Host Range Differences among Xenotropic Type C Retroviruses Isolated from Mouse Kidney Cell Cultures

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SUMMARY

By co-cultivation procedures, infectious xenotropic type C viruses have been recovered from kidney cells of several strains of mice. They have host-range patterns which place them into separate subgroups. In cells cultivated from one NZB kidney, two biologically different xenotropic type C retroviruses were found. One, X-NZB/K-1, infects and replicates well in human and mink fibroblast cells but does not induce foci in mink S+L- cells with good efficiency. The other, X-NZB/K-2, infects and replicates well in mink but not human fibroblast cells, and induces foci readily in mink S+L- cells. Cross-infection studies indicate that these viruses, classified as xenotropic by host range and envelope properties, are genetically stable.

Type C retroviruses are inherited in the genomes of many animal species and have probably been conserved in nature for millions of years (Chattopadhyay et al., 1974; Todaro, 1975; Levy, 1978). Their preservation in the host suggests that they could be required for normal development in the species (Levy, 1978; Varnier et al., 1981). The house mouse (Mus musculus) offers an excellent model for studying this possibility since this animal has as many as three different classes of endogenous type C retroviruses: ecotropic, xenotropic and amphotropic. While sharing some proteins and nucleic acid sequences, these three classes can be distinguished by biological, serological and genetic differences (Gross, 1970; Gardner, 1978; Levy, 1978).

Since multiple copies of the X-tropic type C retrovirus exist in the mouse genome (Chattopadhyay et al., 1976), we wished to determine whether each provirus could code for distinct X-tropic viruses with specific biological functions in the host. Observations in other laboratories have suggested that X-tropic viruses could be separated by serological or genetic differences (Stephenson et al., 1974; Callahan et al., 1974). We have looked for biological differences by examining the host range properties of X-tropic viruses. Most of the cell cultures used were maintained in Eagle's minimal essential medium (EMEM) (Flow Laboratories) with 5 to 10% foetal bovine serum (FBS), 1% glutamine (2 mM) and antibiotics (penicillin, 250 U/ml; streptomycin, 250 μg/ml). For focus formation assays in normal rat kidney cells (NRK), EMEM supplemented with 5% heated (56 °C for 30 min) calf serum, was employed. Cat and mouse S+L- cell lines, originally obtained from P. Allen, were maintained in RPMI 1640 medium (Flow Laboratories) with 10% FBS. Cells used for virus assays were pretreated with 2 ml of diethylaminoethyl-dextran (25 μg/ml for 30 min) or polybrene (10 μg/ml) to increase their sensitivity to virus infection (Duc-Nguyen, 1968; Varnier & Levy, 1979).

For our studies, cells cultured from two kidneys from one 3-month-old female mouse from each strain were mixed with human foreskin (HuF), mink lung (ATTC, CCL 64), dog osteosarcoma (D-17) and rabbit cornea (SIRC; ATTC, CCL 60). These cell lines are cultivated routinely in our laboratory. Approximately 2 × 10^5 cells from each line were used for the coculture. After three weekly cell transfers, the supernatants from these cultures were removed, filtered and passed weekly for 3 to 4 additional weeks in the non-mouse parental cells originally used for cocultivation. Subsequently, the viruses selected by this procedure were assayed by standard procedures for their ability to induce foci in S+L- cells (Fischinger et al., 1974; Peebles, 1975). The infected parental cell lines and the mouse kidney cells were also mixed with the B4 clone of the NRK-Harvey line to obtain X-tropic virus pseudotypes of a mouse sarcoma virus (MSV) (Levy et al., 1975a). These pseudotypes were titrated by focus formation on NRK and HuF cells.

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The co-cultivation of kidney cells with heterologous cell lines was used in attempts to select different X-tropic virus subtypes with preferential growth in one type of host cell. Table 1 presents the titres of mouse X-tropic type C retroviruses produced by mouse kidney (MK) cells alone and by animal cell lines used to propagate X-tropic viruses. These studies indicated that only kidney cells from NZB, NZY and C57BL/6 mice spontaneously released X-tropic viruses detectable by our assays. The co-cultivation of the kidney cells with HuF cells enhanced recovery of the NZB and NZY viruses, but did not reveal any X-tropic viruses in the previously negative cultures. Cells from NZW, NIH Swiss and C57BL/10 mice mixed with HuF cells remained virus-negative. In contrast, cultivation of the kidney cells with dog and mink lung cells demonstrated the presence of viruses in many kidney cultures. Dog cells were the most universal indicators for detection of the X-tropic viruses. Mink lung cells, cultivated with MK cells, led to release of a virus from NIH Swiss, but not from NZW cells. The recovery of viruses from NIH Swiss tissue confirms previous observations that this strain spontaneously produces X-tropic viruses (Levy, 1973). Rabbit cells were the least sensitive for X-tropic virus isolation, although they permitted a substantial replication of the C57BL/10 X-tropic virus isolate. These results indicate that X-tropic viruses can be isolated from the kidneys of six mouse strains and that virus detection can, in some cases, only be achieved with certain animal cells.

