Monoclonal Antibodies Specific for the Bundle-Forming Pilus of Enteropathogenic *Escherichia coli*

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The bundle-forming pilus (BFP) produced by enteropathogenic *Escherichia coli* (EPEC) is associated with the presence of a large EPEC adherence factor plasmid and the formation of localized adherence clusters on tissue culture cells. Three mouse monoclonal antibodies (ICA2, ICA3, and ICA4) were produced against BFP purified from EPEC B171 (O111:NM). These monoclonal antibodies reacted in immunoblots with different epitopes of the 19.5-kDa bundlin subunit of BFP of heterologous EPEC. These reagents could serve as diagnostic tools for the identification of EPEC as well as for studying the role of BfpA in the interaction of EPEC with eukaryotic cells.

Enteropathogenic *Escherichia coli* (EPEC) continues to be an important cause of infantile diarrhea in developing countries (7, 10, 16, 26). EPEC encompasses the classical serotypes of *E. coli* that have been epidemiologically implicated in diarrheal disease and that lack the ability to elaborate heat-labile or heat-stable enterotoxins (26). Most epidemic EPEC strains of certain serotypes isolated from children with intestinal infections adhere to tissue culture cells in tight clusters or microcolonies in a pattern termed localized adherence (LA) (6, 10, 26, 36). The LA phenotype is associated with the presence of a 50- to 60-MDa plasmid (2), referred to as the EPEC adherence factor (EAF) plasmid (27, 31). Both the LA and the EAF plasmid are useful epidemiological markers and indicators of EPEC pathogenicity in vivo (26).

The mechanisms by which EPEC causes diarrhea are just beginning to be understood (25). Intimate adhesion to the intestinal mucosa and effacement of the epithelial microvilli, the so-called attaching and effacing (A/E) lesion, are important features of EPEC pathogenicity (21, 30). EPEC intimate attachment to the eukaryotic cell is attributed to a 94-kDa outer membrane protein termed intimin, identified as the product of the *eaeA* (*E. coli* attaching and effacing) chromosomal locus in strain E2348/69 (O127:H6) (21). Another chromosomal locus termed *eaeB*, located 5 kbp downstream of the *eaeA* gene, was characterized and found to be associated with the formation of A/E lesions and to be essential for signal transduction in epithelial cells (8, 11). Cytoskeleton rearrangements, increases in intracellular calcium, and tyrosine phosphorylation are consequences of EPEC infection, at least in in vitro studies (3, 23, 28, 35).

A 35-kbp genetic chromosomal locus of enterocyte effacement (LEE) containing several regions implicated in the formation of the A/E lesions has been identified in EPEC (29). The LEE is also conserved among other species that produce the A/E lesions, namely, enterohemorrhagic *E. coli*, RDEC-1 (an *E. coli* pathogen of rabbits), *Citrobacter freundii* biotype 4280, and *Hafnia alvei* (29). Within the LEE, four genes have been identified whose predicted protein sequences are similar to components of a recently described secretory pathway (type

III) responsible for exporting proteins lacking a typical signal sequence (20). These genes were designated *sepA*, *sepB*, *sepC*, and *sepD* (*sep* for secretion of *E. coli* proteins). A *sepB* mutant derivative of EPEC E2348/69, CVD 452, was defective in secretion of several polypeptides, formation of A/E lesions, and protein tyrosine phosphorylation in tissue culture cells, suggesting that EPEC Sep proteins are components of a type III secretory apparatus necessary for the export of virulence determinants (20).

Recently, an inducible bundle-forming pilus (BFP) encoded by the EAF plasmid was described in EPEC exhibiting LA (14). Expression of the BFP and the presence of the gene encoding the bundlin structural subunit (*bfpA*) have been correlated with the LA property (13, 14). Although some workers reported that fimbriae are not involved in the adherence of EPEC to HEp-2 cells (37), other studies suggested that several fimbrial adhesins are necessary for adherence and manifestation of A/E lesions (12, 15, 22). For instance, rod-like pili may serve initially to tether bacteria to the epithelial cell surface while the BFP expressed by EPEC growing on this surface participate in the formation of the bacterial microcolony by forming bundles that link one bacterium to another (14, 15, 22). Although the contribution of BFP to EPEC pathogenesis remains uncertain, the correlation between BFP expression and LA and the sequence similarities between BfpA and the type IV pili of some gram-negative pathogens suggest an association of BFP with virulence (13, 14, 18).

