these antisera are not specific for each type of P fimbria, as was shown by Korhonen et al. (8) and de Ree et al. (4) in ELISA studies. Nevertheless, Ørskov and Ørskov (12) were able to use these polyclonal antisera in crossed immunoelectrophoresis and crossed-line immunoelectrophoresis as very sensitive methods to discriminate between serologically different fimbriae. With these methods they were able to demonstrate seven serologically different fimbriae (F7 to F12) on clinical isolates. However, crossed-immunoelectrophoresis technique for F-antigen determination is cumbersome and can only be carried out in highly specialized laboratories. The main purpose of this study was to develop a simple and fast method that uses the recently produced specific MAbs to determine the serologically different P fimbriae on clinical isolates of *E. coli*.

TABLE 4. Coagglutination of MAbs with wild-type and cloned strains

Bacterial strain	Coagglutination[a] of:		
	$F7_1$ MAb M6-3	$F7_2$ MAb M2-1	F11 MAb M7-6
C1212	+	++	−
C1254	−	−	−
C1018	−	−	−
C1976	−	−	−
C1979	−	−	−
SP133	−	−	++
SP101	−	−	++
AM1727(pPIL110-70)	+	−	−
AM1727(pPIL110-37)	−	+++	−
AM1727(pANN921)	−	−	−
AM1727(pPIL288-10)	−	−	−
AM1727(pPIL291-15)	−	−	+++
AM1727(pRHU845)	−	−	−
AM1727(pDC1)	−	−	+++

[a] Agglutination was read within 1 min: −, no agglutination; +, weak agglutination; ++, agglutination; +++, strong agglutination.

TABLE 5. Reaction of MAbs with wild-type and cloned *E. coli* strains in an ELISA with whole bacteria as an antigen

Bacterial strain	Reaction[a] of:						
	$F7_1$ MAb M6-8	$F7_2$ MAb M2-1	F8 MAb M17-9	F9 MAb M4-7	F11 MAb M7-6	F12 MAb M14-5	F13 (Pap) MAb M8-16
C1212	8	8					
C1254			8				
C1018				6			
C1976							
C1979						9	
C134							5
SP7			4				
SP133					8		
SP101					7		
J96							8
AM1727(pPIL110-70)	9						
AM1727(pPIL110-37)		9					
AM1727(pANN921)			8				
AM1727(pPIL288-10)				9			
AM1727(pPIL291-15)					9		
AM1727(pRHU845)							9
AM1727(pDC1)					9		

[a] Expressed as A_{490} units; 1 unit equals an A_{490} of 0.29.

The first method to be tested was a plate agglutination assay. All the MAbs agglutinated the homologous cloned strains, except for AM1727 (pPIL288-10) (Table 3). This strain showed autoagglutination, which was probably caused by the extremely high fimbria expression of this cloned strain (2). Only MAb M6-8 weakly agglutinated the homologous wild-type strain, whereas the other MAbs did not show any reaction with the wild-type strains. This result can be explained by the lower number of fimbriae present on wild-type strains than on cloned strains, a fact which was recently demonstrated for the F9 and F11 wild-type and cloned strains by electron microscopy (2, 3). Furthermore, in a slide agglutination test the MAbs did not agglutinate the wild-type strains. From these results, we concluded that direct agglutination is not sensitive enough for screening the

P fimbriae on wild-type strains. Therefore, we tried to amplify agglutination by coating the MAbs to protein A of an *S. aureus* suspension. A disadvantage of this method is that only IgG MAbs could be used. Because all the F9 MAbs were of the IgM subclass (4), these MAbs could not be used in this method. The other MAbs tested in this assay agglutinated the homologous cloned strains, and the F11 MAb even agglutinated strain AM1727 (pDC1) (Table 4). In contrast to the results of the plate agglutination assay, almost all the MAbs agglutinated homologous wild-type strains in the coagglutination assay. The only exception was the F11 MAb M7-6, which did not agglutinate the homologous wild-type strain C1976, probably because of the very low number of F11 fimbriae on this wild-type strain (3). However, the F11 MAb did agglutinate some other wild-type strains. From these results, we concluded that the coagglutination test is more sensitive than the plate agglutination test but still has the disadvantage that only IgG MAbs can be used. Another drawback of this method is the difficulty of reading the results in an objective way.

