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Recognition of Enteropathogenic *Escherichia coli* Virulence Determinants by Human Colostrum and Serum Antibodies

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Human colostra and sera collected from Mexican mothers and their children at birth and 6 months thereafter were studied for the presence of antibodies against the bundle-forming pilus and several chromosomal virulence gene products (intimin and secreted proteins EspA and EspB) of enteropathogenic *Escherichia coli* (EPEC). Among 21 colostrum samples studied, 76, 71.5, 57, and 47% of them contained immunoglobulin A (IgA) antibodies against EspA, intimin, EspB, and BfpA, respectively. Interestingly, there was a difference in IgG response to EPEC antigens between the sera from neonates and sera from the same children 6 months later. While the number of neonates reacting to Esps and intimin diminished when they reached 6 months of age, those reacting with BfpA increased from 9 to 71%. Intimin from an enterohemorrhagic *E. coli* strain was also recognized by most of the samples reacting with EPEC intimin. These data suggest that Bfp and Esps elicit an antibody response during the early days of life of neonates and support the value of breast-feeding in areas of the world where bacterial diarrheal infections are endemic.

Among the bacterial causes of childhood diarrhea in the developing world, a large proportion of morbidity and mortality is attributed to enteropathogenic *Escherichia coli* (EPEC) (13, 18, 27). This organism possesses a repertoire of plasmid- and chromosomally encoded virulence factors that act in concert to facilitate colonization of the small bowel, leading to disruption of the enterocyte cell membrane integrity (27). This histopathology, known as the attaching and effacing lesion, is also a characteristic of other enteric pathogens, namely, enterohemorrhagic *E. coli* (EHEC), *Citrobacter rodentium*, *Hafnia alvei*, and the rabbit-pathogenic *E. coli* RDEC-1. The attaching and effacing lesion results from the intimate contact by the bacteria and activation of several chromosomal gene products that interact with components of the host cell, leading to protein phosphorylation and destruction of the cell membrane (27). These genes are clustered in a pathogenicity island called the locus of enterocyte effacement (LEE) (26). LEE-encoded determinants include intimin, a 94-kDa outer membrane protein involved in intimate cell attachment (20); a translocated intimin receptor called Tir (21); and the EPEC-secreted proteins (EspA, EspB, EspD, and EspF) responsible for signal transduction (19, 26), which are secreted through a type III secretion system apparatus, also encoded in the LEE (26). EspA is thought to form a pilus structure necessary for translocation of effector molecules Tir and EspB into eukaryotic cells (22).

Adherence of EPEC to the small intestine and tissue culture cells is a characteristic feature of epidemic strains (reviewed in references 18, 27, and 30). Once the bacteria associate with their target cell through various surface appendages such as pili or EspA-containing fibers and intimin (15, 20, 22, 27), they replicate in situ, aggregating and forming tight microcolonies kept together through highly hydrophobic filamentous ultrastructures composed of bundle-forming pili (BFP) (14). This mode of adherence is referred to as the localized adherence pattern (30). The BFP are composed of a structural bundlin subunit, BfpA (19.5 kDa), which is highly homologous to the toxin-coregulated pilus of *Vibrio cholerae*, longus of enterotoxigenic *E. coli*, and other type IV pili (17).

Several studies in the past have shown that sera from human volunteers experimentally infected with EPEC as well as human colostrum react mainly against intimin (2, 5, 6, 9, 10, 24). However, the methods available then did not assay for BFP or Esps, which are produced under defined bacterial growth conditions. Jarvis et al. demonstrated that Esps were recognized by human convalescent serum from a volunteer experimentally infected with EPEC (19). In a different study, individuals who were experimentally fed with EPEC and then rechallenged with homologous or heterologous EPEC developed protective antibodies against the lipopolysaccharide O antigen and intimin but not against bundlin (11). Bieber et al. demonstrated that a BFP-deficient mutant did not provoke diarrhea in human volunteers as did the wild type (3), confirming previous observations that BFP are a colonization or clustering factor, important for virulence (14). A recent investigation showed that Brazilian children who had been naturally infected with EPEC developed immunoglobulin G (IgG) antibodies against BfpA, intimin, and Esps produced by heterologous EPEC (25).