Following this initial study, we focused on the X-tropic type C viruses expressed by NZB kidney cells. The viruses recovered after the co-cultivation of the NZB kidney cells with human, mink and dog cells were characterized by four different assay systems involving focus formation in S+L−, NRK and HuF cells. In these studies we found that the X-tropic virus, isolated by passage in human cells, produced foci only in the cat and not in the mink S+L− cell lines. The X-tropic virus spontaneously released from NZB kidney cells alone had a similar focus-forming ability, although it has sometimes induced foci in mink S+L− cells with a very poor efficiency. This human cell-grown virus isolate has been tentatively identified as X-NZB/K-1. This virus probably was the initial NZB isolate (Levy & Pincus, 1970; Levy, 1973), since it appears to be the predominant type produced spontaneously by the NZB kidney cells. A different isolate was selected by growth of the X-tropic virus in mink lung cells. This X-tropic virus induced foci in both S+L− cell lines, but its MSV pseudotype was unable to transform human fibroblasts; this isolate we have tentatively called X-NZB/K-2. Dog cells appeared to permit the replication of both these putative X-tropic virus subtypes (Table 2).

The two NZB retroviruses were both neutralized by antiserum against the NZB X-tropic virus and by normal mouse sera, which contains the anti-X-tropic virus neutralizing factor (Levy et al., 1975b). They were not neutralized by antiserum against the ecotropic AKR virus (data not shown). More sensitive immunological assays, such as kinetics of neutralization and the use of monoclonal antibodies may be helpful in defining any subtle antigenic differences between the envelope proteins of these X-tropic virus subtypes.

Cross-infection experiments ruled out the possibility that modification of the viruses occurred following passage in heterologous cells. The two different X-tropic virus subtypes were
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Table 2. Recovery of X-tropic viruses from NZB kidney cells*

<table>
<thead>
<tr>
<th>Cell cultures used for co-cultivation</th>
<th>Focus formation in</th>
<th>Proposed X-tropic virus subtype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mink S+L−</td>
<td>Cat S+L−</td>
</tr>
<tr>
<td>None</td>
<td>None</td>
<td>2:1</td>
</tr>
<tr>
<td>Human</td>
<td>−</td>
<td>2:5</td>
</tr>
<tr>
<td>Mink</td>
<td>1:8</td>
<td>1:6</td>
</tr>
</tbody>
</table>

* See legend to Table 1 for procedure. Data reflect focus-formation titres (log10) by X-tropic viruses in S+L− cell lines (Fischinger et al., 1974; Peebles, 1975) and by their MSV pseudotypes in rat (NRK) and human (HuF) cells (Levy et al., 1975a).

Table 3. Stability of host range properties of the NZB X-tropic virus subtypes*

<table>
<thead>
<tr>
<th>X-tropic virus subtype</th>
<th>Cell cultures used for propagation</th>
<th>Focus formation in</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NRK</td>
<td>HuF</td>
</tr>
<tr>
<td>X-NZB/K-1</td>
<td>Human</td>
<td>3:0</td>
</tr>
<tr>
<td>X-NZB/K-1</td>
<td>Mink</td>
<td>2:9</td>
</tr>
<tr>
<td>X-NZB/K-1</td>
<td>Dog</td>
<td>3:5</td>
</tr>
<tr>
<td>X-NZB/K-2</td>
<td>Human</td>
<td>−</td>
</tr>
<tr>
<td>X-NZB/K-2</td>
<td>Mink</td>
<td>2:2</td>
</tr>
<tr>
<td>X-NZB/K-2</td>
<td>Dog</td>
<td>2:9</td>
</tr>
</tbody>
</table>

* X-tropic MSV preparations, containing the X-tropic virus subtype and its MSV pseudotype, were propagated for 3 weeks in the animal cell cultures listed. The supernatants were harvested, filtered and titrated in the cell lines listed by procedures listed in text. Titre is given as log10 focus-forming units/ml. Titres of less than 0:4 are shown as −.

Propagated for over 3 weeks in human, mink and dog cultures and then their host range properties were again evaluated (Table 3). Similarly, the X-tropic virus pseudotypes of MSV were propagated (Table 3). The MSV pseudotype of the X-NZB/K-1 virus retained the ability to transform rat and human cells. Despite its ability to replicate in mink lung cells, the X-NZB/K-1 virus did not acquire the property of forming foci in mink S+L− cells. The reason for this discrepancy is not yet known, but appears related to the sensitivity of the clone of mink S+L− cells used and the virus subtype. We have recently been able to induce foci in an early passage clone of mink S+L− cells with high-titred X-NZB/K-1 virus, but the virus had a titre of up to 1000-fold more in the other indicator cell lines. The X-NZB/K-2 virus, initially recovered by growth in mink cells, appeared restricted in its replication in human cells and its pseudotype was still unable to transform human cells after its propagation in dog cells (Table 3). Moreover, as noted previously, this virus, even after passage through dog cells, continued to differ from X-NZB/K-1 in its ability to form foci readily in mink S+L− cells. We also cannot conclude that an absolute restriction in replication of X-NZB/K-2 virus in the human cells exists, since relatively low titres of virus were employed. Nevertheless, the data indicate distinct differences in host range properties between these two NZB kidney X-tropic viruses.

Our results substantiate those of Elder et al. (1980), who described two different X-tropic viruses in NZB mice. One, spontaneously expressed by NZB spleen and lung cells, possessed a unique surface glycoprotein (gp70) similar to the gp70 circulating in the blood of all mouse strains tested. The other X-tropic virus, induced from NZB embryo fibroblasts with halogenated pyrimidines, had a gp70 similar to that of X-tropic viruses isolated from several other mouse strains. Since both our isolates were released spontaneously from the same tissue, an NZB kidney, we present the first evidence that two different X-tropic viruses can be expressed simultaneously in one mouse organ. The relationship of our NZB X-tropic viruses to the other NZB viruses recovered from spleen, lung and embryo cells awaits further analysis. All these observations strongly suggest a heterogeneity of X-tropic type C retroviruses expressed in mice and even in the same mouse tissue.
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REFERENCES


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