JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1993, p. 558–564
0095-1137/93/030558-07$02.00/0

Vol. 31, No. 3

Characterization of Monoclonal Antibodies against Putative Colonization Factors of Enterotoxigenic *Escherichia coli* and Their Use in an Epidemiological Study

GLORIA INÉS VIBOUD,[1,2] NORMA BINSZTEIN,[2] AND ANN-MARI SVENNERHOLM[1]*

Department of Medical Microbiology and Immunology, University of Göteborg, Guldhedsgatan 10, S-41346 Göteborg, Sweden,[1] and Instituto Nacional de Microbiología "Carlos G. Malbrán," Velez Sarsfield 563, 1281 Buenos Aires, Argentina[2]

Received 26 October 1992/Accepted 11 December 1992

Monoclonal antibodies (MAbs) against five putative colonization factors (PCFs), i.e., colonization factor antigen (CFA)/III, coli surface antigen (CS)7 and CS17, PCFO159, and PCFO166 of enterotoxigenic *Escherichia coli* (ETEC), were produced. Hybridomas (one each) producing specific antibodies against the respective PCFs were selected. All the MAbs reacted with the corresponding fimbriae but not with CFA/I, CFA/II, or CFA/IV or the heterologous PCFs in bacterial agglutination and enzyme-linked immunosorbent assays (ELISAs). In immunoelectron microscopy these MAbs bound along the fimbriae, and they also reacted with the corresponding subunits in immunoblots. The five MAbs were used to evaluate the prevalence of CFA/III, CS7, CS17, PCFO159, and PCFO166 in ETEC strains isolated from children with diarrhea in Argentina. One hundred five ETEC isolates negative for CFA/I, CFA/II, and CFA/IV were tested in slide agglutination or in a dot blot test for spontaneously agglutinating strains; positive results were confirmed by inhibition ELISAs. It was found that 27% of the CFA-negative ETEC strains carried one of the PCFs. The sensitivity of slide agglutination with these MAbs was similar to that with specific polyclonal antisera; however, the specificity was higher. PCFO166 was found in 9.5% of the strains tested, mainly in ETEC of serogroup O78 producing heat-stable toxin alone. CS17 and CS7 were identified in 6.7 and 5.7%, respectively, of strains producing heat-labile toxin only, most of which belonged to serogroup O114. PCFO159 was found in 3.8% of the isolates tested, whereas CFA/III was detected in only one ETEC strain.

Enterotoxigenic *Escherichia coli* (ETEC) is an important cause of diarrhea, especially in children in developing countries (1, 4). The bacteria colonize the intestine by means of various colonization factor antigens (CFAs) and produce a heat-stable enterotoxin (ST) or a heat-labile enterotoxin (LT) or both. The best-characterized CFAs in human ETEC strains are CFA/I, CFA/II, and CFA/IV. CFA/I is a single antigen (11), while CFA/II has been shown to consist of three fimbrial antigens (31), the so-called *E. coli* surface antigens CS1, CS2, and CS3; CFA/IV is also an antigen complex comprising CS4, CS5, and CS6 (35). In a number of epidemiological studies in different countries, these CFAs have been found in 30 to 75% of clinical ETEC isolates (3, 5, 12). In addition, a number of putative colonization factors (PCFs) have been described and characterized in some detail, e.g., CFA/III, CS7, CS17, PCFO159, and PCFO166 (14, 15, 24, 25, 34). Other such factors are 2230, PCFO148, PCFO9, and 8786 (2, 6, 13, 18).

CFA/III, CS17, PCFO159, and PCFO166 are rod-like fimbriae of about 6 to 8 nm in diameter; CS7, on the other hand, has a helical structure similar to that of CS5 and is 3.5 to 6.5 nm wide (14, 15, 24, 25, 34). CFA/III, CS7, and PCFO159 each consist of one major fimbrial subunit (18, 21.5, and 19 kDa, respectively) (14, 15, 34). Two polypeptide bands of the same intensity are seen when PCFO166 is examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (17 and 15.5 kDa) (24), whereas one prominent band (17.5 kDa) together with a weak band (15.5 kDa) are seen in the case of CS17 (25). For expression, all the different PCFs require plasmid genes which usually are associated with enterotoxin-encoding genes (24, 25, 34).

Using a nonligated intestine experimental animal model (RITARD), we have shown that *E. coli* organisms expressing PCFO159, CS17, CS7, and CFA/III colonize rabbit intestine whereas bacteria expressing PCFO166 do not (33). However, bacteria expressing PCFO166 have been shown to adhere to human enterocytes in vitro (23).

Previous surveys of ETEC have shown that most of the CFAs or PCFs are associated with particular O serogroups and enterotoxin types (3, 26). Thus, PCFO159 has been found only in ST- and LT-producing strains of serotype O159:H4 or O159:H20 (26, 27, 34), and CFA/III has been found only in LT-producing strains of serotype O25:H16 or O25:H− (15, 26, 27). On the other hand, the other PCFs have been expressed in a number of different ETEC strains belonging to various serotypes, i.e., CS17 in LT-producing strains of serotypes O8:H9, O114:H21, and O167:H5 (25, 26); CS7 in LT-producing strains of serotypes O114:H49 or O114:H− and O103:H49 (26); and PCFO166 in ST- or LT- and ST-producing ETEC of serogroups O166, O20, O71, O78, and O98 (24, 26).