There is a lack of information regarding the antibody immune response to specific bacterial virulence determinants in children living in areas where intestinal diarrheal disease due to EPEC is endemic. What antigens are protective during the early days of life? Is there long-lasting immunity to heterologous strains? Are antibodies against a particular antigen transmitted through umbilical cord and maternal colostrum actually present all through the first 6 months of age? Is there a correlation between the presence of antibodies and lack of enteric infection? These are some of the important questions that need to be addressed in order to propose candidates for vaccines against EPEC infection. Towards these aims, in the present study we sought to investigate what EPEC antigens are recognized by antibodies present in human colostra and in sera from mothers and their newborns at the time of birth and what

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antibodies remain or are raised after 6 months of life. The presence of antibodies against EPEC antigens in colostrum supports the value of breast-feeding among children living in areas of the world where bacterial infections in general are endemic. Thus, it may be possible to use these proteins as targets for vaccine development, immunizing mothers and protecting neonates against EPEC infection.

MATERIALS AND METHODS

Bacterial strains and growth conditions. EPEC strains used were B171 (O111:NM), ICB34 (O55:H6), and E2348/69 (O127:H6) (14, 16, 25). B171-4 and JPN-15 are plasmidless derivatives of B171 and E2348/69, respectively, which do not produce BFP (14, 16) and were used as negative controls. EHEC strain 352A (O26:NM, Stx1) (4) was used to determine the reactivity of human samples against EHEC intimin. pET-BfpA, pET-EspA, and pET-EspB are recombinant *E. coli* BL-21 strains carrying the pET28a+ plasmid (Novagen) containing the *bfpA*, *espA*, and *espB* genes, respectively. All pET strains were kindly provided by Gad Frankel (Imperial College of Science, Technology and Medicine, London, United Kingdom). The strains were grown overnight at 37°C in Dulbecco's minimal essential medium (Life Technologies, Grand Island, N.Y.) to promote production of BFP and Esp (16, 19). pET strains were grown in Luria broth with the appropriate antibiotics as indicated below.

Human colostra and sera. Colostrum and serum were obtained from 21 healthy pregnant women (16 to 33 years old) who attended the Hospital de Subzona "Manuel Avila Camacho" in Martínez de la Torre, Veracruz, Mexico, to deliver their babies. This hospital provides free health care to low-income families. The samples were obtained within 24 h after birth. Blood was obtained from the umbilical cord of the newborn children and 6 months thereafter by venous puncture. Parents gave full consent for participation of the children in the study. The sera and colostra were kept at −20°C for further testing.

Rabbit antisera. Rabbit anti-BFP was described earlier (14), and anti-intimin antibodies were produced by immunization of a rabbit with intimin obtained from pCVD450 (28). Polyclonal anti-EspA and anti-EspB antisera were a kind gift of Gad Frankel. All antisera were used in immunoblottings and enzyme-linked immunosorbent assay (ELISA) as described below.

Reactivity to BfpA and intimin. To determine the presence of BfpA and intimin-reacting antibodies, whole-cell extracts of B171 were reacted with sera or colostra by immunoblotting as previously described (25). Whole-cell extracts of JPN-15 and EPEC strain B171-4 grown in L broth were used as negative controls. Due to the homology between EPEC and EHEC intimins, bacterial extracts of EHEC strain 352A were also reacted with the child sera. Briefly, whole-cell extracts of B171 were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis in 16% acrylamide gels (23) and electroblotted onto nitrocellulose membranes (Millipore). After blocking with 3% defatted milk in phosphate-buffered saline containing 0.5% Tween 20 (PBS-T), the blots were reacted with a 1:1,000 dilution of the rabbit antiserum described above, a 1:25 dilution of colostrum, or a 1:50 dilution of the human serum for 1 h at 37°C. The blots were washed with PBS-T and reacted with goat anti-rabbit IgG or goat anti-human IgG, IgM, or IgA conjugated to alkaline phosphatase for 1 h at 37°C. After washing, the reaction was developed with a mixture of nitroblue tetrazolium and 5-bromo-4-chloro-3-indolylphosphate (Sigma).

Isolation of EPEC-secreted proteins and reactivity. EPEC-secreted proteins were prepared from E2348/69 as previously described (19) and reacted with child sera by immunoblotting (25). In short, E2348/69 and ICB34 were grown overnight at 37°C in Dulbecco's minimal essential medium without fetal bovine serum. A 1:20 dilution was made in fresh medium and incubated for an additional 5 h with shaking. After centrifugation at 14,000 × *g* for 10 min, the supernatant was filtered through a 0.45-μm-pore-size filter (Nalgene), and aprotinin (0.5 μg/ml), EDTA (0.5 mM), and phenylmethylsulfonyl fluoride (50 μg/ml) were added to inhibit protease activity. The supernatant was concentrated 500-fold in an Amicon filtration unit under nitrogen pressure. Esps were visualized by Coomassie blue staining in 12% polyacrylamide gels and also reacted with human samples as described above.

