Oncogenic Retrovirus from Spontaneous Murine Osteomas. I. Isolation and Biological Characterization

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SUMMARY

Spontaneous osteomas in strain 101 mice, a strain which has a high incidence of benign bone tumours, harbour numerous C-type virus-like particles with pleomorphic characteristics. A cell-free extract from osteomas from two mice induced bone tumours, together with osteopetrosis and lymphomas, in newborn mice of the low incidence NMRI strain after a latent period of 12 to 15 months. When C3H embryo fibroblasts were infected with the osteoma extract, the resulting cell line produced virus (OA MuLVc) with a high titre. OA MuLVc was cloned by serial endpoint dilution and NIH 3T3 cells were productively infected. The resulting virus was named OA MuLVN. OA MuLVc and OA MuLVN also induced bone tumours, osteopetrosis and lymphomas 12 to 15 months after injection into newborn NMRI mice. The isolated virus showed typical characteristics of the murine retrovirus group. Fv-1 host range restriction assays classified the viruses as N-ecotropic and XC-positive. Tryptic p30 peptide analysis and RNase T1 fingerprint analysis of OA MuLVc and OA MuLVN indicated that OA MuLVc contains an Akv-like virus as well as additional components, whereas OA MuLVN is closely related to Akv, but not identical to it. Serological analysis of the envelope proteins using monoclonal antibodies also showed the virus to be similar, but not identical, to Akv virus.

INTRODUCTION

A number of strains of mice have been observed to have a high incidence of spontaneous osteomas, for example AKR mice with prolonged lifespan after thymectomy (63% in females; Furth, 1946) and CF-1 mice (41% in females; Vaughan, 1973). The most frequent location of osteomas in CF-1 mice is the skull (Charles & Turusov, 1974) whereas the most common sites in AKR mice are the femoral and iliac bones (Furth, 1946). We have observed spontaneous osteomas in 70% of old female strain 101 mice (unpublished results). The preferential location of these osteomas was similar to that in AKR mice, in the os ileum and the diaphysis of the long bones. Finkel and co-workers (Finkel et al., 1973; Finkel & Reilly, 1973) isolated the osteoma-inducing RFB virus from osteomas of the CF-1 mouse but did not further characterize this virus. We have isolated an oncogenic retrovirus from spontaneous osteomas in strain 101 mice. The biological and biochemical characteristics of this virus are described here.

METHODS

Mice. Mice of the inbred 101 strain and random-bred NMRI strain were obtained from the breeding colony of the Institut für Biologie, GSF. The specific pathogen-free mice (Erfle & Neumann, 1980) were kept under an arti-
ficial day/night regimen and given water and pellets ad libitum. The recipient NMRI strain has a spontaneous osteoma and osteopetrosis incidence of less than 1%.

Cells and medium. C3H and BALB/3T3 mouse embryo cells were a gift from Dr R. H. Bassin, NCI, Bethesda, Md., U.S.A., mink CCL 64 cells were obtained from the American Type Culture Collection, XC rat cells and NIH 3T3 cells were a gift from Dr R. C. Gallo, NCI, Bethesda, Md., U.S.A. The cell lines were grown in Dulbecco's MEM with 10% heat-inactivated (56 °C, 30 min) foetal calf serum (Gibco) in a humidified atmosphere with 5% CO₂. Subconfluent cultures were passaged twice weekly. Mycoplasma contamination was routinely checked applying the method described by Chen (1977).

Antisera and viruses. A panel of eight monoclonal antibodies, specific for murine leukaemia envelope proteins gp70 and pl5E epitopes, were purchased from New England Nuclear. The specificity of these antibodies has been described (O'Donnell & Nowinski, 1980; Lostrom et al., 1979). AKR murine leukaemia virus (MuLV) (Akv) (Rowe et al., 1971) was purchased from Electro-Nucleonics, Bethesda, Md., U.S.A.

Preparation of cell-free osteoma extract. Parts of the lower lumbar vertebral column, pelvis and hind legs of two adult female 101 mice which had spontaneous osteomas were homogenized in a mortar in 20 ml of phosphate-buffered saline (PBS) pH 7.2. The bone suspensions were clarified by centrifugation. The supernatants were filtered through a 0.22 μm Millipore filter and designated osteoma extract. Integrity of the filters was controlled by filtration of an Escherichia coli suspension onto sheep blood agar plates.

