INFECTION AND IMMUNITY, June 1980, p. 1019-1027
0019-9567/80/06-1019/09$02.00/0

Vol. 28, No. 3

Species Specificity of In Vitro *Escherichia coli* Adherence to Host Intestinal Cell Membranes and Its Correlation with In Vivo Colonization and Infectivity

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We have previously described an in vitro assay for examining the mucosal adherence of a rabbit diarrheagenic *Escherichia coli*, RDEC-1. That assay defined the in vitro characteristics of RDEC-1 adherence to brush borders isolated from rabbit ileal epithelial cells. The present study was conducted to examine the species specificity of both in vitro RDEC-1 adherence and in vivo infectivity of RDEC-1 and to compare these specificities. Species specificity in vitro adherence was examined by using brush borders prepared from intestinal epithelial cells of rats, guinea pigs, and rabbits, as well as from a surgically resected specimen of human ileum. Strain RDEC-1 adherence to rabbit brush borders in vitro was significantly greater ($P < 0.001$) than its adherence to brush borders from any of the other species. Regional specificity of in vitro adherence of RDEC-1 to ileal segments of rabbit intestinal mucosa was also demonstrated. There was significantly greater adherence of RDEC-1 to rabbit ileal brush borders as compared to rabbit jejunal brush borders ($P < 0.05$). In vivo infectivity was assessed by inoculating RDEC-1 into rats, guinea pigs, and rabbits. RDEC-1 elicited diarrhea in all inoculated rabbits with the mean onset of illness occurring 5 days after inoculation. In contrast, none of the RDEC-1-inoculated rats or guinea pigs developed diarrhea. Furthermore, colonization studies in these animals revealed that RDEC-1 heavily colonized the ileum and cecum (10^9 RDEC-1 colony-forming units/g of tissue) of rabbits; however, only minimal colonization was observed in guinea pigs and rats. In conclusion, the correlation between in vitro adherence and in vivo infectivity that we have observed suggests that the presence of receptors, specific for bacteria, on the surface of the host intestinal mucosa determines species susceptibility to enteric colonization and infectivity by certain strains of enteropathogenic *E. coli*.

Adherence of enteropathogenic bacteria to the mucosal surface of intestinal epithelial cells is becoming increasingly recognized as an important determinant of virulence for some enteric organisms. Adherence of pathogenic *Escherichia coli* appears to facilitate the organism's ability to colonize the small bowel by allowing the pathogens to replicate while resisting the clearing action of intestinal peristalsis. Enteric pathogens whose ability to adhere to isolated intestinal epithelial cells has been documented include the porcine K88-positive *E. coli* (21, 24, 29, 38), the bovine K99-positive *E. coli* (5), lapine *E. coli* RDEC-1 (6, 7), the human enterotoxigenic *E. coli* (13, 28), and *Vibrio cholera* (22, 23). Other infectious processes which have been associated with mucosal adherence include the gonococcal (31, 32) and *E. coli* infections of the urogenital tract (25) and the streptococcal infections of the oral cavity (2) and heart valves (18).

In the case of the enteropathogens, attempts to infect adult members of species other than original host species have largely failed (4, 11). One hypothesis which explains the species specificity of these pathogens is that the intestinal mucosa contains organism-specific receptors which mediate bacterial adherence by interacting with complementary host-specific structures on the surface of the organisms. This adherence may in turn promote colonization of the bowel.

The present studies were conducted to determine whether in vitro adherence of *E. coli* to isolated intestinal brush borders from several species correlates with the species specificity of in vivo colonization and infectivity. For our studies, we chose the rabbit model of diarrhea reported by Cantey and Blake (6). They described a fatal diarrhea in young rabbits caused by *E. coli* RDEC-1 (O15:NM), which appears to be an unusual organism among virulent enteric *E. coli*

strains in that it is noninvasive and does not synthesize either a classical heat-stable or heat-labile *E. coli* toxin. This organism was found to adhere to the surface of the ileum and the cecum and to multiply in these areas. In this study, in vitro RDEC-1 adherence to intestinal brush borders from three animal species (rabbit, rat, and guinea pig) was quantitated, and the results were compared with the in vivo ability of RDEC-1 to colonize and elicit diarrhea in these animals. In addition, in vitro adherence of RDEC-1 to brush borders from human ileum was examined, although studies of human infectivity were not performed.

(This work was presented in part at the annual meeting of the American Gastroenterological Association in New Orleans, La., May 1979.)

