JOURNAL OF VIROLOGY, Feb. 1985, p. 391–398
0022-538X/85/020391-08$02.00/0

Vol. 53, No. 2

Reovirus Serotype 1 Intestinal Infection: a Novel Replicative Cycle with Ileal Disease

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Received 6 September 1984/Accepted 30 October 1984

After oral inoculation, reovirus serotype 1 strain Lang was shown to specifically infect the epithelial cells of the ileum, while sparing the epithelial cells in the duodenum, jejunum, and colon. The initial site of replication was localized in cells of the crypts of Lieberkühn adjacent to Peyer's patches. Virus was subsequently found by immunoperoxidase staining in cells migrating up the crypt-villus complex throughout the ileum. The severity of the pathological changes in the ileum was proportional to the concentration of the viral inoculum. This site-specific infection of the ileum by reovirus may provide a model for diseases that are restricted to specific sites in the intestine.

The gastrointestinal (GI) tract is a portal of entry for a wide variety of viruses which cause systemic infection. These include poliovirus, coxsackievirus, echovirus, hepatitis A virus, coronavirus, and reovirus. Although only a few of these cause overt GI disease, infection of the GI tract as evidenced by the persistent excretion of virus in feces is an essential part of transmission of infection. However, for these viruses the precise mechanism of pathogenesis in the GI tract is not known (4, 12, 28).

To study GI viral pathogenesis, we used the mammalian reovirus serotype 1 strain Lang (reo 1/L). Reovirus serotypes 1, 2, and 3 were initially isolated from humans or mammals with mild gastroenteritis and are classified as enteric viruses (23, 24). Reo 1/L is uniquely suited for these studies because the virus is well characterized (13). The virus consists of two protein capsids surrounding a genome of 10 segments of double-stranded RNA (16). The virus is resistant to intestinal proteolytic enzymes and is a prototype for viruses that cause systemic disease after entry from the GI tract (25). Previous studies in mice have identified the microfold (M) cells located in the dome epithelium of Peyer's patches as the site of specific virus binding and entry into the host (34, 35). In this study, we have defined the site of intestinal infection in mice to be the epithelial cells located in the crypts of Lieberkühn of the ileum. The severity of intestinal infection is directly proportional to the initial inoculum of infectious virus.

MATERIALS AND METHODS

Mice. Female A/J mice (8 to 10 weeks of age; Jackson Laboratories, Bar Harbor, Maine) were fed a house diet ad libitum (Purina, St. Louis Mo.). All mice were checked for the presence of antibody before inoculation, kept in a reovirus-free colony, and tested for the presence of secretory and serum antibody on a weekly basis by enzyme-linked immunosorbent assay (29). The mice had no evidence of antibodies to reovirus before inoculation.

Virus. Reo 1/L previously has been described (13, 21). A second passaged stock of reo 1/L was purified by a modification of published techniques by substituting ultrasonic disruption (Branson ultrasonic 200) for homogenization of cells (21).

Mouse inoculation. Mice were inoculated with 0.2 ml of reo 1/L suspended in sterile saline containing gelatin. Mice were inoculated perorally (p.o.) under light anesthesia with 10^8, 10^9, or 10^{10} PFU of reo 1/L by passing a fine catheter (PE-50 polyethylene tubing; Clay Adams, Parsippany, N.J.) into the stomach. Alternatively, virus was inoculated into the duodenum of anesthestized mice via a 30-gauge needle attached to a tuberculin syringe. Mice were observed for evidence of diarrhea or systemic illness until 20 days after inoculation.

Virus titers in intestinal tissue. Mice were killed on days 1, 3, and 6 after p.o. inoculation of reo 1/L. Intestines were harvested from the gastroduodenal junction to the distal colon. The small intestine was separated from the colon and divided into three segments approximating the duodenum, jejunum, and ileum. The intestinal contents were removed from the colon and segments of small intestine by washing each lumen with 1 ml of phosphate-buffered saline (pH 7.4), and then each piece of intestine was suspended in 1 ml of saline containing 2% gelatin (pH 7.4). Intestinal tissues were prepared for titration by sonic disruption. The virus titer was determined on L-cell monolayers.

Pathological specimens. Mice were sacrificed on days 1, 2, 3, 5, 7, 9, 15, and 20 after infection. All specimens were placed in Bouin fixative and examined by routine histology and immunocytochemistry. Viral antigen was localized by a modification of an avidin-biotin immunoperoxidase technique as described by Kornstein et al. (18). For electronmicroscopic examination of immunoperoxidase-positive specimens, the cover slip of immunoperoxidase-stained tissue sections was removed, and the tissue was infiltrated with Epon. The Epon-impregnated sections were fused to an inverted beam capsule containing polymerized Epon, and serial sections were cut and examined for peroxidase reaction product or virus (6).