Virus preparation. C3H mouse embryo fibroblast cells were infected with osteoma extract after treatment with DEAE-dextran (25 μg/ml) (Duc-Nguyen, 1968) for 30 min. The supernatants of the virus-producing cell line, hereafter named OA MuLVc, were centrifuged through 20% sucrose at 100000 g for 1.5 h (SW27, 4 °C). The virus pellets were suspended in 200 μl TN (0-01 M-Tris–HCl pH 7-4, 0-15 m-NaCl, 3 mM-EDTA), layered on a 20 to 50% (w/w) sucrose gradient and centrifuged overnight at 200000 g (SW41, 4 °C). The gradient fractions containing 36 to 40% sucrose were collected and centrifuged at 150000 g for 1.5 h (SW41, 4 °C). OA MuLVc was cloned by three cycles of serial endpoint dilution using NIH 3T3 cells. Supernatants of the resulting cell line were concentrated as described and named OA MuLVN. The virus pellets were resuspended in 0-01 M-Tris–HCl pH 7-4, 0-15 m-NaCl and served as virus stock for further assays.

Oncogenicity assay. Newborn female mice were used for oncogenicity tests. Fifteen mice of the NMRI strain were injected intraperitoneally with the original cell-free osteoma extract. Twenty-four NMRI mice were given concentrated, sucrose gradient-purified OA MuLVc (cell culture supernatant). Ten NMRI mice were injected with cell-free supernatant from the NIH 3T3 cell line which had been productively infected with OA MuLVc. The data from control mice in previous and parallel experiments were included with data from mock-treated control animals for the evaluation of the oncogenicity assays. Mock-treated animals had been injected with TN buffer.

All animals were checked on 6 days of the week, and the observation period lasted at least 400 days. Moribund animals were killed. All dead and killed animals were autopsied. Each carcass was examined by X-ray.

The following criteria had to be fulfilled for the diagnosis malignant lymphoma to be entered in the statistics. These were either enlargement of one of the following organs to the stated minimum dimensions: peripheral lymph nodes, 10 mm; thymus, 10 mm; mesenterial lymph node, 20 × 5 mm; spleen, 30 mm; left lobe of liver, 30 mm, or abundant effusions in the thoracic or abdominal cavity, plus in all cases histological confirmation of the diagnosis. The lesions were classified according to Dunn (1954).

The criteria for the diagnosis osteoma were: tumour with a diameter of at least 1 mm in the X-ray photograph, plus histological confirmation in cases of doubt. Similarly the diagnosis osteopetrosis was only included in the statistics when the lesions were clearly detectable in the X-ray photograph. The major sites for osteopetrosis were the lower vertebrae, femurs and the os ilium.

Host range studies. NIH 3T3, BALB/3T3 (4 × 10⁴ cells/well) and mink CCL64 cells (8 × 10⁴ cells/well) were grown in cluster plates (Costar, 4 × 6 wells). Twenty-four h later the cells were treated with DEAE-dextran for 30 min and then infected with 0-2 ml aliquots of serial tenfold dilutions of the osteoma extract, OA MuLVc or OA MuLVN, respectively. Thirty min after infection, 0-8 ml of complete medium containing 4 μg/ml Polybrene was added. Twenty-four h later the cells were assayed for the production of MuLV group-specific antigen (p30) applying the indirect immunoperoxidase method according to Nexo (1977), and 3 to 4 days later for syncytium formation by the reverse XC plaque assay (Rowe et al., 1970).

Electron microscopy. OA MuLVc-producing cell cultures and samples of spontaneous and OA MuLVc-induced osteomas were fixed in cacodylate-buffered 3% glutaraldehyde, followed by post-fixation in chrome-osmium. After dehydration the specimens were embedded in Epon 812. Thin sections were cut with a diamond knife on a Reichert Om U3 ultramicrotome, stained with uranyl acetate and lead citrate, and examined in a Zeiss EM 10 CR electron microscope.