MATERIALS AND METHODS

Preparation of intestinal brush borders. (i) RBB. Male New Zealand white rabbits weighing between 1 and 3 kg were anesthetized by intravenous injection of pentabarbital (65 mg/kg). A 30- to 50-cm segment of distal ileum was excised and immediately rinsed with cold (4°C) EDTA buffer (5 mM ethylenediaminetetraacetate, 5 mM Na_2HPO_4-NaH_2PO_4, pH 7.5). All further procedures were done at 4°C. The ileum was opened, the mucosal surface was scraped with glass slides, and the scrapings were placed in EDTA buffer. Rabbit brush borders (RBB) were prepared from these mucosal scrapings by the method of Donaldson et al. (9), which utilized differential centrifugation. Brush border preparations were subjected to repeated slow-speed centrifugations until nuclear contamination was essentially eliminated. The final brush border fraction was resuspended in phosphate-buffered saline (PBS) (0.145 M NaCl, 0.01 M Na_2HPO_4-NaH_2PO_4, pH 7.0). Purity of the brush border preparations was assessed by examination under phase microscopy and by following the enrichment of alkaline phosphatase activity by the method of Ray (34) (Table 1). Brush borders were prepared fresh daily for each assay. Protein was estimated by the method of Lowry et al. (26), and brush borders were diluted to a final concentration of 1 mg/ml. The number of RBB per mg of brush border protein was determined by counting in a hemocytometer under phase microscopy (×600) and found to average 2.65 (± 0.25) × 10^7 RBB/mg (mean ± standard error [SE]).

(ii) Rat and guinea pig brush borders. Rats weighing between 200 and 250 g and guinea pigs weighing between 250 and 300 g, obtained from colonies maintained by the Division of Veterinary Medicine at the Walter Reed Army Institute of Research, were sacrificed by cervical dislocation. The abdomens were opened, and 30-cm ileal segments were quickly rinsed with cold EDTA buffer (40 ml). The intestinal mucosa was harvested by scraping these intact bowel segments with a glass rod with moderate external pressure, and the scrapings were placed in cold EDTA buffer. The remaining steps in the isolation of the brush borders were identical to those used for rabbits. Purity of the brush border preparations was again documented by phase microscopy and by measuring the enrichment of brush border alkaline phosphatase activity (Table 1). These preparations were also resuspended in PBS to a final concentration of 1 mg/ml, and the number of brush borders per mg of protein was determined.

(iii) Human brush borders. A specimen of human ileum was obtained at surgery from the normal margins of a segment resected for an adenocarcinoma of the cecum. Only tissue which was normal upon gross pathological examination was utilized for the brush border preparation. The sample was rinsed with 0.9% NaCl (pH 7.0) at 4°C, and the mucosal surface was carefully dissected away from the muscularis mucosa. The mucosa was placed into EDTA buffer (50 mg, wet weight, per ml) and minced into 2-mm^3 pieces with scissors. The remaining steps in the isolation of human brush borders were carried out in accordance with the method of Houghton and McCarthy (20). The final brush border pellet was resuspended to 1 mg/ml in PBS.

Bacterial strains. *E. coli* RDEC-1 (O15:K?:NM) was isolated by J. R. Cantey (Veterans Administration Hospital, Charleston, S.C.) from laboratory rabbits that spontaneously developed diarrhea. HS (O undetermined:H4) was isolated from a normal human fecal sample and served as a nonadherent, nonpathogenic control strain (7).

Nalidixic acid-resistant (NalR) isolates of both these strains were harvested after overnight incubation at 37°C on nutrient agar containing 50 μg of nalidixic acid per ml of agar. These NalR isolates were utilized for all the in vitro adherence assays and the in vivo feeding experiments. It has been our experience that the intestinal microflora of control rabbits and guinea pigs is devoid of *E. coli*, whereas the intestinal microflora of control rats contains abundant quantities of *E. coli*. Hence, MacConkey agar plates containing nalidixic acid were used to detect the extent of colonization of rat intestinal segments by the inoculated organisms.

Bacterial cultures used for adherence experiments were grown overnight in Penassay broth (Difco Laboratories, Detroit, Mich.) at 37°C in screw-top culture tubes. These suspensions were pelleted at 2,400 × *g* at 23°C for 5 min, washed twice in PBS, and resuspended in 2 ml of PBS. The final concentrations of these bacteria were measured by plating serial 10-fold dilutions of the PBS suspensions on MacConkey agar plates and determining the number of colony-forming units (CFU) per ml after an overnight incubation at 37°C. The final concentrations of bacteria used in these studies were between 2 × 10^9 and 4 × 10^9 per ml.

