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Intestinal M Cells: A Pathway for Entry of Reovirus into the Host

Abstract. *Thirty minutes after inoculation of reovirus type 1 into the intestinal lumen of the mouse, viruses were found adhering to the surface of intestinal M cells but not other epithelial cells. Within 1 hour, viruses were seen in the M cell cytoplasm and were associated with mononuclear cells in the intercellular space adjacent to the M cell. These findings suggest that M cells are the site where reovirus penetrates the intestinal epithelium.*

After their ingestion, enteric viruses move rapidly through the upper alimentary tract and into the lumen of the small intestine. Certain viruses, such as the human rotaviruses (the major cause of infantile viral gastroenteritis), infect intestinal epithelial cells and cause local inflammation and diarrhea (*1*). Many other viruses, including enteroviruses and mammalian reoviruses, enter the systemic circulation through the gastrointestinal tract during the early stages of infection but cause few if any local symptoms (*2, 3*). The factors that determine whether a particular virus will cause local infection or will simply "pass through" the gastrointestinal tract are not known. We have been using mammalian reoviruses to study the pathogenesis of systemic viral infections (*4, 5*). Reoviruses are icosahedral viruses consisting of segmented double-stranded RNA surrounded by a double capsid (shell) containing eight polypeptides (*6–8*).

We recently focused on the primary events determining entry of reoviruses from the gastrointestinal tract into the systemic circulation of mice (*5*). In examining the early stages of infection with type 1 reovirus after peroral inoculation, we noted a striking enlargement of Peyer's patches. This was accompanied by the rapid appearance of infectious virus in mesenteric lymph nodes, suggesting transport to this site (*9*). For these reasons we studied the anatomic localization of reovirus early in infection after its introduction directly into the gastrointestinal tract.

We now report that reovirus type 1 spares intestinal epithelial cells with the exception of the microfold or "M" cells, a population of specialized epithelial cells that overlie Peyer's patches (*10, 11*). M cells have been shown to transport macromolecules, such as horseradish peroxidase and ferritin, from the intestinal lumen across the epithelial barrier to the intercellular space, permitting their uptake by mucosal mononuclear cells (*11, 12*). We now present evidence that entry of reovirus type 1 into the intestinal lymphatics and ultimately the systemic circulation is similarly determined by a specific interaction between reovirus and M cells.

We inoculated reovirus type 1 into closed ileal loops of live suckling mice, which develop the most severe disease in response to virus and which were used in our prior studies of viral entry and spread (*5*). The closed-loop model was selected so that the earliest events of viral attachment and entry could be examined and to achieve a high concentration of virus in the lumen (*13*). The experiments were performed prior to closure of the intestine to macromolecular transport, which occurs at about 18 days of age (*14*).

Closed loops of ileum (1 to 2 cm in length) were constructed in ether-anesthetized BALB/c mice (7 to 10 days old) by suture ligation. We placed the distal ligature 1 to 1.5 cm proximal to the ileocecal valve, taking care to leave the mesenteric blood supply intact. Reovirus type 1 was grown in mouse L cells and purified (*7*). In order to facilitate visualization of virus, a large inoculum (5.6×10^8 plaque-forming units in a volume of 0.02 ml) was injected into the loop with a 26-gauge needle. The loops were removed 30 minutes or 1 hour after virus inoculation and processed for electron microscopy (*15*).

Absorptive cells were the predominant lining cell and were easily seen in all sections. Although an exhaustive search was performed, no viruses were detected adhering to or within absorptive cells lining Peyer's patches or absorptive and goblet cells lining adjacent villi. A few free viruses were occasionally found in the lumen, adjacent to microvilli. In contrast, many viruses were readily seen

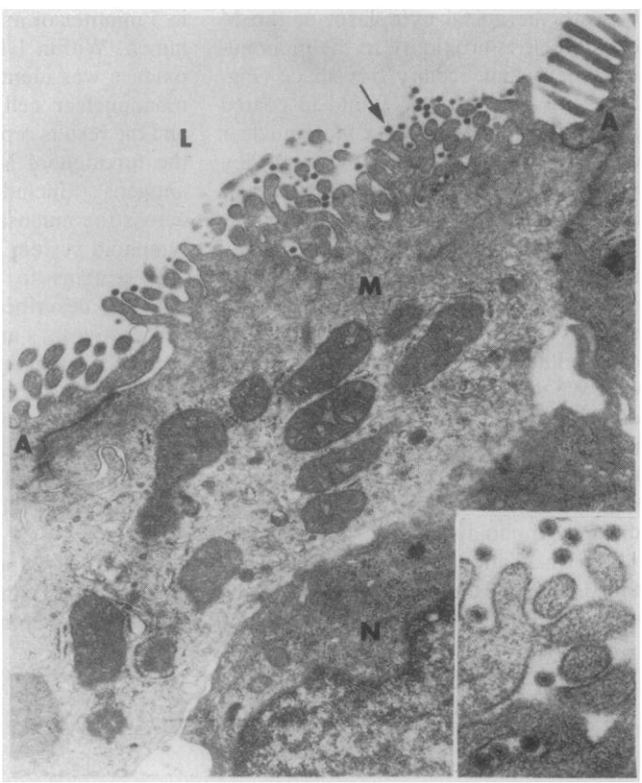

Fig. 1. Events occurring in a closed ileal loop 30 minutes after inoculation with reovirus type 1. The electron micrograph shows an M cell (*M*) with reovirus type 1 (arrow) adhering to its luminal surface. The M cell is bordered by two absorptive cells (*A*) without adherent viruses and envelops a mononuclear cell (*N*) (×13,400). The intestinal lumen (*L*) is indicated. Inset: higher magnification of M cell surface with adherent virus particles (×38,800).

