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Roles of Different Coli Surface Antigens of Colonization Factor Antigen II in Colonization by and Protective Immunogenicity of Enterotoxigenic *Escherichia coli* in Rabbits

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The roles of the subcomponents of colonization factor antigen II, the coli surface antigens CS1, CS2, and CS3, as colonization factors and protective antigens was studied in a nonligated rabbit intestine model (RITARD). Infection with enterotoxigenic *Escherichia coli* (ETEC) carrying CS3 alone or CS1 plus CS3 induced diarrhea in most (80%) of the rabbits, whereas nonenterotoxigenic strains expressing CS1 or CS2 rarely induced diarrhea. Strains carrying CS1, CS2, or CS3 alone were all shed in stools for a significantly longer period than normal fecal flora-type *E. coli*. Initial infection with ETEC positive for CS1 plus CS3 induced significant protection against disease caused by reinfection with a highly diarrheagenic dose of the homologous strain; rabbits previously infected with serotype-heterologous, nontoxigenic bacteria carrying CS1 only were also protected against this challenge, whereas no such protection was induced by serogroup-homologous *E. coli* carrying CS2 only. Animals previously infected with CS1-, CS3-, or CS1-plus-CS3-positive bacteria excreted the CS1-plus-CS3 challenge strain for a significantly shorter period than did "nonimmunized" rabbits, whereas initial infection with bacteria carrying CS2 only did not result in such reduced shedding. Monoclonal antibodies against CS1, CS2, or CS3 all protected against experimental infection with ETEC carrying the corresponding CS factor. These results suggest that all the subcomponents of colonization factor antigen II are colonization factors and may induce anticolonization immunity.

Diarrheal disease caused by enterotoxigenic *Escherichia coli* (ETEC) is a major health problem in children in developing countries (4). The bacteria cause disease by colonizing the small intestine and elaborating a heat-labile enterotoxin (LT) or a heat-stable enterotoxin (ST) or both. Colonization is usually associated with adhesion of the bacteria to specific receptors on the intestinal mucosa by means of antigenically specific fimbriae (12).

For ETEC strains isolated from humans, several distinct types of fimbrial antigens have been described; the hitherto best-characterized ones are colonization factor antigens I and II (CFA/I and CFA/II) and the PCF8775 antigen recently renamed CFA/IV (6, 9, 28). The various CFAs are usually fimbriae that mediate mannose-resistant hemagglutination (MRHA) of different species of erythrocytes. Whereas CFA/I seems to be a homogeneous antigen, CFA/II and CFA/IV have each been shown to consist of more than one antigen component. For CFA/II, these components are called coli surface-associated antigens CS1, CS2, and CS3 (5, 21), and for CFA/IV, they are called CS4, CS5, and CS6 (28). CFA/II is usually composed of one or two CS antigens. In non-O6 strains, CS3 is generally the only one expressed, although one O139 strain that expresses both CS1 and CS3 has been described (17). Depending on biotype, O6 strains usually produce CS1 and CS3 or CS2 and CS3 (5, 21); a few strains have also been reported which carry only CS2. Whereas CS1 and CS2 exist as thicker (6 nm in diameter), rigid fimbriae, CS3 consists of thin (2 to 3 nm in diameter), flexible fibrillar fimbriae (13).

All three CS components of CFA/II are immunogenic and give rise to immunologically distinct immune responses (13). Intraintestinal administration of CFA/II-positive *E. coli* has induced highly significant protection both in rabbits (3) and in humans (11) against subsequent challenge with serotype-heterologous ETEC carrying the homologous CFA/II.

The present study was undertaken to evaluate the roles of the different CS antigens of the CFA/II complex in colonization and disease and in the protective immunogenicity of ETEC bacteria. This included determination of the capacity of *E. coli* carrying CS1, CS2, or CS3 alone to colonize the intestine in a nonligated rabbit intestine model (RITARD) (22). We also studied whether intestinal infection by these strains could induce protection against colonization and disease resulting from subsequent challenge with CFA/II-carrying ETEC and whether monoclonal antibodies (MAbs) against CS1, CS2, and CS3 could protect against experimental diarrhea induced by CS-positive ETEC strains.

MATERIALS AND METHODS

Animals. New Zealand White rabbits of both sexes were used. Their weights ranged from 1.7 to 2.2 kg when experiments began.