In other studies in which only CFA/I, CFA/II, and CFA/IV were tested, many ETEC organisms without CFAs were found (3, 5, 12). Since an ETEC vaccine for use in humans should contain the most prevalent CFAs (32), epidemiological studies of the different PCFs in ETEC isolated in various geographical areas should be undertaken. In this report, we describe the production and characterization of monoclonal antibodies (MAbs) against CFA/III, CS7, CS17, PCFO159, and PCFO166 and evaluate their usefulness for

* Corresponding author.

determination of the prevalence of these PCFs in fresh ETEC isolates from children with diarrhea in Argentina.

MATERIALS AND METHODS

Bacterial strains and culture conditions. The following reference strains from the Colindale strain collection (kindly provided by M. M. McConnell, Colindale, London, United Kingdom) were used in the different tests: 31-10A (LT+, CFA/III+, CS6+), 31-10B (LT−, CFA/III−, CS6−) (27), 334A (ST+, LT+, CS7+), 334C (ST−, LT−, CS7−) (7, 14), E20738A (LT+, CS17+), E20738B (LT−, CS17−) (25), 350C1A (ST+, LT+, PCFO159+), 350C1B (ST−, LT−, PCFO159−) (27, 34), E7476A (ST+, PCFO166+), E7476B (ST−, PCFO166−) (24). In addition, 105 ETEC strains that were negative for CFA/I, CFA/II, and CFA/IV and that had been isolated from children with diarrhea in different parts of Argentina were tested (3). The ETEC isolates, which had been maintained at −70°C in Trypticase soy broth (Difco, Detroit, Mich.) supplemented with 15% of glycerol, were cultured on CFA agar containing bile salts (24). Immediately before the analyses for PCFs, all strains were retested for enterotoxin production, since the loss of enterotoxigenicity is usually associated with the loss of CFAs (24, 34); all the strains produced an enterotoxin at the time of these analyses.

Purified antigens. CFA/III, CS7, CS17, PCFO159, and PCFO166 were purified as previously described for CFA fimbriae (10); further purifications were performed with cesium chloride gradients (17) or by affinity chromatography on Octyl Sepharose (15).

Polyclonal antisera. Antisera to CFA/III, CS7, CS17, PCFO159, and PCFO166 were produced by immunizing rabbits with the reference strains mentioned above and then absorbing the sera with the corresponding PCF-deficient mutants. Some sera were also produced by immunization with purified fimbriae.

Production of MAbs. MAbs against CFA/III, CS7, CS17, PCFO159, and PCFO166 were produced as described previously (8) by immunizing BALB/c mice with the respective purified fimbrial preparation. An initial intraperitoneal injection with 4 μg of purified antigen in Freund's complete adjuvant was followed by two intravenous injections with 4 μg of antigen without adjuvant 7 and 9 weeks later. Spleen cells from immunized mice were fused with exponentially grown F/0 myeloma cells by the aid of polyethylene glycol. Fused cells were distributed in eight microtiter plates (~750 wells; Nunc A/S, Roskilde, Denmark), and stable hybrid cells were selected by growth in Iscove medium–10% fetal calf serum containing hypoxanthine-aminopterine-thymidine. Fifteen days after fusion, culture fluids were tested for the production of specific antibodies against the respective purified fimbriae in an enzyme-linked immunosorbent assay (ELISA). In the initial screening, usually 10 to 20 hybrids producing antibodies in significant titers against the purified fimbriae were selected. Of these, three stable anti-PCF antibody-producing hybridomas were cloned and expanded by cultivation in 100 ml of Iscove medium–10% fetal calf serum in tissue culture bottles (Nunc). Culture fluids from established anti-PCF antibody-secreting hybridomas were harvested and frozen in aliquots at −30°C until further tested, and the specific anti-PCF-antibody-producing cells were frozen in liquid nitrogen for long-term storage (21).

Determination of the isotype and Ig concentration of MAbs. The isotypes of the different MAbs were determined by means of single radial immunodiffusion with mouse immunoglobulin (Ig) isotype-specific antisera (IgM, IgA, IgG1, IgG2a, IgG2b, and IgG3) by the method of Mancini et al. (22). The Ig concentration was determined by the same method by assaying an appropriate dilution of the MAb in isotype-specific anti-mouse Ig; preparations with known contents of the respective mouse Ig isotype were used as standards.

SDS-PAGE and immunoblotting. SDS-PAGE was carried out according to the method of Laemmli (19). Bacterial heat extracts (60°C, 20 min) and purified fimbrial preparations were tested by SDS-PAGE with 16% acrylamide. Protein profiles immobilized on nitrocellulose sheets were either stained with amido black (0.1%) or reacted with the anti-PCF MAbs. Bound antibodies were detected by using anti-mouse Ig labelled with peroxidase. Filters were developed with hydrogen peroxide substrate and 4-chloro-naphthol chromogen (Bio-Rad).

