C-type Virus Particles in Pig Kidney Cell Lines

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Pig kidney cells of the PS or PK2a line have proved useful for the study of group B arboviruses (Westaway, 1966; de Madrid & Porterfield, 1969). While following the course of arbovirus replication in PS cells by thin section electron microscopy, it became clear that the PS cell line was carrying a non-cytopathic virus morphologically indistinguishable from the oncogenic C-type virus particles of murine leukaemias (Bernhard, 1960; Dalton, 1962). The PS cell line is known to be chronically infected with swine fever virus (Shimizu et al. 1969), and to avoid this possible complication, attention was next turned to another pig kidney cell line, PK 15, which is known to be free from swine-fever infection. Numerous C-type particles were found in thin sections prepared from control PK 15 cells. To exclude the possibility that both PS and PK 15 cells had become infected, possibly with a murine virus, during handling in this Institute, a fresh supply of PK 15 cells was obtained from the American Type Culture Collection (designation CCL 33, passage 133). Typical C-type particles were found in the cells as received, and were numerous in later subcultures of these cells. Two further pig kidney cell lines (IB-RS-2 and SK6) were also obtained for examination (Table 1) and C-type particles were present in both. No C-type particles could be seen in preparations made from primary or secondary cultures of pig kidney cells.

Random sections of cell pellets, prepared by a combined glutaraldehyde-osmium tetroxide technique (Hirsch & Fedorko, 1968), revealed extracellular rounded particles 95 to 115 nm. in diameter in relation to at least 25 % of the cell profiles from PS, PK 15 and IB-RS-2 cultures (Fig. 1). Such particles were much less numerous in the SK6 cells. Irrespective of the cell line, the particles had a well-defined envelope with unit membrane structure. This was separated by a zone of low density from a prominent core or nucleoid of high electron density, which was rather irregular in outline, often showed a fibrillar substructure and measured 60 to 85 nm. in diameter (Fig. 2a). Particles budding outwards from the cell surfaces were frequently found (Fig. 2b) and, occasionally, budding particles were seen within cytoplasmic vacuoles. C-type particles were numerous in sections prepared from pellet supernatant medium removed from PK 15 bottle cultures.

The significance of the presence of C-type virus particles in all of four pig cell lines examined, as well as the origin and identity of the virus or viruses concerned, have yet to be determined. The finding by electron microscopy of previously unsuspected C-type virus infections of stable murine cell lines has been well documented (Kindig & Kirsten, 1967). Very recently such particles were first reported by Breese (1970) in two porcine cell lines, notably after inoculation with other test viruses. The causal relationship between certain C-type virus infections of mice and the development of leukaemia and lymphoid tumours is well established (Moloney, 1960). There is growing evidence of a similar basis for leukaemia in other mammals, including the cat (Jarrett et al. 1964; Rickard et al. 1969), the guinea-pig (Opler, 1967) and in cattle (Dutcher et al. 1967; Miller et al. 1969). Naturally occurring leukaemia-lymphosarcoma disease in swine seems to be sporadic and comparatively uncommon (Koller, Olson & Gillette, 1970), and no definite identification of C-type particles in tissues from normal or diseased swine is known to us. Four litters of newborn mice of the Balb/C strain, inoculated intracerebrally and subcutaneously with preparations of C-type particles.
Fig. 1. Group of C-type virus particles as found in the intercellular spaces of sectioned PK15 cultures.

Fig. 2. Details of particles at high magnification. (a) Mature extracellular forms; (b) developmental form, budding from a cell surface in a PS line culture.
from PK 15 cells, have remained healthy over a period of 10 months. We have not attempted to transmit the virus to piglets.

It is possible that the particles present in pig kidney cell lines originated, at one or more points in time, in bovine serum used in the culture media; although none could be identified in pelleted foetal bovine serum as used in this laboratory, nor could C-type particles be found with the electron microscope in sections of human diploid cells (WI-38 strain) grown in the same medium as the PS and PK 15 cultures. Our attempts deliberately to infect WI-38 cells with C-type virus from the PK 15 cultures have so far also been negative. The findings summarized in Table I suggest that some widely used porcine cell lines are often contaminated with C-type particles; this should be borne in mind when they are required for virus research or vaccine production.

<table>
<thead>
<tr>
<th>Cells</th>
<th>Source</th>
<th>Passage level</th>
<th>C-type particles</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS</td>
<td>E.G. Westaway</td>
<td>&gt; 200</td>
<td>++</td>
</tr>
<tr>
<td>PK 15</td>
<td>Weybridge</td>
<td>&gt; 150</td>
<td>++</td>
</tr>
<tr>
<td>PK 15 (CCL33)</td>
<td>A.T.C.C.</td>
<td>133</td>
<td>++</td>
</tr>
<tr>
<td>IB-RS-2 (clone 60)</td>
<td>Pirbright</td>
<td>98</td>
<td>++</td>
</tr>
<tr>
<td>SK6</td>
<td>Royal Veterinary College</td>
<td>&gt; 160</td>
<td>+</td>
</tr>
<tr>
<td>Pig kidney</td>
<td>Royal Veterinary College</td>
<td>Primary and secondary cultures</td>
<td>0</td>
</tr>
</tbody>
</table>

The PK 15 cells used in this study were received from the Central Veterinary Laboratory, Weybridge; the IB-RS-2 line, originally established in Brazil, was obtained from the Animal Virus Research Institute, Pirbright, Surrey; and the SK6 line was kindly supplied by Dr L. Kasza of the Royal Veterinary College, London.

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