Bacteria. ETEC strains E1392-75 (O6:K15:H16; $CS1^+$ $CS3^+$ ST^+ LT^+), isolated from a patient in Hong Kong, and 278485 (O6:K15:H16; $CS2^+$ $CS3^+$ ST^+ LT^+), originally isolated from a patient in Bangladesh, were used (1, 18). In addition, *E. coli* E19446 (O139:H28; $CS3^+$ ST^+ LT^+), 60R936 (O139:H28; $CS1^+$ ST^- LT^-; derived from strain E24377 [$MRHA^-$] and containing plasmid NTP176 encoding CS1, CS2, and ampicillin resistance), 58R957 (O6:H16; $CS2^+$ ST^- LT^-; derived from G176 and containing plasmid NTP176), and 58R602 (O139:H28; CS^- ST^- LT^-; resistant to sulfonamide, as well as streptomycin) were used; the origins and characterization of these strains and the inserted plasmids have been described elsewhere (16, 17, 19). The results of previous infection (24) with two additional control

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strains, i.e., the CS-negative mutants E11881B (ST^- LT^-) and E11881D (ST^+ LT^+) of strain E11881 (O25:H42; $CS4^+$ $CS6^+$), were also included. Stock cultures of all strains were suspended in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) with 15% (wt/vol) glycerol and stored as multiple aliquots at −70°C until used.

Strains were grown in Casamino Acids-yeast extract medium (9) at 37°C for 6 to 7 h and then inoculated on CFA agar (8) and grown at 37°C overnight. Strains harboring antibiotic resistance plasmids were inoculated on CFA agar plates containing the appropriate antibiotic at a final concentration of 100 μg/ml. The bacteria were harvested and washed in saline, and the cultures were adjusted to the desired bacterial concentration by optical density measurements. In each experiment, the bacterial concentration was also checked by a viable-cell count on blood agar plates after serial dilution in saline.

Antigens. CFA/II (CS1 plus CS3) was purified from strain 1392-75, CS2 was purified from strain 58R957, and CS3 was purified from an O78 *E. coli* strain by the methods described by Evans et al. (7) or Klemm et al. (10).

Antibodies. MAbs against the different CS antigens of CFA/II were produced as previously described (15) by immunizing BALB/c mice with purified CFA/II (CS1 plus CS3) or CS2. Three hybridomas producing high levels of specific antibodies against CS1, CS2, or CS3 were cloned and propagated either by production of ascitic fluid or by the dialysis tubing method (20). All three MAbs were of the IgG1 isotype, and the antibody preparations used had comparable enzyme-linked immunosorbent assay (ELISA) titers against the homologous CS factors, i.e., 1/200,000 to 1/500,000 (15).

Agglutination tests. MRHA of bovine erythrocytes was performed as previously described (8). The presence of different CS components on ETEC bacteria was ascertained by slide agglutination, using the MAbs against CS1, CS2, and CS3.

RITARD model. The reversible intestinal tie adult rabbit diarrhea (RITARD) model described previously (22) was used with some modifications (3). In short, the cecum was brought out through a midline incision and ligated permanently as close to the ileal-cecal junction as possible. A 10-ml sample of the bacterial inoculum was then injected into the duodenum, the ileum having first been obstructed with an umbilical tape tie about 5 cm proximal to the mesoappendix. The ileal tie was gently removed 2 h after the bacterial injection. The same technique was used for rechallenging surviving rabbits 14 days after the initial infection; all animals were sacrificed within 7 days after the last infection.

Monitoring of disease. During the 14 days after the initial infection, rabbits were observed several times daily for diarrhea and other symtoms of disease or for death; animals were considered to have diarrhea if they passed two or more loose stools or one or more watery stools per day. Rectal swabs were collected daily from each animal and plated on blood agar for identification of the challenge organism. The challenge strains were identified by typical *E. coli* colony morphology, MRHA of bovine erythrocytes, and slide agglutination, using MAbs against the different CS components of CFA/II. For detection of enterotoxin-producing bacteria, LT or ST production or both were determined in a direct culture-in-plate GM1-ELISA as described previously (24). In short, six individual colonies from each blood agar plate were inoculated into individual wells of GM1-coated microtiter plates containing Casamino Acids-yeast extract medium with 45 μg of lincomycin per ml and 2.5 mg of glucose per ml. After the plates were incubated at 37°C overnight with shaking, individual colonies producing LT or ST were identified by means of GM1-ELISA methods (26, 27). Excretion of the toxin-negative, CS-negative strain 58R602 was monitored by plating rectal swabs on Luria broth agar plates containing streptomycin and sulfonamide each at a final concentration of 250 μg/ml; Antibiotic Sensitivity Medium (Biodisk AB, Solna, Sweden) agar plates containing these drugs at similar concentrations were also used.

