Indian J Med Res 115, February 2002, pp 37-45

Cytoskeletal changes during poliovirus infection in an intestinal cell line

Gagandeep Kang, Prabha Desikan & Minnie Mathan

The Wellcome Trust Research Laboratory, Department of Gastrointestinal Sciences, Christian Medical College & Hospital, Vellore, India

Received July 25, 2001

Background & objectives: **Although polioviral replication has been extensively studied, cytoskeletal changes in the host cell during poliovirus replication have not been extensively investigated. We studied the ultrastructural and cytoskeletal changes in host cells during poliovirus infection.**

Methods: **Fluorescence staining of filamentous actin with a fluorescein-isothiocynate labelled mycotoxin, in the absence and presence of microfilament inhibitors cytochalasins B and D, and electron microscopy were used to investigate the role and fate of actin microfilaments during poliovirus infection, morphogenesis and release in an intestinal cell line, HRT-18.**

Results: **At 10 h post-infection, fluorescence staining of actin showed focal areas of fluorescence in the cytoplasm. By 16 h, these became more prominent and increased in number, and by 18-22 h they coalesced to enclose areas of the cytoplasm. These changes in the actin profile were confirmed by electron microscopy, where small actin bundles appeared in association with vesicles, increased in size, number and thickness, enclosed areas of cytoplasm with numerous vesicles and were finally seen in association with crystalline arrays of virus near the periphery of the cells. The addition of microfilament inhibitors cytochalasins B and D, after the initial period of adsorption resulted in complete inhibition of changes in the actin profile and of viral release, indicating that microfilament inhibitors prevented both polymerization of actin and movement of the virus within the cell.**

Interpretation & conclusion: **In poliovirus infection, both intracellular movement and release of virus appear to be related to cytoskeletal changes, particularly involving actin microfilaments.**

Key words Cytochalasins - electron microscopy - fluorescence - microfilaments - morphogenesis - poliovirus

Polioviruses are non-enveloped, icosahedral RNA viruses which infect susceptible cells to result in a cytopathic effect (CPE), production of progeny virus and finally cellular death. As an obligate intracellular pathogen, poliovirus requires many host cell functions for its replication. The initial step in infection is attachment of the virion to domain 1 of the poliovirus receptor embedded in the plasma membrane[1]. This is followed by penetration and uncoating of the viral RNA genome. The entry of the virus into the host cell causes dramatic changes in cellular metabolism and characteristic morphological changes are seen in infected eukaryotic cells[1-4].

Within an hour of infection of HeLa cells, margination of chromatin is seen, where the nuclear material loses its normally homogeneous

microscopic texture and accumulates along the inside of the nuclear envelope[2]. About 3 h after infection, membranous vesicles appear in the cytoplasm, beginning first in the perinuclear area and spreading outwards until the entire cytoplasm is involved[3,4]. Cellular RNA, protein and DNA synthesis begin to decline within a few hours of infection. Attempts have been made to correlate CPE with distribution of viral proteins in host organelles, but the results have not been definitive[3]. A number of techniques including the use of infectious clones, site-specific and randomly mutagenized viral mutants and the development of *in vitro* assay systems have provided insights into the mechanisms of viral RNA replication, protein synthesis and processing and the regulation of these processes[5], but these studies did not focus on morphological changes in host cells. A recent report of poliovirus infection in Caco-2 cells, a human enterocytic cell line, indicates that apoptotic processes may be involved in host cell responses to polioviral infection[6].

The spreading of membranous vesicles, containing viral proteins[3,4], through the cytoplasm is associated with changes in permeability of the plasma membrane, which is followed by leakage of intracellular components and shrivelling of the entire cell. Crystalline arrays of virus are often found in the cytoplasm during the late stages of infection, first in the perinuclear region and then in the periphery, before being released by the rupture of the cell[7]. Whether the virions move passively within the cytosol, using the regular streams that exist within a cell, or generate a movement of their own is yet to be elucidated.

Entry into, movement and multiplication within intestinal epithelial cells are the first steps in the development of disease due to a wide variety of organisms, including viruses, bacteria and parasites. In organisms such as *Shigella, Listeria* and enteropathogenic and enterohaemorrhagic *Escherichia coli*, it has been shown that major alterations of the cytoskeleton of the host cell take place with attachment, entry and invasion[8-11]. Studies using 7-nitrobenz-2-oxa-1, 3-diazole-phallicidin (NBD-phallicidin) and fluorescein-isothiocynate labelled phalloidin (FITC phalloidin) have shown that condensations of filamentous actin are formed under the plasma membrane when the bacteria attach to and enter the cells and that actin plays a major role in the intra- and intercellular spread of bacteria[9,10,12]. Entry and spread of bacteria that induce changes in the actin profile of the host cell can be prevented by the use of microfilament inhibitors[13,14]. In viruses, microfilament inhibitors have been used to demonstrate the role of actin in viral release[15,16].