Electron microscopy. Bacterial cells grown on CFA agar with bile salts were harvested and applied on Formvar-carbon-coated grids (200 mesh). The coated grids were placed on drops with the respective MAb (diluted 1/2 in 1% bovine serum albumin [BSA] in phosphate-buffered saline [PBS] containing 0.05% Tween 20) for 15 min and subsequently on drops with gold-labelled anti-mouse Ig (Amersham International, Amersham, United Kingdom) diluted 1/10 in 1% BSA in PBS-Tween before negative staining with 1% ammonium molybdate was performed. Grids were then examined with an electron microscope (JEOL Ltd., Tokyo, Japan). For control purposes, all PCF-positive reference strains were tested for unspecific binding of gold particles with an irrelevant MAb against ST.

Serum agglutination test. Each strain was diluted in PBS to a concentration of 10^{10} organisms per ml. The suspension was applied in drops of 10 μl on a glass slide. To each drop, 10 μl of the respective MAb was added. The appearance of macroscopically visible agglutinates within 2 min was regarded as a positive reaction. Bacteria mixed with PBS were used as negative controls.

Dot blot test. All the strains that agglutinated spontaneously, i.e., in PBS, were analyzed for reactivity with specific anti-PCF MAbs in a dot blot test (3). Briefly, nitrocellulose filter paper (HAWP; 0.45-μm pore size; Millipore Corp., Bedford, Mass.) was soaked in saline solution, and 2 μl of bacterial heat extract (60°C, 20 min) was applied on the filter paper and left at 37°C for 60 min. The paper was washed several times with gentle agitation, and crude extracts containing PCF were visualized by ELISA with the appropriate MAb. Stained dots on a white background indicated positive results (3).

Inhibition ELISA. The capacity of different strains (in a concentration of 10^{10} bacteria per ml and fivefold dilutions thereof) to inhibit the binding of anti-PCF MAbs to the homologous solid-phase bound purified fimbriae was analyzed as previously described (21). Strains that inhibited binding of a MAb by ≥50% were regarded as positive for the corresponding PCF.

Serotyping. The ETEC strains were serotyped by Ida Ørskov at the International Escherichia and Klebsiella Centre, Statens Seruminstitut, Copenhagen, Denmark.

RESULTS

From a number of different hybridomas producing antibodies against the PCFs CFA/III, CS7, CS17, PCFO159, and PCFO166, one stable clone producing specific antibodies in high titers against each factor was selected for further

TABLE 1. Properties of Mabs raised against PCFs

MAb	Isotype	Ig concn (μg/ml)	ELISA titer[a]
CFA/III-3:3	IgG1	130	4,000
CS7-5:2	IgG1	93	600
CS17-8:1	IgG1	103	3,000
PCFO159-5:1	IgG2b	80	4,000
PCFO166-1:6	IgG1	78	4,000

[a] Titers against the homologous solid-phase-bound purified fimbrial antigen were determined as described previously (21).

studies. The isotype, Ig concentration, and ELISA titer of the MAbs against purified fimbriae are listed in Table 1.

The specificity of the MAbs for the corresponding PCFs was tested by various methods. The capacity of the MAbs to agglutinate bacteria expressing CFAs and PCFs was tested on glass slides. All the selected MAbs agglutinated ETEC expressing the homologous fimbriae but not bacteria possessing any of the established ETEC CFAs or any of the heterologous PCFs or the corresponding PCF-deficient strains. Furthermore, none of the MAbs agglutinated normal flora *E. coli* or heterologous enteropathogens, e.g., *Salmonella*, *Shigella*, or *Campylobacter* strains (data not shown). The same results were obtained when the different MAbs were tested for reactivity with the homologous and heterologous fimbriae in ELISAs. Thus, whereas the different MAbs reacted in ELISA titers varying between 600 and 4,000 against the homologous fimbriae (Table 1), none of the MAbs had a titer against a heterologous antigen that exceeded 5, i.e., the reciprocal of the initial dilution tested.

Crude heat extract preparations of the reference strains, i.e., the PCF-positive strains and the corresponding PCF-negative mutants, were subjected to SDS-PAGE and tested for reactivity with the different MAbs. As shown in Fig. 1, all five MAbs reacted with crude extracts of the strains expressing homologous fimbriae, giving a single band in the case of CFA/III, CS7, and PCFO159 or two bands in the case of PCFO166, which were of molecular weights similar to those of the bands developed when using the corresponding purified antigen preparations. The MAb against CS17 reacted with two bands of the crude extract preparation, one distinct band (17.5 kDa) and a much weaker one (15.5 kDa); however, only the most prominent band of CS17 (17 kDa) was revealed when using the purified fimbriae. In no instance were any significant bands developed with either of the MAbs when using crude extracts of the PCF-negative mutants.

The ability of the different MAbs to bind to native pili was directly demonstrated by immunogold labelling of whole bacteria. As shown by immunoelectron microscopy (Fig. 2), the colloidal gold particles were in all instances distributed along the length of the pili on bacteria bearing the corresponding PCF, indicating that all the MAbs recognize epitopes that are distributed along the pilus structures. On the other hand, an irrelevant MAb (anti-ST) did not bind to any of the five different PCF-positive reference strains (data not shown).