Passive protection tests. The capacity of MAbs against different CS factors to protect against experimental infection with CFA/II-positive ETEC (strains E1392-75 and 278485) was evaluated in ligated small bowel loops in rabbits as previously described (2). The MAbs were tested at a final concentration corresponding to 10,000 times the ELISA titer against the homologous CS factor (see below). Serial 10-fold dilutions of bacterial cultures, adjusted to 10^{10} organisms per ml, were incubated together with the different anti-CS MAbs at room temperature for 1 h; the mixtures were then injected into four 5-cm loops randomly positioned in four different animals. The animals were sacrificed after 18 h, and the bacterial dose causing half-maximum fluid accumulation (ED_{50}) in the loops was determined. The protection factor was calculated as the ratio between the geometric mean ED_{50} value in the presence of MAb and that in phosphate-buffered saline.

Serological analyses. All rabbits were bled immediately before the initial infection, on the day of rechallenge 14 days after the initial infection, and then on the day of sacrifice 5 to 7 days after the second infection. Serum samples were taken and stored at −30°C until used. Antibodies against LT were determined by using the GM1-ELISA as previously described (23). Antibodies against purified CFA/II (CS1 plus CS3, CS3, or CS2) were determined by ELISA as previously described (15); plates were coated by incubating 1 to 5 μg of purified fimbriae per ml at 37°C overnight. Sera were tested in fivefold serial dilutions, and anti-rabbit immunoglobulin-horseradish peroxidase (Dakopatts, Copenhagen, Denmark) was used as the enzyme conjugate. The endpoint titer was determined as the reciprocal value of the interpolated dilution giving an A_{450} of 0.2 above the background when the enzyme was reacted with its substrate for 20 min.

RESULTS

Diarrheal response and colonizing ability. The capacity of various *E. coli* strains carrying CS1, CS2, or CS3 alone and ETEC expressing CS1 plus CS3 to induce diarrhea and to colonize the intestine was studied in rabbits infected in the RITARD model (Table 1). Groups of animals were infected by injecting 10^{10} or 10^{11} bacteria directly into the duodenum of each rabbit; in previous studies, challenge doses between 1×10^{10} and 5×10^{11} CFA-positive ETEC organisms were found to be diarrheagenic (3, 24). Most of the rabbits infected with enterotoxigenic bacteria carrying CS1 plus CS3 or CS3 alone, i.e., strains E1392-75 and E19466, developed diarrhea (Table 1). The disease was more severe and lasted for a longer period when 10^{11} rather than 10^{10} organisms were used for the infection, although this difference was statistically significant ($P < 0.01$) only for strain E19466. Rabbits given enterotoxin-negative *E. coli* expressing CS1 or CS2 alone or lacking CS factors only rarely developed diarrhea (Table 1).

All three strains expressing CS1, CS2, or CS3 alone seemed to colonize and multiply in the rabbit intestine, as judged by fecal excretion of the infecting organisms (Table 1). Thus, the mean times of shedding of the different CS-

TABLE 1. Influence of CFA/II CS antigens and enterotoxin production on the ability of *E. coli* to cause diarrhea and to colonize in the RITARD model

Infecting strain	CFA/II antigen	Enterotoxins	Bacterial dose	Diarrheal response		Bacterial excretion	
				No. of ill rabbits/no. infected	Duration (days)[a]	No. of rabbits excreting for >2 days/no. infected	Duration (days)[a]
Test strains							
E1392-75 (O6:K15:H16)	CS1 + CS3	ST + LT	10^{10}	4/4	1.3 ± 0.2	4/4	6.6 ± 1.7
			10^{11}	5/7	2.7 ± 1.0	7/7	7.1 ± 1.0
E19466 (O139:H28)	CS3	ST + LT	10^{10}	4/6	3.0 ± 0.3	6/6	8.2 ± 1.5
			10^{11}	4/4	6.8 ± 1.4[b]	4/4	≥7.3 ± 1.3[b]
60R936 (O139:H28)	CS1		10^{11}	1/7	0.1 ± 0.1	6/7	4.1 ± 0.4
589R957 (O6:H16)	CS2		10^{11}	2/8	0.3 ± 0.2	8/8	6.7 ± 0.9
Control strains							
58R602 (O139:H28)			10^{11}	0/4	0	2/4	2.9 ± 0.4
E11881B (O25:H42)[c]			10^{11}	2/14	0.2 ± 0.1	5/12	2.8 ± 0.3
E11881D (O25:H42)[c]		ST + LT	10^{11}	2/4	0.5 ± 0.3	1/4	2.3 ± 0.3