In this study, electron microscopy and fluorescence staining with and without microfilament inhibitors were used to investigate the role and fate of actin microfilaments during poliovirus infection, morphogenesis and release in an intestinal cell line, HRT-18, since morphological and cytoskeletal changes in host cells during poliovirus infection have not been extensively investigated previously.

Material & Methods

Time of viral release by HRT-18 cells: Monolayers of HRT-18 cells, a continuous cell line derived from a human ileocaecal adenocarcinoma (a gift from J.R. Patel, Sheffield, UK), grown in 25 cm^2 tissue culture flasks (Corning, Sigma Chemical Co, St. Louis, MO, USA) were infected with type 1 poliovirus (Mahoney strain, a gift from T.J. John, CMC Hospital Vellore) at 50 pfu/ml. One hour after infection the cells were washed three times in phosphate-buffered saline (PBS, *p*H 7.2) and fresh RPMI 1640 medium supplemented with 10 per cent foetal calf serum (Gibco BRL, Gaithersberg, MD, USA) added. A 0.5 ml volume of medium was removed at 0, 1, 2, and subsequently at 2 hourly intervals for 24 h after infection and frozen at -70°C until assay. Each sample was inoculated into 2 tubes of primary monkey kidney (PMK) cells, allowed to adsorb for 1 h and then washed 3 times with PBS before changing the medium. The PMK cells were incubated at 37°C and observed daily for cytopathic effect (CPE). CPE was graded as 1+ to 4+ depending on the percentage of cells affected by the cytopathic process (1+ 25%, 2+ 50%, 3+ 50-90%, 4+ almost all cells affected, many detached and floating in the medium).

For the studies in which microfilament inhibitors cytochalasins B and D (Sigma Chemical Co., St. Louis, MO, USA) were used, the HRT-18 cells were infected as above, washed, and fresh RPMI 1640 medium containing a final concentration of 20 μg/ml of cytochalasin D was added. The flasks were incubated and a 0.5 ml volume removed at 6, 10, 14, 18 and 20 h after infection and assayed for released virus in PMK cells as described above.

Fluorescence actin staining: Monolayers of HRT-18 cells were grown on multispot test slides (ICN Laboratories, Costa Mesa, CA, USA). They were infected with 50 pfu/ml of poliovirus type 1 in duplicate. One hour after infection, the cells were washed 3 times with PBS and fresh medium added. The slides were then incubated for 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 h in a moist chamber at 37°C with 5 per cent carbon dioxide. The slides were stained with FITC labelled phalloidin (Sigma Chemical Co., St. Louis, MO, USA) as described[8]. Briefly, the cells on the slides were washed 3 times with PBS, fixed with 3 per cent formalin as recommended in the original protocol for fluorescence staining of actin[8] and then permeabilised with 0.1 per cent Triton X-100. They were washed 3 times with PBS and then treated with FITC labelled phalloidin at a concentration of 5 μg/ml for 20 min and examined for areas of fluorescence under an Olympus BHTU fluorescence microscope at 40X magnification.

For the studies using microfilament inhibitors, monolayers of HRT-18 cells were grown on multispot test slides and infected with type 1 poliovirus at a titre of 50 pfu/cell in duplicate. One hour after adsorption, medium containing a final concentration of 20 μg/ml of cytochalasin B or 2 μg/ml of cytochalasin D, was added. The slides were then incubated for 6, 10, 12, 14, 18 and 20 h respectively at 37°C in a moist chamber with 5 per cent carbon dioxide, and processed and examined as described above.

Transmission electron microscopy: Monolayers of HRT-18 cells were grown in tissue flasks, infected with 50 pfu/ml of poliovirus type 1 and incubated at 37°C for time periods from 2 to 24 h. The monolayers were fixed with 3 per cent glutaraldehyde for 1 h at 40°C and washed with 0.1 M sodium cacodylate buffer (*p*H 7.2) for half an hour at 40°C. Post fixation was carried out with 1 per cent osmium tetroxide (in 0.2 M sodium cacodylate buffer, *p*H 7.4) for 30 min at 40°C. The cells were scraped with a rubber policeman and centrifuged at 3000 rpm. 2 per cent agar was added at 60°C and again centrifuged at 10,000 rpm for one minute and the pellet in agar was kept overnight at 40°C. Dehydration of the pellet was carried out in graded ethanol, and it was then cleared with propylene oxide. After infiltration with pure resin, the pellet was embedded with resin in a silicon mould and the resin allowed to polymerise for 48 h at 60°C. 60-90 nm thick sections were made and etched with 3 per cent hydrogen peroxide in methanol for 5 min. They were washed with methanol followed by distilled water and stained with uranyl acetate. Contrast was enhanced by staining with lead citrate. The grids were examined in a Philips 301C microscope (Eindhoven, Netherlands).