The capacity of the different MAbs to detect PCFs, i.e., CFA/III, CS7, CS17, PCFO159, and PCFO166, in clinical ETEC isolates was also analyzed with 105 ETEC strains that had been shown to be negative for CFA/I, CFA/II, and CFA/IV. These ETEC strains had been isolated from children with diarrhea in seven different parts of Argentina

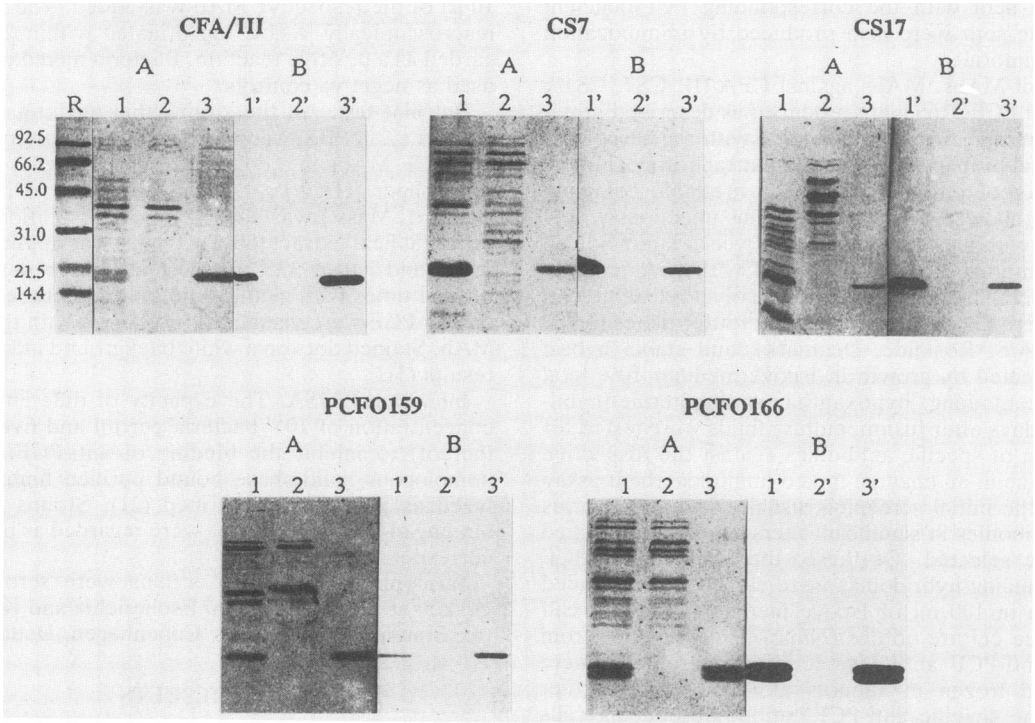

FIG. 1. SDS-PAGE (A) and immunoblotting (B) of bacterial heat extracts and purified fimbriae, using amido black (0.1%) staining for demonstration of the blotted protein bands, and the corresponding MAbs for immunological demonstration of the respective fimbrial subunits. R, low-molecular-mass references (in kilodaltons); 1 and 1', reference strain expressing the fimbriae; 2 and 2', corresponding fimbria-negative mutant; 3 and 3', purified fimbriae.