Biochemical studies. Sedimentation analysis of [³H]uridine-labelled OA MuLVc RNA was performed as described previously (Erfle et al., 1979). 70S RNA of Akv was used as a marker. Reverse transcriptase (RT) activity was determined as described previously (Erfle et al., 1978) in concentrated fractions of a 20 to 50% linear sucrose gradient of OA MuLVc (Goodman & Spiegelman, 1971). The reaction was stopped after 90 min
incubation with 1 ml of 10% TCA. The template-primer specificity was studied in 100-fold concentrated OA MuLVc using 10 μg/ml of poly(A)n, (dT)12-18, poly(dA)n, (dT)12-18, poly(C)n, (dG)12-18 or poly(Cm)n, (dG)12-18 (Boehringer, Mannheim) and 10 μCi of the appropriate labelled deoxyribonucleotide triphosphates [3H]dTTP (50 Ci/mmol) or [3H]dGTP (16 Ci/mmol) from New England Nuclear (Baltimore & Smoler, 1971). Divalent cation preference was assayed using various concentrations of Mn²⁺ and Mg²⁺ (Scolnick et al., 1970; Baltimore & Smoler, 1971).

**SDS PAGE.** Virus proteins were examined on polyacrylamide gels using OA MuLVc labelled with [³H]glucosamine hydrochloride (30 Ci/mmol) and [³H]leucine (110 Ci/mmol) (Amersham) added to the culture medium (Ihle et al., 1973; Oroszlan et al., 1971).

Sucrose density gradient-purified OA MuLVc was subjected to SDS-PAGE in 15% polyacrylamide according to Laemmli (1970). A set of eight marker polypeptides (molecular weights: 12600, 14300, 21500, 30000, 37000, 43000, 55000, 68000) was run in parallel (Weber & Osborn, 1969). The gel was subjected to pre-electrophoresis at 60 V, then to electrophoresis at 200 V. Individual gel lanes were cut with a razor blade grid and the slices eluted in 0-5% SDS overnight. Radioactivity of the slices was counted in a Kontron β-counter.

**Tryptic peptide analysis.** Peptide mapping of the viral p30 proteins from OA MuLVc and Akv was carried out after 1³S-iolabelling according to Greenwood et al. (1963) and carboxymethylation of the viral proteins (Waxdal et al., 1968). The separation of tryptic peptides was carried out on cellulose-coated thin-layer plates by electrophoresis at pH 1-9 in 2 M-acetic acid and 0-6 M-formic acid (20 V/cm, 0 °C, 1-5 to 2 h) and subsequent chromatography in n-butanol:acetic acid:water:pyridine (16:2.5:10:12.5) as described by Bates et al. (1975).

**Serological analysis.** ELISAs were carried out for the serological analysis of antigenic determinants of the env gene products of OA MuLVc and OA MuLVx using the following eight monoclonal antibodies: anti-gp70 a e and anti-pl5E a-c (Stone & Nowinski, 1980). Microtitre plates (Dynatech, Plochingen, F.R.G.) were coated (200 μl/well) with OA MuLVc, OA MuLVx or Akv each containing 2 μg/ml in 50 mM-carbonate buffer pH 9.6 for 2 h at 37 °C. After washing of the plates with PBS, 200 μl of serial antibody dilutions in PBS with 2% bovine serum albumin (BSA) were added, starting with 1 ng IgG/ml. The plates were washed again with PBS, and 200 μl of horseradish peroxidase-labelled rabbit anti-mouse IgG (10 μg/ml) was added to each well and incubated for 2 h at 37 °C. After washing with PBS, the colour development was initiated by adding 200 μl substrate (0.4 mg O-phenylenediamine hydrochloride, 0.005% H₂O₂ in 7.74 g/l citric acid and 17.93 g/l Na₂HPO₄, pH 6.0) and the reaction was followed photometrically at 15, 30, 45 and 60 min by reading at 450 nm in a Titertek Multiskan (Flow Laboratories). Backgrounds, generally ranging from 0.02 to 0.1 A₅₅₀, were subtracted from the test readings. The data obtained at 45 min were used for the evaluation.

**RNase T₁ fingerprints.** The analysis of RNase T₁-resistant OA MuLVc and OA MuLVx oligonucleotides by two-dimensional gel electrophoresis was carried out as described in detail previously (Pedersen & Haseltine, 1980; Pedersen et al., 1980, 1981).