Estimation of infected intestinal cells. The number of infected epithelial cells in pathological specimens was estimated by counting antigen-positive and -negative cells in at least three separate crypt-villus complexes. For mice infected with 10^{10} PFU, the number of infected cells was estimated by counting the number of antigen-positive and -negative cells in the region of the ileum perforation on three

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TABLE 1. Infectious virus in various segments of intestine[a]

Segment of bowel	Titer of reo 1 (PFU [$\log_{10}$]/mg) at postinfection day:		
	1	3	6
Duodenum	4.69 ± 0.43[b]	3.53 ± 1.07[d]	0.68 ± 1.24[c]
Jejunum	4.73 ± 0.21[b]	3.73 ± 0.56[d]	0.99 ± 1.21[c]
Ileum	5.55 ± 0.16	4.07 ± 0.56	3.12 ± 0.82
Colon	4.04 ± 1.96[d]	4.20 ± 0.51[d]	0.70 ± 1.06[c]

[a] Adult A/J mice were inoculated p.o. with 10^8 PFU of reo 1/L. The mice were sacrificed on days 1, 3, and 6 after inoculation, and the intestine was removed for virus titration. The titer is the geometric mean of three to six specimens and is expressed relative to the protein content of the specimen. The viral titer of each segment of bowel was compared with the titer in the ileum. Significance is noted by superscripts: [b], $P < 0.02$; [c], $P < 0.01$; [d], P is not significant.

separate slides. The percentage of antigen-positive cells was calculated as follows: (number of cells that are antigen positive/total number of cells in the crypt or villus) × 100.

Intestinal epithelial cell separation. Intestinal epithelial cells were eluted from the mucosa by a modification of the method of Weiser (33). Mice were sacrificed on days 4 and 6 after p.o. inoculation. The small intestine was harvested from the gastroduodenal junction to the distal colon and washed with 2 ml of ice-cold phosphate-buffered saline (pH 7.4). The small intestine then was everted over a glass rod and incubated for 15 min at 37°C in Joklik's modification of Eagle minimum essential media for spinner culture (calcium free; pH 7.4), containing 1.5 mM EDTA (complete MEM). The eluted cells were removed from the intestine by gentle aspiration with a Pasteur pipette. The intestine was resuspended in complete MEM, and the procedure was repeated. Five eluted cell fractions were combined, washed twice in phosphate-buffered saline, and counted. The cells obtained were single cell suspensions of villus epithelial cells. The intestinal epithelial cells that remained attached to the submucosa after elution were examined by light microscopy of the residual intestine.

Preparation of rabbit antireovirus antibody. Hyperimmune antireovirus serum was produced in rabbits by standard immunization protocols. The gammaglobulin fraction was obtained by precipitation with ammonium sulfate and was passaged through a staphyloccocal protein A column (14). The antibody fraction eluted from the staphylococcal protein A column was absorbed with mouse liver powder (20 mg/ml; Sigma Chemical Co., St. Louis, Mo.) for 1 h at room temperature. Antireovirus antibody was absorbed with purified reovirus (10^{11} particles per ml) to remove antibody specific to reovirus.

Infectious center assay. Infectious center assays were performed on isolated intestinal cells by modifying the technique of Ahmed and Graham (2). Isolated intestinal cells were incubated with hyperimmune antireovirus rabbit immunoglobulin G for 1 h and then washed twice with phosphate-buffered saline to remove excess antibody. These cells were mixed in serial dilution with 7×10^5 L cells and incubated at 37°C for 60 min in fibronectin-coated plates to permit cells to adhere; the cultures were overlaid with nutrient agar, and plaques were scored.

Virus titration. The titer of reo 1/L was determined in 12-well dishes (Costar). L cells (7×10^5 per well) were incubated in Costar dishes overnight at 37°C. The medium was removed, and virus was incubated with the L cells for 1 h at 37°C. A 1.5-ml nutrient layer consisting of medium 199 (Irvine Scientific, Irvine, Calif.) supplemented with 5% fetal bovine serum, 1% glutamine, 5 μg of gentamicin per ml, 40 U of Mycostatin (nystatin; E. R. Squibb & Sons, Princeton, N.J.) per ml, and 1% agar (Sigma), was placed in each well. A second nutrient layer (1 ml) was added on day 3 of incubation, and a final layer was added on day 6. The final layer contained medium 199 supplemented with 5 μg of gentamicin per ml, 40 Units of Mycostatin per ml, 0.02% neutral red dye, and 1% agar. L-cell monolayers were examined for viral plaques 24 h later.

Protein concentration of intestinal specimens. The concentration of protein in specimens of intestine was determined by the Bradford dye-binding method (Bio-Rad Laboratories, Richmond, Calif.) (8).

Statistical analysis. The Student t test was used to determine the significance of viral titers in the ileum compared with other segments of bowel and the number of immunoperoxidase-positive cells in the crypts of Lieberkühn compared with the number of immunoperoxidase-positive cells in the villi.