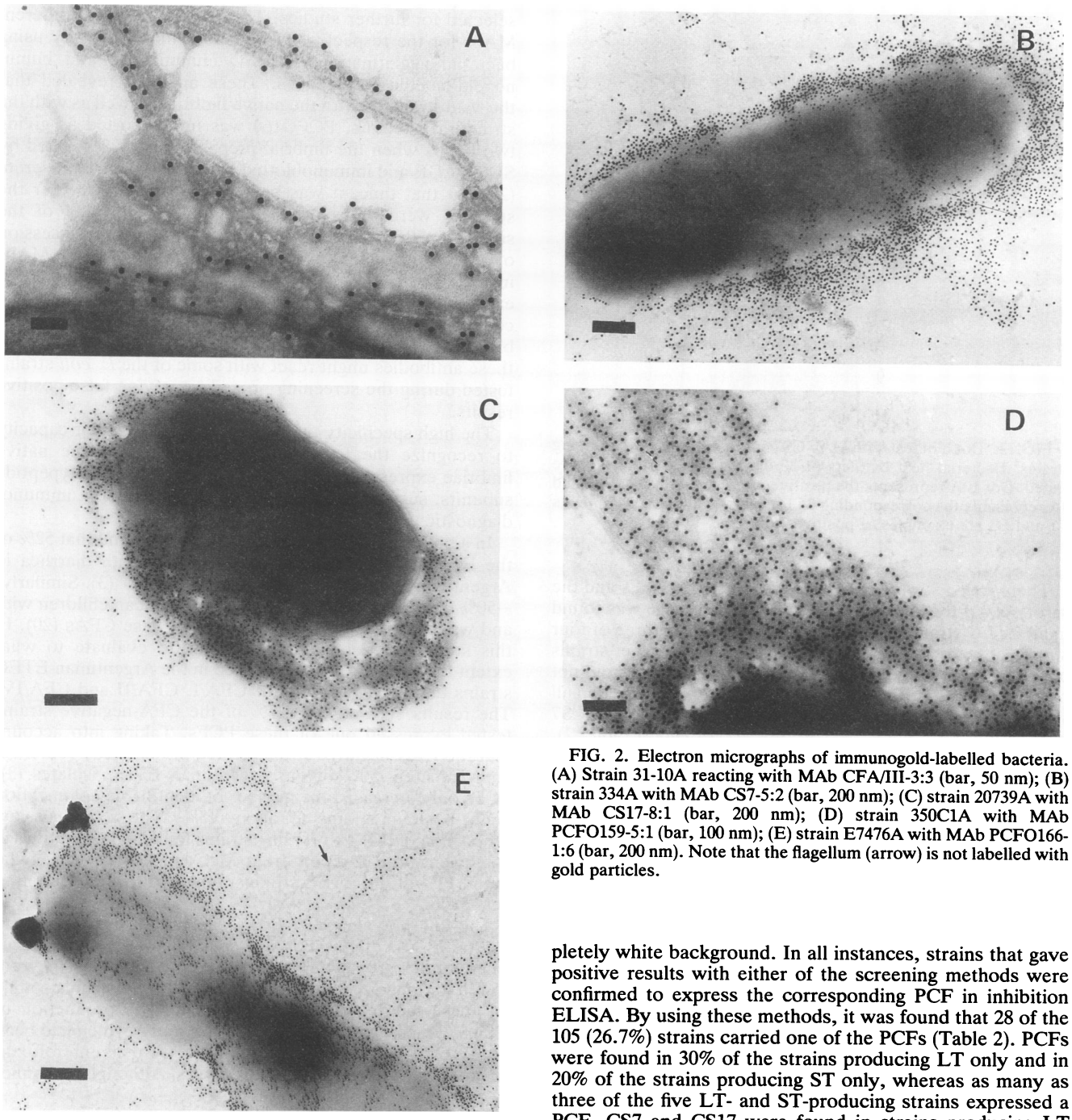

FIG. 2. Electron micrographs of immunogold-labelled bacteria. (A) Strain 31-10A reacting with MAb CFA/III-3:3 (bar, 50 nm); (B) strain 334A with MAb CS7-5:2 (bar, 200 nm); (C) strain 20739A with MAb CS17-8:1 (bar, 200 nm); (D) strain 350C1A with MAb PCFO159-5:1 (bar, 100 nm); (E) strain E7476A with MAb PCFO166-1:6 (bar, 200 nm). Note that the flagellum (arrow) is not labelled with gold particles.

during 18 months. The strains belonged to 28 different typeable serogroups, and 50 of them produced LT, 50 produced ST, and 5 produced both toxins. After the strains were grown on CFA agar in the presence of bile salts, they were tested for the respective PCF by slide agglutination with the different MAbs. Those strains that agglutinated spontaneously in PBS (28 strains) were screened for the different PCFs by a dot blot test. As exemplified in Fig. 3, positive results in the latter test were seen as strongly stained dots when the MAbs were used, whereas preparations lacking fimbriae were indistinguishable from a completely white background. In all instances, strains that gave positive results with either of the screening methods were confirmed to express the corresponding PCF in inhibition ELISA. By using these methods, it was found that 28 of the 105 (26.7%) strains carried one of the PCFs (Table 2). PCFs were found in 30% of the strains producing LT only and in 20% of the strains producing ST only, whereas as many as three of the five LT- and ST-producing strains expressed a PCF. CS7 and CS17 were found in strains producing LT only, whereas PCFO166 was expressed in strains that produced ST alone. PCFO159 was found in LT- or ST-producing ETEC strains (Table 2).

For control purposes, the strains were also tested for the five different PCFs by using specific polyclonal antisera, i.e., sera raised against purified fimbriae or whole bacteria and absorbed with the corresponding PCF-negative strain. All strains that agglutinated with the MAbs also reacted with the corresponding polyclonal antisera; in addition, two strains agglutinated with antiserum only. When these two strains were tested in immunodiffusion against polyclonal antisera (30), no identity band with the corresponding fimbriae was revealed, indicating falsely positive results.

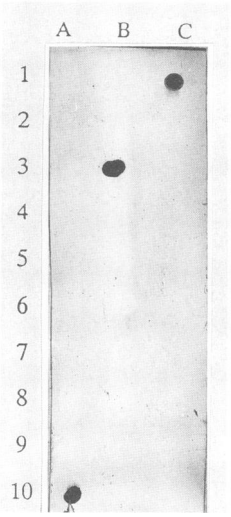

FIG. 3. Dot blot test with MAb CS7-5:2 to detect CS7-expressing strains. Bacterial heat extracts of 30 clinical ETEC isolates were tested. Dot 10A represents the positive reference strain (334A), and 9A represents the corresponding PCF-negative mutant (334C). Dots C1 and B3 are examples of positive reactions.

There was a strong association between the PCFs and the serotypes of the strains studied. Thus, PCFO166 was found in all ETEC strains of serotype O78:H− and in three of four strains of serotype O78:H18 (Table 2), whereas other strains of the same serogroup but with different H antigens did not produce these fimbriae. Similarly, CS17 was expressed in all strains of serotype O114:H21 and O114:H−, whereas CS7 was found in strains of serotype O114:H49 (Table 2). PCFO159 fimbriae, on the other hand, were expressed in ETEC belonging to different O groups, i.e., two LT- and ST-producing ETEC strains of serotype O159:H4 as well as one LT- and ST-producing strain of serotype O102:H10.

The remaining strains, which were negative for the PCFs tested, belonged to many different O groups, i.e., 7, 8, 9, 12, 15, 20, 25, 27, 29, 68, 78, 89, 92, 112ab, 133, 148, 153, 159, 160, 166, 170, and 172.

