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Characterization of Xenotropic and Dual-tropic Type C Retroviruses Isolated from Abelson Tumour

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SUMMARY

Tumours induced in Balb/c mice by Abelson virus complex were found to contain a xenotropic virus (A-X-MuLV) and an NB-tropic, dual-tropic virus (NBX), in addition to the Moloney leukaemia virus (M-MuLV) and the defective, transforming Abelson virus genome. Both A-X-MuLV and NBX virus were presumably present in a genomically masked form and could be recovered only by co-cultivation of tumour cells with permissive cells. Only about 0.87% and 0.13% of the viruses in the co-culture supernatant represented A-X-MuLV and NBX virus respectively; the majority were M-MuLV. The NBX virus acted more efficiently than the HIX virus (Fischinger et al., 1975) as helper to rescue murine sarcoma virus (MSV) from S^L^- cells of hamster, rat and mouse origin, whereas the converse was true for those of cat and human origin. The interference and neutralization patterns suggested that the NBX virus was an env gene recombinant between A-X-MuLV and M-MuLV. The fact that NBX virus cross-reacted in radioimmunoassays with gp70s of both M-MuLV and Balb:virus-2 provides evidence for the recombinant nature of the NBX gp70-coding gene which was probably derived from both M-MuLV and a virus similar to Balb:virus-2 or A-X-MuLV. The presence of a unique antigenic determinant on the gp70 of NBX virus is also suggested. Both A-X-MuLV and NBX virus cross-reacted with type-specific p12s of M-MuLV and Balb:virus-2, suggesting that the gag gene coding for p12 of NBX virus was derived from the A-X-MuLV, which was itself a recombinant, its p12-coding gene being derived from both Balb:virus-2-like virus and M-MuLV. The NBX virus was not oncogenic when tested in newborn Balb/c mice.

INTRODUCTION

Three classes of murine type C retroviruses (MuLV) have been distinguished by host range properties. These include ecotropic viruses (E-MuLV) which preferentially replicate in mouse cells, xenotropic viruses (X-MuLV) which preferentially replicate in cells from heterologous species other than mice (for review, see Levy, 1978) and a group of dual-tropic MuLV showing host ranges of both E-MuLV and X-MuLV. Increasing evidence suggests that generation of dual-tropic MuLV involving genetic recombination between E-MuLV and X-MuLV is implicated on leukaemic processes.

Fischinger et al. (1975) reported the isolation of a virus, designated HIX, with hybrid envelope properties of NB-tropic Moloney-MuLV (M-MuLV) and X-MuLV. Hartley et al. (1977) described MCF (mink cell focus-inducing) viruses with the combined host range properties.
properties of N-tropic E-MuLV and X-MuLV. These are present in thymuses of preleukaemic and leukaemic AKR mice, lymphomas of Akv-1- or Akv-2-congenic National Institutes of Health (NIH) Swiss mice, and the thymus of a preleukaemic C58 mouse. Hiai et al. (1977) detected in the thymus of hairless (hr/hr) mice a 'polytropic virus', which has some similarities to the MCF virus. Biochemical and serological properties, as well as the broad host range property, indicated that these arose by a recombinational event within the env gene between E-MuLV and X-MuLV (Elder et al., 1977; Rommelaere et al., 1978; Faller et al., 1978; Fischinger et al., 1978a). Another group of viruses with a wide host range have been isolated from wild mice in California (Rasheed et al., 1976; Hartley & Rowe, 1976; Gardner, 1978) and because of their unique viral interference pattern and immunological characteristics, they are assigned a separate entity designated 'amphotropic viruses' (Hartley & Rowe, 1976; Levy, 1978).

Abelson-MuLV complex was isolated from a Balb/c mouse that had been treated with prednisolone and inoculated with M-MuLV (Abelson & Rabstein, 1970). It transforms NIH/3T3 cells (Scher & Siegler, 1975) and lymphoid cells of both bone marrow (Boss et al., 1979) and foetal mouse liver (Rosenberg et al., 1975). The virus complex induces non-thymic lymphomas in mice with a short latent period (3 to 5 weeks) and consists of a defective transforming virus and a helper M-MuLV (Scher & Siegler, 1975). In this article, we report the presence in the Abelson tumour of two additional MuLVs: an X-MuLV, designated A-X-MuLV, and a dual-tropic MuLV, designated NBX. These have been analysed for their host range, viral interference and neutralization patterns, oncogenic potential, and antigenic relationships to various E-MuLV and X-MuLV in terms of cross-reactivities by competitive p12 and gp70 radioimmunoassays. The NBX virus appears to be a recombinant of M-MuLV and X-MuLV with unique biological and subviral antigenic properties.