RESULTS

Infectious virus is predominantly found in the ileum. Previous studies demonstrated that reo 1/L binds to M cells overlying Peyer's patches and that it is transported through them into the host (34, 35). Nevertheless, electron micrographs show no evidence of reovirus replication in M cells (34, 35). Furthermore, the number of M cells, ca. 10% of the population of cells in the dome epithelium of Peyer's patches, is probably insufficient to support the titer of virus obtained after reo 1/L infection (Table 1; 17, 20, 25, 30). Our goal in these experiments was to define the precise location of intestinal infection for reo 1. The total virus present in specific regions of the intestine was determined by viral titrations of segments of bowel corresponding to the duodenum, jejunum, ileum, and colon (Table 1). Virus was detected in all segments of the intestine on postinfection days 1 through 6. On day 1 postinoculation, viral titers in the ileum were significantly ($P < 0.02$) higher than those obtained in other segments of small bowel. In general, viral titers decreased dramatically in all segments of intestine except in the ileum by day 6 postinoculation. In the ileum, the titer of virus was ca. 2 to 3 $\log_{10}$ greater in the other segments of bowel 6 days after infection ($P < 0.01$) (Table 1). These results suggested that reo 1 is specifically associated with cells in the ileum.

TABLE 2. Infectious center assay[a]

Bowel segment	Eluted cells per 10^4 cells on day:		retained cells (PFU/ml) on day:	
	4	6	4	6
Duodenum	<1	<1	NT	<10
Jejunum	<1	<1	NT	<10
Ileum	10	10	1.5×10^4	8.3×10^2
Colon[b]	<1	<1	7.5×10^3	<10

[a] Intestinal epithelial cells from the villus were separated into a single cell suspension, and the titer for virus was determined as indicated in the text. In the eluted cells, infectious virus was only found in the ileum on days 4 and 6 after infection. There was no infectious virus present in the villus cells obtained from the duodenum, jejunum, and colon. The titer of virus obtained from the retained cells is expressed as PFU per milliliter of lysate. NT, Not tested.

[b] Eluted cells in the colon are surface epithelium cells, and the retained cells are the glands. In other segments of intestine, the eluted cells represent the epithelial cells of the villus, and the retained cells represent the crypt epithelial cells.