Preparation and reactivity to His-tagged BfpA, EspA, and EspB. *E. coli* BL-21 carrying pET28a+ plasmids with *bfpA*, *espA*, and *espB* genes were grown overnight at 37°C in Luria broth supplemented with kanamycin (30 μg/ml) and 0.2% glucose. A 1:100 dilution was made in fresh medium containing 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) and incubated at 37°C with constant shaking for 2 h. When the culture reached an optical density value at 600 nm of 1.0, the bacterial pellet was obtained by centrifugation at 5,000 × *g* for 10 min at 4°C and resuspended in 4 M NaCl before sonication. The suspension was centrifuged at 39,000 × *g* for 20 min, and the supernatant was stored at −20°C until further use. BL-21(pET28a+) was used as a negative control in all experiments. After confirmation of the presence of EspA and EspB with the specific sera, EspA and EspB sonicates were mixed and reacted simultaneously with child sera or colostra by immunoblotting as described above.

ELISA. A standard ELISA was used to titrate human serum and colostrum antibodies against EPEC virulence determinants. Briefly, 96-well Immunolon ELISA II plates (Dynatech) were coated with EPEC His-tagged antigens adjusted to the same concentration in carbonate buffer (pH 9.6). After overnight incubation at 4°C, the plates were washed and blocked for 2 h with phosphate-buffered saline containing 1% bovine serum albumin. After washing with PBS-T, the plates were first incubated for 1 h with 1:1,000 dilutions of rabbit anti-BfpA, anti-EspA, or anti-EspB antisera. Goat anti-rabbit IgG conjugated to alkaline phosphatase was added for 1 h followed by addition of the phosphatase substrate, and the development of color was read at an optical density of 405 nm in an ELISA reader. Sonicates of pET28a+ were used as negative controls. After all the conditions were set up, the immobilized antigens were reacted with twofold dilutions of the child sera or colostra, and bound antibodies were detected with anti-human IgA, IgG, or IgM antibodies conjugated to alkaline phosphatase as described above.

Statistical analysis. Statistical analysis of antibody reactivities from sera from the different groups obtained by ELISA was done using the Kruskal-Wallis test (29).

TABLE 1. Immunoblotting reactivities of colostra and sera against EPEC antigens

Sample (n = 21)	No. of samples reactive to EPEC antigens (%)				
	BfpA	EspA	EspB	Int_{EPEC}	Int_{EHEC}
Colostrum	11 (52)	16 (76)	12 (57)	15 (71)	17 (81)
Maternal serum	8 (38)	18 (86)	19 (90)	19 (90)	16 (76)
Neonate serum	2 (9)	9 (43)	18 (86)	15 (71)	21 (100)
Infant serum	15 (71)	5 (24)	13 (62)	9 (43)	12 (57)

RESULTS

Colostrum IgA antibodies against BfpA. Among the 21 colostrum samples examined individually, 11 (47%) contained IgA but not IgG antibodies against BfpA, as determined by immunoblotting of sonicate extracts of pET-BfpA and confirmed by reactivity to whole lysates of B171. These results showed that antibodies recognized BfpA from two different EPEC serotypes, suggesting the presence of common epitopes among these antigens. No reactivity with the 19.5-kDa bundlin was observed when these colostra were reacted with whole-cell extracts of B171-4 or pET28a+. Reactivity against BfpA was further confirmed by ELISA as described below (Tables 1 and 2).

Serum reactivity to BfpA. Antibodies against BfpA were found in the serum of all three groups of samples but were more frequently found among infants than among mothers or newborns. Interestingly, 15 out of 21 (71%) sera from 6-month-old children (who were all breast-fed) reacted with bundlin compared with 2 out of 21 (10%) sera from the same children at birth. We do not discard the possibility that anti-BfpA antibodies were developed by active immunization with EPEC antigens present in the environment. Only 8 of the 21 maternal sera were shown to react with BfpA. In all groups, only IgG anti-BfpA reactive antibodies and not IgA or IgM antibodies were found (Table 1).