Bacterial adherence assays. The in vitro adherence assay was conducted as previously described (7). Briefly, 50 μl of freshly prepared brush borders (10^5 brush borders) were mixed with a 20-μl suspension of either strain RDEC or HS (10^7 *E. coli*) and 30 μl of PBS. These mixtures were allowed to incubate for 15 min with shaking at 23°C. The tubes were shaken with a Vortex mixer to disperse aggregates of coagglutinated bacteria and brush borders. Bacterial adherence to the dispersed brush borders was quantitated by observation under phase microscopy at a magnification of ×600. The number of *E. coli* adhering to each of 40 brush borders was counted, and the results were

reported as the mean number of organisms adhering per brush border.

Indirect immunofluorescent studies. Two-micrometer sections from freshly sacrificed rabbit, rat, or guinea pig ileum were cut by using a cryostat set at −15°C. The sections were placed onto frozen glass slides and fixed to them by blowing warm air over the surface of the slide for 3 min. Next, a few drops of either RDEC or HS were placed directly onto the tissue specimen and allowed to incubate at 37°C in a moist chamber. After 15 min, the excess bacteria were washed off the slides with three 5-min changes of PBS. Each sample was then fixed for 20 min in absolute methanol, dried in a forced air incubator for 40 min, fixed again in 0.05 N HCl for 5 min followed by two 5-min changes in PBS. Next, a drop of either anti-RDEC-1 or HS serum, which was produced in rabbits, was placed onto the tissue and allowed to incubate for 20 min. Excess serum was removed by two changes of PBS. A few drops of fluorescein-conjugated goat anti-rabbit immunoglobulin (Bio-Rad Laboratories, Richmond, Calif.), containing nonconjugated rhodamine as a counterstain, was layered over the tissue sample and allowed to react for 20 min in a moist chamber, followed by removal of excess stain by four changes of PBS at 5-min intervals. Each specimen was then examined for the presence of fluorescein-labeled organisms with a Zeiss microscope.

In vivo feeding experiments. Rats (200 g), guinea pigs (250 g), and rabbits (1 kg) were inoculated via the orogastric route with nalidixic acid-resistant strains of either RDEC-1 or HS in volumes of 2.5, 5, or 10 ml, respectively. The oral bolus contained a 1:1 mixture of 10% $NaHCO_3$ and a suspension of bacteria grown in Penassay medium. Control animals received a 1:1 mixture of 10% $NaHCO_3$ and sterile Penassay medium. These animals were individually caged and observed daily for diarrhea. The presence of diarrhea was scored as firm pellet, 0; soft pellets, +; and loose stools, ++. The animals were sacrificed during the ++ stage of diarrhea or 10 days after inoculation. The rats and guinea pigs were sacrificed by cervical dislocation, and the rabbits were overdosed with pentobarbital. Their abdomens were quickly rinsed with 95% ethanol, and a laparotomy was performed. One-cm-long segments of jejunum and ileum, together with their contents, plus a section (1 by 1 cm^2) of cecal wall with adherent cecal contents were aseptically excised, weighed, and placed into a sterile ground glass homogenizer containing 2 ml of sterile saline. After homogenization, a 0.1-ml portion was removed, serially diluted (10-fold) in normal saline, and then plated onto standard MacConkey agar (rabbits and guinea pigs) or onto MacConkey agar containing nalidixic acid (50 μg/ml) for quantitation of bacterial counts (CFU/ml). Colonization of each bowel segment was expressed as the CFU per gram of tissue. Several colonies were serotyped to document the presence of RDEC-1 or HS by using antisera produced in rabbits against each of the bacterial strains.

RESULTS

Species specificity of in vitro RDEC-1 adherence. The species specificity of RDEC-1 adherence was investigated by incubating RDEC-1 with intestinal brush borders prepared from four rats, two guinea pigs, one rabbit, and a single human. When more than one animal was used, the intestinal scrapings were pooled to give a comparable yield of brush borders. The equivalent purity of these preparations was documented by assaying the enrichment of brush border alkaline phosphatase activity, counting the number of brush borders per milligram of protein, and verifying their morphological integrity under phase microscopy. The four preparations were morphologically of similar purity (>90%), had a comparable number of brush borders per milligram of protein, and were similarly enriched in brush border alkaline phosphatase activity (Table 1).