The last method to be tested was a whole-bacterium ELISA. During the course of this study, more MAbs were available than in the former two assays. Since these two assays were found to be unsatisfactory for our purposes, we did not test the more recently obtained MAbs in the agglutination assays, but we did test the whole series of available MAbs in the ELISA. All the MAbs tested in this method reacted strongly with the fimbriae on the homologous cloned strains (Table 5). The F11 MAb M7-6 recognized the Clegg fimbriae on strain AM1727 (pDC1), as was also shown with the coagglutination assay (Table 4). From these results, we concluded that Clegg fimbriae are probably identical to F11 fimbriae. All the MAbs also recognized the fimbriae on the homologous wild-type strains with this method, except, again, for the F11 MAb M7-6. However, the F11 MAb M7-6 did detect the fimbriae on the wild-type strains SP133 and SP101. So, this MAb could still be useful for the determination of F11 fimbriae on wild-type strains. The reaction of the F11 MAb M7-6 in the coagglutination assay and the whole-bacterium ELISA with strains SP133 and SP101 strongly suggested that these strains express F11 fimbriae. Similarly, as in the coagglutination test, the negative reaction of the F11 Mab M7-6 with the fimbriae on the homologous wild-type strain C1976 was probably caused by the very low number of F11 fimbriae on this strain (3).

The recombinant strain AM1727 (pANN921) codes for F8 fimbriae, which were cloned by J. Hacker (manuscript in preparation) from the wild-type strain 2980 (serotype O18:K5:H^-) described by Jann et al. (7). MAb M17-9, which was raised against the purified F8 fimbriae from the recombinant strain, reacted as expected with the wild-type F8 reference strain C1254 and also with strain SP7. So, the latter strain also expresses F8 fimbriae.

The F12 MAb M14-5 recognized the F12 fimbriae on the homologous wild-type strain C1979 but not the F12 fimbriae on the wild-type strain C134. This result could mean that the F12 fimbriae on strain C134 are expressed in very low numbers or that these F12 fimbriae are different from the F12 fimbriae on strain C1979. This last explanation would mean that there are two kinds of F12 fimbria. This possibility has already been suggested by the crossed-immunoelectrophoresis results for strain C1979 of Ørskov and Ørskov (12). Further studies are required before F12 fimbriae can be distinguished into $F12_1$ and $F12_2$ fimbriae.

From the results of the whole-bacterium ELISA we concluded that this method is the best one for the detection of P fimbriae on wild-type *E. coli* strains. This whole-bacterium ELISA is not more sensitive than the coagglutination method but has two major advantages: (i) all the immunoglobulins can be used in the ELISA, whereas in the coagglutination test only IgG MAbs can be used; (ii) the results of the ELISA can be read in an objective way, in contrast to the results of the coagglutination test.

In a fimbria ELISA we have demonstrated that MAb M2-1 reacted with $F7_2$ and F9 fimbriae (4; see Table 2). However, the reaction with the F9 fimbriae could neither be shown in the coagglutination test nor in the whole-bacterium ELISA. This result was probably caused by the weak reaction of MAb M2-1 with the F9 fimbriae.

Only the fimbriae of three SP strains (SP7, SP133, and SP101) could be determined with the MAbs. All SP strains were, just like the reference strains for fimbriae F7 to F13, isolated from urinary tract infections, and all expressed P fimbriae (14). However, the serotype of some SP strains (e.g., SP57 and SP88, O157:K^-) is not common among uropathogenic strains (17), and so it is possible that these strains also express rare fimbriae. Preliminary data on a limited number of our collection of uropathogenic *E. coli* strains showed that we could determine the P fimbriae on 80% of the strains with the MAbs used in this study. So, fimbriae F7 to F13 are probably the most common fimbriae among uropathogenic *E. coli*

With the whole-bacterium ELISA and the MAbs against the various P fimbriae we have developed an easy and fast method for the determination of P fimbriae among uropathogenic *E. coli*. With this method we have planned to do epidemiological studies on the distribution of P fimbriae among wild-type *E. coli* strains and to extend the O:K:H serotype with the F serotype.

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