For many laboratories in developing countries, the use of radioactive DNA probes or tissue culture assays for identification of EPEC in childhood diarrhea is not feasible. Recently, a PCR-based assay to detect the *bfpA* gene of EPEC was reported (17). The fluorescent-actin staining test, which detects polymerized actin at the sites of A/E lesions produced by EPEC adhering to tissue culture cells (23), does not necessarily correlate with the presence of the EAF plasmid or LA (10, 38). Since BFP appears to be a common antigen of EPEC harboring the EAF plasmid and exhibiting LA, our objective was to develop monoclonal antibodies (MAbs) which could be used as reagents in epidemiological studies of EPEC infection. Furthermore, these reagents would be useful in mapping antigenic and functional domains of BFP to study its significance in the adherence and in vivo pathogenesis of EPEC infection.

Toward this aim, BFP was purified from EPEC strain B171 (O111:NM), an isolate from an outbreak of infantile diarrhea

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Anti-BFP

FIG. 1. Immunoblotting analysis of whole-cell lysates of heterogeneous EPEC to detect the bundlin subunit of BFP by using polyclonal anti-BFP antibodies. Lanes: 1, 281; 2, E40; 3, E18; 4, 110AC; 5, 015-1; 6, 5328; 7, B171; 8, E2348/69; 9, purified BFP. The positions of molecular mass markers (in kilodaltons) are shown to the left of the gel.

in Seattle, Washington (32), by procedures described earlier (14) and was used for MAb production and screening (33). Female BALB/c mice were immunized with 25 μ g of BFP four times at weekly intervals as described previously (34). Protein concentration was estimated by the method of Bradford (4). Spleen cells from two immunized BALB/c mice were fused with SP2/O myeloma cells (5). Bacteria were cultured on heart infusion agar (Gibco) overnight at 37°C, washed in phosphate-buffered saline (PBS) (Sigma; pH 7.2), and used in an enzyme-linked immunosorbent assay (ELISA) as previously described (1). Supernatant fluids harvested from hybridomas were tested in an ELISA (34) for reactivities to BFP (1 μ g/ml), EPEC B171, and *E. coli* K-12. The hybridomas secreting antibodies specific for BFP were cloned twice by limiting dilutions. The ascitic fluids produced in pristane-primed mice were diluted in PBS-Tween 20 (0.05%) containing 0.1% bovine serum albumin and screened for reactivities to BFP and other antigens in an ELISA. Purified Shiga toxin (9) and monoclonal (MAb) ICL8 of immunoglobulin G1 (IgG1) isotype specific for lipopolysaccharide of *Shigella dysenteriae* type 1 (34) were used to check for nonspecific reactions. To determine the isotypes of MAbs, supernatants obtained from hybridoma serum-free-medium were tested in an ELISA (5).

Three specific and stable clones ICA2, ICA3, and ICA4 of the IgG1 isotype were obtained. The titers of ascitic fluids corresponding to the four MAbs varied between 1.5×10^5 and 1.6×10^7 against BFP. All MAbs reacted in an ELISA with EPEC strains B171, E2348/69, and 281 (O111:NM). They did not react with *E. coli* K-12 or with Shiga toxin (data not shown).

To assess the usefulness of these MAbs in the identification of *bfpA*⁺ EPEC isolates, whole-cell lysates of EAF⁺, *bfpA*⁺, and LA⁺ EPEC strains were reacted with these MAbs in immunoblots as previously described (13). The strains were grown overnight at 37°C in Eagle's minimal essential medium supplemented with 10% fetal bovine serum for enhanced production of BFP (13, 14). One milliliter of this culture was spun and the cell pellet was boiled in sodium dodecyl sulfate-polyacrylamide gel sample buffer for 5 min (24). Following electrophoresis in 16% polyacrylamide gels and electroblotting (39), the membranes were reacted with a rabbit polyclonal antibody (used as positive control) or mouse MAbs (ascitic fluids at 1:250 dilutions) against BFP and then with species-specific secondary antibodies conjugated to alkaline phosphatase (Sigma, St. Louis, Mo.). The reaction was visualized by a mixture of 5-bromo-4-chloro-3-indolylphosphate and Nitro Blue Tetrazolium (Sigma). Rabbit polyclonal anti-BFP serum recognized a polypeptide in heterologous EPEC that migrated with an apparent molecular mass of 19.5 kDa, which corre-

TABLE 1. Bacterial strains and their reactivities with anti-BFP MAbs in immunoblots^a