The adherence of RDEC-1 and HS to these brush borders is displayed in Table 2. The adherence of RDEC-1 to RBB was clearly distinguishable both from the adherence to brush borders from other species and from the adherence of the control strain (HS) to RBB. RDEC-1's adherence to the RBB was at least 13-fold greater than its adherence to rat, guinea pig, or human brush borders ($P < 0.001$). Moreover, the adherence of RDEC-1 to RBB was eight times greater than that of the control strain (HS) to RBB ($P < 0.001$). The adherence of RDEC-1 to

TABLE 1. *Equivalence and purity of intestinal brush border preparations*[a]

Species	No. of brush borders per mg of protein	Alkaline phosphatase activity		Enrichment
		Homogenate	Brush border	
Rat	4.68×10^7	0.103[b]	2.425	23
Guinea pig	2.74×10^7	0.293	4.116	14
Rabbit	2.76×10^7	0.078	1.800	23
Human	3.07×10^7	0.107	1.875	18

[a] Each data point represents values obtained from a single preparation of brush borders assayed in duplicate.

[b] Nanomoles of paranitrophenolphosphate hydrolyzed per minute per microgram of protein.

TABLE 2. *Species specificity of RDEC-1 adherence to isolated intestinal brush borders*[a]

Species	Intestinal segment	Mean no. of adherent *E. coli* per brush border	
		RDEC-1	HS
Rat	Ileum	0.9 ± 0.3	0.8 ± 0.2
Guinea pig	Ileum	0.1 ± 0.1	0.0 ± 0.0
Rabbit	Ileum	11.9 ± 1.2	1.5 ± 0.8
Human	Ileum	0.2 ± 0.1	0.4 ± 0.1

[a] Duplicate samples from each brush border preparation were assayed, and the mean number of organisms adhering per brush border ± 1 standard error was reported. The ratio of bacteria to brush borders ranged from 35:1 to 60:1.

all three types of non-RBB and the adherence of the control strain to all four types of brush borders tested were similar and minimal.

To assure that the concentration of RDEC-1 used in the above experiment was not limiting its adherence capability, we varied the ratio of RDEC-1 to brush borders from 1:1 to greater than 1,000:1. As can be seen from Fig. 1, the adherence of RDEC-1 to RBB occurs in a dose-dependent manner with negligible adherence seen at a ratio of one organism to one brush border and apparent saturation of the brush border receptors occurring above a ratio of 100:1. In contrast, adherence of RDEC-1 to either guinea pig or rat brush borders did not increase at any of the concentrations used. Linear regression analysis of the data in Fig. 1 using a double-reciprocal Lineweaver-Burk plot revealed an apparent saturation (V_{max}) of 45 RDEC-1 per RBB within 15 min (correlation coefficient of 0.99).

Immunofluorescent studies confirmed the adherence specificity of RDEC-1 for rabbit tissue and extended the observation to a degree of subcellular specificity. Figure 2A shows RDEC-1 adhering to the mucosal surface of rabbit ileal villi. Mucosal RDEC-1 adherence was observed from the base to the tips of each villus. Careful examination revealed that RDEC-1 only adhered to the apical portion of the villus epithelial cells. RDEC-1 were never observed adhering along the basolateral margins of the villus epithelial cells, to any portion of crypt epithelial cells, or to cells in the lamina propria, the muscularis mucosa, or the serosa. In contrast to the results in the rabbit, we were unable to detect RDEC-1 adhering, other than randomly and sparsely, to any portion of rat or guinea pig ileal mucosa (Fig. 2B and C). The nonadherent control *E. coli* strain, HS, also failed to adhere specifically to any area, but instead was randomly dispersed over the entire section of rabbit ileum.