[a] Arithmetic mean ± the standard error for the whole group of rabbits.
[b] All rabbits died of diarrhea 3 to 10 days after infection.
[c] Results of infection with this control strain have previously been reported (24).

positive strains in stools were considerably longer than the mean time of shedding of either of the CS-negative control strains (Table 1) or of the nonenterotoxigenic, non-MRHA fecal *E. coli* isolated from normal human fecal flora, which was 2.1 days (14). The mean time of shedding of enterotoxigenic bacteria carrying CS3 alone did not differ significantly from that of ETEC expressing CS3 together with CS1 (Table 1; $P > 0.1$). In addition, the nontoxigenic strain that expressed CS2 only was excreted for a period similar to that for the CS1-plus-CS3-positive E1392-75 strain, whereas the toxin-negative strain carrying CS1 only was excreted for a somewhat shorter period.

Protective immunity. The protective effect in the RITARD model of an initial infection with *E. coli* bacteria expressing the different CS components of CFA/II against reinfection with CFA/II-carrying ETEC was studied. Rabbits that recovered from the initial infection with the different strains were rechallenged 14 days later with 10^{11} bacteria of strain E1392-75. It was found that rabbits initially infected with 10^{10} or 10^{11} E1392-75 organisms were completely protected against disease resulting from reinfection with the homologous strain (Table 2). Thus, none of the eight animals tested developed diarrhea after reinfection with a challenge organism that caused disease in most (9 of 11) of the previously noninfected rabbits (Table 1). Similarly, none of the rabbits previously infected with serotype-heterologous bacteria carrying CS1 only developed diarrhea in response to reinfection. However, some of the animals previously given serotype-heterologous bacteria carrying CS3 only or serotype-homologous bacteria carrying CS2 only developed diarrhea (Table 2).

The protective effect of an initial infection with the various CS-positive strains against subsequent colonization with the strain used for reinfection was also studied. It was found that an "immunizing" infection with bacteria carrying CS1 plus CS3 or CS3 or CS1 alone induced protection against excretion of the challenge strain in all reinfected rabbits (Table 2). Thus, whereas previously noninfected rabbits excreted the challenge organism (10^{11} bacteria of strain E1392-75) for a mean of 7.1 days, the same challenge organism given to rabbits previously infected with CS1- or CS3-expressing organisms was shed for only 2.2 to 3.0 days, i.e., for a significantly ($P < 0.01$) shorter period than from "nonimmunized" animals. This effect on the shedding of the challenge strain could not be ascribed to the previous surgical manipulation of the intestine since rabbits initially infected with the bacteria carrying CS2 only excreted the challenge strain for a significantly longer period than rabbits immunized with bacteria expressing CS1 plus CS3 (the Student *t* test, $P < 0.01$) or CS1 ($P < 0.01$) or CS3 ($P < 0.01$) only.

TABLE 2. Protection against challenge with a $CS1^+$ $CS3^+$ ST-LT-producing strain (1392-75) resulting from initial infection with *E. coli* strains which carry CS1, CS2, or CS3

Immunizing infection[a]			Challenge infection[b]		
			Diarrheal response		
Strain	CFA/II antigen	Enterotoxin	No. of ill rabbits/ no. infected (P)	Duration (days)	Duration (days) of bacterial excretion (P)
E1392-75	CS1 + CS3	ST + LT	0/8 (<0.001)[c]	0	2.4 ± 0.3[d] (<0.001)[e]
E19466	CS3	ST + LT	2/6 (0.07)[c]	0.3	3.0 ± 0 (<0.001)[e]
60R936	CS1		0/5 (<0.01)[c]	0	2.2 ± 0.3 (<0.001)[e]
59R957	CS2		4/7 (>0.1)[c]	<1[f]	4.8 ± 0.3[g] (>0.1)

[a] The immunizing infection consisted of 10^{10} or 10^{11} bacteria of the different strains.
[b] Fourteen days after the initial infection, 10^{11} organisms of strain E1392-75 were given.
[c] In comparison with the frequency of diarrhea in rabbits after initial infection with strain E1392-75; Fischer exact test.
[d] Arithmetic mean ± the standard error for the whole group of rabbits.
[e] In comparison with excretion of strain E1392-75 in previously noninfected rabbits; the Student *t* test.
[f] Two rabbits died within 24 h and one died within 72 h after reinfection.
[g] Two rabbits that died within 24 h after reinfection are not included.

