Influence of the Cytoskeleton on the Expression of a Mouse Hepatitis Virus (MHV-3) in Peritoneal Macrophages: Acute and Persistent Infection

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SUMMARY

The effects of anti-cytokinetic drugs on virus production, formation of syncytia, cell surface changes and lysosomal damage were examined during mouse hepatitis virus 3 (MHV-3) infection of mouse peritoneal macrophages. Colchicine and vinblastine caused no detectable effect on the infectious process. In the presence of cytochalasin B the acute, highly cytopathogenic interaction that MHV-3 establishes with macrophages was converted into one which persisted for several days. Under these conditions the cell surface changes induced by the infection were maintained unaltered but cell fusion was reduced and no significant lysosomal damage was detectable.

Mouse hepatitis virus 3 (MHV-3) is known to cause acute focal hepatic necrosis in young mice of susceptible strains (Dick et al., 1956) but it can also be harboured in the latent state (Sabesin, 1972) and a chronic steady-state infection can be experimentally induced in the animal (Virelizier et al., 1975). Cultured mouse peritoneal macrophages are highly susceptible to MHV-3 and the resulting in vitro infection evolves rapidly with formation of large syncytia which are characterized by extensive lysosomal damage (Allison & Mallucci, 1965; Mallucci, 1965, 1966). The mechanisms whereby cell fusion is induced in the course of a virus infection are not well understood, but structural alterations of the cell surface are of obvious importance (Poste & Pasternak, 1978; Knutton, 1980). In chick fibroblasts infected with Newcastle disease virus (NDV), this is indicated by both an alteration of the membrane-operated uptake of metabolic precursors and an altered response to the agglutinating effect of plant lectins (Rott et al., 1975; Reeve et al., 1975). The existence of functional associations between surface macromolecules and cytokinetic elements (Ash & Singer, 1976; Sundquist & Ehrnst, 1976; Toh & Hard, 1977; Schreiner et al., 1977; Koch & Smith, 1978; Flanagan & Koch, 1978; Thom et al., 1979; Hoesli et al., 1980) may also be of importance in the evolution of some virus infections as it has been observed that translation of vesicular stomatitis virus (VSV) requires ribosomal association with the cytoskeletal framework (Cervera et al., 1981) and that macromolecular changes at the cell exterior can affect the organization of microfilament structures (Mallucci & Wells, 1976; Wells & Mallucci, 1978). On the other hand, since cytoskeletal elements can play a role in the control and display of cell surface macromolecular components (De Petris, 1974; Nicolson, 1975; Yahara & Edelman, 1975; Sundquist & Ehrnst, 1976; Edelman, 1976), a primary alteration of the cytoskeleton may lead to structural and functional changes of the surface membrane, which may in turn alter the assembly and release of membrane-bound (enveloped) viruses and modify the cytopathic effect that they cause. In the work reported here, we have followed the production of virus and the fusion process in macrophages infected with MHV-3 and treated with cytoskeleton-disrupting drugs, and found that disruption of microtubules had no detectable effects on the infectious process but that when microfilament structures were impaired the infection no longer evolved acutely as virus continued to be produced at a constant rate over several days. Under these conditions the fusion process was reduced and no significant lysosomal damage was detectable.

Stock MHV-3 virus was grown in macrophages from Swiss NIH mice and had a titre of 10⁶.⁸ TCID₅₀/ml. The cells were collected without previous stimulation to prevent involvement of the phagolysosomal systems prior to infection, and were cultured at 34 °C in Eagle's BHK medium supplemented with 20% inactivated foetal calf serum and containing 10 IU heparin/ml. After overnight incubation the cultures were thoroughly rinsed and the mononuclear cells which remained attached to the glass were used. No contamination with fibroblasts was observed.
Fig. 1. Growth of MHV-3 virus in the presence of anti-cytokinetic drugs. Δ, Colchicine; □, vinblastine; ●, cytochalasin B; ○, control.

After infection the cells were washed and returned to the incubator in medium without heparin. Antibodies to MHV-3 were raised in 6- to 7-week-old male Swiss NIH mice injected subcutaneously with $10^3$ TCID$_{50}$ of virus and challenged intraperitoneally with the same dose after 10 days. Sera were collected 4 weeks later, and pooled and assayed for neutralizing activity. Virus antigen was detected by indirect immunofluorescence using immune and control sera and fluorescein-labelled goat anti-mouse IgG preadsorbed on macrophage cultures (Mallucci, 1965). For acid phosphatase staining, an azo-dye-based method was used (Barka, 1960) and cells were counterstained with Mayer's haemalum nuclear stain. Colchicine and vinblastine sulphate (Sigma) were used at a final concentration of $10^{-6}$ M, a dose shown to cause loss of microtubule structure within 30 min as determined by specific immunofluorescence (Wells & Mallucci, 1978). Cytochalasin B (ICI) was used at a concentration of 5 µg/ml in 0.1% dimethyl sulphoxide (DMSO). Controls consisted of cells treated with 0.1% DMSO.