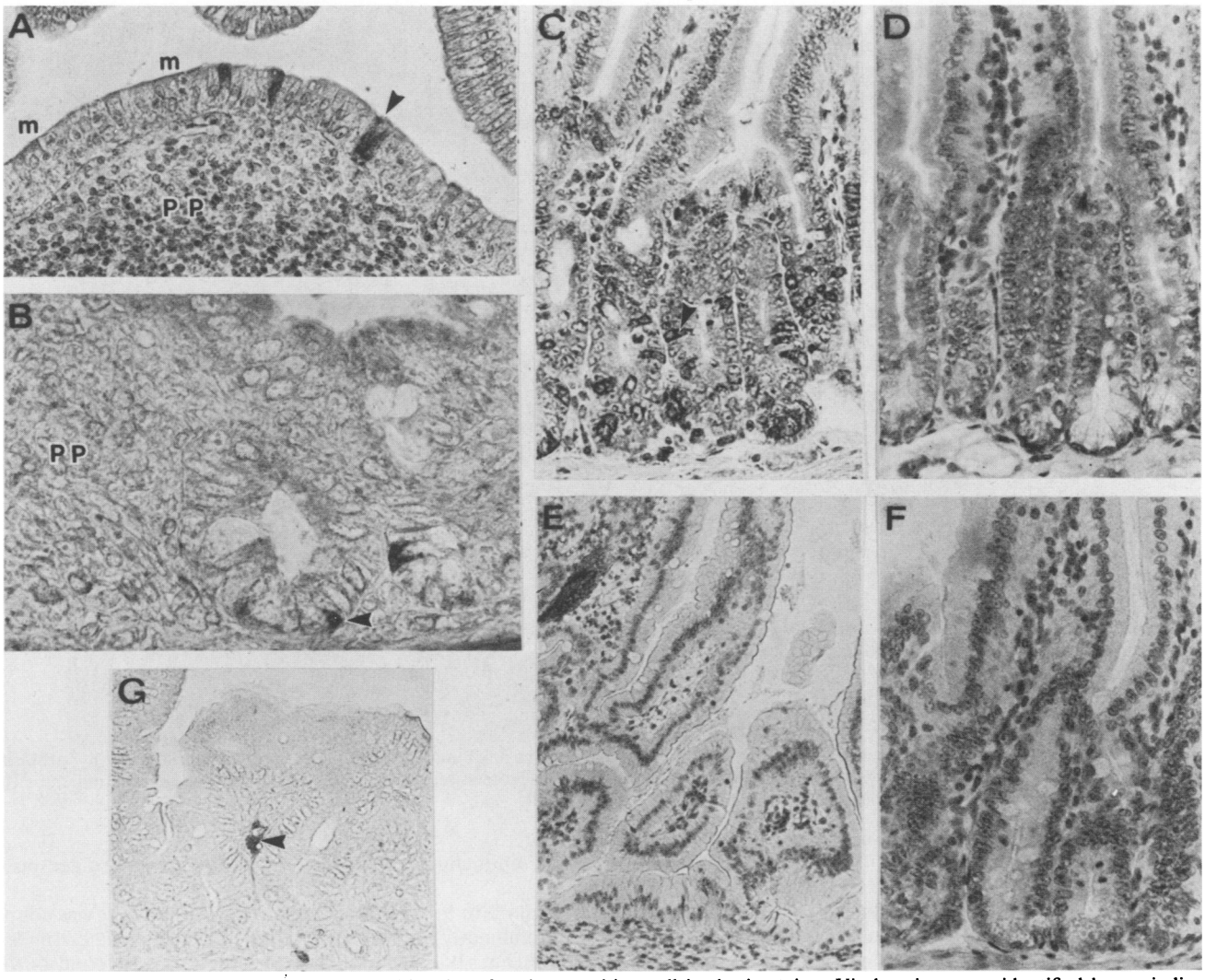

FIG. 1. Photomicrographs, showing the initial site of antigen-positive cell in the intestine. Viral antigen was identified by an indirect immunoperoxidase technique with biotinylated antibody and avidin-peroxidase. The antigen-positive cells are darkened by the reaction product. The arrows point to representative cells. (A) At 48 h after inoculation, reovirus antigen-positive intestinal cells were seen in the dome epithelial cells overlying Peyer's patches (PP). The localization of M cells was confirmed by 1-μm plastic sections (m) (×150) and (B) in adjacent crypts (×300). (C) At 5 days after inoculation, the number of antigen-positive cells in the crypts greatly exceeded those in the villi (×300). (G) At 20 days after inoculation, the intestine appeared normal morphologically, and antigen-positive cells could only be found in mononuclear cells in the lamina propria (×300). Controls: D, in the absence of reovirus-specific antibody no staining was observed; E, staining a known antigen-positive specimen was lost when antireovirus rabbit antibody was absorbed with purified reo 1/L; F, no staining was seen in an uninfected specimen (×300).

To determine whether infectious virus localized to the ileum as a result of either absorption to cell surfaces or replication in epithelial cells, infectious center assays were performed on an epithelial cell fraction (2, 33). We chose days 4 and 6 to examine isolated epithelial cells since the initial inoculum of virus should no longer be present on these days (15). Single cell suspensions of intestinal cells were treated with rabbit antireovirus antiserum to neutralize any virus adherent to the cell surfaces. Pathological examination of the eluted cells showed that they represent the villus cell population (data not shown). A total of 7.2×10^5 cells were eluted from the ileum, and we estimated that 0.1% was infected with reovirus (Table 2). We lysed the epithelial cell population by sonication and determined the titer of virus in the lysed cells. Approximately 7 PFU was present per infected cell, yielding a titer of 5.0×10^3 PFU for the isolated villus cell population.

TABLE 3. Effect of initial inoculum of pathology[a]

Titer	No. of mice		
	Ileitis	Diarrhea	Death
1×10^{10}	++++ (4/5)	0/10	5/10
5×10^9	+++ (2/5)	0/10	0/10
1×10^9	++ (2/5)	0/10	0/10
1×10^8	+ (1/5)	0/5	0/5
1×10^6	− (0/5)	0/5	0/5

[a] A/J mice were inoculated with 10^6 to 10^{10} PFU of reo 1/L p.o. and observed for 6 days. Five mice in each group were sacrificed for examination of pathology. The pathology was scored in the following manner: ++++, loss of crypt-villus complex and perforation of the ileum; +++, crypt hyperplasia, villus blunting, loss of nuclear polarity, and vacuolization of epithelial cells; ++, two of the criteria listed in +++; +, one of the criteria listed in +++. Diarrhea was evaluated by the capacity to express a liquid or semiformed stool during the observation period. The number within parentheses represent the number of mice with microscopic evidence of ileal disease in each group.