### RESULTS

**Pathological findings in the original osteoma-bearing strain 101 mice**

The two female 101 mice bearing spontaneous osteomas showed the following pathological findings. Mouse no. 1 was 636 days old, and in the X-ray photograph had osteomas at the right femur, diaphysis, and at the left os ilium. Bone histology (lower thoracic and upper lumbar vertebrae) showed focal thickening of trabeculae, focal osteonecrosis and one focus of fibrous-osseous lesion. Other findings were malignant lymphoma (Dunn Type B) and trabecular carcinoma of the liver. Mouse no. 2 was 638 days old, and in the X-ray photograph had two osteomas at the right os ilium and three small osteomas of the ribs. Bone histology (lumbar vertebrae) showed multifocal thickening of trabeculae and focal osteonecrosis. Other findings were multifocal fibrous-osseous lesions and neoplastic nodules of the liver.

The cell-free osteoma extract used for the inoculation of newborn NMRI mice and the infection of C3H mouse embryo fibroblasts was prepared from the pelvis and bones of the lower legs of both mice and the lower lumbar vertebrae of mouse no. 1.

**Oncogenicity assay**

The results are summarized in Table 1. Osteomas, osteopetrosis and lymphomas were induced after injection into newborn NMRI mice of (i) the cell-free extract prepared from the osteoma-containing skeletal parts of the strain 101 mice, (ii) the cell culture supernatant from C3H
Fig. 1. X-ray photograph of OA MuLV-infected and control NMRI mice. (a) OA MuLV<sub>c</sub>-induced osteoma of the left scapula (336-day-old mouse), (b) OA MuLV<sub>c</sub>-induced osteopetrosis of the lower vertebral column and femurs (344-day-old mouse), (c) control mouse (557 days old).

fibroblasts infected with the extract (OA MuLV<sub>c</sub>), and (iii) the biologically cloned OA MuLV<sub>N</sub>. The cell-free extracts, OA MuLV<sub>c</sub> and OA MuLV<sub>N</sub> induced osteomas in 27% (4/15), 18% (4/22) and 20% (2/10), respectively, of the infected mice (Fig. 1a). Osteopetrosis was seen in 73% (11/15), 64% (14/22) and 60% (6/10), respectively, of the infected mice and developed preferentially in the femurs, the os ileum and the lower part of the vertebral column (Fig. 1b). In addition, focal osteonecrosis was diagnosed in the animals that developed osteopetrosis. Malignant lymphomas (lymphoblastic type, Dunn type B or type A) were observed in 67% (10/15), 86% (19/22) and 60% (6/10) of mice, respectively. Three different control experiments were carried out including a total of 158 mice. The only lesions observed in these control animals during the period of observation (441 to 532 days) were the 8% (4/50) of lymphomas which developed in mice in experiment 2. No osteomas or osteopetrosis were seen in the control mice (Table 1). For comparison, an X-ray photograph of 557-day-old NMRI control mouse is shown in Fig. 1(c).

*Host range studies*

The infectivity of OA MuLV<sub>c</sub> and OA MuLV<sub>N</sub> was examined using NIH 3T3 cells carrying the <i>Fv-1</i><sup>n</sup><sup>°</sup> genotype, BALB/3T3 cells carrying the <i>Fv-1</i><sup>b</sup><sup>°</sup> genotype and mink CCL 64 cells, by the determination of p30 antigen production and XC syncytial plaque formation. As shown in Table 2 the titre of the osteoma extract was about 150-fold higher in NIH 3T3 cells as compared to BALB/3T3 cells. OA MuLV<sub>c</sub> was also infectious for mink cells, but only at a low titre. The titres obtained by the XC plaque assays were one to two orders of magnitude lower than the titres obtained by the method of p30 determination. Mink cells infected with OA MuLV<sub>c</sub> did not show foci at the concentration of virus used. Furthermore, the mink lung cells infected with OA MuLV<sub>c</sub> were negative for p30 expression when assayed after two or three additional passages.

