The Mode of Entry of Vaccinia Virus into L Cells

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SUMMARY

Electron microscopy of L cells infected with vaccinia virus at a high multiplicity indicated that virus cores entered the cytoplasm, during a 15 min period of adsorption at 37 °C, as a result of fusion between the plasma membrane and the envelopes of virus particles. It is suggested that for vertebrate poxviruses this, rather than phagocytosis, could be the effective mode of entry leading to infection.

Recent studies in this laboratory showed that when L cells are infected with vaccinia virus, RNA and protein synthesis commence with exceptional rapidity, virus-specific polypeptides being detectable only 20 min after infection (Metz & Esteban, 1972; Esteban & Metz, 1973). It is generally accepted that when poxviruses infect animal cells phagocytosis of the particles (‘viropexis’) is an obligatory initial step (Dales, 1965; McAuslan, 1969). We have now re-examined by electron microscopy features of the adsorption and penetration of vaccinia virus into cultured cells, and our findings indicate the occurrence of direct fusion between the envelope of attached virus particles and the plasma membrane of the cell.

L cells at a concentration of 1.0 × 10^7/ml were infected in suspension with vaccinia virus (strain WR) at a multiplicity of about 500 particles/cell, for 15 min at 37 °C in Puck’s saline A with 20 mM-Mg^2+ and 2 % and foetal calf serum. Immediately following the adsorption period, designated zero-time, the cells were diluted to 1.0 × 10^9/ml in tris-buffered Eagle’s medium (pH 7.2) containing 5 % calf serum. Cultures were shaken at 37 °C, and samples of 2 to 3 ml withdrawn at intervals for fixation. Samples for electron microscopy were taken at zero-time and at hourly intervals up to 4 h thereafter, together with uninfected controls at each point. The cells were sedimented, washed briefly by re-suspension in ice-cold Ringer’s solution and pelleted (< 500 g for 1 min); they were fixed by re-suspension for 30 min in a freshly made ice-cold mixture of glutaraldehyde and osmium tetroxide, washed, and post-fixed with uranyl acetate according to the schedule of Hirsch & Fedorko (1968). Epon-embedded cell pellets were sectioned, mainly as interrupted serial runs to obtain good sampling, and examined with the electron microscope after staining with uranyl acetate and lead citrate. In surveying the cells attention was paid particularly to the relationships and fine structure of vaccinia virus observed (a) as mature extracellular virus particles; (b) as naked cores within the cell; (c) as virus particles undergoing ingestion, or contained in cytoplasmic phagosomes.

(a) In all samples fixed from zero-time up to 4 h after infection numerous poxvirus particles were in close proximity to the external surface of the cells. They exhibited the usual range of profiles, depending upon plane of section; presenting an internal core, paired lateral bodies and an external membrane or envelope. The virus envelope, due to the high contrast obtained with simultaneous glutaraldehyde and osmium tetroxide fixation, showed conspicuous triple-layering of the unit membrane with an irregular coating of electron-dense material on its outer face. Occasional particles seemed to lie in more intimate contact with the plasma membrane; only the coating of the virus envelope separating the two membranes (Fig. 1).
Typical poxvirus cores were identifiable in the cytoplasm of all but a few of the cells examined, at each time point after infection including those taken at zero-time. Evidently large numbers of virus particles had effectively transferred their cores into the cytoplasm during the 15 min period of adsorption. Cores were round, oval or rectangular in outline, and measured up to 300 nm in the long axis (Figs. 5, 6). Each possessed a well defined dense
wall with a diffuse zone of radiating projections around it, and the centre was filled with filamentous material presumed to be virus nucleoprotein. Core profiles in the sections gave no indication of any regular association with cytoplasmic membranes, vesicles or other organelles; but in the cells fixed at zero-time their distribution was predominantly marginal, i.e. most were located close to the plasma membrane or within large pseudopodium-like extensions of the cell periphery. At the later stages cores were more widely distributed, and some were found close to the nuclear envelope.