Colostrum and serum reactivity to Esps. EspA (25 kDa) was recognized by IgA and IgG antibodies in 16 (76%) and 2 (9.5%) of the colostra, respectively. Among the sera from mothers, neonates, and infants, anti-EspA antibodies were found in 18 (85.7%), 9 (43%), and 5 (24%), respectively (Tables 1 and 2). No anti-EspA IgM antibodies were detected in any of the child sera (data not shown). On the other hand, EspB (38 kDa) was detected by IgA antibodies in 12 (57%) colostra, while EspB-reactive IgG antibodies were found in 5 (24%) colostra. Anti-EspB IgG antibodies were present in 19 (90%), 18 (85%), and 13 (62%) of sera from mothers, neonates, and infants, respectively. There was a significant difference between reactivity of colostra and reactivity of neonate sera against EspA or EspB ($P < 0.001$). Reactivities to EspA

TABLE 2. Reactivity of colostra to EPEC antigens by ELISA and immunoblotting[a]

Sample no.	BfpA		EspA		EspB	
	E	Ib	E	Ib	E	Ib
1	−	−	16	+	16	+
2	−	+	−	+	16	+
3	40	+	−	+	−	−
4	80	−	−	+	−	+
5	320	+	−	−	−	+
6	80	+	256	+	8	+
7	−	−	−	−	−	−
8	160	+	8	+	−	−
9	80	+	16	+	16	+
10	40	−	256	+	−	−
11	320	+	16	+	−	−
12	−	−	−	+	−	−
13	160	+	−	−	128	+
14	−	−	32	+	−	−
15	−	+	256	+	128	+
16	−	−	32	+	32	+
17	−	+	−	−	−	−
18	−	−	256	+	64	+
19	40	+	128	−	32	+
20	−	−	16	+	−	−
21	−	−	16	+	8	+

[a] The numbers in columns E (ELISA) represent the titer of each sample expressed as the reciprocal of the dilution at which a positive reaction was observed (for BfpA, 1:10 to 1:20 = negative, 1:40 to 1:80 = weak positive, and >1:160 = positive; for EspA and EspB, 1:2 to 1:4 = negative, 1:8 to 1:16 = weak positive, and >1:32 = positive). Dilutions with absorbance values below 0.2 were considered negative. Ib, immunoblotting.

and EspB were also confirmed when total Esps obtained from ICB34 were used as the antigens.

Colostrum and serum reactivity to EPEC and EHEC intimins. Intimin from EPEC strain B171 (Int_{EPEC}) was detected by 15 (71.5%) colostra, whereas 19 (90%), 15 (71.5%), and 9 (43%) of maternal, neonate, and infant sera reacted with Int_{EPEC}, respectively (Table 1). Significant differences were observed only between sera from mothers and sera from infants ($P < 0.001$). Since EHEC also produces a class of intimin (Int_{EHEC}), human samples were reacted with whole-cell extracts of EHEC strain 352A. Both Int_{EPEC} and Int_{EHEC} reacted with rabbit anti-intimin antibodies, confirming the shared antigenicity between these proteins (reference 1 and data not shown). Among all human samples tested, 17 (81%), 16 (76%), 21 (100%), and 12 (57%) of the colostrum, maternal, neonatal, and infant samples, respectively, reacted with Int_{EHEC}. A slightly larger number of the sera reacted more strongly with Int_{EHEC} than with Int_{EPEC}.

Titration of reactive antibodies present in colostra against BfpA and Esps. All colostrum samples were reacted with His-tagged BfpA, EspA, and EspB proteins by ELISA starting at a 1:2 dilution (for EspA and EspB) or a 1:10 dilution (for BfpA), followed by serial twofold dilutions. The dilution with an absorbance value higher than 0.3 (for BfpA) or 0.2 (for Esps) was considered positive, whereas the dilution with an absorbance value below 0.3 or 0.2, respectively, was considered negative. This criterion was based on the absorbance value for the negative controls employed. The titer for each sample is expressed as the reciprocal of the lowest dilution at which an absorbance equal to or higher than 0.3 was reached.