TABLE 3. Passive protective effect of different anti-CS MAbs alone and in combination against challenge with graded doses of CFA/II-carrying ST-LT-producing *E. coli* strains

MAb[a]	Challenge strain			
	E1392-75 (CS1 + CS3)		278485 (CS2 + CS3)	
	ED_{50}	PF[b]	ED_{50}	PF
Anti-CS1	6×10^9	4.5	8×10^8	1
Anti-CS2	NT[c]		6×10^9	10
Anti-CS3	4×10^9	3	2×10^9	3
Anti-CS1 + anti-CS3	3×10^{10}	25	NT	
Anti-CS2 + anti-CS3	NT		13×10^9	20
None (PBS)[d]	1.3×10^9		6×10^8	

[a] All MAbs were tested at a dilution corresponding to 10,000 times the ELISA titer against homologous CFA/II.
[b] PF, Protection factor.
[c] NT, Not tested.
[d] PBS, Phosphate-buffered saline.

Passive protection analyses. We also evaluated the capacity of MAbs against the different CS factors, alone and in combination, to provide protection against experimental infection with ETEC expressing CS1 plus CS3 or CS2 plus CS3 in rabbit small-bowel loops. All the MAbs afforded some protection against fluid accumulation in the loops induced by bacteria expressing the corresponding CS component, whereas no effect was seen with heterologous MAbs (Table 3). Combined administration of anti-CS1 and anti-CS3 MAbs resulted in considerably better protection than that obtained with each antibody alone against challenge with the CS1-plus-CS3-positive bacteria (Table 3); doubling the amount of anti-CS1 or anti-CS3 antibody in the loops did not result in such enhanced protection. Similarly, combined administration of anti-CS2 and anti-CS3 MAbs gave stronger protection against challenge with CS2-plus-CS3-positive organisms than that obtained with corresponding amounts of one antibody preparation alone.

Serological analyses. The CFA antibody responses in serum after the immunizing and challenge infections in the different groups of animals were also compared. Initial infection with either of the test strains resulted in significant antibody responses against the homologous but not the heterologous CS antigens. The antibody responses against CFA/II (CS1 plus CS3) induced by organisms positive for CS1 only, CS3 only, and CS1 plus CS3 were comparable, whereas responses against CS2 in rabbits infected with CS2 bacteria were somewhat lower (Table 4). The challenge infection resulted in enhanced CFA/II responses in all rabbits previously primed with corresponding CS antigens. In no instance did initial infection with LT-producing organisms result in significant anti-LT antibody responses in serum (data not shown).

DISCUSSION

In a previous study (3) we have shown that CFA/II-carrying ETEC bacteria are capable of colonizing the intestine and causing diarrhea in rabbits infected in the RITARD model. We now extend this observation by showing that all the individual CS components of the CFA/II complex are colonizing factors in the rabbit intestine. Thus, strains carrying either of the CS antigens alone or in combination were excreted for a significantly longer period in stools than *E. coli* isolated from normal fecal flora (14) or CS-negative mutants derived from CFA/II- (Table 1) or CFA/IV- (24) positive ETEC strains. The mean times of shedding the CS1-, CS2-, or CS3-positive organisms in stools were comparable to those previously found for ETEC strains carrying either CS4 plus CS6 or CS6 alone (24). Enterotoxin production did not seem to affect the excretion time of CS-positive organisms. These results are analogous to previous findings showing that the mean excretion time for enterotoxigenic (ST plus LT) bacteria carrying CS6 only was comparable to that for corresponding nonenterotoxigenic mutant strains (24).