METHODS

Cell cultures. Cultures of NIH Swiss (NIH), Balb/c and (C3H × C57BL/6)F1 mouse embryo (ME), dog thymus (A7573), normal rat kidney (NRK) and duck embryo cells were provided by Biotech, Rockville, Md., U.S.A. Rat, feline embryo, horse skin, human embryo (PTA), mink lung, rhesus monkey kidney, rabbit cornea (SIRC) and SC-1 cells have been described previously (Chang et al., 1981). S+L- cells of Kirsten-murine sarcoma (MSV)-transformed human osteosarcoma (KHOS) (Rhim et al., 1975), Moloney MSV-transformed mink lung (S+L- mink) (Peebles, 1975), feline embryo kidney (81/CCC) (Fischinger et al., 1974), hamster tumour (HT-1) (Huebner et al., 1966; Chang et al., 1971) and mouse 3T3FL (D56) (Bassin et al., 1971), and Harvey murine sarcoma virus-transformed NRK (H-MSV/NRK) (Levy, 1973) were all described previously. The Abelson virus-transformed, non-producer NIH/3T3 cell line (ANN-1) was obtained from Dr C. D. Scher (Harvard Medical School, Boston, Ma, U.S.A.). Cell cultures were grown in RPMI 1640 medium supplemented with 10% foetal bovine serum and antibiotics.

Viruses. The Abelson virus complex in the form of a 20% Balb/c mouse tumour extract (stock no. PPV2D) was obtained from Dr M. Potter of this laboratory, who prepared it from Balb/c mice injected with Dr L. S. Rabstein's (Microbiological Associates, Bethesda, Md., U.S.A.) original stock at the 3rd passage level. This was injected into newborn Balb/c mice and tumours arising were serially passaged by transplanting minced tumour tissues in syngeneic mice. For the experiments in this report, the tumours were used at the 6th to 8th passages in vivo. Another tumour, induced by a stock virus prepared by a further 2 passages of PPV2D was also tested. The dual-tropic viruses, HIX (stock HIX/FEF VPS) and MCF (AKR247 strain), were obtained from Drs P. J. Fischinger and J. W. Hartley respectively (NIH, Bethesda, Md., U.S.A.). The amphotropic virus, 4070A, was obtained from Dr J. W. Hartley. The E-MuLVs (Moloney, Rauscher and AKR viruses) and X-MuLVs (Balb: virus-
Dual-tropic virus from Abelson tumour

2 and NZB-X-MuLV) were previously described (Chang et al., 1981). Various MSV pseudotypes were prepared by co-cultivation of virus-infected FE cells with 81/CCC or S+L- mink cells.

**Preparation of tumors for virus isolation.** A 20% extract of a tumor was prepared by homogenization in balanced salt solution with a glass tissue grinder. A portion of tumour tissue was cut into small pieces (1 mm diam.) which were explanted on Falcon plastic culture dishes (50 mm diam.) some of which also received $3 \times 10^5$ FE or mink lung cells for co-cultivation. These cultures were refed 3 days later. The culture supernatant was collected and filtered at day 6 for virus titration.

**Helper activity test.** Various MSV non-producer cells ($3 \times 10^5$/plate) were either directly infected with HIX or NBX virus ($5 \times 10^4$ infectious units) 1 day after culture in medium containing polybrene (6 µg/ml), or co-cultivated with $5 \times 10^5$ NIH-ME cells, which were infected with HIX or NBX virus and producing $5 \times 10^4$ infectious units per ml culture supernatant. These cultures were passaged once at day 4 and the supernatant collected at day 7 in the case of direct infection, or passaged twice at days 4 and 8, and the supernatant collected at day 10 in the case of co-cultivation. NIH-ME cells were used for titration of MSV by focus formation.

**Virus assays.** Cell cultures were plated in medium containing polybrene (6 µg/ml) and inoculated with virus next day. The cultures were carried up to 21 days with subcultures. Virus infectivity was measured by its ability to induce MuLV gs antigen which is detectable by complement fixation (CF) test using broad reacting rat anti-MSV serum, and by reverse transcriptase (RT) assay of the culture supernatants (Chang et al., 1981). The XC test was also used for E-MuLV.