Results

The medium taken from the poliovirus infected HRT-18 cells at 1, 2, 4 and 6 h produced no CPE in the PMK cells, indicating that viral release had not taken place at these time periods. The medium taken at 8 h showed 1+ CPE at 3 days indicating that a small number on viruses was being released at this time. Medium removed at 10 h post-infection showed 2+ CPE on the first day, and 4+ CPE by the second day. Medium removed at later time periods showed 4+ CPE from the first day. All mock-infected controls inoculated with plain medium showed no CPE.

On fluorescence actin staining of HRT-18 cells, the uninfected cells had scanty, uniform or amorphous fluorescence, mainly at the periphery and occasionally in the perinuclear region (Fig. 1a). No significant changes were seen till 10-12 h post infection, when small, focal areas of filamentous fluorescence appeared in the cytoplasm (Fig. 1b). Between 12-16 h, these focal areas increased in number and became more prominent,

especially in the perinuclear region (Fig. 1c). By about 18-22 h, the focal fluorescent areas appeared to coalesce and enclose areas of the cytoplasm with bright areas of high fluorescence adjacent to some parts of the periphery of the cells (Fig. 1d, Table).

Using transmission electron microscopy, very few polymerised actin bundles were seen in the uninfected cells. They were scattered mainly near the periphery of the cells and were also seen near the nucleus (Fig. 2a). For 8-10 h post infection, no significant changes were detectable in the cells. Nuclear changes started to appear at 8-10 h post-infection, with the nuclei appearing dark and with margination of the nuclear chromatin. Small vesicles appeared in the cytoplasm and small bundles of actin were seen scattered among them (Fig. 2b). Clumps of polyribosomes were seen in the cytoplasm. At 12 h post infection, the vesicles were seen to increase in number and also enlarge in size. They occupied a large part of the cytoplasm and pushed the nucleus to one side of the cell. The actin bundles had increased in number and were markedly thicker (Fig. 2c). By about 14-16 h post-infection, the actin bundles appeared still thicker, longer and more prominent, and appeared to enclose small areas of the cytoplasm, each bundle surrounding a number of vesicles (Fig. 2d). The cells had enlarged in size, had a number of vacuoles and showed chromatin condensation and degenerative changes. Between 18 and 22 h of incubation, the cells showed marked degenerative changes. Crystalline arrays of viruses were seen, some close to the periphery of the cell with very prominent actin bundles surrounding them and near the periphery of the cells (Fig. 2e, f, Table).

Microfilament inhibitors, cytochalasins B and D were used to study the role of actin in viral transport and release. These microfilament inhibitors were added after the initial period of adsorption, such that viral entry into the HRT-18 cells would not be affected. Up to 20 h post infection the cells appeared uniformly stained, with no areas of brighter fluorescence, indicating that both microfilament inhibitors prevented polymerization of actin and movement of the virus within the cell (Fig. 1e). On electron microscopy, cells appeared morphologically normal, with no vesicle formation or actin accumulation (Fig. 2g). The inoculated PMK cells also showed no CPE, indicating that poliovirus was not released by HRT-18 cells incubated with cytochalasins B and D containing media for upto 20 h post-infection.

Discussion

Most studies on the morphogenesis of poliovirus have been carried out in HeLa cells where changes begin to appear at 2 h post-infection and maximal viral multiplication occurs at 6 h[4,7,17-19]. In this study, we have used an intestinal cell line, HRT-18 where maximal replication occurs at and after 12 h, indicating that poliovirus type 1 replicates more slowly in this cell line than in HeLa cells. These

Table. Electron microscopic changes and fluorescence actin staining after injection of HRT-18 cells by poliovirus type 1

Time (h) Post-infection	Viral release into medium	Electron microscopy	Fluorescence actin staining
10	-	No changes	Scanty, uniform fluorescence
10-14	+	Margination of nuclear chromatin, small vesicles appear with scattered small actin bundles	Cytoplasm has areas of focal fluorescence
14-18	++	Vesicles and actin bundles increase in size, number pushing nucleus to periphery	Perinuclear focal fluorescence seen
18-22	++	Actin bundles thicker, enclosing areas of cytoplasm	Foci coalesce, enclosing areas of cytoplasm
22-24	++	Crystalline arrays of virus near periphery, marked degenerative changes	Increased fluorescence at periphery