The multiplication of MHV-3 was followed in macrophages inoculated at a multiplicity of 0.5 TCID$_{50}$/cell. Colchicine, vinblastine and cytochalasin B were added when virus adsorption had been completed and the inoculum replaced with fresh medium. Fig. 1 shows that the pattern of virus production was not altered by colchicine or vinblastine but was remarkably changed by cytochalasin B. When cells were exposed to this drug there was no decline of infectivity after the initial peak but virus continued to be produced at a steady level. Although virus remained detectable for 10 to 14 days the observations in Fig. 1 were made over days 1 to 5, as on more prolonged incubation there occurred a varying degree of cell loss due to the enucleating effect of cytochalasin B, especially on single cells. Some of the morphological changes after infection are shown in Fig. 2. In the absence of drugs, the blebs and projections (filopodia) appeared shortly after infection and cells acquired an enlarged, flattened shape (Fig. 2a). Cell–cell contact was readily followed by fusion with the formation of vast cytoplasmic masses (Fig. 2b). Colchicine or vinblastine did not alter these events but the effect of cytochalasin B, which allowed virus replication to continue, was of interest. Blebbing was again an early event but filopodia were absent. Contact between cells was only occasionally preceded by the establishment of bridges (Fig. 2c) and cells which had come into contact proceeded, though less promptly, to form syncytia. These remained of a relatively small size and had a compact 'three-dimensional' appearance with persisting blebbing (Fig. 2d). Immunofluorescent staining showed that in cultures exposed to cytochalasin B, both individual cells and polykaryocytes were producing virus antigen throughout the time of observation. Investigations on cell surface activity, as
Fig. 2. Cytopathic changes during MHV-3 infection. (a) Early changes at 6 h post-infection; note cell spreading, cytoplasmic projections and surface blebbing. (b) Extensive syncytia formation 36 h post-infection. (c,d) Cells treated with cytochalasin B at 1 and 5 days post-infection respectively; note the persistence of the smaller and more compact syncytia. (e) Surface of early polykaryocyte (10 h post-infection) with diffuse and intense microvillar formations. (f) Infected cell treated with cytochalasin B; the microvilli are very diffuse and of irregular size, and the smaller projections on their surface (arrows) are of a size compatible with formations of viral origin. (a to d) Interference contrast microscopy; (e, f) scanning electron microscopy. Bar markers in (a to d) and in (e, f) represent 10 μm and 0.5 μm respectively.
expressed by finer morphological changes, showed that numerous microvilli developed in the untreated cultures as an immediate result of the infection (Fig. 2e), while at a more advanced stage the density of the microvilli decreased and diffuse disorganization of the membrane ensued. In contrast, the surface of macrophages exposed to cytochalasin B became richly dense with microvilli of irregular shape and size which persisted throughout the period of culture and virus production (Fig. 2f). Since a striking aspect of the cytopathic damage caused by MHV-3 in macrophages is an early increase in the permeability of the lysosomal membrane followed by massive lysosomal disruption (Allison & Mallucci, 1965; Mallucci, 1966), we examined the involvement of these organelles and found that in the presence of cytochalasin B, a considerable degree of lysosomal integrity had been retained both in single cells and in polykaryocytes.

Studies on parainfluenza simian virus 5 (SV5) in different cell systems have led to the suggestion that the surface membrane may have a role in determining whether a virus infection will develop in an acute or in a moderate form (Holmes & Choppin, 1966). More recently, it has been shown that conditions which affect the macromolecular arrangement of cell surface components can alter maturation and release of NDV (Reeve et al., 1975), and that substances with the ability to involve surface membrane-operated processes can alter the cytopathogenicity of SV5 virus (Rott et al., 1975). Cytoskeleton-disrupting drugs have also been used to investigate the evolution of infections caused by some enveloped RNA viruses. In the case of NDV no alteration of virus production has been observed (Genty & Bussereau, 1980) but various changes have been reported in other systems (Joseph & Oldstone, 1974; Menna et al., 1975; Panem, 1977). We found that depolymerization of the microtubular system by colchicine or vinblastine produced no effects. It must be considered, however, that in the case of coronaviruses, virus maturation occurs within cytoplasmic vesicles which are exported to the surface (Ferreira & Manaker, 1965; Becker et al., 1967; Oshiro et al., 1971) and virus envelope proteins may not become an integral part of the plasma membrane. Thus, the altered arrangement of surface macromolecules that microtubular disruption induces would be of no impediment to a process such as cell fusion, which may be favoured by the ensuing displacement of surface macromolecules. On the other hand, when the actomyosin system was impaired by cytochalasin B, the acute, highly cytopathic interaction that MHV-3 establishes with macrophages was changed into one which had the character of a steady-state infection. Under these conditions the infected cells did fuse but polykaryocytes, though numerous, were of relatively small dimensions and maintained the rounding imposed by the drug on the individual cells. The fact that infected cells did not acquire a flat shape even after fusion may offer an explanation for the minor involvement, if any, of the lysosomal system, and it could be speculated that extensive lysosomal disruption in MHV-3 infection results from the fusion of lysosomes with a 'collapsed' plasma membrane.

Modification of cellular parameters rather than comparison between different cell systems is a new approach to gain insight into the mechanisms which determine whether a virus infection will be expressed in an acute or in a moderate, persistent form. The results presented above show the relevance of experimentally induced changes in the actomyosin system in the evolution of a virus infection, but events which occur during movement, growth, antigenic pressure on cell surface determinants (including virus antigens), and other conditions which affect the interplay between the cell surface and the cytoskeleton may also alter the fate of a cell–virus interaction.

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REFERENCES


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