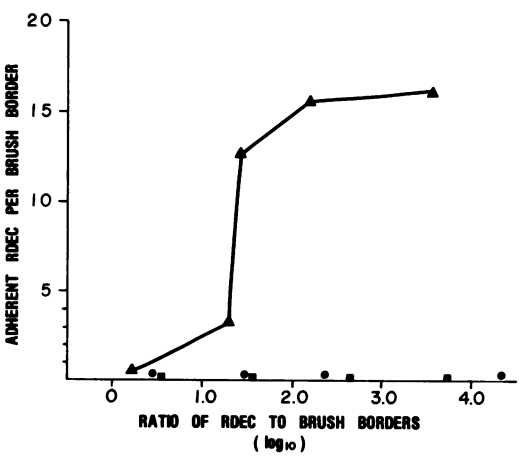
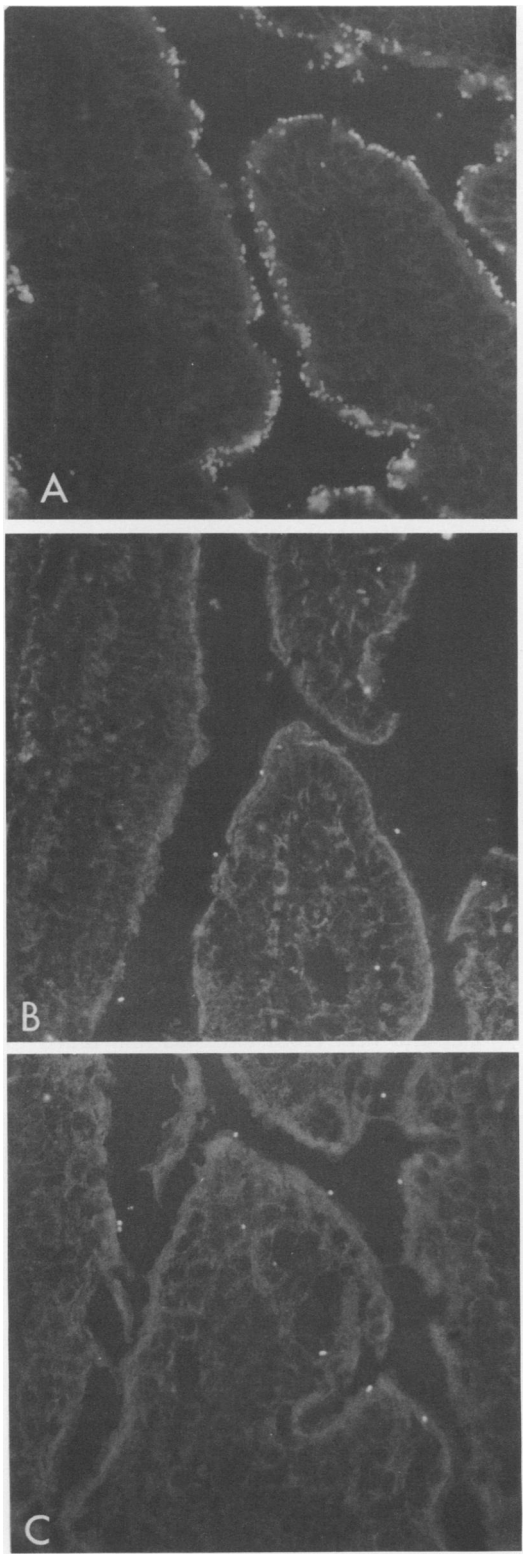


FIG. 1. *Species specificity of in vitro RDEC-1 adherence to intestinal brush borders. Rabbit (▲), guinea pig (■), and rat (●) ileal brush borders where incubated for 15 min at 23°C with varying concentrations of RDEC-1. Adherence was monitored under phase microscopy by determining the number of RDEC-1 adherent per brush border. Each point represents at least three separate experiments performed in duplicate.*

Regional specificity of in vitro RDEC-1 adherence. To examine whether the in vitro adherence of RDEC-1 to rabbit intestinal mucosa displays a regional preference, we examined the ability of RDEC-1 to adhere to ileal and jejunal brush borders. This was performed by making two separate but parallel brush border preparations from the same rabbit, one derived from the final 30 cm of terminal ileum and the other from the initial 30 cm of proximal small bowel (duodenum and jejunum). These experiments were repeated with five separate rabbits. The mean number of adherent RDEC-1 on ileal brush borders (10.7 ± 1.2) was significantly greater (Student's paired t test, $P < 0.05$) than that of RDEC-1 adherent to jejunal brush borders (6.1 ± 1.2).

Specificity of in vitro colonization and infectivity. Correlation of in vitro adherence of RDEC-1 with in vivo colonization of the intestinal tract was investigated by orally inoculating rats, guinea pigs, and rabbits with either sterile Penassay broth (PAB) or broth containing known numbers of viable RDEC-1 or HS. The animals were observed for a period of up to 10 days, during which time they were checked daily for diarrhea. After sacrifice, *E. coli* growth along the intestinal tract was quantitated and selected colonies were serotyped to document the presence of RDEC-1 or HS. The results of this experiment are displayed in Table 3.