Systematic search was made through a considerable number of apparent virus-cell attachment sites, to ascertain possible means by which many cores had gained rapid entry to the cell cytoplasm. In infected cells fixed at zero-time profiles were found indicating clearly the occurrence of a direct virus to cell fusion phenomenon in which continuity was established, at a point of contact, between the virus envelope and the plasma membrane (Figs. 2, 3, 4). This, in effect, brought the interior of such particles into contact with the peripheral cell cytoplasm. To avoid the problem of confusing mere superimposition of membranes for genuine fusion, images that showed only blurring at the point of contact were regarded as inconclusive; only those in which continuity of the triple layering of the two membranes could be clearly traced, in the plane of the section, were rated as indicating fusion. An example is seen in Fig. 3, depicting the site of fusion between a vaccinia particle and a projection of the cell margin: the virus core has expanded already into an oval form typical of naked cores, but the paired lateral bodies are still evident on either side of the core. The impression was gained that lateral bodies dispersed soon after fusion; traces of only one remained in the fused particle shown in Fig. 4. Incorporation of the virus envelope into the cell membrane is suggested by images such as these, but the possibility of its being shed as an empty vesicle after release of the core is not excluded. In the present survey, images showing definite fusion were confined to cells fixed immediately after the 15 min adsorption period. A few less conclusive examples were located in the sample taken 1 h after infection, but none were found in the later samples.

(c) In infected cells taken at zero-time no profiles showing vaccinia particles undergoing phagocytosis were found, nor were poxvirus particles seen within closed phagosomes in the cell cytoplasm. However, in samples examined from 1 h to 4 h after infection such profiles occurred quite often and their frequency appeared to increase somewhat with time. The intravacuolar vaccinia particle shown in Fig. 7 was evidently ingested along with a C-type particle from the cell surface: such particles are of course a familiar feature of murine cell lines, and in the present study they occurred equally in both the control and vaccinia-infected cultures.

That animal viruses may release their nucleoprotein into the cytoplasm of susceptible

Figs. 1 to 6. Sections from L cells fixed at zero-time, i.e. immediately after virus adsorption.
Fig. 1. A typical intact vaccinia particle in contact with the cell surface.
Fig. 2. Virus attachment site with an indication of localized loss of integrity in closely apposed portions of the plasma membrane and a vaccinia envelope; interpreted as commencing membrane fusion.
Figs. 3, 4. Sections through vaccinia particles fused with the cell membrane; showing continuity of their envelopes with the plasma membrane, expanded core morphology and (Fig. 3) paired lateral bodies.
Figs. 5, 6. Free vaccinia cores in the cell cytoplasm, showing the characteristic oval and rectangular profiles with external projections.
Fig. 7. An intraphagosomal vaccinia particle, in an L cell fixed following 1 h of incubation after virus adsorption.
host cells by a mechanism involving membrane fusion was first proposed for the myxoviruses (Hoyle, 1962); and this has been amply substantiated by electron microscopy (Meiselman, Kohn & Danon, 1967; Morgan & Howe, 1968; Morgan & Rose, 1968; Dourmashkin & Tyrrell, 1970). Penetration of cells by direct membrane fusion has now been attributed to a variety of other enveloped viruses some of which, like myxoviruses, also cause syncytium formation: they include herpes simplex virus (Morgan, Rose & Mednis, 1968), vesicular stomatitis virus (Heine & Schnaitman, 1971), simian foamy virus (Dermott & Samuels, 1973) and, most recently, an insect pox-like virus (Granados, 1973). Thus, the finding that vaccinia virus can enter L cells by membrane fusion as well as by phagocytosis brings the vertebrate poxviruses into line with other major groups in which both modes of entry have been reported. It does not follow that membrane fusion is necessarily the normal form of entry leading to infection, but in our view it is quite likely to be so. It may be significant that at the earliest time of observation, with numerous cores already detectable in the cytoplasm, there was morphological evidence for fusion but none for phagocytosis, while at later times the reverse was the case. Phagocytosis is a somewhat non-specific phenomenon, and it would be surprising not to find a proportion of virus particles from the inoculum being engulfed and digested along with other particulate matter from the cell surface; whereas it may be supposed that fusion between a virus envelope and the plasma membrane would hardly take place other than as a specific event of functional significance. In addition, penetration by membrane fusion avoids entirely the problem of transferring uncoated virus cores, intact, from the hostile environment of a phagolysosome into the surrounding cytoplasm. The case for ‘viropexis’ as an obligatory step in poxvirus infections has rested mainly on the demonstrability of phagocytic profiles using electron microscopy, and the reversible arrest of penetration by pre-treatment of cells with sodium fluoride (Dales & Kajioka, 1964). This does not exclude the direct virus-cell fusion mechanism; and in view of the present findings it appears likely that the phenomenon was previously overlooked. A conceivable compromise would be that sometimes, after phagocytosis, poxvirus cores may be transferred to the cytoplasm prior to lysosomal fusion with the phagosome, or even that the lysosomal response might be inhibited as occurs following ingestion of certain bacterial pathogens (Armstrong & Hart, 1971); but there is inadequate evidence to sustain such hypotheses at present.

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REFERENCES


Short communications


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