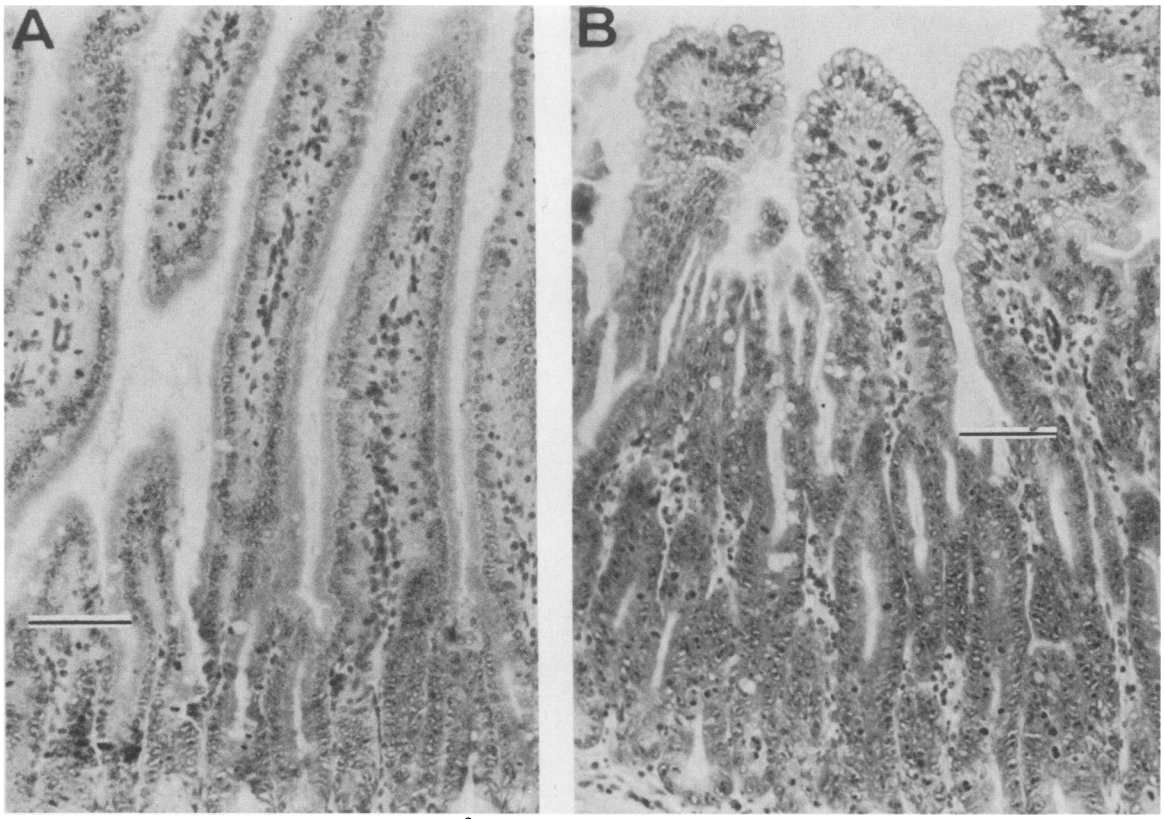

FIG. 2. (A) Normal ileum; (B) mice inoculated with 10^8 PFU had evidence of inflammatory cells in the lamina propria, crypt hyperplasia, and a loss of nuclear polarity of the epithelial cells. There is evidence of vacuolization of the villus cells. The line marker indicates the demarcation between the crypt and villus cells.

The quantity of virus present in the retained cell population in various bowel segments was determined by sonic disruption of the intestine remaining after elution of villus cells. Retained cells were found in clumps, and thus these cells were not incubated with antireovirus antibody since antibody might not have access to all cell surfaces and would be unable to neutralize virus. Infectious virus was associated with the retained cell population of the colon and ileum on day 4 but only in the ileum on day 6 (Table 2). The total titer of virus in the retained cells represented more than 50% of the total virus (retained and eluted) associated with cells on these days. We could not find infectious virus in the duodenum or jejunum in the retained cell population on day 6. Hence, virus was associated with both the eluted and retained cell populations in the ileum.

Immunoperoxidase staining for reovirus antigen localizes infected epithelial cells to the ileum. Virus titration of specific regions of intestine revealed an association of reo 1/L with the villus and crypt cell populations of the ileum. To determine which type of intestinal cell was infected by reo 1/L, mice were inoculated with 5×10^9 PFU of reo 1/L and euthanized at various times postinfection. The small intestine was removed and stained by an avidin-biotin complex immunoperoxidase method for viral antigen. Infected epithelial cells were first identified at 48 h postinoculation (Fig. 1). The epithelial cells which were positive for reovirus antigen initially were located in the basal crypt cells and in the dome epithelium associated with Peyer's patches (Fig. 1A and B). Similar to the results of the infectious center assay, infected cells comprised a small proportion (<1%) of the epithelial cells. Occasional mononuclear cells beneath the dome epithelium and in Peyer's patches were antigen positive.

From 2 to 9 days after infection, viral antigen was found predominantly in the crypt epithelial cells located in the ileum (Fig. 1C). No evidence of infection was found in the duodenum or proximal jejunum, even in sites adjacent to Peyer's patches. At the junction of the distal jejunum and ileum, isolated crypt cells exhibited staining for antigen. Finally, at 20 days postinfection, when infectious virus could no longer be recovered ($<10^3$ PFU/ml) from the intestine, antigen was present in macrophages in the lamina propria of the ileum but not in any intestinal epithelial cells (Fig. 1G).

