Brazilian Journal of Medical and Biological Research (1997) 30: 1187-1190
ISSN 0100-879X Short Communication

Rotavirus and reovirus interaction with mouse peritoneal resident phagocytic cells

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Abstract

Rotaviruses and reoviruses are involved in human and animal diseases. It is known that both viruses penetrate the gastrointestinal tract but their interaction with phagocytic cells is unknown. To study this interaction, peritoneal resident phagocytic cells were used and rotavirus and reovirus replication in peritoneal phagocytic cells was observed. However, rotavirus replication in these cells led to the production of defective particles since MA-104 cells inoculated with rotavirus phagocytic cell lysate did not show any evidence of virus replication. On the basis of these results, we suggest that, although reovirus dissemination may be helped by these phagocytic cells, these cells may control rotavirus infection and probably contribute to the prevention of its dissemination.

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Received November 12, 1996
Accepted August 27, 1997

Key words
- Rotavirus
- Reovirus
- Phagocytic cells

Rotavirus is a frequent cause of human and animal diarrhea worldwide. It is known that these viruses penetrate the gastrointestinal tract and replicate in enterocytes (absorptive cells) causing their destruction, sloughing and replacement by immature cells with reduced absorptive and digestive capacity, with the consequent occurrence of diarrhea. An important consequence for humans and animals is dehydration, which can be fatal if not treated.

Rotavirus infection seems to be limited to the gastrointestinal tract and, although some investigators mention the presence of viral antigens at other sites of the body (1-3), there is no convincing study demonstrating rotavirus dissemination after replication in enterocytes. Anatomopathological studies have demonstrated that the destruction of enterocytes by rotaviruses is self limited with complete recovery of villous epithelial morphology and function although the interaction between the virus and the host defense system is poorly understood.

It is known that specific antibodies are produced in response to rotavirus intestinal replication and also that after acute infection immunocompetent individuals show a good lymphoproliferative response to rotavirus antigens *in vitro* (4). There are few animal experiments studying host defense mechanisms against rotavirus. Some investigators describe the presence of rotavirus particles in phagocytes of the intestinal lamina pro-

pria (5-7), but they did not indicate if these viruses are destroyed by these phagocytes.

Under natural circumstances reoviruses behave primarily as enteric viruses. Reovirus serotype 1 replicates in intestinal absorptive cells. Moreover, after oral inoculation, reovirus serotypes 1 and 3 adhere to the intestinal M cells (8,9) and are transported across these cells into the Peyer's patches. Infectious viruses are observed in mesenteric lymph nodes although their interaction with phagocytic cells has not been described (10).

The objective of the present study was to determine rotavirus and reovirus interaction with peritoneal resident phagocytic cells *in vitro*. Rotavirus was used as a model of localized infection limited to the gastrointestinal tract while reovirus was used as a model of systemic infection.

Peritoneal phagocytic cells were obtained from adult Swiss mice (40-45 days old) after peritoneal lavage with 10 ml of growth medium (RPMI 1640 medium containing 1 M HEPES, 100 IU/ml penicillin, 2.5 µg/ml gentamicin, and 2.5 µg/ml amphotericin B) supplemented with 20% fetal bovine serum. A cell suspension containing 10^6 cells per ml of growth medium was distributed into Leighton tubes containing glass coverslips (2 ml per tube) and incubated at 37ºC for 1 h. After incubation, the supernatant was discarded, and the cell culture was washed with RPMI medium and maintained in growth medium at 37ºC.

The peritoneal cells obtained were tested for phagocytic activity 24 and 48 h after harvesting. The cell monolayers were incubated with a 1% (w/v) suspension of *Saccharomyces cerevisiae* for 60 min at 37ºC. After incubation the coverslips were washed and fixed for 10 min in methanol for subsequent staining with Giemsa. After staining the coverslips were mounted on slides with Canadian Balsam and observed by light microscopy.

For phagocytic cell inoculation simian rotavirus (strain SA-11) and reovirus serotype 1 were used, both adapted to MA-104 cells with a multiplicity of infection (MOI) of approximately 10. The phagocytic cell cultures were inoculated in one experimental test 24 h after being obtained and 48 h later in the other experiment. An inoculum volume of 0.5 ml per tube was used and the cells were incubated at 37ºC for 2 h for virus

