An Ultrastructural Examination of Non-lymphoid Host Cells of Moloney Murine Leukaemia Virus of CFW/D Mice

By Peter C. Freebeck,* Roderick MacLeod and Timothy O'Brien

Department of Biology and Department of Genetics and Development, School of Basic Medical Sciences, University of Illinois, Urbana, Illinois 61801, U.S.A.

(Accepted 22 September 1983)

SUMMARY

An ultrastructural examination of various tissues from CFW/D mice, neonatally injected with Moloney murine leukaemia virus (Mo-MuLV) revealed the presence of type C virus particles budding from the membranes of various bone marrow-derived cells, pancreatic acinar cells, beta cells, and submandibular gland acinar cells. These observations indicate that Mo-MuLV has the potential to replicate in non-lymphoid cell types even when acquired post-partum. In the pancreas, a large accumulation of immature and mature viral particles was observed between the cell membranes of the acinar cells and the basal lamina. A similar accumulation of viral particles was observed in the lumina of the acinus of submandibular glands. The role of murine leukaemia viruses as potential mediators of aberrations of non-haemopoietic tissues and an alternative mode of viral transmission are discussed.

INTRODUCTION

The pathologies associated with murine leukaemia virus infections have been related directly or indirectly to aberrations of the haematopoietic system. A productive retrovirus infection is dependent upon the presence of permissive host cells and this condition of permissiveness is thought to vary during the course of host maturation and cellular differentiation (Jaenisch, 1980). Previous studies have shown that the tropism of Moloney murine leukaemia virus (Mo-MuLV) is limited to the lymphoid lineage if the virus is acquired postnatally (Jaenisch et al., 1975; Jaenisch, 1976, 1980), yet recent evidence indicates that non-lymphoid cells may also support a productive viral infection (Simon et al., 1982).

The insertion of the Mo-MuLV proviral genome into the germ line of BALB/c mice (BALB/Mo; Jaenisch, 1976) results in a productive viral infection within a variety of cell-types, lymphoid and non-lymphoid alike (Simon et al., 1982). The productive viral infection within the non-lymphoid cells occurs even though infectious virus is not activated until at least the first week following the birth of BALB/Mo mice (Jaenisch et al., 1981). One possible interpretation is that the proviral genome is indeed activated by mid-gestation in embryonic BALB/Mo mice but at levels not easily detectable. Equally plausible is an interpretation assuming that mice acquiring Mo-MuLV following birth do contain non-lymphoid cells infected with Mo-MuLV but again at levels not easily detected.

The purpose of our study was to determine whether any non-lymphoid cells in mice acquiring Mo-MuLV postnatally support a productive type C viral infection, confirming recent evidence that Mo-MuLV replicates in non-haematopoietic tissues.

We undertook a detailed ultrastructural study of various tissues of CFW/D mice infected postnatally with Mo-MuLV, and the results of our investigations indicate that not only is type C virus capable of replicating in non-lymphoid cell types, but that it replicates actively in cells that are of vital importance to the normal physiology of the mouse.

* Mailing address: 847 East 57th Street, Chicago, Illinois 60637, U.S.A.
Fig. 1. Typical type C virus particle budding from the plasma membrane of a bone marrow reticulocyte. Arrow indicates virus particle. Bar marker represents 0.5 μm.

Fig. 2. Arrow indicates a virus particle budding from the membrane of a bone marrow leukocyte with the ultrastructural characteristics of a neutrophil. Bar marker represents 0.5 μm.
Non-lymphoid tropism of Mo-MuLV

METHODS

Mouse strain. The outbred CFW/D mice used in this study were bred from a stock kindly donated by Dr Alex McCarter, University of Western Ontario, London, Ontario, Canada.

Virus. Stock viral inoculum was produced by infecting cultured thymus–bone marrow (TB) cells (Ball et al., 1973) with Mo-MuLV at a multiplicity of infection of 0.5 and incubated at 34 °C in a Forma Scientific (5% CO₂) incubator for 3 days. The culture medium was removed and the infected TB cells were overlayed with fresh complete medium. This medium was harvested 24 h later, filtered through a 0.45 μm membrane (Millipore), and stored in 4 ml glass vials in a Kelvinator Ultracold deep-freeze at −80 °C. This virus-containing supernatant fluid had a virus titre of approximately 2 × 10⁵ infectious units per ml as measured by the 15F focus forming assay (McCarter et al., 1977).

Injection procedure. Mice were injected within 24 h of birth using a tuberculin syringe fitted with a 25-gauge needle. A 0.1 ml sample of virus-containing solution was introduced through the hind-limb muscle into the peritoneal cavity. Uninjected controls were included in all experiments.

Kidney fluorescence. Frozen kidneys were sectioned at approximately 5.0 μm using an International Cryostat. They were acetone-fixed for 30 s, stained with fluorescein-conjugated goat anti-Mo-MuLV antibodies for 30 min, washed for 30 min in a continuous flow of phosphate-buffered saline (pH 7.2), and then examined with a Leitz Ortholux fluorescence microscope.