In control rabbits given sterile PAB, no *E. coli* were present at sacrifice in any of the bowel segments tested. In the rabbits fed the nonpathogenic strain, HS, essentially no HS could be recovered from the small bowel (jejunum and ileum), and only low recoveries were made from the cecum (10^5 HS/g of tissue). In contrast to these two groups, rabbits fed strain RDEC-1 developed heavy colonization of both the ileum and cecum. Cecal colonization by RDEC-1 in these animals was threefold greater than ileal colonization and 10,000-fold greater than cecal colonization in the HS-infected rabbits. The high mean growth in the jejunum of these animals ($10^{7.8}$ RDEC-1/g of tissue) was the result of a high value from a single animal which had $10^{8.5}$ RDEC-1/g of tissue while two of the rabbits



had only $10^{2.8}$ and $10^{3.1}$ RDEC-1/g of tissue and two rabbits had no jejunal colonization at all. Thus jejunal colonization by RDEC-1 in these rabbits was highly variable, but usually much lower than that in the ileum and cecum of these rabbits. All experimental rabbits infected with RDEC-1 contracted diarrhea with a mean onset occurring 5 days after feeding.

In control guinea pigs given sterile PAB, no *E. coli* were isolated from the jejunum or ileum, and only 1 of 10 animals had *E. coli* present in its cecum. Similarly, no *E. coli* were recovered from the jejunum or ileum of guinea pigs inoculated with RDEC-1 or HS, and only minimal growth of these organisms was detected in the guinea pig cecum. In addition, RDEC-1 failed to elicit diarrhea in any of these animals.

Past studies had shown that control rats differed from control rabbits and guinea pigs in that there was endogenous *E. coli* growth in all of their intestinal segments, with heavy growth being particularly noted in the cecum. Therefore, to determine the extent of either RDEC-1 or HS colonization in the presence of this high *E. coli* background growth, we plated samples of intestinal homogenates onto MacConkey agar plates containing nalidixic acid (50 μg/ml), and the results in Table 3 for rats are expressed as growth on nalidixic acid-containing plates. These plates permitted growth of either of the inoculated nalidixic acid-resistant RDEC-1 or HS, but not growth of endogenous nonresistant *E. coli* strains. Under these conditions, no NalR *E. coli* growth could be detected in any of the intestinal segments studied in the control rats. Similarly, no *E. coli* growth was observed in the jejunum or ileum of the animals inoculated with NalR HS, and minimal growth was observed in the same segments in the rats fed NalR RDEC-1. Growth in the cecum was also minimal and equivalent in both groups. Again RDEC-1 failed to elicit diarrhea in any of the rats.

DISCUSSION

E. coli have been shown to induce diarrhea by at least two different mechanisms (11, 19). Some strains have the ability to invade the epithelial cells of the colon, produce mucosal damage, and cause a dysentery-like illness. *E. coli* RDEC-1, the strain studied in this paper, has not been demonstrated to be an invasive organism despite an extensive morphological study (37). Other *E.*

FIG. 2. *Immunofluorescent detection of the species specificity of RDEC-1 adherence to the ileal mucosal surface. (A) RDEC-1 adhering to rabbit ileum; (B) RDEC-1 nonadherence to rat ileum; and (C) guinea pig ileum.* ×400.

TABLE 3. *In vivo colonization by RDEC-1 of rabbit, guinea pig, and rat intestine*

Species	Inoculum	No. of animals inoculated	Colonization[a]		
			Jejunum	Ileum	Cecum
Rabbits	Sterile PAB	4	0	0	0
	HS PAB[b]	6	1.11 ± 1.11	0	5.11 ± 4.74
	RDEC-1 PAB[c]	6	7.83 ± 7.82	8.63 ± 8.33	9.23 ± 7.95
Guinea pigs	Sterile PAB	10	0	0	2.40 ± 2.40
	HS PAB	6	0	0	3.63 ± 3.54
	RDEC-1 PAB	10	0	0	3.82 ± 3.80
Rats[d]	Sterile PAB	8	0	0	0
	HS PAB	7	0	0	1.23 ± 1.17
	RDEC-1 PAB	8	0.95 ± 0.90	1.46 ± 1.46	2.54 ± 2.47

