Extracellular Release of Enveloped Vaccinia Virus from Mouse Nasal Epithelial Cells in vivo

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SUMMARY

The release of vaccinia virus from mouse nasal epithelial cells infected in vivo was studied by electron microscopy. Intracellular naked vaccinia virus was enwrapped by Golgi membranes to form a double membrane intermediate. The outer membrane of the intermediate presumably fused with the plasma membrane, releasing extracellular enveloped virus. No signs of simple naked virus budding at the plasma membrane were observed. The majority of extracellular virus was enveloped and not naked.

A considerable amount of data has accumulated in recent years demonstrating the mechanisms by which vaccinia virus is released from infected cells in culture. Infectious intracellular naked vaccinia virus (INV) is either wrapped in a double membrane intermediate of which the outer membrane in turn fuses with the plasma membrane, releasing a virion enclosed in a single envelope (Ichihashi et al., 1971; Morgan, 1976; Payne & Kristensson, 1979) or by simple budding of INV at the plasma membrane (Tsutsui, 1983; Tsutsui et al., 1983). The extracellular enveloped vaccinia (EEV) virion is biologically significant. The envelope contains glycoproteins and the vaccinia virus haemagglutinin which are not found in INV (Payne, 1978, 1979). The release of EEV has been shown to be responsible for the spread of infection in cell cultures (Payne, 1980). Indirect results have also implicated EEV in virus dissemination in infected mice, since antibody to envelope antigens protects mice against an otherwise lethal infection (Payne, 1980). The present communication demonstrates for the first time that EEV virus is produced in vivo in infected mice.

Six-week-old female Swiss albino mice (NMRI strain, ALAB, Stockholm, Sweden) were infected by dropwise nasal instillation of 0.1 ml IHD-J vaccinia virus containing 1 × 10⁸ p.f.u. The vaccinia strain was previously passaged seven times on RK-13 cells. At various days post-infection, the mice were perfused through the heart with 5% glutaraldehyde in Sörensen’s phosphate buffer. Sections through the nasal cavity were post-fixed in osmium tetroxide, alcohol-dehydrated and embedded in Epon. Light microscopy was performed on 1 μm-thick sections stained with toluidine blue. Samples from three mice infected for 5 days were selected for ultrastructural studies. Ultrathin sections from these specimens were stained with lead citrate and uranyl acetate.

Numerous typical vaccinia immature and mature (INV) virions were observed in squamous epithelial cells of the nasal cavity (Fig. 1a). INV virions were often seen in association with the Golgi apparatus (Fig. 1b). The virions appeared to be enwrapped to different degrees either by cisternae or by chains of vesicles. Many virions enwrapped by a double membrane structure were dispersed in the cytoplasm (Fig. 1a, c). INV virions were often attached to intracellular membranes, but were only rarely aligned underneath the plasma membrane. There were no signs of any budding process. A few mature virions with a single membrane (envelope) were observed attached at the outer surface of the cell (Fig. 2). Most free extracellular virions were surrounded by a single envelope (EEV).

An intermediate stage showing the process by which double membrane virions were released in the form of single membrane EEV was never observed. This may be due to the rapidity with which this stage is started and completed. Nevertheless, it seems reasonable to assume that this...
Fig. 1. (a) Infected epithelial cell showing immature (IM) and mature (M) virions. Groups of the latter are attached to multivesicular bodies (MB) and three virions are enclosed by a double membrane (arrows and inset). (b) Virions associated with the Golgi apparatus. (c) Numerous double membrane-enclosed viral particles in the cytoplasm. Bar markers represent 500 nm.
stage is achieved through fusion of the outer membrane of the double membrane virion with the plasma membrane followed by exgulfment of a virion enclosed by a single envelope (EEV). This would be analogous to our observations in vaccinia virus-infected cultured cells (Payne & Kristensson, 1979).

The present work reports for the first time the mechanism of vaccinia virus release from infected cells in an intact animal. It is noteworthy, firstly, that in infected mouse nasal tissue the large majority of extracellular virus was enveloped and not naked. This indicates that virus release is not by cell disruption as is often supposed. Secondly, there were no signs of a simple budding process at the plasma membrane as observed in FL cells (Tsutsui, 1983; Tsutsui et al., 1983). Virus was released instead by conversion of INV to a double membrane virion that functions as a transport intermediate in the release of virus surrounded by a single envelope (EEV). This is clearly analogous to the release process described in RK-13 cell cultures (Payne & Kristensson, 1979).

The demonstration of the vaccinia release process in vivo via a double membrane intermediate is significant for two reasons. (i) The previously studied release in vitro of vaccinia virus (Payne & Kristensson, 1979) is not a laboratory artefact but rather faithfully reflects the in vivo process.
(ii) EEV and not INV is probably responsible for virus dissemination in vivo, since large numbers of EEV virions are now known to be released and antibody to the envelope antigens protects against lethal infection whereas antibody to INV does not (Payne, 1980).

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REFERENCES


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