Electron microscopy. Tissues were removed from mice at intervals ranging from 30 days to 10 months post-infection, sliced into 1 mm cubes, and fixed immediately in 3.5% glutaraldehyde/0.1 M-sodium cacodylate buffer (pH 7.4) at room temperature for 2 h. Following a 1 h wash in 0.1 M-sodium cacodylate (pH 7.4), the tissues were postfixed in 1% OsO₄/0.1 M-sodium cacodylate (pH 7.4) for 1 h, dehydrated in a graded acetone series, and embedded in either Epon 812 or Spurr’s low viscosity resin. Ultrathin sections were stained with aqueous uranyl acetate followed by lead citrate and then examined with a Siemens 102 transmission electron microscope.

RESULTS

The criteria used to establish a successful Mo-MuLV infection included the detection of Mo-MuLV antigens by antibody-specific immunofluorescence in kidney sections. Specific immunofluorescence was observed uniformly within the glomeruli of mice injected with Mo-MuLV but never within the uninjected mice serving as controls. Furthermore, mice injected with Mo-MuLV following birth developed tumours at 8 to 10 months of age whereas tumour development was absent within control mice.
Ultrastructural examination of lymphoid tissues of Mo-MuLV-infected mice revealed the presence of typical type C virus particles. Fig. 1 and 2, respectively, show virus budding from the plasma membrane of a bone marrow reticulocyte and a bone marrow leukocyte. Virus particles were also observed budding into the lumina of the cytoplasmic cisternae of bone marrow.
megakaryocytes, and inside the lumina, aggregates of both immature and mature particles were present (Fig. 3).

An examination of the ultrastructure of non-lymphoid cells within the pancreas and the submandibular glands of infected animals also revealed type C virus. In the pancreas, numerous virus particles were found present between the plasma membranes of the pancreatic acinar cells and the basal lamina. Virus particles were also present within the acinar cell rough endoplasmic reticulum (Fig. 4). Several of these particles had an immature morphology, and some had the distinct appearance of being in the process of budding. In the islets of Langerhans, virus particles were seen in close association with beta cells (Fig. 5). A large number of the virus particles observed in this location had an immature morphology, suggesting that they had replicated in the beta cells. The profile of a budding virus shown in the delineated area in Fig. 5, as magnified in the inset, further substantiated this conclusion.

Numerous immature and mature viral particles were also observed in association with the submandibular glands of infected mice. A large accumulation of virus particles, many of which were immature, were in the lumina of the acinar cells of this secretory gland (Fig. 6). Virus particles were also observed budding from the plasma membranes of these cell types (Fig. 7).

**DISCUSSION**

The purpose of this study was to determine whether Mo-MuLV, when injected into neonatal CFW/D mice, replicated in cells other than those of bone marrow origin. Previous studies used the techniques of molecular hybridization *in vitro* with nucleic acids extracted from whole organs. It is now recognized that this technique lacks the sensitivity required to detect viral replication within the non-lymphoid cells of BALB/Mo mice (Simon *et al.*, 1982). It is possible that our ultrastructural analysis provided the necessary sensitivity, lacking in techniques *in vitro*, to detect an acquired viral infection within non-lymphoid cells of mice.
Of major significance to this study was the finding that viral replication occurred in cells of the endocrine and exocrine system. In the pancreas, the large accumulations of immature virus particles between the plasma membranes of acinar cells and the basal lamina, and the presence
Non-lymphoid tropism of Mo-MuLV

of immature particles in the intracellular vacuoles of acinar cells gave every indication that viral replication had occurred in these cells. The observation of a virus budding into a cytoplasmic vesicle of a pancreatic beta cell further substantiated our conclusion that viral replication does occur in non-lymphoid cells. Additional evidence for non-lymphoid tropism of Mo-MuLV was established when budding virus was observed in acinar cells of the submandibular glands. It was also interesting to note that large numbers of viral particles aggregated in the lumina and cell junctions of these cell types.

Breast feeding is thought to be the major route of postnatal horizontal transmission of Mo-MuLV and of murine leukaemia viruses in general (Gardner et al., 1979; Jaenisch, 1980). Our description of viral replication within cells of the submandibular glands of mice infected postnatally provides an alternative pathway by which Mo-MuLV may be transmitted among susceptible hosts.

The presence of budding and immature viral particles within cells of the pancreas and submandibular glands demonstrates that CFW/D mice, acquiring Mo-MuLV postnatally, can support a productive type C viral infection within non-lymphoid cells. Although our ultrastructural observations, coupled with results obtained by Simon et al., (1982), indicate that Mo-MuLV can replicate in cells other than lymphoid, the origin of the particles we observed is still undetermined. The possibility exists that the viral particles were endogenously derived. That a retrovirus such as Mo-MuLV may replicate in lymphoid and non-lymphoid organs alike calls our attention to the following: non-lymphoid tissues supporting a productive retrovirus infection may serve to transmit virus to susceptible hosts, and retrovirus replication within non-lymphoid tissues may produce, either directly or indirectly, a state of disease at the site of replication.

REFERENCES

(Received 10 August 1983)