Strain	LA ^b	<i>bfpA</i> probe ^b	Anti-BFP (polyclonal)	Reactivity ^c		
				MAb (isotype)		
				ICA2 (IgG1)	ICA3 (IgG1)	ICA4 (IgG1)
B171	+	+	+	+	+	+
B171(LB) ^d	+	+	—	—	—	—
E2348/69	+	+	+	+	+	+
JPN15 ^e	—	—	—	—	—	—
HS4(pMAR7) ^f	+	+	+	+	+	+
004-1	+	+	+	+	+	+
54-5	+	+	+	—	—	—
O15-1	+	+	+	—	+	+
034-01	+	+	+	+/-	+	+
040-01	+	+	+	+/-	+	+
167A	+	+	+	+	+	+
146A	+	+	—	+	+	+
118B	+	+	+	+/-	+	+
80A	+	+	+	+	+	+
110AC	+	+	+	—	+	+
37A	+	+	+	+	+	+
6Mex	+	+	+	+	+	+
281	+	+	+	+	+	+
5328	+	+	+	—	+	+
3336-54	+	+	+	—	+	+
659-79	+	+	+	+	+	+
2517-80	+	+	+	—	+	+
5513-56	+	+	+	+	+	+
E18	—	+	+/-	—	—	—
E40	—	+	—	—	—	—
<i>E. coli</i> K-12 DH5 α	—	—	—	—	—	—
<i>E. coli</i> HS4	—	—	—	—	—	—
ETEC E9034A	—	—	—	—	—	—

^a All strains were grown in Eagle's minimal essential medium at 37°C. Whole-cell lysates were electrophoresed and analyzed by immunoblotting.

^b The positivity (+) or negativity (—) of LA and the *bfpA* probe is indicated.

^c Symbols: +, strong reaction; +/-, weak reaction; —, negative.

^d B171 grown in Luria broth.

^e JPN15 is a plasmidless derivative of E2348/69.

^f HS4(pMAR7) is an *E. coli* K-12 strain carrying the 60-MDa EAF plasmid.

sponds to BfpA (Fig. 1). It did not react with EPEC E40 which is a LA[—] *bfpA*⁺ strain (Fig. 1; Table 1). Thus, the phenotypic expression of BfpA is a useful marker of LA positivity. This antiserum reacted with all but one LA⁺ *bfpA*⁺ EPEC strains,

FIG. 2. Immunoblotting analysis of whole-cell lysates of heterogeneous EPEC to detect the bundlin subunit of BFP by using MAb ICA3. Lanes: 1, purified BFP from strain B171; 2, 034-01; 3, 040-01; 4, 167A; 5, 146A; 6, 54-5; 7, 118B; 8, 110AC; 9, 015-1; 10, prestained mass standards; 11, *E. coli* K-12 strain DH5 α ; 12, *E. coli* K-12 strain HS4(pMAR7); 13, E2348/69; 14, B171; 15, B171 grown in Luria broth; 16, JPN15; 17, ETEC E9034A. The positivity (+) or negativity (—) of LA and the presence (+) or absence (—) of reactivity with MAb ICA3 are shown under the gel.

FIG. 3. Immunoblotting analysis of whole-cell lysates of heterologous EPEC to detect the bundlin subunit of BFP by using MAb ICA4. Lanes: 1, 6Mex; 2, 37A; 3, 281; 4, 5328; 5, 3336-54; 6, HS4; 7, 659-79; 8, 2517-80; 9, 5513-56; 10, 004-1; 11, 80A. The positivity (+) or negativity (-) of LA and the presence (+) or absence (-) of reactivity with MAb ICA4 are shown under the gel.

suggesting the existence of serotype-specific epitopes on BFP. The lack of reactivity may also be explained by the loss of an epitope caused by genetic variation within the *bfpA* sequence or by the loss of DNA sequences. We have previously reported size variations (18 to 21 kDa) among the pilin subunits of BFP (13). Thus, it is possible that certain epitopes are not universal among EPEC BFPs.

MAbs ICA3 and ICA4 showed identical reactivities: they recognized the bundlin subunit (19.5 kDa) in 19 of 20 (95%) LA⁺ wild-type EPEC strains (Fig. 2 and 3 and Table 1). The epitope recognized by MAb ICA2 appears to be widely distributed but less common among EPEC BFPs, since only 14 of 20 (70%) LA⁺ strains reacted with ICA2 (Table 1). These Western blot (immunoblot) results were confirmed in repeated experiments. No reactivity was observed by using these MAbs with *E. coli* K-12 or with non-EPEC *E. coli* (Fig. 2; Table 1). These data demonstrate the presence of common and less common epitopes among BFP produced by heterologous EPEC.