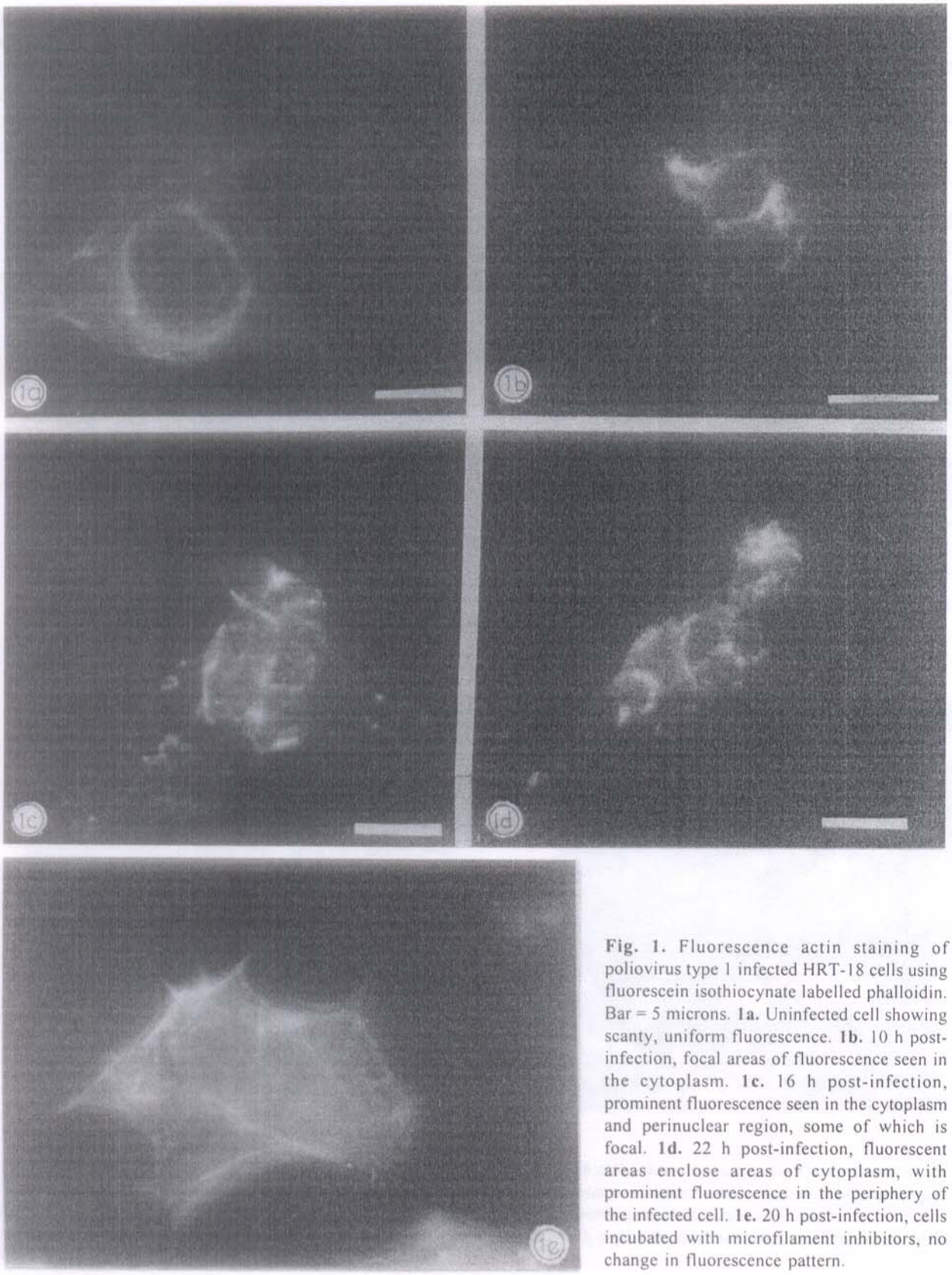


Fig. 1. Fluorescence actin staining of poliovirus type 1 infected HRT-18 cells using fluorescein isothiocynate labelled phalloidin. Bar = 5 microns. **1a.** Uninfected cell showing scanty, uniform fluorescence. **1b.** 10 h post-infection, focal areas of fluorescence seen in the cytoplasm. **1c.** 16 h post-infection, prominent fluorescence seen in the cytoplasm and perinuclear region, some of which is focal. **1d.** 22 h post-infection, fluorescent areas enclose areas of cytoplasm, with prominent fluorescence in the periphery of the infected cell. **1e.** 20 h post-infection, cells incubated with microfilament inhibitors, no change in fluorescence pattern.

results are confirmed by fluorescence and electron microscopic studies where changes first appeared at 8-10 h post-infection and were maximal at 20-22 h, and correlate well with studies in Caco-2 cells, another differentiated cell line, where morphological and apoptotic changes appeared at 8 hours[6].