During the entire course of the infection in the ileum, a greater number of crypt epithelial cells than villus epithelial cells were found to contain viral antigens (Fig. 1C). By counting stained versus nonstained cells in the ileum, an estimated 12.2 ± 3% of the epithelial cells located in the crypts were antigen positive compared with 0.37 ± 0.2% of the epithelial cells of the villi. This difference is statistically significant ($P < 0.015$).

Reovirus does not replicate in M cells. M cells, which are located in the epithelium overlying Peyer's patches, did not demonstrate viral antigen by immunoperoxidase staining. Virus particles were not found in M cells in electron micrographs obtained 2 days after infection; however, viral particles were visible in the electron micrographs of intestinal epithelial cells that were positive by immunoperoxidase staining. This observation suggests that virus does not replicate in M cells.

Pathological changes due to reo 1/L infection are restricted to the ileum. To determine the role of the initial dose of virus

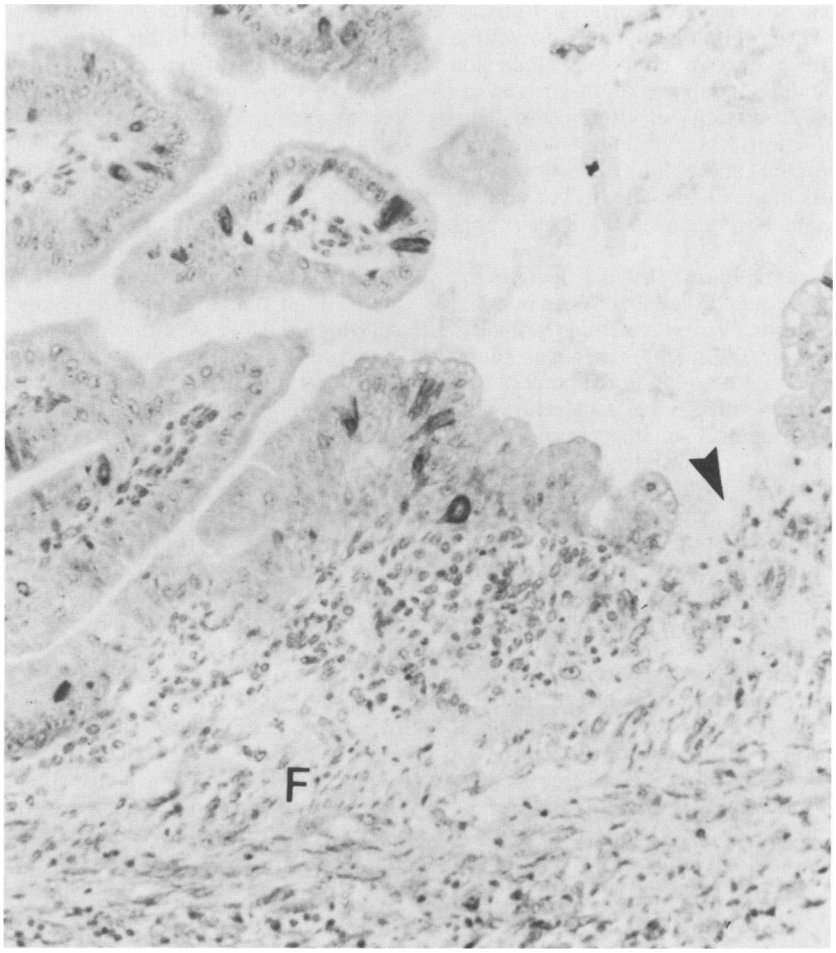

FIG. 3. In mice infected with 10^{10} PFU of reovirus, perforation or incipient perforation of the intestine is found in the ileum. Near sites of perforation, there was a complete absence of the normal crypt-villus architecture, leaving the lamina propria covered by a single layer of epithelium. Fibroblast proliferation is evident (F). Exposure of the lamina propria to intestinal contents can be seen at the right-hand border of the photomicrograph (arrow) (×450).