DISCUSSION

In this report, we have described the production and characterization of MAbs against CFA/III, CS7, CS17, PCFO159, and PCFO166. In each case, only one MAb that agglutinated bacteria expressing the corresponding PCF was selected for further studies. The specificity of the different MAbs for the respective PCFs was demonstrated by using bacterial agglutination, ELISA, immunoblot, and immunogold-labelling techniques. These analyses revealed that the MAbs reacted with the native fimbriae as well as with the structural subunits. PCFO166 was first reported as giving two bands when the fimbrial preparation was separated by SDS-PAGE and immunoblotted with a polyclonal antiserum (24). At that time, it was not possible to say whether the subunits were antigenically related. The reaction of the subunits with the specific MAb demonstrates the possession of a common epitope. The advantage of using the MAbs instead of specific polyclonal antisera to test for PCF-expressing bacteria is that rabbit or other animal sera may contain antibodies against *E. coli* antigens (e.g., outer membrane proteins and O antigens) already before immunization; these antibodies might react with some of the *E. coli* strains tested during the screening procedure, giving false-positive results.

The high specificity of the MAbs as well as their capacity to recognize the homologous PCFs, both as the native fimbriae expressed on the bacteria and as their polypeptide subunits, suggest that the MAbs may be useful in immunodiagnostic tests.

In a previous epidemiological study, we found that 52% of the ETEC strains isolated from children with diarrhea in Argentina carried CFA/I, CFA/II, or CFA/IV (3). Similarly, ~50% of ETEC strains isolated from Mexican children with and without diarrhea expressed one of these CFAs (20). In this study, we used different MAbs to evaluate to what extent the five PCFs are expressed in the Argentinian ETEC strains that were negative for CFA/I, CFA/II and CFA/IV. The results showed that 27% of the CFA-negative strains tested expressed one of these PCFs. Taking into account that the CFA-negative strains tested in this study represent approximately 50% of the Argentinian ETEC isolates (3), PCFs only increased the number of identifiable colonization factor-positive strains to about 13%. The relatively low proportion of PCF-expressing strains found in this study is in agreement with a report from McConnell et al. (26), who found 17% of the ETEC strains in their culture collection to bear PCF.

The low prevalence of PCFs can be attributed to many factors. A loss of plasmids that code for the PCFs can occur when strains are subcultured many times or when they are stored for long periods (29). However, most plasmids coding for these factors usually also code for the production of toxins (24, 34), and the strains in this study, which had been subjected to only a few subculturings, were all enterotoxin-positive at the time of the PCF analyses. Although we cannot

TABLE 2. Presence of PCFs in relationship to serotype in 105 ETEC isolates from children in Argentina

PCF	No. of strains producing:			Total (%) (n = 105)	Serotype(s)[a]
	LT	ST	LT and ST		
PCFO166	0	10	0	10 (9.5)	O78:H− (6), O78:H18 (3), O128:H27
CS17	7	0	0	7 (6.7)	O114:H21 (3), O114:H− (2), O128:H21, O12:H41
CS7	6	0	0	6 (5.7)	O114:H49 (2), O128:H49, rough:H49, O78:H10, O127:H40.
PCFO159	1	0	3	4 (3.8)	O159:H4 (2), O102:H10 (2)
CFA/III	1	0	0	1 (0.9)	O?:H−
Total no. of positive strains	15	10	3	28 (26.7)	

[a] Parentheses indicate the number of strains from each serotype, if more than one.

rule out occasional losses of PCFs without losses of toxin (29), the facts that the PCFs have been found to be closely associated with certain serotypes and that almost all the strains belonging to these serotypes possess the corresponding fimbriae suggest that the low prevalence of PCF-carrying strains was not due to the loss of PCF-encoding genes.

In the present study, we found PCFs in strains of serotypes that so far had not been associated with any CFAs in Argentinian isolates, i.e., O78:H18 and -H− and O114:H49, -H21, and -H−. The low prevalence of CFA/III-bearing strains in this study, which is in agreement with previous findings (27), is due to the fact that the O25 serogroup, which is related to CFA/III, is very rare among Argentinian ETEC isolates. Despite the fact that CFA/III-expressing strains have hitherto been detected exclusively in CS6-positive strains producing LT only (15, 23, 27), we previously found that none of the CS6-positive, LT-producing ETEC strains that we isolated in Argentina expressed CFA/III (16).

It has been suggested that an ETEC vaccine for humans should contain the most prevalent CFAs (32). Therefore, it is important to know the prevalence of these factors in ETEC in various geographical areas. The MAbs described in this article may be used in different epidemiological studies in which the prevalence of PCFs on ETEC will be evaluated.

Since expression of one or more adhesins is probably required to enable colonization of ETEC in the small intestine, it is likely that a substantial number of ETEC strains may possess fimbriae other than the CFAs and the PCFs of the present study, e.g., PCFO9, PCFO148, 2230, 8776, or some colonization factor which has not yet been identified. We were not able to test for the above-mentioned factors, since the reference strains were not available at the time of the study. However, when strains lacking CFAs and PCFs were tested for adhesion to intestinal epithelial cells and in electron microscopy to search for "new" colonization factors, an ETEC strain which expressed typical rod-like fimbriae which were immunologically distinct from all previously reported adhesive factors in humans was found (36), indicating the presence of a hitherto-not-described type of adhesin.