In general, for each antigen tested a high correlation between the results obtained by immunoblotting and those obtained by ELISA was observed (Table 2). Among 11 colostra that reacted with BfpA by immunoblotting, 9 were positive by ELISA. Nevertheless, a few colostrum samples (samples 2, 3, 15, 17, and 19) showed positivity against BfpA in immunoblots but were weakly positive or negative by ELISA. This difference may be attributed to recognition of epitopes exposed after denaturation of proteins in immunoblots. Colostrum sample 4 showed a 1:80 titer in ELISA (considered weakly positive) but was negative in immunoblots for BfpA.

For EspA and EspB, all samples which showed reactivity in ELISA with titers between 1:16 and 1:256 also reacted positively in immunoblots (Table 2). Among 16 samples positive by immunoblotting for EspA, only 4 samples (colostrum samples 6, 10, 15, and 18) showed titers equal to or higher than 1:128. Colostrum sample 19 (with a titer of 1:128 in ELISA) did not react with EspA in immunoblots. Four colostra (samples 2, 3, 4, and 12) failed to react to EspA in ELISA. When reactivity to EspB was analyzed, except for colostra 4 and 5, which were negative in ELISA, all samples reacting with EspB by immunoblotting reacted in ELISA with titers in the range of 1:8 to 1:128. Only two samples (colostra 13 and 15) showed titers of 1:128 (Table 2).

Titration of BfpA-reactive antibodies in sera. Since most sera from neonates did not react with BfpA, we were particularly interested in determining serum reactivity against BfpA by ELISA of all sera from infants that had previously reacted with BfpA by immunoblotting. There was a perfect correlation between the results obtained by immunoblotting and those obtained by ELISA (data not shown). Titers against BfpA were generally high (1:80 to 1:320) in all the sera tested. These results suggest the possibility that these children had been exposed to EPEC producing this antigen during the first 6 months of life, since no anti-BfpA antibodies were readily detected at birth in most cases.

DISCUSSION

The development of protective antibodies during the course of infection and thereafter remains an interesting issue to address. Although some of the molecular determinants of EPEC pathogenicity are known, little information is available regarding the actual protective role of its virulence determinants during naturally occurring EPEC infections in children. In this study, we determined that colostra and sera obtained from 21 mothers and their infants living in a semiurban area of Central East Mexico contained, to differing extents, antibodies reactive to defined virulence determinants of EPEC, such as BFP, intimin, and EPEC-secreted proteins EspA and EspB. Generally, the presence of antibodies against a particular antigen is considered an indicator that an individual has been naturally or experimentally exposed to that antigen (2, 5, 6, 24). Thus, we first determined which samples reacted with each of these antigens in immunoblots. We determined the class of reacting Igs using different secondary antibodies against human IgA, IgG, and IgM. Reactive antibodies in colostra were generally of the IgA class, while serum antibodies reacting with EPEC antigens were of the IgG class. Cravioto et al. (9) found IgA immunoglobulins in colostrum that reacted with EPEC antigens, particularly with intimin.

We detected reactivity against all antigens tested, although the frequency of reactivity varied depending on the antigen and the source of the sera. When colostrum samples were analyzed for reactivity, anti-EspA and anti-intimin (EPEC and EHEC) IgA antibodies were the most frequently found, followed by anti-BfpA and anti-EspB IgA antibodies. Why some antigens are more frequently reactive with some colostra is unknown, but it is most likely due to the intrinsic biochemical and antigenic properties of each bacterial product tested and

probably reflects differences in each individual's innate immune response. Nevertheless, the results suggest that passive immunization against these EPEC antigens can be achieved through breast-feeding.

Likewise, a difference in reactivities to EPEC antigens was found among sera from mothers, neonates, and infants. The profile of antigen recognition by maternal sera was similar to that of colostra, although some differences were observed. This could be due to quantitative differences in the concentration of reactive IgA or IgG present in colostrum or serum, respectively. Most maternal sera recognized EspA, EspB, and intimins, while BfpA was the antigen eliciting the least reaction. A marked difference in reactivities existed between sera from neonates and sera obtained 6 months later from the same children. On one hand, sera from neonates most frequently recognized EPEC antigens in the order Int_{EPEC} (100%), EspB (85%), Int_{EHEC} (71%), and EspA (43%), while BfpA (9.5%) was the antigen eliciting the least reaction. Reactive antibodies present in the sera of neonates are most likely transferred by their mothers through the umbilical cord (2, 6). On the other hand, sera obtained 6 months later from these same children showed a different profile of antigen recognition. These children most frequently exhibited antibodies against BfpA (71%), followed by EspB (62%), Int_{EHEC} (57%), Int_{EPEC} (43%), and EspA (24%). High titers of antibodies against BfpA were more often found in sera from infants than in colostra or sera from mothers and neonates. The fact that neonates develop antibodies against BfpA during the first 6 months of life suggests only that these children may have been exposed to EPEC antigens and consequently have produced antibodies to colonization factors produced in vivo, such as BFP (2, 6). This shift in antigen recognition is noteworthy and suggests that exposure to diarrheal pathogens such as EPEC during the first 6 months of life probably elicits a specific immune response to antigenic variations found among virulence products of distinct EPEC serotypes (1, 16). It can be speculated that the different antigens studied here are produced in vivo at different intervals during the infection process and that some antigens appear upon contact with mucus components and signaling by environmental factors present on the intestinal epithelia. Other antigens may be repressed once the bacteria have established themselves in the small intestine (12). Thus, the length of exposure to each particular antigen during a natural infection may reflect differences in the profiles of antigen recognition observed between neonates and infants.