Mouse MAbs against BFP were reacted with BFP produced by EPEC B171 and E2348/69 to determine their reactivities to native epitopes by immunoelectron microscopy and gold labeling as previously described (13, 14). Although the MAbs reacted with the denatured bundlin subunit in immunoblots, none of them reacted with native epitopes of BFP produced by these strains (Fig. 4). Other workers have found that some surface proteins (for example, flagellin) had to be heat denatured or unfolded in order to show serological activity with the antibody (19). Thus, it is possible that the epitopes recognized by anti-BFP MAbs become accessible to antibody only under denaturing conditions. Anti-BFP MAbs were selected on the basis of their reactivities in an ELISA which employed BFP, which was also denatured during purification.

To test the effects of BFP MAbs on the adherence of EPEC to HEp-2 cells, different dilutions of antibodies (1:10, 1:25, 1:50, and 1:100) were incubated with heterologous EPEC strains E2348/69, B171, and 281 for 20 min prior to the HEp-2 adherence assay described above. The mixture was added to HEp-2 cells containing the same dilutions of antibodies. After 3 h of infection, the wells were washed and the cell-associated bacteria were visualized by Giemsa staining. The difference in the number (or size) of bacterial clusters per cell or in the number of infected cells in the wells containing antibodies was recorded and compared with the value for the positive control which contained no antisera. One hundred cells per sample were randomly counted to enumerate cell infectivity in the absence or presence of MAbs. Of the three anti-BFP MAbs produced, only ICA2 affected the LA pattern exhibited by E2348/69 in a dose-dependent manner. None or only a few small bacterial clusters (two to four bacteria) and a few individual bacteria adhering diffusively were seen at the highest

FIG. 4. Electron micrograph showing BFP expressed by EPEC E2348/69 and reacted with MAb ICA1 and 10-nm-diameter gold particles conjugated to protein A. No gold labelling is seen on BFP or the bacterial surface. Bar, 0.35 μ m.

concentration of ICA2 used (1:10) (data not shown). The number and the size of bacterial clusters increased with the dilution of ICA2. However, the effect of this MAb is intriguing, since it did not recognize BFP by immunogold labelling.

In summary, we have developed MABs with broad reactivities against BFPs of heterologous EPEC serotypes, which could aid in the identification of BFP-producing EPEC isolated from clinical specimens, especially in less well-equipped laboratories in developing countries. In addition, these reagents could prove useful in studying the functions of BFP antigenic determinants and the interaction of BFP with eukaryotic cells.