ACKNOWLEDGMENTS

We gratefully acknowledge the skilled technical assistance of Ella Olofsson and Gudrun Wiklund. We thank R. Notario, A. M. Picandet, M. Vergara, E. Patrito, O. Nader, D. Maurel, and M. Szefner for supplying the *E. coli* strains. We also thank Moyra McConnell for critically reviewing the manuscript.

Financial support was obtained from the Swedish Agency for Research Cooperation with Developing Countries (SAREC), the Secretariat of Science and Technology (SECYT), Argentina, and the Swedish Medical Research Council (grant 16X-09084).

REFERENCES

1. **Agüero, M. E., L. Reyes, V. Prado, I. Ørskov, F. Ørskov, and F. C. Cabello.** 1985. Enterotoxigenic *Escherichia coli* in a population of infants with diarrhea in Chile. J. Clin. Microbiol. **22:**576–581.
2. **Aubel, D., A. Darfeuille-Michaud, and B. Joly.** 1991. New adhesive factor (antigen 8786) on a human enterotoxigenic *Escherichia coli* O117:H4 strain isolated in Africa. Infect. Immun. **59:**1290–1299.
3. **Binsztein, N., M. J. Jouve, G. I. Viboud, L. L. Moral, M. Rivas, I. Ørskov, C. Århén, and A.-M. Svennerholm.** 1991. Colonization factors of enterotoxigenic *Escherichia coli* isolated from children with diarrhea in Argentina. J. Clin. Microbiol. **29:**1893–1898.
4. **Black, R. E.** 1986. The epidemiology of cholera and enterotoxigenic E. coli diarrheal disease, p. 23–32. *In* J. Holmgren, A. Lindberg, and R. Möllby (ed.), Development of vaccines and drugs against diarrhea. 11th Nobel Conference. Studentlitteratur, Lund, Sweden.
5. **Changchawalit, S., P. Echeverria, D. N. Taylor, U. Leksomboon, C. Tirapat, B. Eampokalap, and B. Rowe.** 1984. Colonization factors associated with enterotoxigenic *Escherichia coli* isolated in Thailand. Infect. Immun. **45:**525–527.
6. **Darfeuille-Michaud, A., C. Forestier, B. Joly, and R. Cluzel.** 1986. Identification of a nonfimbrial adhesive factor of an enterotoxigenic *Escherichia coli* strain. Infect. Immun. **52:**468–475.
7. **Deneke, C. F., G. M. Thorne, and S. L. Gorbach.** 1981. Serotypes of attachment pili of enterotoxigenic *Escherichia coli* isolated from humans. Infect. Immun. **32:**1254–1260.
8. **De St. Groth, S. F., and D. Scheidegger.** 1980. Production of monoclonal antibodies. Strategy and tactics. J. Immunol. Methods **35:**1–212.
9. **Evans, D. G., and D. J. Evans, Jr.** 1978. New surface-associated heat-labile colonization factor antigen (CFA/II) produced by enterotoxigenic *Escherichia coli* of serogroups O6 and O8. Infect. Immun. **21:**638–647.
10. **Evans, D. G., D. J. Evans, Jr., S. Clegg, and J. A. Pauley.** 1979. Purification and characterization of the CFA/I antigen of enterotoxigenic *Escherichia coli*. Infect. Immun. **25:**738–748.
11. **Evans, D. G., R. P. Silver, D. J. Evans, Jr., D. G. Chase, and S. L. Gorbach.** 1975. Plasmid-controlled colonization factor associated with virulence in *Escherichia coli* enterotoxigenic for humans. Infect. Immun. **12:**656–667.
12. **Gotherfors, L., C. Åhrén, B. Stoll, D. K. Barua, F. Orskov, M. A. Salek, and A.-M. Svennerholm.** 1985. Presence of colonization factor antigens on fresh isolates of fecal *Escherichia coli*: a prospective study. J. Infect. Dis. **152:**1128–1133.
13. **Heuzenroeder, M. W., T. R. Elliot, C. J. Thomas, R. Halter, and P. A. Manning.** 1990. A new fimbrial type (PCFO9) on enterotoxigenic *Escherichia coli* O9:H− LT+ isolated from a case of diarrhea in central Australia. FEMS Microbiol. Lett. **66:**55–60.
14. **Hibberd, M. L., M. M. McConnell, A. M. Field, and B. Rowe.** 1990. The fimbriae of human enterotoxigenic *Escherichia coli* strain 334 are related to CS5 fimbriae. J. Gen. Microbiol. **136:**2449–2456.
15. **Honda, T., M. Arita, and T. Miwatani.** 1984. Characterization of new hydrophobic pili of human enterotoxigenic *Escherichia coli*: a possible new colonization factor. Infect. Immun. **43:**959–965.
16. **Jouve, M., N. Binsztein, G. I. Viboud, M. Vergara, I. Ørskov, and A.-M. Svennerholm.** Colonization factor antigens and putative colonization factors in enterotoxigenic *Escherichia coli* isolated from children with and without diarrhea: a prospective study. Submitted for publication.
17. **Knutton, S., D. R. Lloyd, D. C. A. Candy, and A. S. McNeish.** 1984. Ultrastructural study of adhesion of enterotoxigenic *Escherichia coli* to erythrocytes and human intestinal epithelial cells. Infect. Immun. **44:**519–527.
18. **Knutton, S., D. R. Lloyd, and A. S. McNeish.** 1987. Identification of a new fimbrial structure in enterotoxigenic *Escherichia coli* (ETEC) serotype O148:H28 which adheres to human intestinal mucosa: a potentially new human ETEC colonization factor. Infect. Immun. **55:**86–92.
19. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
20. **López-Vidal, Y., J. J. Calva, A. Trujillo, A. Ponce de Léon, A. Ramos, A.-M. Svennerholm, and G. M. Ruiz-Palácios.** 1990. Enterotoxins and adhesins of enterotoxigenic *Escherichia coli*: are they risk factors for acute diarrhea in the community? J. Infect. Dis. **162:**442–447.
21. **Lopez-Vidal, Y., P. Klemm, and A.-M. Svennerholm.** 1988. Monoclonal antibodies against different epitopes on colonization factor antigen I of enterotoxin-producing *Escherichia coli*. J. Clin. Microbiol. **26:**1967–1972.
22. **Mancini, G., A. O. Carbonara, and J. F. Heremans.** 1965. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry **2:**235–254.