**Viral interference and neutralization tests.** These were as described by Chang et al. (1981) except that SC-1 and FE cells were used for replication of MuLVs before test for interference with various MSV pseudotypes, and S+L- mink cells were used for neutralization tests. For interference tests, the cells preinfected with MuLV were passaged *in vitro* for 2 to 3 weeks when $10^5$ infectious units were being produced as detected by CF and RT assays. The antisera against X-MuLV, dual-tropic viruses (NBX, HIX and MCF) and amphotropic (4070A) virus were prepared by immunization of NZW rabbits with virus-infected SIRC cells.

**Competitive radioimmunoassays.** The procedure described previously (Chang et al., 1981) was followed. All competing viruses were purified and concentrated by banding twice in a sucrose gradient and were disrupted with Triton X-100 before competitive radioimmunoassay with the purified and $^{125}$I-labelled p12 or gp70, each tested against its own specific antiserum prepared in NZW rabbits. The p12 and gp70 proteins were purified according to the published procedures of Barbacid et al. (1976) and Strand & August (1973).

**RESULTS**

Isolation of MuLVs from Abelson tumours

The 20% extract of Abelson tumour from Balb/c mice was inoculated in various dilutions on NIH-ME, FE and mink lung cells. As shown in Table 1, NIH-ME cells revealed the presence of $10^3$ to $10^6$ infectious units of MuLV, while FE and mink lung cells showed no detectable virus. When the tumour cells were cultured alone, the supernatant fluid also had $10^3$ to $10^6$ infectious units of MuLV as titrated on NIH-ME cells, but none on FE and mink lung cells. However, the supernatants of tumour cell and FE or mink lung cell co-cultivation plates showed the presence of not only $10^5$ to $10^6$ infectious units of MuLV in ME cells, but also $10^3$ to $10^4$ infectious units of MuLV in FE and mink lung cells. These results indicated the presence of E-MuLV and X-MuLV and/or dual-tropic viruses, in addition to the defective
Table 1. Recovery of MuLV from Abelson tumours

<table>
<thead>
<tr>
<th>Source of inoculum</th>
<th>Virus titre* in NIH-ME cells</th>
<th>Virus titre in FE cells</th>
<th>Virus titre in Mink lung cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumour extracts (20%)</td>
<td>$10^5$-$10^6$</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Tumour culture supernatants</td>
<td>$10^5$-$10^6$</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Tumour and FE (or mink lung) cells co-cultivation supernatants</td>
<td>$10^5$-$10^6$</td>
<td>$10^3$-$10^4$</td>
<td>$10^3$-$10^4$</td>
</tr>
</tbody>
</table>

* Titres were expressed as infectious unit/ml as determined by gs antigen production (CF test) or virion-associated RT assay on the infected culture supernatants.

Abelson virus itself in the tumour. The culture supernatant containing a mixture of these viruses was further passaged in FE and NIH-ME cells by a limiting dilution procedure to isolate clones of viruses. By cloning four times in NIH-ME cells, an XC-positive E-MuLV was isolated, which was designated A-E-MuLV. This virus showed NB-tropism as it replicated equally well on NIH-ME (Fv-I'n), Balb/c-ME (Fv-I'nb) and (C3H x C57BL/6)F1-ME (Fv-I'nb) cells but not on FE or other non-murine cells. As the Abelson virus preparation was originally derived from M-MuLV stock, it is quite possible that the isolated A-E-MuLV represents a clone of M-MuLV. This is confirmed by competitive radio-immunoassays (vide infra). The viruses present in the co-cultivation plates were purified first by a limiting dilution procedure (twice) in FE cells followed by cloning in 81/CCC cells. The viruses produced by 30 transformed 81/CCC cell foci randomly picked were further propagated in FE cells before testing for their infectivity in ME and FE cells. Out of these 30 virus clones, 26 replicated in FE but not ME cells. One of these 26 clones was further purified (twice) by a limiting dilution procedure in FE cells. This virus was subsequently shown to be XC-negative and xenotropic, i.e. able to replicate in various heterologous cells, including rat, feline, dog, horse, human, mink, monkey, rabbit and duck, but not mouse cells. This virus was designated A-X-MuLV. The remaining four clones of virus replicated in both FE and NIH-ME cells (i.e. dual-tropic), and one of these viruses was further cloned by limit dilution in FE cells. This virus, designated NBX, was XC-negative and exhibited a host range including both of those of A-E-MuLV and A-X-MuLV. Unlike MCF virus, NBX virus did not cause cytopathic effect on mink lung cell culture. The NB-tropism of NBX virus clearly distinguishes it from the N-tropism of amphotropic and MCF viruses except some strains of the latter that are NB-tropic (Troxler et al., 1977; Vogt, 1979; Kontor & Krueger, 1979). Viruses similar to A-E-MuLV, A-X-MuLV and NBX have been successfully re-isolated from a tumour induced by the Abelson virus complex at a different passage level.