[a] Mean $\log_{10}$ *E. coli* growth per g of tissue ± 1 standard error.
[b] Inoculum contained 1.6×10^9 to 2.9×10^9 HS per ml of PAB.
[c] Inoculum contained 0.6×10^9 to 0.9×10^9 RDEC-1 per ml of PAB.
[d] Quantitation of *E. coli* growth in rats was obtained from MacConkey agar plates containing 50 μg of nalidixic acid per ml (see text).

coli have the capacity to elaborate enterotoxin(s) which cause the small bowel to secrete water and electrolytes. This mechanism has been suggested for RDEC-1, and preliminary evidence suggests that this organism synthesizes and secretes a small amount of *Shigella dysenteriae*-like enterotoxin (A. D. O'Brien, M. R. Thompson, J. R. Cantey, and S. B. Formal., Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, B103, p. 32). Adherence of organisms to the mucosal surface may be important for virulence by either of the above pathogenic mechanisms in that it may enhance the organisms' ability to colonize the bowel by allowing them to replicate while resisting the clearing action of peristalsis. Adherence may also increase the efficiency of toxin delivery by minimizing the distance between the mucosal surface and the pathogen. Based on results reported here and in earlier studies (7), we believe the adherence mechanism is important for RDEC-1 colonization.

At present, the molecular basis of bacterial adherence remains incompletely defined, but in theory must result from the mutual recognition and interaction of surface structures on both the bacteria and host cells. Current terminology defines the adherent structure on the bacterial surface as an adhesin and the complementary structure on the host epithelium as a bacterial receptor.

Although not all piliated *E. coli* are adherent, RDEC-1, which is the subject of this paper, is typical of adherent *E. coli* (8, 10, 21, 29, 36) in that it possesses abundant pili revealed by electron microscopic examination. Furthermore, recent studies in our laboratory have shown that genetic transfer of RDEC-1 pili correlates with transfer of adherence properties, whereas phenotypic suppression of pili on RDEC-1 has been correlated with loss of the adherence capacity of RDEC-1 (C. P. Cheney, P. A. Schad, E. C. Boedeker, and S. B. Formal, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, B24, p. 19).

With respect to host receptors for these bacteria, our in vitro adherence data reported here reveal three important characteristics about the adherence of RDEC-1. First, the adherence of RDEC-1 to intestinal brush borders is a species-specific event, occurring with rabbit but not with guinea pig, rat, or human brush borders. Attempts to detect low-affinity host receptor activity for RDEC-1 on brush borders from guinea pigs and rats by increasing the ratio of RDEC-1 per brush border also failed to elicit adherence. Second, there appears to be a gradient in the distribution of RDEC-1 receptors along the mucosal surface of the rabbit small bowel, with more receptors localized distally in the ileum than proximally in the jejunum. This finding is in contrast to that of a similar experiment reported by Isaacson et al., who used porcine jejunal and ileal epithelial cells and failed to detect any regional differences in the content of host receptors for two adherent, piliated *E. coli* strains (21). Finally, the in vitro immunofluorescent studies suggest that host receptors for RDEC-1 are selectively localized on the brush border surface of rabbit ileal epithelial cells and are not present on the basolateral membranes of epithelial cells or on any of the other membranes of the underlying tissue. This remarkable degree of species, tissue, and cell specificity possessed by RDEC-1 indicates that the rabbit intestinal mucosa contains receptors specific for RDEC-1, which are not present in other mammalian species.

Although the chemical nature of the RDEC-1 receptor on the RBB is presently unknown, the likely candidates include the intestinal mucus, the glycocalyx, and the major constituents of the brush border membrane, including phospholipids, glycolipids, and glycoproteins. It is also pos-

sible that bacterial receptors may be present on more than one of the above brush border constituents, as is seen in the case of blood group antigens A, B, and O, which are present simultaneously on cell membrane glycolipids and glycoproteins and in mucus (33).

With respect to the receptor activity which we have observed, we do not believe that the mucus or glycocalyx layers are essential for adherence because our preparation of brush borders involves extensive washing with EDTA solutions which removes these layers from the organelles (unpublished electron microscopic observations). Hence, we feel that the intrinsic membrane components are responsible for the in vitro adherence which we have observed. The glycolipids are possible candidates because brush border membranes from several species have been shown to possess a higher percentage of glycolipid than that found in any other mammalian cell membranes (14, 15). In addition, mammalian intestinal brush border membranes contain at least 12 major proteins and glycoproteins which could also serve as the RDEC-1 receptors (1, 12, 16, 27). If either of these intrinsic membrane components were the receptors for RDEC-1 then the species specificity demonstrated by RDEC-1 might be attributable to their unique oligosaccharide structures or to other antigenic differences in these glycosylated molecules.