*Electron microscopy*

Ultrastructural examination of the tissues of a spontaneous osteoma (Fig. 2) and of an osteoma induced by the original cell-free osteoma extract showed the presence of numerous budding, immature and mature C-type-like virus particles. Budding of virus particles was observed from cell membranes mainly from osteocytes and only occasionally from osteoblasts. The
Table 1. Oncogenicity of osteoma-derived MuLV in NMRI mice

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Inoculum</th>
<th>Number of animals</th>
<th>Observation period (days)</th>
<th>Lymphoma incidence</th>
<th>Latent period (days)</th>
<th>Animals with osteoma</th>
<th>Mean age (days)</th>
<th>Osteoma*</th>
<th>Animals with OP</th>
<th>Mean age (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Osteoma extract</td>
<td>15</td>
<td>441</td>
<td>67% (10/15)</td>
<td>350 ± 74</td>
<td>27% (4/15)</td>
<td>381 ± 45</td>
<td></td>
<td>73% (11/15)</td>
<td>356 ± 45</td>
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<tr>
<td></td>
<td>Control†</td>
<td>100</td>
<td>441</td>
<td>0% (0/100)</td>
<td>-</td>
<td>0% (0/100)</td>
<td>-</td>
<td></td>
<td>0% (0/100)</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>OA MuLV&lt;sub&gt;c&lt;/sub&gt;</td>
<td>22</td>
<td>532</td>
<td>86% (19/22)</td>
<td>347 ± 77</td>
<td>18% (4/22)</td>
<td>389 ± 33</td>
<td></td>
<td>64% (14/22)</td>
<td>393 ± 75</td>
</tr>
<tr>
<td></td>
<td>Control‡</td>
<td>8</td>
<td>532</td>
<td>0% (0/8)</td>
<td>-</td>
<td>0% (0/8)</td>
<td>-</td>
<td></td>
<td>0% (0/8)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Control§</td>
<td>50</td>
<td>532</td>
<td>8% (4/50)</td>
<td>365 ± 157</td>
<td>0% (0/50)</td>
<td>-</td>
<td></td>
<td>0% (0/50)</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>OA MuLV&lt;sub&gt;N&lt;/sub&gt;</td>
<td>10</td>
<td>447</td>
<td>60% (6/10)</td>
<td>404 ± 64</td>
<td>20% (2/10)</td>
<td>389 ± 53</td>
<td></td>
<td>60% (6/10)</td>
<td>404 ± 64</td>
</tr>
</tbody>
</table>

* Single or multiple osteomas; diameter of tumour at least 1 mm.
† Pooled data of two experiments carried out previously and in parallel respectively.
‡ Mock-treated animals were injected with the equivalent amount of TN buffer.
§ Data of an experiment carried out in parallel.
pericellular space of osteocyte-containing lacunae was often filled with numerous immature and mature C-type-like particles. The morphology of the mature virus particles was somewhat different from that of typical C-type particles. They were pleomorphic and possessed a very large...
and not clearly defined dense core which often filled nearly the whole space within the outer envelope of the particles. The entire diameters of the particles varied from approximately 90 to 150 nm.

The morphological characteristics of the virus particles observed in osteomas were also observed in virus particles found in C3H mouse fibroblast cells productively infected with OA MuLV_C.

**Biochemical properties**

The tritiated, RNase-sensitive nucleic acid of OA MuLV_C was found in the 70S region of the sedimentation gradient.

Reverse transcriptase activity was found in a sucrose density gradient of OA MuLV_C at a density of 1.17 g/ml. The enzyme utilized poly(A)_n.(dT)_{12-18}, poly(C)_n.(dG)_{12-18}, poly(Cm)_n.(dG)_{12-18}, and poly(dA)_n.(dT)_{12-18} at a ratio of 80:5:3:1, and Mn^{2+} as divalent cation.

Tritiated OA MuLV_C showed a protein pattern characteristic of murine C-type retroviruses. It showed the presence of p10, p12, p15, p30 and gp70 proteins.

Tryptic fingerprint analysis of OA MuLV_C p30 showed a high degree of homology in comparison to the p30 of Akv, but some of the spots differed in intensity.