Both the fluorescence and the electron microscopic studies reported here show that cytoskeletal changes occur in HRT-18 cells infected with poliovirus type 1. The cytoskeleton of most eukaryotic cells consists of three types of protein fibres-microfilaments, microtubules and

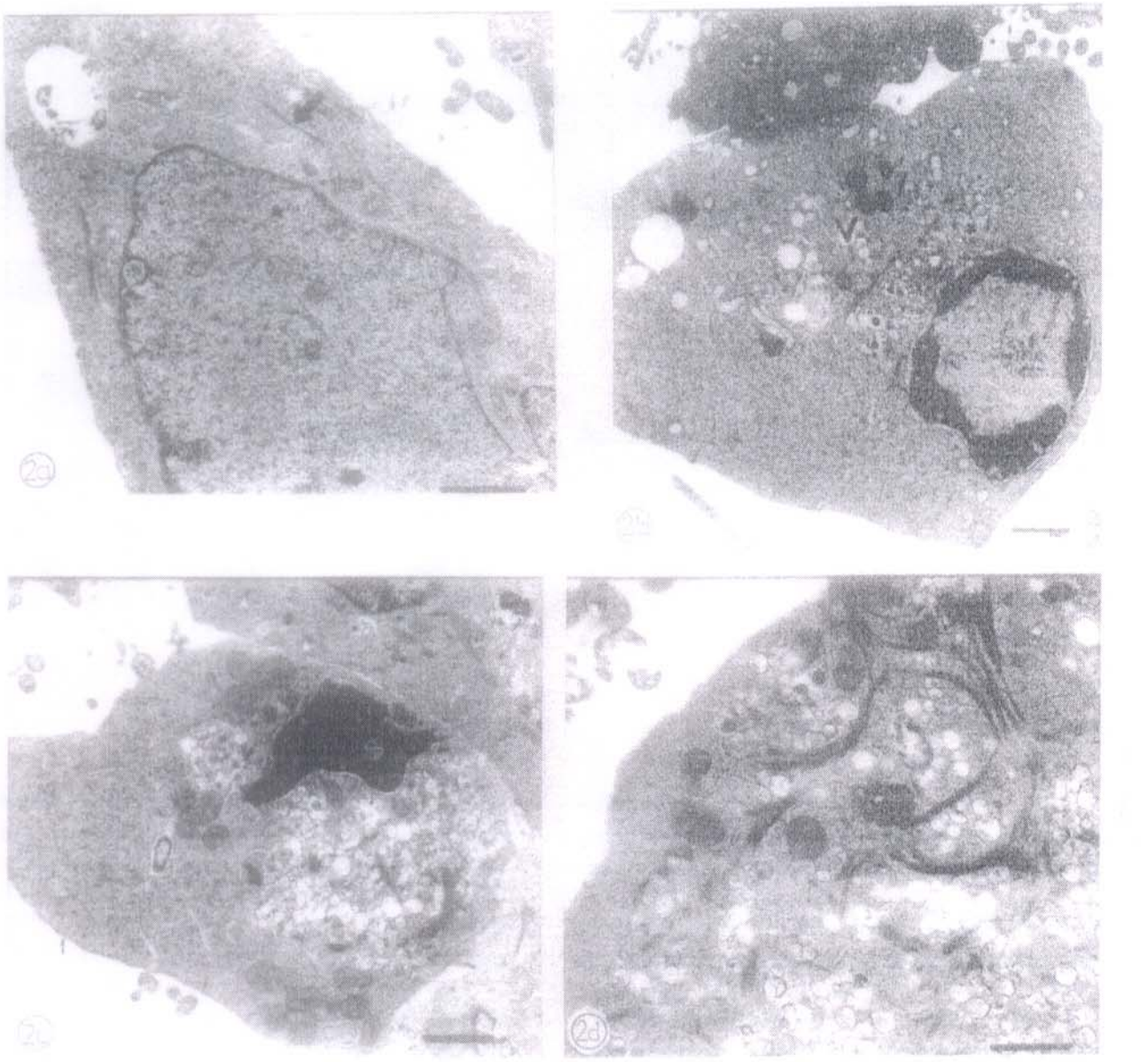
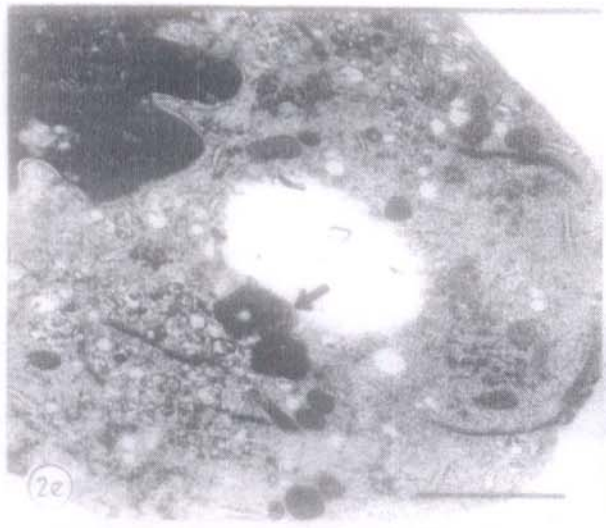
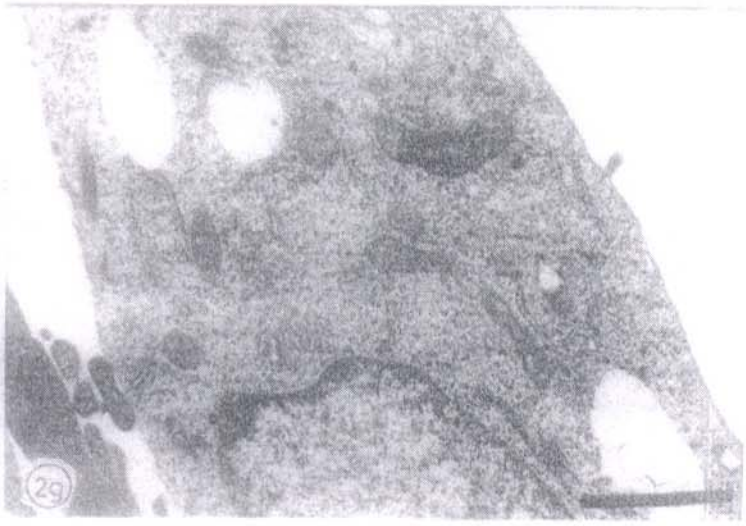