**Helper activity of NBX virus**

Since these host range properties of NBX virus were similar to those reported for HIX virus (Fischinger et al., 1975), a comparative study of these viruses for their helper activity for MSV genome rescue from various animal cells was conducted. MSV non-producer cell lines were directly superinfected with these viruses or co-cultivated with NIH-ME cells replicating these viruses (Table 2). The results of co-cultivation indicated that the helper activity of HIX virus was low in HT-1 (hamster) ($1.0 \log_{10}$ f.f.u./ml) and moderate in 81/CCC (cat), H-MSV/NRK (rat), KHOS (human) and D56 (mouse) cells (2.48 to 3.6 $\log_{10}$ f.f.u./ml), whereas that of NBX virus was high in HT-1, H-MSV/NRK and D56 cells (4.18 to 5.4 $\log_{10}$ f.f.u./ml), moderate in 81/CCC (2.45 $\log_{10}$ f.f.u./ml) and low in KHOS (1.0 $\log_{10}$ f.f.u./ml). The results of direct superinfection showed more subtle differences owing to its lower efficiency of infection and MSV genome rescue. Nevertheless, NBX virus
Table 2. Helper activity by direct infection or co-cultivation methods

<table>
<thead>
<tr>
<th>Animal origin</th>
<th>Non-producers used for MSV rescue</th>
<th>MSV (log_{10} f.f.u./ml)* recovered after superinfection with†</th>
<th>MSV (log_{10} f.f.u./ml)* recovered after co-cultivation with NIH-ME cells‡ carrying</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat</td>
<td>81/CCC</td>
<td>2.08</td>
<td>2.45</td>
</tr>
<tr>
<td>Hamster</td>
<td>HT-1</td>
<td>1.40</td>
<td>5.40</td>
</tr>
<tr>
<td>Rat</td>
<td>H-MSV/NRK</td>
<td>5.08</td>
<td>4.70</td>
</tr>
<tr>
<td>Human</td>
<td>KHOS</td>
<td>0.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Mouse</td>
<td>D56</td>
<td>5.08</td>
<td>4.18</td>
</tr>
</tbody>
</table>

* Titrated on NIH-ME cells (titration in SC-1 and NRK cells gave similar results).
† Multiplicity of infection was 0.17.
‡ These cells were producing $5 \times 10^4$ infectious virus/ml at the time of co-cultivation.

Table 3. Neutralization tests

<table>
<thead>
<tr>
<th>Antiserum against*</th>
<th>Serum dilutions</th>
<th>M-MuLV</th>
<th>A-X-MuLV</th>
<th>NBX</th>
<th>HIX</th>
<th>MCF</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-MuLV</td>
<td>320</td>
<td>&lt;0.001†</td>
<td>1.0</td>
<td>0.02</td>
<td>0.25</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>640</td>
<td>1.0</td>
<td>0.20</td>
<td>0.90</td>
<td>1.00</td>
<td>1.0</td>
</tr>
<tr>
<td>Balb: virus-2</td>
<td>320</td>
<td>1.0</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>160</td>
<td>1.0</td>
<td>0.20</td>
<td>0.05</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>NBX</td>
<td>160</td>
<td>1.0</td>
<td>0.01</td>
<td>&lt;0.001</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>320</td>
<td>1.0</td>
<td>0.20</td>
<td>0.06</td>
<td>0.68</td>
<td>1.0</td>
</tr>
<tr>
<td>HIX</td>
<td>80</td>
<td>1.0</td>
<td>0.14</td>
<td>0.006</td>
<td>0.05</td>
<td>1.0</td>
</tr>
<tr>
<td>MCF</td>
<td>160</td>
<td>1.0</td>
<td>0.30</td>
<td>0.25</td>
<td>0.80</td>
<td>1.0</td>
</tr>
</tbody>
</table>

* All sera, except anti-M-MuLV serum, were derived from rabbits immunized with infected SIRC cells. The anti-M-MuLV serum was from Fischer rat carrying MSV(M-MuLV)-induced tumour.
† Efficiency of MSV focus induction relative to that of mock-treated control.

showed a marked helper activity in H-MSV/NRK and D56 cells, whereas HIX virus showed greatly reduced efficiencies in all except 81/CCC cells.