**Serological characterization**

Serological characterization of the antigenic determinants of OA MuLV_C and OA MuLV_N envelope proteins was carried out using a panel of eight monoclonal antibodies reacting with five epitopes of the gp70 glycoprotein and with three epitopes of the p15E protein of ecotropic MuLV. Akv was used as reference. Reactivity of all antibodies could be detected with OA MuLV_C, OA MuLV_N and Akv (Fig. 3). OA MuLV_C and OA MuLV_N exhibited the characteristics of N-ecotropic MuLV. The three antibodies to p15E which recognize class-and group-specific antigenic determinants of the p15E protein reacted similarly with OA MuLV_C, OA MuLV_N and Akv. Although p15E epitopes showed lower affinity to the respective antibodies than did OA MuLV_C and OA MuLV_N, the common serological pattern of the three p15E epitopes showed that a close relationship exists between both OA MuLV_C and OA MuLV_N, and the Akv-type class and group. The pattern of reactions of the gp70 epitopes showed considerable differences. In particular, the gp70^b, gp70^c and gp70^e epitopes of OA MuLV_C showed a lower affinity for their respective antibodies than did those of Akv. The pattern of OA MuLV_N gp70 epitopes was similar to those of OA MuLV_C, except gp70^a, which reacted more like that of Akv, and gp70^e which showed a distinctly different reaction.

**RNase T1 fingerprints of OA MuLV_C and OA MuLV_N RNAs**

Fig. 4(a, b) shows the RNase T1 fingerprints of 70S RNAs isolated from OA MuLV_C and OA MuLV_N respectively. The analysis of the two fingerprints showed the same major pattern in both. Whereas the OA MuLV_N pattern was composed of oligonucleotides having a uniform intensity, the OA MuLV_C RNA gave a pattern of non-uniform intensity. The fingerprint of the OA MuLV_C RNA also contained a number of weakly labelled oligonucleotides that are not detected in the OA MuLV_N RNA. This demonstrates that the OA MuLV_C was a heterogeneous stock, out of which the OA MuLV_N was isolated as a single replication-competent virus.

In order to study the relationship of OA MuLV_N to other murine leukaemia viruses, we compared the RNase T1 fingerprint of RNA from this virus with a prototype virus, Akv. The RNase T1 fingerprint of RNA from this virus has been analysed in detail (Pedersen & Haseltine, 1980) and a precise oligonucleotide map was established from the complete provirus nucleotide sequence (Etzerodt et al., 1984). Fig. 4(c) shows a schematic comparison of RNase T1 fingerprints of OA MuLV_N and Akv virus RNAs. This comparison showed that the two virus RNAs are very closely related, sharing the majority of the approximately 60 oligonucleotides resolved by this technique. Two oligonucleotides of unimolar intensity were detected as unique to the OA
DISCUSSION

We describe here the isolation and partial characterization of an osteoma-inducing retrovirus isolated from the skeletons of old osteoma-bearing 101 strain mice. The data obtained support the concept of the viral aetiology of this type of bone tumour which was first recognized by cell-free transmission studies in CF-1 mice (Finkel et al., 1976). CF-1 mice, like 101 mice, show a fairly high natural incidence of osteomas of about 20% (Finkel et al., 1973). The original cell-free osteoma extract induced osteomas, osteopetrosis and malignant lymphomas in NMRI mice. The same neoplasms were induced by injection of OA MuLVc which was harvested from C3H mouse embryo fibroblasts productively infected with the osteoma extract, and by OA MuLVN virus derived from the OA MuLVc virus stock by serial endpoint dilution in NIH 3T3 cells.

The relationship between virus-induced neoplasms in lymphoreticular tissue and bone malformations has been observed in other studies, for example the induction of myelofibrosis...
Fig. 4. RNase T₁ fingerprint analysis of viral RNA of (a) 70S RNA of OA MuLV<sub>c</sub>, (b) 70S RNA of OA MuLV<sub>N</sub>. (c) Schematic comparison of the RNase T₁ fingerprint of OA MuLV<sub>N</sub> with the RNase T₁ fingerprint of Akv (Pedersen & Haseltine, 1980); open circles represent oligonucleotides shared with the genome of Akv virus, solid circles represent oligonucleotides not present in the Akv virus genome, and cross-hatched circles represent Akv oligonucleotides missing in the OA MuLV<sub>N</sub> RNA.

and new bone formation after infection of rats with Rauscher leukaemia virus (van Gorp & Swaen, 1969) and the induction of osteopetrosis together with nephroblastoma and lymphoid leukosis in chickens with a myeloblastosis-associated virus [MAV-2(O)] (Smith & Moscovici, 1969). The induction of osteopetrosis together with reticular cell neoplasms (type B) by a virus isolated from sarcoma 37 in BALB/c mice has also been reported (Merwin & Redmon, 1969). Osteoma associated with malignant lymphoma has been described to occur spontaneously in CF-1 mice (Breslow et al., 1974) and also in thymectomized AKR mice (Furth, 1946). In the strain 101 mice both tumours can be found together, but the frequency of spontaneous osteoma is much higher (74%) than the frequency of malignant lymphoma (40%).