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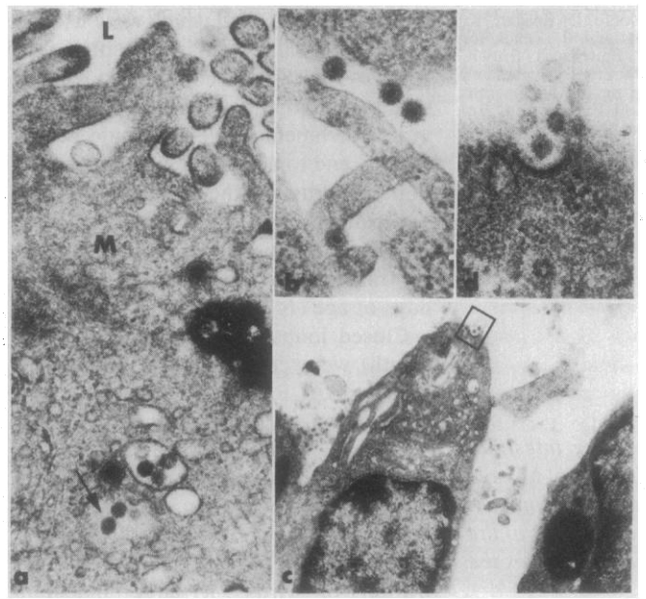

Fig. 2. Events occurring in a closed ileal loop 1 hour after inoculation. (a) M cell containing reovirus type 1 particles (arrow) within cytoplasmic vesicles (×36,600). (b) Viruses in the central extracellular space enclosed by an M cell (×47,600). (c) Viruses in a coated pit of a mononuclear cell in the extracellular space enveloped by an M cell (×10,000). (d) Higher magnification of the enclosed area in (c), showing the coated pit containing reovirus (×49,000).

adhering to all the M cells identified in the samples removed 30 minutes after inoculation (Fig. 1). These particles, with the characteristic double capsid of reovirus, adhered to the surface projections as well as to the stunted microvilli and were also present between the microfolds (Fig. 1). Some of the particles between the microfolds appeared to have been incorporated into the extreme apical cytoplasm of the M cell.

There were many reovirus particles within smooth-surfaced vesicles in the M cell cytoplasm in the tissue collected 1 hour after inoculation (Fig. 2a). A few were found free in the intercellular space beneath the apical cytoplasm of the M cells, in close proximity to the mononuclear cells that occupy this space (Fig. 2b). Viruses were also found in coated pits on the surface of these mononuclear cells (Fig. 2, c and d). However, they were not identified in the mononuclear cell cytoplasm.

It appears that reovirus type 1 initially adheres to the surface of the M cell, is endocytosed into the cell, traverses the cell in vesicles, and is liberated into the extracellular space enveloped by the M cell. We do not know whether the viruses adherent to the coated pits of the mononuclear cells subsequently enter the cells by endocytosis, facilitating their transport into Peyer's patches or more distant lymphoid tissue.

The association of reovirus type 1 with the M cell appears to be specific. Viruses were not found on the luminal surface of goblet cells and were only occasionally found adjacent to the luminal surface of absorptive cells.

The M cell is characterized by many surface microfolds, irregular stunted microvilli, prominent vesicles in the apical cytoplasm, and attenuated cytoplasmic processes that extend laterally, enfolding mononuclear cells within a central hollow (*10*). Bockman and Cooper (*12*) showed uptake of ferritin in the mouse by specialized epithelial cells (presumably M cells) overlying Peyer's patches and by cells in Peyer's patches. Owen (*11*) demonstrated uptake of horseradish peroxidase in the mouse by M cells within 5 minutes of its injection into the ileal lumen. Within 1 hour, horseradish peroxidase was identified in the underlying mononuclear cells. Those experiments and the results reported here suggest that the function of M cells is to transport antigens (including microorganisms) across the mucosal barrier, allowing the lymphoid system access to them.

It remains to be seen whether the pathway described in this report represents reovirus's major route of entry or a clearance pathway used by the host to eliminate the virus. Since the M cell transports "antigens" to Peyer's patches and since infectious virus rapidly appears there, it is likely that the pathway described is used by reovirus to enter the host.

Numerous experimental and clinical observations, although not focusing on M cells, have pointed to Peyer's patches as sites of localization of microbes including both viruses (polio, coxsackie, reovirus) and bacteria (*Salmonella enteritides, Salmonella typhi, Mycobacterium tuberculosis*) (*2, 16*). The fact that M cells transport protein antigens as well as infectious microorganisms across the mucosal epithelial barrier and deliver them directly to mononuclear cells (lymphocytes or monocytes) suggests that M cells play a central role in delivering foreign proteins and microorganisms to the host and its immune system.

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