It should be added that the NBX virus could act as a helper for recovering the defective Abelson virus from non-producer ANN-1 cells. The recovered pseudotype Abelson-MuLV(NBX) could transform NIH/3T3 cells (data not shown).

Viral interference patterns

The NBX, HIX and MCF viruses showed a complete reciprocal interference pattern, indicating that these belong to the class of dual-tropic MuLV, whereas the ecotropic A-E-MuLV and amphotropic 4070A viruses, each comprised a separate entity. The A-X-MuLV replicating in FE cells interfered with the replication of NBX, HIX and MCF virus, but the NBX virus replicating in FE cells could only effect a partial interference with the replication of A-X-MuLV. Such a one-way interference was also observed in the interference relationship between E-MuLV and dual-tropic viruses. For example, A-E-MuLV replicating in SC-1 cells interfered efficiently with NBX and MCF viruses (but not HIX virus), and the dual-tropic viruses replicating in SC-1 cells interfered with M-MuLV only partially or none at all (data not shown).

Neutralization tests

The results of neutralization tests are summarized in Table 3. It is apparent that NBX, HIX and MCF viruses share cross-reacting envelope antigens as manifested by extensive
cross-neutralizations. It should be pointed out, however, that MCF virus was slightly different from NBX and HIX viruses in its neutralization pattern. Although the anti-M-MuLV serum neutralized NBX and HIX viruses (indices: 0.02 and 0.25 respectively) it did not neutralize MCF virus. Conversely, the antisera against NBX, HIX or MCF viruses did not neutralize M-MuLV. The antisera against the xenotropic Balb: virus-2 did neutralize NBX, HIX and MCF viruses as well as A-X-MuLV, whereas the antisera against NBX, HIX and MCF viruses did not neutralize A-X-MuLV.

These results suggest that the envelope antigens (responsible for neutralization reaction) of NBX and HIX viruses were partially derived from X-MuLV and M-MuLV, whereas those of MCF virus were from X-MuLV and an antigen from an endogenous (AKR-type) E-MuLV. As reported by others (Levy et al., 1975; Fischinger et al., 1976), a serum pool from old NZB mice also exhibited neutralization activities (titres 80 to 160) against A-X-MuLV and NBX as well as HIX and MCF viruses (data not shown). No neutralizing activity against M-MuLV was detected with this serum. These facts indicate again the presence in dual-tropic viruses of an envelope antigenic determinant specific to X-MuLV. Rabbit anti-AT124-X-MuLV and anti-NZB-X-MuLV sera also neutralized efficiently A-X-MuLV and NBX virus, but not at all the A-E-MuLV (data not shown).

**Competitive radioimmunoassays**

In order to analyse further the recombinant properties of NBX, competitive radio-immunoassays were carried out using type-specific p12 antisera and antisera of gp70 derived from E-MuLVs and X-MuLVs (Fig. 1). The A-E-MuLV showed a pattern of competitive inhibition similar to that of M-MuLV both in p12 and gp70 reactions, indicating its identity with M-MuLV.

The A-X-MuLV (at 100 µg dose level) competed with Balb: virus-2 and M-MuLV p12s (30% and 70% inhibition respectively), but scarcely with p12s of Rauscher (R)-MuLV, AKR-MuLV or NZB-X-MuLV (the 30% inhibition was considered significant since the standard error was less than 5% and the results were reproducible). This would suggest the possibility of occurrence of a cross-over within the **gag** gene (p12 region) of M-MuLV and Balb: virus-2. The group-specific envelope antigen present in A-X-MuLV showed cross-reactive competitive inhibition against gp70 of M-MuLV (72%), R-MuLV (62%), AKR-MuLV (70%) and Balb: virus-2 (55%) but not against that of NZB-X-MuLV.