in the development of symptomatic bowel disease and pathological changes in the intestine, we administered virus either p.o. or by inoculation directly into the duodenum of 8- to 12-week-old A/J mice (Jackson Laboratory). We used both approaches since we were uncertain of the effect of gastric contents on reo 1/L infectivity; both techniques resulted in a similar pathological response. Results are shown (Table 3) for the mice inoculated p.o. with 10^6, 10^8, and 10^{10} PFU. At doses of 10^6 and 10^8 PFU per mouse, there were no gross pathological changes in the intestine. However, mice inoculated with 10^8 to 10^9 PFU had microscopic evidence of infection, consisting of inflammatory cells in the lamina propria, hyperplasia of the crypts, and loss of nuclear polarity from days 2 to 14 (Fig. 2). The alterations in the crypt-villus ratio and loss of nuclear polarity are similar to alterations from lesions described in other forms of nonbacterial gastroenteritis (27). Inoculation of 10^{10} PFU per mouse resulted in a fatal outcome for 5 of 10 mice by day 5. The five mice that died and three of the five still alive on day 5 had evidence of fibroblast proliferation and fibrosis in the lamina propria, as well as peritonitis (Fig. 3). All mice that died had transmural perforation of the ileum. In the three surviving mice with peritonitis, the lamina propria and muscularis were still intact, suggesting that the lesion might have resolved. An estimated 20.3 ± 2% of the epithelial cells in the area of the perforation were antigen positive, strongly suggesting that the virus played a direct role in the induction of this lesion. None of the mice inoculated with the above doses of virus developed diarrhea, consistent with the localized nature of the inflammatory process in the intestine.

DISCUSSION

We have shown that reo 1/L selectively replicates in the crypt epithelial cells of the ileum and that the proximal small intestine is infected. The association of virus with intestinal cells was pursued by three separate techniques: (i) titration of virus from segments of intestine corresponding to duodenum, jejunum, ileum, and colon; (ii) infectious center assay of isolated intestinal epithelial cells; and (iii) immunoperoxidase localization of virus antigen. Titration of virus from segments of intestine obtained on day 1 postinfection revealed a greater localization of virus in the ileum as compared with other segments of small bowel. However, viral titers in the ileum were significantly different than viral titers in the duodenum and jejunum but were not significantly different from titers obtained in the colon on day 1 postinfection. The difference in viral titers in the ileum compared with all other segments of bowel on day 3 postinfection was not statistically significant. We hypothesize that the apparent association of virus with the ileum on day 1

postinfection and the lack of association on day 3 postinfection represent transit of the initial inoculum through the gastrointestinal tract. However, we cannot exclude the possibility that specific binding or replication of virus in the ileum accounts for the higher quantity of virus in the ileum on day 1. On day 6 postinfection, when viral titers were quite low in the duodenum, jejunum, and colon, there was still a significant quantity of virus in the ileum. The titer of virus in the ileum on day 6 postinfection suggests that virus replicates in the ileum.

The infectious center assay confirms that the ileum is the primary site of infection and that epithelial cells are infected on days 4 and 6 postinoculation. Virus was found primarily in cells eluted from the ileum. Although virus was found associated with the retained cell population of the colon on day 4 after infection, this may reflect virus adherent to the cell surface since we did not neutralize adherent virus with antireovirus antibody on retained cells. Indeed, by day 6, we could no longer find infectious virus associated with the colonic crypt cells, yet infectious virus still was associated with the ileal crypts, suggesting that infection was restricted to the ileum. Further support of this hypothesis was provided by immunoperoxidase techniques, in that reovirus antigen was only found in the ileum and not in other segments of the intestine. These results indicate that, in the intestine, reo 1 replication is restricted to the ileum.

The results obtained with immunoperoxidase staining of infected ileum further suggest that the principal site of epithelial-cell infection in the ileum is in the crypts of Lieberkühn. It is improbable that the extensive concentration of infected cells in the crypt is due to preferential exposure of the crypt epithelial cells to virus during a viremia, since the villus is the region with the greatest vascularity (3). In addition, the localization of the infection in regions adjacent to the Peyer's patch on day 2 suggests that the plexus of lymphatics in the lamina propria is the route of transmission for the initial epithelial infection. In recent experiments we have found that intravenous administration of reo 1/L results in the same specific pathological changes in the ileum as p.o. inoculation or inoculation directly into the duodenum; hence, it is likely that tropism of the virus is dependent upon specific receptors located on the basal lateral membranes of crypt cells (D. Rubin and A. Anderson, manuscript in preparation). Wolf and co-workers have found that a small percentage of intestinal epithelial cells bind reo 1 to their luminal surface (34). However, these investigators did not find entry of reo 1 into these cells. Therefore, it is unlikely that the initial infection of crypt cells adjacent to Peyers' patches is due to entry of virus from the luminal surface of crypt cells. At later times postinfection, when crypt cells throughout the ileum have evidence of virus antigen, it is possible that a viremia may account for the distribution of infected cells (Fig. 4). Alternatively, virus may be widely distributed and absorbed to intestinal crypt cells in the GI tract, but only the basal crypt cells in the ileum may be capable of supporting reo 1/L replication.