The present study was in part undertaken to evaluate whether all the different CS components of CFA/II are protective antigens and whether CS3, which is present on the majority of CFA/II-positive strains, could be used alone in a future ETEC vaccine providing effective immunity against CFA/II-positive organisms. Our results showing significantly reduced excretion of the CS1-plus-CS3-positive challenge strain in all rabbits who had previously been infected with bacteria carrying either or both of these CS factors strongly suggest that both CS1 and CS3 are capable of inducing anticolonization immunity. Such immunity was seemingly not induced by homologous O antigen, since initial infection with the O6 strain carrying CS2 did not result in significantly reduced shedding of the serogroup-homologous strain used for rechallenge.

The finding of slightly better protection against disease induced by the CS1-plus-CS3-positive challenge strain as a result of previous infection with the strain carrying CS1 only compared with infection with the strain carrying CS3 only does not necessarily imply that CS1 is a more potent

TABLE 4. Antibody responses in serum against different CS antigens of CFA/II before and after an initial infection with *E. coli* strains carrying different CS factors and after rechallenge with strain E1392-75 (CS1 plus CS3)

Immunizing strain[a]	Antibody titer								
	Day 0[b]			Day 14[c]			Day 21[c]		
	CS1 + CS3	CS3	CS2	CS1 + CS3	CS3	CS2	CS1 + CS3	CS3	CS2
E1392-75(CS1 + CS3)	40	50	<5	1,100	500	<5	4,300	2,700	<10
E19446(CS3)	35	30	<5	1,000	1,000	<5	10,300	11,000	<10
60R936(CS1)	25	30	<5	700	70	20	4,300	300	<10
58R957(CS2)	40	30	10	<50	<50	300	1,200	500	250

[a] The immunizing infection consisted of 10^{10} or 10^{11} bacteria of the different strains; animals were bled immediately before (day 0) and then 14 days after the infection. For the challenge infection, 10^{11} bacteria of strain E1392-75 were given 14 days after the initial infection and animals were bled 7 days after the reinfection.
[b] Titer of pooled sera from all animals in the group.
[c] Geometric mean of titer of all sera in the group.

protective antigen than CS3. Thus, strains carrying CS1 or CS3 only were equally effective in inducing protection against colonization of the challenge bacteria, and both strains induced comparable serum antibody responses after oral infection. Instead, the difference might be ascribed to the relatively small number of animals studied. Another problem in comparing the protective immunogenicities of the different CS factors is the fact that only one strain, representing each individual CS factor, was used in these studies. Although the concentrations of CS antigen expressed by the CS1 and CS3 strains in vitro were comparable, as determined by ELISA inhibition tests (14), we cannot exclude differences in expression of the two CS factors in vivo.

Our passive protection experiments suggest that anti-CS2 antibodies also may be effective in protecting against bacteria carrying CS2, although we cannot exclude that this protection was due to the agglutinating rather than the antiadhesive properties of the antibodies. However, in a previous study (2) we have shown that Fab fragments of anti-CFA/I antibodies may be effective in preventing fluid accumulation in rabbit ileal loops induced by CFA/I-carrying ETEC, suggesting interference with the binding of the bacteria to the intestinal mucosa.

The findings that anti-CS1 or anti-CS2 antibodies may enhance the protection induced by anti-CS3 alone against challenge with CS1-plus-CS3- or CS2-plus-CS3-positive bacteria are analogous to previous results showing that antibodies with different specificities, e.g., anti-LT and anti-CFA/I (2), may cooperate synergistically in protecting against ETEC expressing both of these virulence factors. These data strongly suggest that a future ETEC vaccine should contain all three CS factors of CFA/II to provide optimal protective efficacy against the different types of CFA/II-positive strains.

Our findings of significant serum antibody responses against the CS factors of the infecting strain and also enhanced immune responses following reinfection with CS antigen-homologous bacteria suggest that intestinal administration of the different CS antigens is effective in inducing significant anti-CS antibody responses, as well as immunologic memory for these responses. Whether this is true only for CS antigens expressed on live bacteria or whether these factors can also be immunogenic in the gut when given in purified form or when present on killed bacteria cannot be evaluated in the RITARD model since inactivated antigens are very poor immunogens in the rabbit intestine (3). Oral administration of purified CFAs to human volunteers has also been very inefficient in eliciting intestinal antibody formation, probably because of proteolytic degradation of the fimbriae (11). We have recently constructed a killed oral ETEC vaccine containing CFA/I and the different CS components of CFA/II that is comparatively resistant to proteolytic degradation (25) and that will be evaluated for the capacity to induce local CFA antibody responses in the guts of human volunteers.

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