Data supporting the hypothesis that host receptors are either glycolipid or glycoprotein in nature has been derived indirectly from hemagglutination and adherence inhibition assays with other *E. coli* adherence systems. For example, Gibbons et al. (17) were able to inhibit adherence of the K88 *E. coli* by using a glycopeptide possessing terminal B-galactosyl residues. Similarly, Bar-Shavit et al. (3) and Ofek et al. (30) were able to inhibit *E. coli* adherence to host cells with D-mannose and its analogs. Although we have previously shown that RDEC-1 adherence to brush border membranes is D-mannose resistant, it is possible that more complex oligosaccharide structures on either glycoproteins or glycolipids may serve as the bacterial receptor for RDEC-1 (7).

Whatever the molecular nature of the RDEC-1 receptor on rabbit ileal epithelial cells proves to be, the species specificity of in vitro adherence clearly correlates with the specificity of in vivo colonization and infectivity. Our results showed that RDEC-1 was only able to elicit diarrhea in rabbits. Furthermore, after the onset of the diarrhea phase, RDEC-1 was found to have heavily colonized the ileal and cecal regions of these rabbits, with minimal colonization found in the jejunum. We believe that the ability of RDEC-1 to adhere to the rabbit intestinal mucosa plays a prominent role in promoting colonization in rabbits. This idea was strengthened by the fact that our nonadherent control strain of *E. coli*, HS, failed to colonize rabbit ileum and the extent of cecal colonization by HS was 10,000-fold less than that seen with RDEC-1. Further evidence emphasizing the role of adherence in the virulence of RDEC-1 was derived from the inability of RDEC-1 to colonize the ileum and cecum of rats and guinea pigs. The demonstrated absence of RDEC-1 receptors on the mucosal surface in these animals is likely to be a factor in the failure of RDEC-1 to colonize their intestines, since a lack of receptors in these animals would lead to rapid clearance of nonadherent RDEC-1.

The hypothesis that the presence of bacterial receptors on host epithelial cell correlates with infectivity and colonization was also suggested by the findings of Rutter et al. (35). They reported that pigs have a genotypic trait governing the adherence of *E. coli* possessing the K88 antigen to their intestinal brush borders and called the genotypes "adhesive" or "nonadhesive." The "adhesive" trait was inherited in a simple Mendelian dominant manner. After challenging these two genotypic variants with a $K88^+$ strain of *E. coli*, they found that 88% of the "adhesive" pigs developed diarrhea while only 8% of the "nonadhesive" pigs showed any clinical symptoms. Furthermore the extent of *E. coli* colonization was 1,000-fold greater in the "adhesive" pigs. We believe that New Zealand white rabbits are "adhesive" for RDEC-1, while guinea pigs, rats, and humans are "nonadhesive." To date, we have not found New Zealand white rabbits which are genotypically " nonadhesive."

The ileal-cecal site of RDEC-1 colonization in rabbits is unique among animal and human *E. coli* infections. This selectivity may in part be explained by the regional adherence specificity of RDEC-1 for the ileum. Adherence assays on isolated jejunal and ileal RBB prepared in parallel demonstrated a nearly 2-fold-greater adherence capacity of the ileal brush borders. However the in vitro differences in RDEC-1 adherence observed between proximal and distal intestine cannot fully explain the marked differences in the extent of colonization seen proximally and distally. Other colonization promoting or inhibiting factors must be involved in the selective colonization of the distal intestine. For example, regional variations in the luminal environment provided by the microflora, the pH, the oxidation-reduction potential, the concentration of nutrients, or the motility patterns in the ileum and cecum may also influence RDEC-1 growth or expression of surface adhesions.

In summary, the presence of receptors specific

for RDEC-1 on the surface of rabbit intestinal mucosa appears to determine the rabbit's species susceptibility to enteric colonization and infectivity by RDEC-1.

ACKNOWLEDGMENTS

This project was supported by the U.S. Army Medical Research and Development Command.

The secretarial expertise of Joseph G. Leak and excellent technical assistance of Melissa K. Diodato are gratefully acknowledged.

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