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REFERENCES

- Albert, M. J., M. Ansaruzzaman, S. M. Faruque, P. K. Neogi, K. Haider, and S. Tzipori. 1991. An ELISA for the detection of localized adherent classic enteropathogenic *Escherichia coli* serogroups. *J. Infect. Dis.* **164**:986–989.
- Baldini, M. M., J. B. Kaper, M. M. Levine, D. C. A. Candy, and H. W. Moon. 1983. Plasmid-mediated adhesion in enteropathogenic *Escherichia coli*. *J. Pediatr. Gastroenterol. Nutr.* **2**:534–538.
- Baldwin, T. J., W. Ward, A. Aitken, S. Knutton, and P. H. Williams. 1991. Elevation of intracellular calcium levels in HEP-2 cells infected with enteropathogenic *Escherichia coli*. *Infect. Immun.* **59**:1599–1604.
- Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye-binding. *Anal. Biochem.* **72**:248–254.
- Carlin, N. I. A., and A. A. Lindberg. 1986. Monoclonal antibodies specific for *Shigella flexneri* lipopolysaccharides: clones binding to type I and type III: 6,7,8 antigens, group 6 antigen, and a core epitope. *Infect. Immun.* **53**:103–109.
- Cravioto, A., R. J. Gross, S. M. Scotland, and B. Rowe. 1979. An adhesive factor found in strains of *Escherichia coli* belonging to the traditional infantile enteropathogenic serotypes. *Curr. Microbiol.* **3**:95–99.
- Cravioto, A., A. Tello, A. Navarro, J. Ruiz, H. Villafán, F. Uribe, and C. Esclava. 1991. Association of *Escherichia coli* HEP-2 adherence patterns with type and duration of diarrhoea. *Lancet* **337**:262–264.
- Donnenberg, S. S., J. Yu, and J. B. Kaper. 1993. A second chromosomal gene necessary for intimate attachment of enteropathogenic *Escherichia coli* to epithelial cells. *J. Bacteriol.* **175**:4670–4680.
- Donohue-Rolfe, A., G. T. Keusch, C. Edson, D. Thorley-Lawson, and M. Jacewicz. 1984. Pathogenesis of *Shigella* diarrhea. IX. Simplified high-yield purification of *Shigella* toxin and characterization of subunit specific monoclonal and polyclonal antibodies. *J. Exp. Med.* **160**:1767–1781.
- Echeverria, P., F. Ørskov, I. Ørskov, S. Knutton, F. Scheutz, J. E. Brown, and U. Lexomboon. 1991. Attaching and effacing enteropathogenic *Escherichia coli* as a cause of infantile diarrhea in Bangkok. *J. Infect. Dis.* **164**:550–554.
- Foubister, V., I. Rosenshine, M. S. Donnenberg, and B. B. Finlay. 1994. The *eaeB* gene of enteropathogenic *Escherichia coli* is necessary for signal transduction in epithelial cells. *Infect. Immun.* **62**:3038–3040.
- Francis, C. L., A. Jerse, J. B. Kaper, and S. Falkow. 1991. Characterization of interactions of enteropathogenic *Escherichia coli* O127:H6 with mammalian cell in vitro. *J. Infect. Dis.* **164**:693–703.
- Girón, J. A., M. S. Donnenberg, W. C. Martin, K. G. Jarvis, and J. B. Kaper. 1993. Distribution of the bundle-forming pilus structural gene (*bfpA*) among enteropathogenic *Escherichia coli* (EPEC). *J. Infect. Dis.* **168**:1037–1041.
- Girón, J. A., A. S. Y. Ho, and G. K. Schoolnik. 1991. An inducible bundle-forming pilus of enteropathogenic *Escherichia coli*. *Science* **254**:710–713.
- Girón, J. A., A. S. Y. Ho, and G. K. Schoolnik. 1993. Characterization of fimbriae produced by enteropathogenic *Escherichia coli*. *J. Bacteriol.* **175**:7391–7403.
- Gomes, T. A. T., M. A. M. Vieira, I. K. Wachsmuth, P. A. Blake, and L. R. Trabulsi. 1989. Serotype-specific prevalence of *Escherichia coli* strains with EPEC adherence factor genes in infants with and without diarrhea in São Paulo, Brazil. *J. Infect. Dis.* **160**:131–135.
- Gunzburg, G. T., N. G. Tornieporth, and L. R. Riley. 1995. Identification of enteropathogenic *Escherichia coli* by PCR-based detection of the bundle-forming pilus gene. *J. Clin. Microbiol.* **33**:1375–1377.
- Hobbs, M., and J. S. Mattick. 1993. Common components in the assembly of type 4 fimbriae, DNA transfer systems, filamentous phage and protein-secretion apparatus: a general system for the formation of surface-associated protein complexes. *Mol. Microbiol.* **10**:233–243.
- Ibrahim, G. F., G. H. Fleet, M. J. Lyons, and R. A. Walker. 1985. Immunological relationship between *Salmonella* flagellin and between these and flagellin from other species of *Enterobacteriaceae*. *Med. Microbiol. Immunol.* **174**:101–113.
- Jarvis, K. G., J. A. Girón, A. E. Jerse, T. K. McDaniel, M. S. Donnenberg, and J. B. Kaper. 1995. Enteropathogenic *Escherichia coli* contains a putative type III secretion system necessary for the export of proteins involved in attaching and effacing lesion formation. *Proc. Natl. Acad. Sci. USA* **92**:7996–8000.
