Rotavirus causes selective vimentin reorganization in monkey kidney CV-1 cells

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The effect of rotavirus infection on cytoskeletal organization was examined in cultured African green monkey kidney (CV-1) cells. Rhesus rotavirus caused significant and selective changes in the organization of the vimentin filament network without having any effect on microtubules or actin. Double-immunofluorescence studies showed that at 6 h post-infection, and in the absence of cytopathic effect, the normal arrays of vimentin fibres radiating from multiple sites around the nucleus were lost. Vimentin fibres became irregularly distributed in the cytoplasm and were totally disrupted in the later stages of infection. Vimentin reorganization occurred independent of extracellular Ca

Rotaviruses are classified in the family Reoviridae, which includes the genera Orthoreovirus, Rotavirus and Orbivirus. These dsRNA viruses have non-enveloped particles about 70 nm in diameter and possess an inner capsid and an outer protein shell with icosahedral symmetry (Bellamy & Both, 1990). Except for their overall size, cytoplasmic replication and segmented genome, the members of this family of RNA viruses show significant differences in their mode of morphogenesis (Bellamy & Both, 1990; Dryden et al., 1993). Rotavirus undergoes a unique assembly process which is limited to the cytoplasm and endoplasmic reticulum (ER) (Bellamy & Both, 1990). Cytoplasmic capsid proteins assemble into single-shelled particles which translocate from the viroplasm into adjacent ER and become transiently enveloped by a membrane from the ER. During the assembly process, the transiently acquired ER membrane envelope is lost and the outer capsid proteins VP7 and VP4 are folded onto the single-shelled particles (Poruchynsky et al., 1991).

Although a considerable amount of information has been obtained about the entry, genetics and assembly process of rotaviruses, no information is as yet available on how rotaviruses alter cellular metabolism or the cytoskeleton during infection, nor is the mechanism by which rotaviruses cause cell injury well established. A role for the cytoskeleton in virus replication has been reported in several studies (Eaton et al., 1987; Lenk & Penman, 1979; Luftig, 1982; Mattion et al., 1992; Mora et al., 1987; Sharpe et al., 1982; Hua & Patton, 1994). At least two non-structural proteins (NS34 and NS53) of rotavirus have been found in association with the cytoskeleton (Hua & Patton, 1994; Mattion et al., 1992). Reovirus has been shown to disrupt and reorganize vimentin filaments without affecting microfilaments or microtubules (Sharpe et al., 1982). Mora et al. (1987) have described a temporary assembly of reovirus products on the cytoskeleton and bluetongue virus particles have been shown to bind to intermediate filaments (Eaton et al., 1987). Reoviruses and adenoviruses associate with microtubules both in vitro and in vivo (Babiss et al., 1979; Defer et al., 1990) and vaccinia virus associates with microfilaments (Hiller et al., 1979). Likewise the poliovirus dsRNA replicon associates with the cytoskeleton during replication (Lenk & Penman, 1979).

Considering the differences in morphogenesis between members of the Reoviridae we decided to examine whether rotavirus infection of CV-1 cells causes reorganization of the major cytoskeletal components (microtubules, actin and intermediate filaments). Semi-confluent CV-1 cells were grown in Lab-Tek multichambers (Nunc) in Eagle’s MEM and were infected with trypsin-activated (10 μg/ml Sigma IX for 30 min at 37°C) plaque-purified rhesus rotavirus (RRV) at an m.o.i. of 0.5. After a 1 h incubation at 37°C the inoculum was removed and the cells were washed once with Eagle’s MEM and then incubated in Eagle’s MEM without trypsin. The cultures were sampled at 3, 6, 8, 9, 12 and 16 h post-infection (p.i.) by rinsing twice in PBS pH 7.4, and fixed in methanol at −20°C for 5 to 6 min. The cells were preincubated with PBS containing 5% normal swine serum (Dakopatts) and 0.3% Triton X-100 (Eastman Kodak) for 15 min at room temperature. The
Fig. 1. Organization of vimentin in uninfected (a) and rotavirus-infected CV-1 cells (b). Immunofluorescence shows bundles of the intermediate filaments radiating from the nuclei in uninfected CV-1 cells. In rotavirus-infected cells this organization of the vimentin network is completely lost at 6 h p.i. (b) Bar markers represent 25 μm.
followed by fixation and immunofluorescence examination. As reported elsewhere, a reduced extracellular Ca\(^{2+}\) level caused a delay in c.p.e. development, but vimentin reorganization could not be prevented, even at 1.4 mM-EGTA (Fig. 4). EGTA had no effect on vimentin organization in uninfected cells under the experimental conditions. These results suggest that vimentin reorganization is neither a result of an increased influx of Ca\(^{2+}\) nor a non-specific phenomenon accompanying cell degeneration.

This study examined the effect of rotavirus infection on the cytoskeleton organization in epithelial cells and shows that rotavirus causes significant and selective changes in the organization of the intermediate filament subunit, vimentin, in CV-1 cells. These changes are similar to those previously described for reovirus (Sharpe et al., 1982). However, the pathological significance of these changes is not known, since the physiological role of vimentin or other intermediate filaments is still not clear (Blomendal & Pieper, 1989). It has been reported that a perinuclear condensation of the vimentin filament network observed early in adenovirus infection appeared to be a cytological marker for cytoplasmic transit of infectious virions within the infected cells (Defer et al., 1990). It has also been suggested that vimentin may play a role in the life cycle of orbiviruses, since bluetongue virus particles were found attached to vimentin filaments of cells (Eaton et al., 1987). Furthermore, it has also been reported that the radially arranged vimentin fibres in monkey kidney cells seem to carry prosomes, which are mRNA-associated ribonucleoprotein particles and cofactors of untranslated free mRNA (Arcangeletti et al., 1992). The exact physiological role of prosomes is not clear, but they are able to discriminate between adenovirus and host cell mRNA in vitro (Horsch et al., 1989), and it has been suggested that they may inhibit cell-free protein synthesis.

Previously, we have observed that rotavirus is assembled in a normal fashion in the ER of neurons (Weclewicz et al., 1993a). Rotavirus not only binds to, but also causes reorganization of, the microtubule-associated protein (MAP2) in neurons (which lack vimentin) (Weclewicz et al., 1993b) suggesting that rotavirus may interact with different cytoskeletal components in different cell types. The roles for such interactions in viral maturation and cytopathology remain to be clarified, but it is interesting to note that
assembly of rotavirus takes place adjacent to, or on the cytoskeleton framework. Further studies will include critical examination of the subviral particles or specific rotavirus proteins that interact with the cytoskeleton.

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References


Fig. 4. Double immunofluorescence showing reorganization of vimentin in rotavirus-infected cells cultivated with 1 mM-EGTA in the maintenance medium from 1 h p.i. to 13 h p.i. (a) viral antigens stained with rabbit antirotavirus antiserum and TRITC-conjugated swine anti-rabbit IgG. (b) Reorganization of vimentin revealed by labelling with anti-vimentin MAb and FITC-conjugated rabbit anti-mouse IgG. Bar markers represent 50 μm.

neurons only support abortive infection with rotavirus (Weclewicz et al., 1993 a) whereas infection of epithelial cells such as CV-1 and MA-104 will yield infectious virus. Recent studies have shown that NS34 and NS53 interact with the cytoskeleton (Mattion et al., 1992; Michelangeli et al., 1991), suggesting that replication and/or capsid assembly of rotavirus takes place adjacent to, or on the cytoskeleton framework. Further studies will include critical examination of the subviral particles or specific rotavirus proteins that interact with the cytoskeleton.

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