African Swine Fever Virus Replication in Porcine Lymphocytes

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SUMMARY

Purified preparations of porcine lymphocytes were infected with three isolates of virulent African swine fever virus (ASFV). Electron microscopy showed the presence of small numbers of mature virus particles in degenerating cells. The titres of infective virus released were low and reached a maximum by 24 h after infection.

African swine fever virus replicates in cells of the monocyte-macrophage series of the pig both in vivo (Moulton & Coggins, 1968a; Colgrove, Haelterman & Coggins, 1969) and in vitro (Malmquist & Hay, 1960; Moulton & Coggins, 1968b), and cultures of pig bone marrow or leukocyte cells are the most sensitive for the titration of ASFV. It has also been suggested that ASFV may replicate in lymphocytes because of the severe lymphoid tissue necrosis seen during acute ASF (Maurer, Griesemer & Jones, 1958) and the demonstration by immunofluorescence of virus antigen in presumptive lymphocytes (Colgrove et al. 1969).

In this study we infected purified populations of unstimulated porcine lymphocytes with virulent isolates of ASFV and showed that the virus replicates in these cells.

Purified lymphocytes were prepared from heparinized (10 i.u./ml) venous blood taken from healthy Large White-Landrace crossbred pigs of 20 to 30 kg weight. After centrifuging at 200 g for 20 min,uffy coat cells were carefully removed and made up to 5 ml per 15 ml of whole blood in Hanks medium containing yeast extract and lactalbumen hydrolysate (LYH). Five ml amounts were centrifuged on Ficoll-Hypaque gradients and the red cells were removed from the lymphocyte band by one cycle of distilled water lysis. Phagocytic cells were removed from the preparation with a magnet after incubation in the presence of carbonyl iron. Cytospin preparations of the final cell suspension which were stained with Giemsa contained ≥ 99% lymphocytes.

Lymphocytes were infected at an m.o.i. of 1 with virulent ASFV (Table 1), incubated for 1 h at 37 °C and washed five times to remove unadsorbed virus. Infected cells (3 x 10⁷) were finally resuspended in 15 ml of RPMI 1640 containing 10% heat-inactivated foetal calf serum and antibiotics and cultured in Falcon plastic flasks. Samples of cells were collected at different times post-infection (p.i.) for virus assay and electron microscopy.

Virus was assayed by inoculating tenfold dilutions of lymphocyte suspensions into 3 to 4-day-old stationary tube cultures of pig bone marrow cells (Plowright, Parker & Staple, 1968). Haemadsorption was observed up to 6 days p.i. and titres expressed as log₁₀ HAD₅₀ (50% haemadsorbing doses) per ml.

For electron microscopy, lymphocytes infected with the Lee or Kirawira isolates of ASFV, were fixed in 1% glutaraldehyde in phosphate buffered saline (PBS) for 20 min at 20 °C, washed twice in PBS and post-fixed in 1.33% OsO₄ in s-collidine buffer. After washing in distilled water, cells were dehydrated through a graded series of alcohols and substituted with propylene oxide and then Epon 812 resin. Ultrathin sections were lightly stained with saturated uranyl acetate followed by lead citrate and viewed in a Philips 301 TEM at 60 or 80 kV. Lymphocytes were readily distinguished from the few contaminating monocytes and neutrophils by their characteristic ultrastructure.
Table I. Replication of virulent isolates of African swine fever virus in cultures of porcine lymphocytes

<table>
<thead>
<tr>
<th>Virus isolate</th>
<th>0 h</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kirawira (African)</td>
<td>3.0</td>
<td>5.5</td>
<td>5.2</td>
<td>5.8</td>
</tr>
<tr>
<td>Lee (African)</td>
<td>0.6</td>
<td>3.8</td>
<td>4.5</td>
<td>4.8</td>
</tr>
<tr>
<td>Lisbon 60 (European)</td>
<td>1.0</td>
<td>3.4</td>
<td>4.1</td>
<td>4.1</td>
</tr>
</tbody>
</table>

* Mean of two experiments.

Fig. 1. Pig lymphocytes infected with ASFV 24 h p.i. (a) Cell on right shows severe cytopathic effect and factor site adjacent to nucleus. (b) Enlargement of factory site showing mature virus particles.

Maximum virus titres for the three isolates tested were reached by 24 h p.i. (Table I) and the titres remained relatively constant over the following 48 h. The electron microscopy studies showed the presence of mature virus particles within lymphocytes (Fig. 1). As early as 24 h there was a high proportion of dead and dying cells compared to control preparations, and this proportion increased over the time course of the experiment. Virus was usually seen in conjunction with membrane debris and dying cells although virus-like particles and possible factory sites were sometimes identified in less degenerate cells. Usually only small numbers of mature virus particles were seen in any one cell and this correlates with the low titres of infective virus found on titration.

Our data show that ASFV replicates in unstimulated lymphocytes and may explain the extensive lymphoid necrosis and lymphopoenia seen during acute ASF. Our electron microscopy results are in contrast to those of Enjuanes, Cubero & Viñuela (1977), who failed to detect ASFV in lymphocytes. However, they were using an extensively passaged ASFV isolate and it may be that such viruses differ in their ability to replicate in lymphocytes. The cytolytic infection of lymphocytes by ASFV may account in part for the poor allergic reaction shown by pigs infected with this virus (Hess, 1971).
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REFERENCES


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