The relatively high frequency of anti-intimin antibodies may reflect the prevalence of EPEC in the environment or the immunogenic nature of this family of antigens found in EPEC and EHEC (1). It is not known why EHEC infections, in particular the hemolytic-uremic syndrome, are restricted to developed countries and not commonly observed in developing countries (27). The fact that individuals living in areas where EPEC infections are endemic develop antibodies against EPEC intimin may explain, in part, the absence of hemolytic-uremic syndrome in countries like Mexico.

As opposed to other investigations conducted in Brazil and Thailand, where the epidemiology of EPEC infections has been extensively studied (15, 18, 27), the burden of EPEC disease in Mexico remains largely unknown. Cravioto et al. conducted several studies to determine the association of HEp-2 cell-adherent *E. coli* in children living in the State of Morelos, located near Mexico City (7, 8). Close to 60% of the fecal specimens obtained from children during the first 2 years of life yielded adherent *E. coli*. Strains with localized adherence were significantly associated with nonbloody acute diarrhea, and 41% of them belonged to the classical EPEC serotypes (7). Based on these reports (7, 8), we cannot discard the possibility that EPEC might also be responsible for acute diarrhea in the region of Mexico studied here. Ongoing efforts are needed to study the prevalence of diarrheagenic *E. coli* in this geographic area of Mexico. Unfortunately, we were not able to follow up the onset of diarrhea in the children studied to verify the presence of EPEC in their stools and correlate this with the presence of antibodies against the EPEC antigens studied because the mothers did not attend a health center or hospital. Nevertheless, among 77% of the children who were breast-fed, only 19% of them had diarrheal episodes in which the etiology was unknown. Thus, breast-feeding is an important factor in protective immunity against diarrheal infections (9, 15, 27). However, since an antibody response is generally considered a marker of infection, it is reasonable to suggest that some of the children were likely exposed to EPEC.

Previous studies have shown that human volunteers who were fed with EPEC produced antibodies against intimin (11, 24). Other investigators detected anti-intimin IgA antibodies in human colostrum which inhibited adherence to cultured cells (5, 6, 9). However, at the time these studies were performed the BFP and EPEC-secreted products were not sought because the methods then available did not assay for BFP or Esps. Thus, those studies did not reveal whether the BFP or Esps are produced in vivo or if antibodies are elicited against these important EPEC determinants. In a recent study, human volunteers who had been fed with EPEC in the past and rechallenged with two EPEC strains of different serotypes did not develop antibodies against BfpA (11). It was suggested that anti-O-antigen antibodies were responsible for a lesser severity of disease in the homologous-rechallenge study group. Anti-intimin antibodies were also found, but no correlation with protection was noted. A more recent report demonstrated that children with diarrheal illness due to EPEC infection develop antibodies against BFP, intimin, and Esps 10 days after the onset of diarrhea (25). These reports support our findings that, during natural EPEC infections, an antibody response is elicited against EPEC virulence determinants.

To summarize, in the present study we demonstrate that human colostra and sera from mothers and their children living in developing countries contain antibodies specific against intimin, secreted proteins EspA and EspB, and BFP, produced by heterologous EPEC serotypes. Our data suggest that BFP and the virulence determinants encoded on the LEE elicit an antibody response in the early days of life of neonates and support the value of breast-feeding in areas of the world where bacterial diarrheal infections are endemic. Further studies are needed to elucidate whether these antibodies are protective.

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