Fig. 2. Transmission electron micrographs of poliovirus type 1 infected HRT-18 cells. Bar = 1 micron. **2a.** Uninfected cell, with very few actin bundles (arrow). **2b.** 10 h post-infection, margination of nuclear chromatin is seen, vesicles (arrow head) have appeared in the cytoplasm with scattered bundles of actin among them. **2c.** 12 h post-infection, vesicles occupy a large part of the cell, displacing the nucleus to one side, actin bundles are thicker and more numerous. **2d.** 16 h post-infection, actin bundles are thicker and appear to enclose small areas of cytoplasm with vesicles.





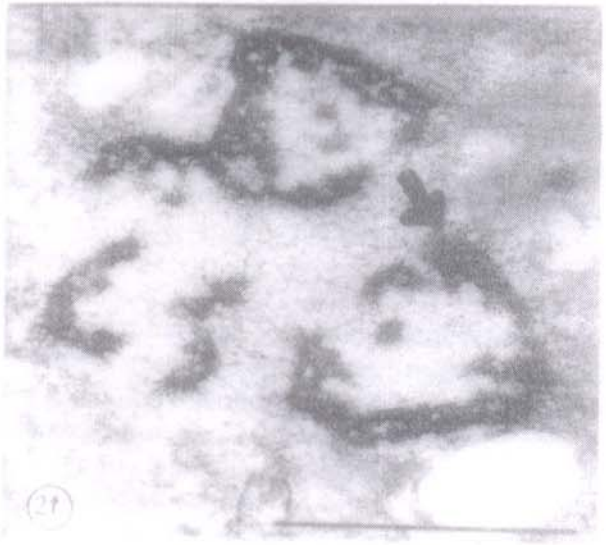


Fig. 2e. 22 h post-infection, crystalline arrays of viral particles seen in the cytoplasm (arrow) with prominent actin bundles near the periphery. **2f.** Large crystalline arrays (arrow) of virus in HRT-18 cells. **2g.** 12 h post-infection, cells incubated with microfilament inhibitors, no vesicle formation, no thick actin bundles seen.

intermediate filaments. The microfilament actin, which is a contractile protein, can polymerise reversibly from a monomeric or globular form (G-actin) to a polymeric or a filamentous form (F-actin)[8-10]. Evidence of the role of actin in viral infections has steadily accumulated over the past decade[15,16,20-26]. In our study, the findings on fluorescence microscopy were consistent with the pattern of morphogenesis of poliovirus[4,7], where the virions move from the perinuclear region to the periphery of the cell. The amount of intracellular fluorescence increased with increase in the time period post infection indicating an increase in the F-actin. This was corroborated by transmission electron microscopy where microfilament bundles were seen in close proximity to the viroplasm, becoming thicker and more prominent as the virions matured and moved towards the periphery of the cell. It is probable that movement of poliovirions occurs through the interaction of the virions with the host cell actin similar to the mechanisms seen in some bacterial infections[10-12]. In Caco-2 cells, the ultrastructural features of polioviral infection also showed condensation of chromatin, intracytoplasmic vacuolation and accumulation of viral particles as seen here in HRT-18 cells[6]. Although association of viral replication complexes with the host cell cytoskeleton is described ultrastructurally in Caco-2 cells[6], the filaments have not been characterised further as done in this study by FITC-phalloidin

staining. There have been few earlier studies on other viruses which document the role of actin in virus release from cells[15,16,23], but none regarding their intracellular transport.