- Jerse, A. E., J. Yu, B. Tall, and J. B. Kaper. 1990. A genetic locus of enteropathogenic *Escherichia coli* necessary for the production of attaching and effacing lesions on tissue culture cells. *Proc. Natl. Acad. Sci. USA* **87**:7839–7843.
- Knutton, S., M. M. Baldini, J. B. Kaper, and A. S. McNeish. 1987. Role of plasmid-encoded adherence factors in adhesion of enteropathogenic *Escherichia coli* to HEP-2 cells. *Infect. Immun.* **55**:78–85.
- Knutton, S., A. D. Phillips, H. R. Smith, R. J. Gross, R. Shaw, P. Watson, and E. Price. 1991. Screening for enteropathogenic *Escherichia coli* in infants with diarrhea by the fluorescent-actin staining test. *Infect. Immun.* **59**:365–371.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* **227**:680–685.
- Law, D. 1994. Adhesion and its role in the virulence of enteropathogenic *Escherichia coli*. *Clin. Microbiol. Rev.* **7**:152–173.
- Levine, M. M. 1987. *Escherichia coli* that cause diarrhea: enterotoxigenic, enteropathogenic, enteroinvasive, enterohemorrhagic, and enteroadherent. *J. Infect. Dis.* **155**:377–389.
- Levine, M. M., J. P. Nataro, H. Karch, M. M. Baldini, J. B. Kaper, R. E. Black, M. L. Clements, and A. D. O'Brien. 1985. The diarrheal response of humans to some classic serotypes of enteropathogenic *Escherichia coli* is dependent on a plasmid encoding an enteroadhesiveness factor. *J. Infect. Dis.* **152**:550–559.
- Manjarrez-Hernández, H. A., B. Ames, L. Sellers, T. J. Baldwin, S. Knutton, P. H. Williams, and A. Aitken. 1991. Purification of a 20-kDa phosphoprotein from epithelial cells and identification of myosin light chain-phosphorylation induced by enteropathogenic *Escherichia coli*. *FEBS Lett.* **292**:121–127.
- McDaniel, T. K., K. G. Jarvis, M. S. Donnenberg, and J. B. Kaper. 1995. A genetic locus of enterocyte effacement conserved among diverse enterobacterial pathogens. *Proc. Natl. Acad. Sci. USA* **92**:1664–1668.
- Moon, H. W., S. C. Whipp, R. A. Argenzio, M. M. Levine, and R. A. Gianella. 1983. Attaching and effacing activities of rabbit and human enteropathogenic *Escherichia coli* in pig and rabbit intestines. *Infect. Immun.* **41**:1340–1351.
- Nataro, J. P., M. M. Baldini, J. B. Kaper, R. E. Black, N. Bravo, and M. M. Levine. 1985. Detection of an adherence factor of enteropathogenic *Escherichia coli* with a DNA probe. *J. Infect. Dis.* **152**:560–565.
- Paulozzi, L. J., K. E. Johnson, L. M. Kamahele, C. R. Clausen, L. W. Riley, and S. D. Helgeson. 1986. Diarrhea associated with adherent enteropathogenic *Escherichia coli* in an infant and toddler center, Seattle, Washington. *Pediatrics* **77**:296–300.
- Qadri, F., T. Azim, A. Chowdhury, T. Hossain, R. B. Sack, and M. J. Albert. 1994. Production, characterization and application of monoclonal antibodies to *Vibrio cholerae* O139 synonym Bengal. *Clin. Diagn. Lab. Immunol.* **1**:51–54.
- Qadri, F., T. Azim, T. Hossain, D. Islam, G. Mondal, S. M. Faruque, and M. J. Albert. 1993. A monoclonal antibody to *Shigella dysenteriae* 13 cross-reacting with Shiga toxin. *FEMS Microbiol. Lett.* **107**:343–348.
- Rosenshine, I., M. S. Donnenberg, J. B. Kaper, and B. B. Finlay. 1992. Signal transduction between enteropathogenic *Escherichia coli* (EPEC) and epithelial cells: EPEC induces tyrosine phosphorylation of host cell proteins to initiate cytoskeletal rearrangement and bacterial uptake. *EMBO J.* **11**:3551–3560.
- Scaletsky, I. C. A., M. L. M. Silva, and L. R. Trabulsi. 1984. Distinctive patterns of adherence of enteropathogenic *Escherichia coli* to HeLa cells. *Infect. Immun.* **45**:534–536.
- Scotland, S. M., J. E. Richmond, and B. Rowe. 1983. Adhesion of enteropathogenic strains of *Escherichia coli* (EPEC) to HEP-2 cells is not dependent on the presence of fimbriae. *FEMS Microbiol. Lett.* **20**:191–195.
- Scotland, S. M., H. R. Smith, and B. Rowe. 1991. *Escherichia coli* O128 strains from infants with diarrhea commonly show localized adhesion and positivity in the fluorescent-actin staining test but do not hybridize with an enteropathogenic *E. coli* adherence factor probe. *Infect. Immun.* **59**:1569–1571.
- Towbin, H., T. Staehelin, and J. Gordon. 1983. Electrophoretic transfer of protein from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA* **76**:4350–4354.