The NBX virus (at 100 µg dose level) showed competitive inhibition against p12s of M-MuLV (80%) and Balb: virus-2 (60%), but less efficiently against those of R-MuLV (22%), AKR-MuLV (15%) and NZB-X-MuLV (15%). This would again indicate, like the case of A-X-MuLV, a possible recombinational event occurring within the p12 **gag** gene of M-MuLV and Balb: virus-2. Therefore, the gene coding for NBX p12 may have been derived from that of A-X-MuLV. The NBX virus competed with an efficiency of 60% inhibition against both M-MuLV and Balb: virus-2 gp70s, suggesting that its **env** gene was partially derived from both of these viruses. Much less inhibition was observed when NBX virus was tested against gp70s of R-MuLV and AKR-MuLV. The finding that the slopes of curves exhibited by NBX virus was much less than those of A-X-MuLV in E-MuLV gp70 assays would suggest that the NBX virus has a unique gp70 antigenic determinant, in addition to its group-specific determinant which was most closely related to those of M-MuLV and Balb: virus-2. Therefore, the NBX virus p12 gene may have been derived from A-X-MuLV and the gp70 gene from both M-MuLV and a virus similar to Balb: virus-2 or A-X-MuLV, but the origin of the gene responsible for encoding the unique gp70 antigenic determinant is not known.
Fig. 1. Competitive radioimmunoassays. Homologous anti-p12 serum versus $^{125}$I-labelled p12 of (a) M-MuLV; (b) R-MuLV; (c) AKR-MuLV; (d) Balb: virus 2; (e) NZB-X-MuLV. Homologous anti-gp70 serum versus $^{125}$I-labelled gp70 of (f) M-MuLV; (g) R-MuLV; (h) AKR-MuLV; (i) Balb: virus 2; (j) NZB-X-MuLV. Competing viruses (Triton X-100-disrupted) were: □, M-MuLV; ○, A-E-MuLV; ◦, R-MuLV; △, A-X-MuLV; ■, NBX; ●, AKR-MuLV; ◆, Balb: virus 2; ▲, NZB-X-MuLV.
Oncogenicity test

The NBX virus was injected into 30 newborn Balb-c mice by intraperitoneal and subcutaneous routes (10^3 to 10^4 infectious units per mouse). During an observation period of more than 12 months, no tumour was produced in these mice.

DISCUSSION

The results presented here show that the tumour induced by Abelson virus complex in Balb/c mice contains at least three classes of retrovirus: an ecotropic A-E-MuLV, a xenotropic A-X-MuLV and a dual-tropic NBX virus. The requirement of co-cultivation of tumour cells with FE or mink lung cells for the isolation of A-X-MuLV and NBX virus suggests that cell-free tumour extracts or tumour cell culture supernatants may contain these viruses in the form of A-X-MuLV(A-E-MuLV) or NBX(A-E-MuLV) pseudotypes, which would be non-infectious on FE or mink lung cells. However, infection of FE or mink lung cells through cell fusion with tumour cells would bypass this form of host range restriction. Such a genomic masking phenomenon has also been reported for HIX and MCF viruses (Fischinger et al., 1978b; Vogt, 1979; Haas & Patch, 1980). Furthermore, as preinfection of cells with A-E-MuLV interfered with the replication of NBX virus (but not vice versa), it is possible that horizontal in vivo spread of NBX virus among tissues would be restricted if these cells were preinfected with A-E-MuLV which comprised the majority of virus population in the inoculum and tumour tissues. Although the infectious virus titre in the tumour-FE (or mink lung) cell co-cultivation supernatant was 10^3 to 10^4 when titrated on FE cells, further passage in FE cells by a limiting dilution procedure, which eliminated phenotypically mixed A-E-MuLV(A-X-MuLV) and A-E-MuLV(NBX), followed by cloning by single focus isolation from infected 81/CCC cells, revealed that the NBX virus comprised only 13% (4/30), while A-X-MuLV accounted for 87% (26/30) of the partially purified population. Considering that the A-E-MuLV in the co-cultivation supernatant was at least 100-fold higher in titre, it may be deduced that the A-X-MuLV and NBX subpopulations appear to be a minority of less than 0.87% and 0.13% respectively, of viruses produced in the co-cultivation supernatant.

Besides the various heterologous cells in which the A-X-MuLV and NBX virus can replicate, NBX virus appears to be able, at the same m.o.i., to infect hamster (HT-1) cells and rescue the MSV genome more efficiently than does the HIX virus. By contrast, HIX virus was more efficient than NBX virus in its ability to replicate in human KHOS cells and rescue the MSV genome. A dual-tropic virus (S/A-1), which is also NB-tropic and able to replicate in hamster cells, has been described (Kontor & Krueger, 1979). The molecular basis of this biological marker deserves further investigation. The results of the interference test for HIX, MCF and amphotropic viruses were consistent with previous reports (Fischinger et al., 1975, 1978a, b; Hartley et al., 1977; Hartley & Rowe, 1976).