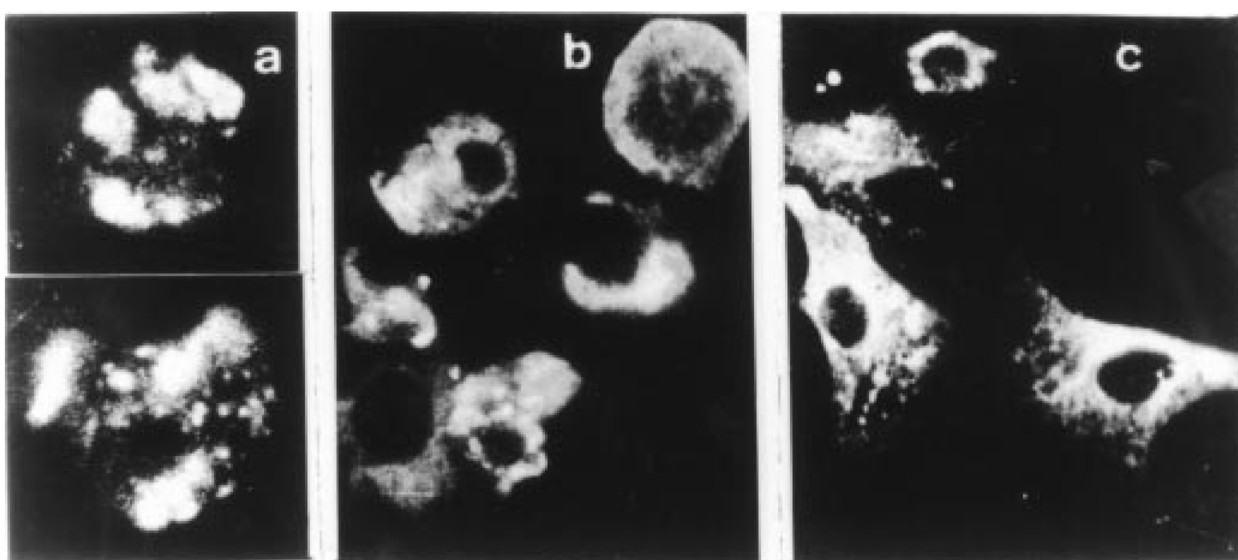

Figure 1 - Peritoneal phagocytic cells (a,b) and MA-104 cells (c) inoculated with rotavirus (a) and reovirus (b,c), and processed by immunofluorescence. *a* and *b*, 400X; *c*, 160X. Characteristic perinuclear fluorescent staining of the infected cells can be observed.

adsorption. After incubation the inoculum was discarded, the cells were washed twice and then maintained in growth medium (RPMI 1640 medium containing 1 M HEPES, 100 IU/ml penicillin, 2.5 µg/ml gentamicin, and 2.5 µg/ml amphotericin B) supplemented with 2% fetal bovine serum.

Virus replication was detected by two different methods: the indirect immunofluorescence test (IF) using a specific immune serum (a rabbit anti-rotavirus and a rabbit anti-reovirus immune serum, prepared as described in Ref. 11) and a labeled fluorescein anti-rabbit immune serum (Pasteur).

The other form of viral replication detection in phagocytic cells was by inoculation of MA-104 cells with the lysate of the infected phagocytic cell. MA-104 cells grown on glass coverslips were inoculated 24 h after trypsinization with 0.5 ml of the infected phagocytic cell lysate and incubated at 37°C for 2 h for viral adsorption. After incubation the inoculum was discarded and the monolayers were washed twice and maintained in MEM Eagle containing 1 M HEPES, 100 IU/ml penicillin, 2.5 µg/ml gentamicin, and 2.5 µg/ml amphotericin B, without bovine serum but supplemented with crystalline trypsin (0.04 mg/ml). Virus replication in the inoculated MA-104 cells was observed by microscopic visualization of suggestive viral cellular cytopathic effect (CPE) and by the observation of specific fluorescence.

In the present study, the peritoneal cells in culture showed the characteristics of phagocytic cells described by Carr (12). They were firmly attached to the glass surface of the coverslips, showed no replication in culture, depended on fetal bovine serum to survive, showed phagocytic activity and had a life time of about 7 days. After inoculation these phagocytic cell cultures showed signs of rotavirus and reovirus replication by IF (Figure 1a,b). The phagocytic cells inoculated with reovirus showed more than 90% of fluorescent cells while those inoculated with rotavirus showed fluorescence in less than 2% of the cells in culture. Different results were observed when MA-104 cells were inoculated with virus phagocytic cell lysate. MA-104 cells inoculated with rotavirus phagocytic cell lysate showed no signs of viral replication until the third passage of the lysate on these cells. Nevertheless, MA-104 cells inoculated with reovirus phagocytic cell lysate showed a characteristic CPE and the presence of cytoplasmic specific fluorescence by IF (Figure 1c).

Phagocytic cells are a group of heterogeneous cells with multiple functions. Some of these cells are located at the points of entry of the organism acting as a barrier against infectious agents, engulfing and generally destroying them. If viruses replicate in these cells, dissemination of the infection may occur since some of these cells migrate throughout the organism. In our experiments the presence of specific fluorescence in phagocytes inoculated with rotavirus and reovirus suggested an intrinsic susceptibility of these cells to both viruses. The replication of reovirus in macrophage cells and the production of infective particles that caused infection in MA-104 cell cultures are consistent with the pathogenicity of these viruses that disseminate throughout the body after penetrating the intestinal mucosa probably inside macrophage cells.

The demonstration of rotavirus replication in phagocytic cells agrees with reports by different investigators who described the presence of rotavirus particles in subepithelial phagocytic cells, suggesting that this may be a mechanism of virus interaction with the immune system (5-7,13,14). The observation of rotavirus replication in phagocytic cells but the absence of viral replication in MA-104 cells inoculated with the lysate of these phagocytic cells suggests the production of defective viral particles. It is possible that, although the replication of rotavirus in phagocytic cells may occur, the production of defective particles could limit its dissemination throughout the body. These results

concerning rotaviruses may explain the experimental findings of Eiden et al. (15) who observed that T lymphocyte-deprived mice did not produce specific antibodies after rotavirus intestinal replication but had the same disease course as control mice (immunocompetent mice). The destruction of rotavirus particles by the phagocytic cells or the production of defective particles by these cells may explain the benign course of the infection in the T lymphocyte-deprived mice.

Studies on rotavirus replication in macrophage cells are in progress in our laboratory to understand the interaction of rotavirus with these cells.

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