The close association of actin bundles with the intracellular virions suggests that nucleation and filamentation of actin may be virus induced. In addition, it is seen that the actin bundles get thicker as the post infection period increases. It is possible that the thickness of the bundles is directly related to the length of time the organisms have spent in contact with the cytoplasm. However, it is not known whether the increased concentration of F-actin is due to recruitment of the pool of F-actin available in the vicinity of the virus, or to *de novo* polymerisation of the actin subunits as is seen in *Shigella*[10,12]. The presence of a receptor for F-actin or interaction with an actin binding protein that would induce formation of bundles may be considered. Alternatively, a viral product interacting directly with actin monomers could induce polymerization or induce rapid changes in the cytosolic conditions within the intermediate vicinity of the virions.

The mechanism of the intracellular viral movement generated by F-actin needs to be studied. It is generally considered that myosin is the driving force of actin based intracellular movements, but some studies on bacteria report a complete absence of myosin associated with the actin[10], and this suggests that either rapid polymerisation of actin or a brisk formation of a gel or bundle is sufficient to generate intracellular movement of virions. It is possible that F-actin itself acts as a structural scaffold to which new building blocks in the form of G-actin or small F-actin units are continually added at the virion actin interface, thereby continually pushing the virions forward.

In this study, the use of both cytochalasin B and D prevented cytoskeletal changes from taking place. Cytochalasin B disrupts microfilaments by inhibiting the polymerization of actin monomers[27]. Cytochalasin D disrupts microfilament function by binding to myosin[28]. In this study, these microfilament inhibitors both prevented movement and release of the virus. In experiments using these compounds, it has been shown that in measles virus and frogvirus 3[15,16] unlike the results reported here, the intracellular movement of viral particles was not affected, but viral release did not occur. It is possible that in poliovirus both the movement and release of virus may be actin dependent, whereas in other viruses, only viral release requires an active role for microfilaments. Further work is required to conclusively prove these findings by quantifying viral proteins in the presence of microfilament inhibitor to demonstrate abortive replication and to define the role of actin in poliovirus infection.

References

1. Mendelsohn CL, Wimmer E, Racaniello VR. Cellular receptor for poliovirus: molecular cloning, nucleotide sequence and expression of a new member of the immunoglobulin superfamily. *Cell* 1989; *56* : 855-65.
2. Franklin RM, Baltimore D. Patterns of macromolecular synthesis in normal and virus infected mammalian cells. *Cold Spring Harbor Quant Biol* 1962; *27* : 175-94.
3. Bienz K, Egger D, Rasser Y, Bossart W. Intracellular distribution of poliovirus proteins and the induction of virus-specific cytoplasmic structures. *Virology* 1983; *131* : 39-48.
4. Dales S, Eggers HJ, Tamm I, Palade GE. Electron microscopic study of the formation of poliovirus. *Virology* 1965; *26* : 379-89.
5. Rueckert RR. Picornaviridae: The viruses and their replication. In : Fields BN, Knipe DM, Howley PM, editors. *Virology*, 3rd ed. New York : Lipincott-Raven 1996; p. 609-54.
6. Ammendolia MG, Tinari A, Calcabrini A, Superti F. Poliovirus infection induces apoptosis in Caco-2 cells. *J Med Virol* 1999; *59* : 122-29.
7. Mattern CFT, Daniel WA. Replication of poliovirus in HeLa cells: Electron microscopic observations. *Virology* 1965; *26* : 646-63.
8. Knutton S, Baldwin T, Williams PH, McNeish AS. Actin accumulation at sites of bacterial adhesion to tissue culture cells: Basis of a new diagnostic test for enteropathogenic and enterohaemorrhagic *Escherichia coli*. *Infect Immun* 1989; *57* : 1290-8.
9. Mounier J, Ryter A, Coquis-Rondon M, Sansonetti PJ. Intracellular and cell-to-cell spread of *Listeria monocytogenes* involves interaction with F-actin in the enterocytelike cell line Caco-2. *Infect Immun* 1990; 58 : 1048-58.
10. Prevost MC, Lesourd M, Arpin M, Vernel F, Mounier J, Hellio R, *et al*. Unipolar reorganisation of F-actin layer at bacterial division and bundling of actin filaments by plastin

correlate with movement of *Shigella flexneri* within HeLa cells. *Infect Immun* 1992; *60* : 4088-99.