To produce ileitis in adult mice, massive doses of virus were necessary. Other investigators have found that adult mice are resistant to reovirus disease (31); however, we inoculated mice with ca. 2 $\log_{10}$ units more infectious virus than had been described previously by Tardieu and co-workers. Therefore, it is difficult to compare our capacity to induce disease with previous studies. The high doses required to produce disease suggest that the A/J mice used in these experiments may be partially resistant to the effects of reo 1/L. This may be due to either a failure of local spread of

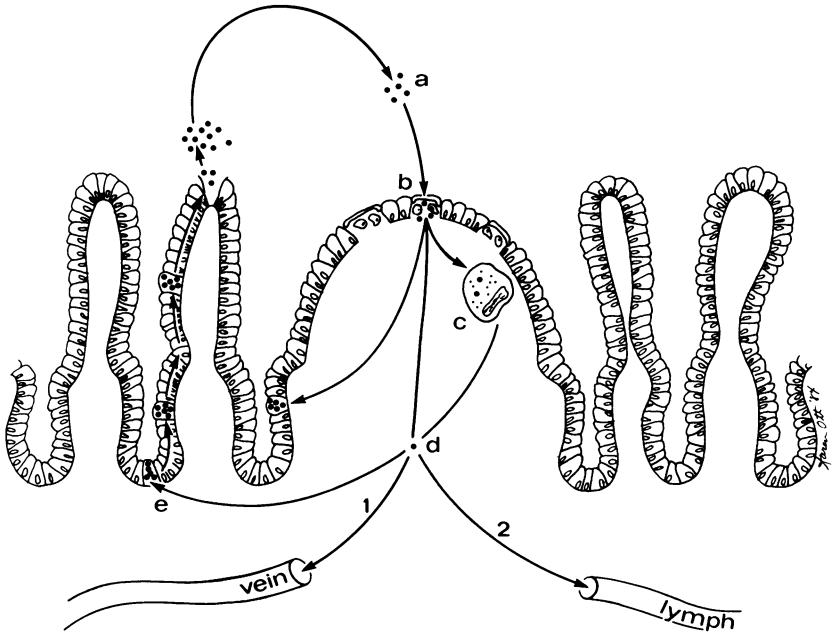

FIG. 4. Schematic diagram, showing the probable route of entry and local or systemic spread of reovirus. Virus (a) in the lumen of the intestine is transported into the host via M cells (b) to the macrophage-rich subepithelial region overlying a Peyer's patch. From the lymphoid follicle, the virus either is endocytosed (c) and carried intracellularly or flows freely in lymph to the basal crypt epithelial cells. Dissemination of virus may occur (d) by vein (no. 1) or by lymphatic efferents (no. 2) to the blood stream where it is circulated to systemic or intestinal sites supportive of virus replication, thereby resulting in infection of crypt cells distant to the Peyer's patch. In the intestine, the infected basal crypt cell (e) migrates toward the villus tip during muturation into an absorptive enterocyte; lysis or exfoliation releases free virus into the intestinal lumen.

infectious virus to susceptible cells or the inability of adult mice to sustain a viremia.

We were particularly intrigued by the pathology seen with high-virus inocula. The remarkable ability of reo 1/L to cause perforation of the ileum may relate to its capacity to infect the undifferentiated progenitor cells at the base of the crypts, thereby destroying the integrity of the crypt-villus complex. The scarring and narrowing of the lumen associated with perforation of the ileum, which was observed in the high-dose reo 1/L infections, resemble the pathological changes of the congenital disease intestinal atresia in humans (11, 32). Infants with intestinal atresia are born with areas of stricture or complete obliteration of the intestinal lumen. Although the etiology is unknown, several animal models have been advanced to explain congenital intestinal atresia. In one model, perforation of the intestine in utero resulted in neonatal atretic lesions (1). Several observations point to an association between intestinal atresia and reo 1: (i) as described above in mice, reo 1 infects the ileum and can cause perforation; (ii) a related reovirus, serotype 3, produces biliary atresia in rodents, and human infants with biliary atresia have antibodies to this virus (5, 18); and (iii) we have been able to infect adult mice during early pregnancy and can demonstrate reovirus antigen in the neonatal intestine (D. Rubin and A. Anderson, unpublished data).

Infection with a site-specific virus, such as reovirus, may be the cause of site-specific disease. It is known that nutrients including vitamins, minerals, and bile salts are absorbed in particular regions of the small intestine (7). Infection of the ileum may affect absorption of nutrients, allow for attachment of a second pathogen and lead to overgrowth of bacteria, or lead to persistent infection (9, 10, 22, 25). Hence, a number of disease states, including chronic diseases and certain types of malabsorption (9, 25), may result from viral infection. Further studies are in progress to examine the role of reovirus in the pathogenesis of intestinal disease.

ACKNOWLEDGMENTS

We thank M. A. Eaton for expert technical assistance, M. Attie, F. Gonzalez-Scarano, E. Eisenberg, N. Nathanson, and S. Cohen for critical review of the manuscript; and B. N. Fields for valuable discussion.

D.H.R. was supported by Public Health Service grant 1 R22 AI18554 from the National Institutes of Health.

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