23. **McConnell, M. M.** 1991. Newly characterized putative colonization factors of human enterotoxigenic *Escherichia coli*, p. 79–85. *In* T. Wadström, H. Mäkelä, A.-M. Svennerholm, and H. Wolfe-Watz (ed.), Molecular pathogenesis of gastrointestinal infections. Plenum, London.
24. **McConnell, M. M., H. Chart, A. M. Field, M. Hibberd, and B. Rowe.** 1989. Characterization of a putative colonization factor (PCFO166) of enterotoxigenic *Escherichia coli* of serogroup O166. J. Gen. Microbiol. **135:**1135–1144.
25. **McConnell, M. M., M. Hibberd, A. M. Field, H. Chart, and B. Rowe.** 1990. Characterization of a new colonization factor (CS17) from a human enterotoxigenic *Escherichia coli* of serotype O114:H21 which produces only heat-labile enterotoxin. J. Infect. Dis. **161:**343–347.
26. **McConnell, M. M., M. L. Hibberd, M. E. Penny, S. M. Scotland, T. Cheasty, and B. Rowe.** 1991. Surveys of human enterotoxigenic *Escherichia coli* from three different geographical areas for possible colonization factors. Epidemiol. Infect. **106:**477–484.
27. **McConnell, M. M., and B. Rowe.** 1989. Prevalence of putative colonization factors CFA/III and PCFO159 in enterotoxigenic *Escherichia coli*. J. Infect. Dis. **159:**582–586.
28. **McConnell, M. M., L. V. Thomas, N. P. Day, and B. Rowe.** 1985. Enzyme-linked immunosorbent assays for the detection of adhesion factor antigens of enterotoxigenic *Escherichia coli*. J. Infect. Dis. **152:**1120–1127.
29. **McConnell, M. M., L. V. Thomas, G. A. Willshaw, H. R. Smith, and B. Rowe.** 1988. Genetic control and properties of coli surface antigens of colonization factor IV (PCF8775) of enterotoxigenic *Escherichia coli*. Infect. Immun. **56:**1974–1980.
30. **Ouchterlony, Ö., and L. Å. Nilson.** 1978. Immunodiffusion and immunoelectrophoresis, p. 1–44. *In* D. M. Weir (ed.), Handbook of experimental immunology, 3rd ed. Blackwell, Oxford.
31. **Smyth, C. J.** 1982. Two mannose-resistant haemagglutinins on enterotoxigenic *Escherichia coli* of serotype O6.K15.H16 or H^- isolated from travellers and infantile diarrhoea. J. Gen. Microbiol. **128:**2081–2096.
32. **Svennerholm, A.-M., J. Holmgren, and D. A. Sack.** 1989. Development of oral vaccines against enterotoxinogenic *Escherichia coli* diarrhoea. Vaccine **7:**196–198.
33. **Svennerholm, A.-M., M. M. McConnell, and G. Wiklund.** Roles of different putative colonization factor antigens in colonization of human enterotoxigenic *Escherichia coli* in rabbits. Microb. Pathog., in press.
34. **Tacket, C. O., D. R. Maneval, and M. M. Levine.** 1987. Purification, morphology, and genetics of a new fimbrial putative colonization factor of enterotoxigenic *Escherichia coli* O159:H4. Infect. Immun. **55:**1063–1069.
35. **Thomas, L. V., M. M. McConnell, B. Rowe, and A. M. Field.** 1985. The possession of three novel coli surface antigens by enterotoxigenic *Escherichia coli* strains positive for the new putative colonization factor PCF8775. J. Gen. Microbiol. **131:** 2319–2326.
36. **Viboud, G. I., N. Binsztein, M. Jouve, and A.-M. Svennerholm.** 1992. New fimbrial putative colonization factor on human enterotoxigenic *Escherichia coli*, D-281, p. 142. Abstr. 92nd Gen. Meet. Am. Soc. Microbiol. 1992. American Society for Microbiology, Washington, D.C.