11. Sanger JM, Sanger JW, Southwick FS. Host cell actin assembly is necessary and likely to provide the propulsive force for intracellular movement of *Listeria monocytogenes*. *Infect Immun* 1992; *60* : 3609-19.
12. Vasselon T, Mounier J, Prevost MC, Hellio R, Sansonetti PJ. Stress fiber-based movement of *Shigella flexneri* within cells. *Infect Immun* 1991; *59* : 1723-32.
13. Donnenberg MS, Donohue-Rolfe A, Keusch GT. A comparison of HEp-2 cell invasion by enteropathogenic and enteroinvasive *Escherichia coli*. *FEMS Microbiol Lett* 1990; *69* : 83-6.
14. Pal T, Newland JW, Tall BD, Formal SB, Hale TL. Intracellular spread of *Shigella flexneri* associated with the kcpA locus and a 140 kilodalton protein. *Infect Immun* 1989; *57* : 477-86.
15. Murti KG, Chen M, Goorha R. Interaction of frog virus 3 with the cytomatrix. III. Role of microfilaments in virus release. *Virology* 1985; *142* : 317-25.
16. Stallcup KC, Raine CS, Fields BN. Cytochalasin B inhibits the maturation of measles virus. *Virology* 1983; *124* : 59-74.
17. Bablanian R, Eggers HJ, Tamm I. Studies on the mechanism of poliovirus-induced cell damage. I. The relation between poliovirus-induced metabolic and morphological alterations in cultured cells. *Virology* 1965; *26* : 100-13.
18. Bablanian R, Eggers HJ, Tamm I. Studies on the mechanism of poliovirus-induced cell damage II. The relation between poliovirus growth and virus-induced morphological changes in cells. *Virology* 1965; *26* : 114-21.
19. Gromeier M, Wetz K. Kinetics of poliovirus uncoating in HeLa cells in a nonacidic environment. *J Virol* 1990; *64* : 3590-7.
20. Babes L, Gaghes A, Orasanu M, Teletin N, Petrasincu DE, Codau M. Effect of prostaglandin E2 (PGE2) and cyclic adenosine monophosphate (cAMP) upon actin cytoskeleton in human pulmonary fibroblasts (ICP-23) infected by measles virus. *Roum Arch Microbiol Immunol* 1992; *51* : 239-51.
21. Bedows E, Rao KMK, Welsh MJ. Fate of microfilaments in Vero cells infected with measles virus and herpes simplex virus type 1. *Mol Cell Biol* 1983; *3* : 712-9.
22. Bonneau AM, Darveau A, Sonenberg N. Effect of viral infection on host protein synthesis and mRNA association with the cytoplasmic cytoskeletal structure. *J Cell Biol* 1985; 100 : 1209-18.
23. Heeg U, Dienes HP, Muller S, Falke D. Involvement of actin-containing microfilaments on HSV induced cytopathology and the influence of inhibitors of glycosylation. *Arch Virol* 1986; *91* : 257-70.
24. Holme TC, Kellie S, Wyke JA, Crawford N. Effect of transformation by Rous sarcoma virus on the character and distribution of actin in Rat-1 fibroblasts: a biochemical and microscopical study. *Br J Cancer* 1986; *53* : 465-76.
25. Jones, NL, Kilpatrick BA. The effects of human cytomegalovirus infection on cytoskeleton-associated polysomes. *Eur J Cell Biol* 1988; *46* : 31-8.
26. Marchisio PC, Cirillo D, Teti A, Zambonin-Zallone A, Tarone G. Rous sarcoma virus-transformed fibroblasts and cells of monocytic origin display a peculiar dot-like organisation of cytoskeletal proteins involved in microfilament-membrane interactions. *Exp Cell Res* 1987; 169 : 202-14.
27. Tannenbaum J, Tanenbaum SW, Godman GC. The binding sites of cytochalasin D. II. Their relationship to hexose transport and to cytochalasin B. *J Cell Physiol* 1977; 91 : 239-48.
28. Puszkin E, Puszkin S, Lo LW, Tanenbaum SW. Binding of cytochalasin D to platelet and muscle myosin. *J Biol Chem* 1973; 248 : 7754-61.

Reprint requests: Dr Gagandeep Kang, Professor of Microbiology, The Wellcome Trust Research Laboratory, Department of Gastrointestinal Sciences, Christian Medical College & Hospital, Vellore 632004, India