These findings raise the question whether the different diseases are caused by one virus type infecting a common target cell (Amsel & Dell, 1972; Loutit & Sansom, 1976) or different target cells (Loutit et al., 1982), or whether they are induced by different virus types. Attempts to separate the lymphoma-inducing activity from that which induced skeletal changes in the sarcoma 37 agent and the MAV-2(O) isolates were not successful (Merwin & Redmon, 1969; Smith & Morgan, 1982). This indicates that a single virus type induced both the skeletal changes and the tumours of the lymphoreticular system (Holmes, 1964). A similar situation was observed in the
experiments described here. OA MuLV_N was biologically cloned from the OA MuLV_C virus stock, but still induced both bone tumours and malignant lymphomas in NMRI mice. The results obtained in the OA MuLV_N oncogenicity experiments gave two further indications that one virus type may be the causative agent of different types of tumours: (i) osteomas in the OA MuLV_N-infected NMRI mice were only detected in lymphomatous moribund animals which were killed before the end of the observation period and (ii) osteomas were only found in animals which also developed severe osteopetrosis. The observation, however, that bone tumour incidences were constantly lower than lymphoma incidences in all the experiments in vivo may be explained by the slower development of bone neoplasms in comparison with lymphoreticular tumours. In contrast to OA MuLV_N, the OA MuLV_C seems to be a complex of more than one virus type. This was first indicated by electron microscopy studies (Marquart, 1979) which showed the presence of typical C-type particles together with C-type-like pleomorphic virus particles resembling the RFB osteoma virus particles described by Finkel et al. (1973).

Host range studies suggest that OA MuLV_C represents a virus stock having OA MuLV_N as the major component. The latter is N-ecotropic and XC-positive and it most likely determined the results obtained with OA MuLV_C in the host range experiments and in the oncogenicity assays. A second virus type which was infectious for mink cells was also detected in the cell culture-grown OA MuLV_C stock. This xenotropic component, however, was not detected in the original osteoma extract, possibly due to abortive infections in the first passage in vitro or because of low levels of virus. Its induction could also be a consequence of infection of the C3H mouse cells with the osteoma extract (Rapp, 1983).

The antigenic reaction of the p15E and gp70 epitopes (Stone & Nowinski, 1980) of OA MuLV_C and OA MuLV_N were compared with that of the non-oncogenic Akv. Whereas the reaction of the group- and class-specific p15E epitopes was similar to that of Akv, the type-specific gp70 epitopes of OA MuLV_N virus revealed a characteristic pattern of reaction distinct from that of Akv, indicating mutations in two gp70 epitope gene regions. The RNase T1 fingerprint analysis of OA MuLV_C RNA showed a non-homogeneous pattern, indicating that this cell line produces a mixture of virus particles. By three rounds of infection at endpoint dilution, OA MuLV_N was isolated from this stock. This cloned virus clearly retains the major RNase T1 oligonucleotide markers of the original stock. It is quite possible, however, that the OA MuLV_C contains additional replication-competent viruses of related structure. RNase T1 fingerprint analysis showed that OA MuLV_N closely resembles Akv, an endogenous virus of the AKR mouse strain (Pedersen & Haseltine, 1980; Pedersen et al., 1982).

The comparative infection of newborn NMRI mice with the prototype N-tropic endogenous virus, Akv, or with two T-cell leukaemia-inducing recombinant viruses from the AKR mouse did not result in the pathological changes in bone tissue as described above (data not shown). The elucidation of the virus genetic determinants of the typical bone tissue pathogenicity of OA MuLV_N, and of the pathway of cellular transformation by this isolate, are the subjects of further studies.

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Oncogenic retrovirus from murine osteomas


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