The significance of the one-way interference phenomena observed is not clear at present but it may be related to the recombinant nature of dual-tropic viruses, causing quantitative (and possibly qualitative) differences in the expression of various env gene products on the infected cells, since dual-tropic viruses (as compared with E-MuLV or X-MuLV) would be expected to express smaller amounts of E-MuLV- or X-MuLV-specific env antigens, resulting in less efficient interference against E-MuLV or X-MuLV. This is corroborated by the findings of neutralization tests where it was shown that the antisera against the ecotropic M-MuLV and xenotropic Balb: virus-2 could neutralize dual-tropic viruses (except that MCF virus was not neutralized by anti-M-MuLV, owing to its AKR origin), but the antisera against dual-tropic viruses could not neutralize M-MuLV or A-X-MuLV (Table 3). Cross-reactions among these viruses could be shown only when higher concentrations of antisera were used.
Dual-tropic virus from Abelson tumour

The results of competitive radioimmunoassays with type-specific p12 antisera suggested a possible recombinant origin of the \textit{gag} gene of A-X-MuLV, i.e. the A-X-MuLV emerged as a result of cross-over between M-MuLV and Balb: virus-2-like X-MuLV within their \textit{gag} genes coding for p12. By contrast the \textit{gag} gene of a strain of X-MuLV was reported to be entirely of endogenous E-MuLV origin (Ihle \textit{et al.}, 1978). The NBX virus \textit{env} gene coding for gp70 was most probably derived from those of M-MuLV and an endogenous Balb: virus-2-like X-MuLV, as a result of a second recombinational event between M-MuLV and A-X-MuLV within the \textit{env} gene. Furthermore, the presence of a unique gp70 antigenic determinant, the origin of which is unknown, was deduced from the finding that, markedly less degrees of competitive inhibition against E-MuLV gp70s were observed for NBX virus as compared with A-X-MuLV. A similar, unique gp70 antigen has also been described for HIX (Fischinger \textit{et al.}, 1978\textit{a}), MCF (Devare \textit{et al.}, 1978; Cloyd \textit{et al.}, 1979) and the spleen focus-forming virus (SFFV) of Friend virus complex (Ruscetti \textit{et al.}, 1978, 1979). The identity or relationship of these unique antigenic determinants needs further investigation. It is possible that, as a result of recombination within the \textit{env} genes, a portion of the gp70 antigenic determinants (epitope) was modified by substitution of amino acids, or by conformational changes in polypeptide chains. Further investigations with monoclonal antibodies and purified subviral components of NBX would be required to elucidate these questions.

The type-specific p12 of HIX and MCF viruses was reported to be exclusively derived from that of M-MuLV and AKR-MuLV respectively, except that two MCF strains (Z-6 and Mo-MCF) derived \textit{gag} gene from M-MuLV-like virus (Devare \textit{et al.}, 1978; Vogt, 1979). By contrast, our present data with NBX virus suggest that its gene for p12 may have been derived from A-X-MuLV, which is probably a recombinant itself, deriving its p12 gene from both Balb: virus-2-like virus and M-MuLV.

Unlike the HIX virus, the NBX virus did not show oncogenicity when injected into neonatal mice. As there are variations in the oncogenic potential among different strains of MCF (Cloyd \textit{et al.}, 1980), it would be necessary to define not only the oncogenicity of NBX- or HIX-like dual-tropic virus from various sources, but also the role played by E-MuLV and X-MuLV existing as phenotypic mixtures, because (i) the process of generation of dual-tropic virus, rather than the presence of the latter itself in the cell, may be important for leukaemogenesis; (ii) the genomically masked HIX virus, represented as a HIX(M-MuLV) pseudotype has a better chance of survival than HIX virus in mice, which have an inhibitory serum factor (Fischinger \textit{et al.}, 1978\textit{b}) and (iii) the type of helper virus for Abelson defective virus would determine the different levels of oncogenic potential of Abelson virus pseudotypes (Scher, 1978; Rosenberg & Baltimore, 1978).

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\textsc{REFERENCES}


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