Serological Characterization of C-type Retroviruses Endogenous to the C57BL/6 Mouse and Isolated in Tumours Induced by Radiation Leukaemia Virus (RadLV-Rs)

By T. ASTIER, B. GUILLEMAIN,* F. LAIGRET, R. MAMOUN AND J. F. DUPLAN

Institut National de la Santé et de la Recherche Médicale, U.117, 229 cours de l’Argonne, 33076 Bordeaux Cedex, France

(Accepted 22 February 1982)

SUMMARY

Radiation leukaemia virus, Rs strain (RadLV-Rs), is a virus complex derived from radiation-induced lymphoma of the C57BL/6 mouse. Several B-ecotropic retroviruses (T1223/B, T98/B and T128/B) were isolated from the RadLV-Rs and further cloned. They were found to be highly leukaemogenic and polymorphic in terms of XC cell fusion activity. To investigate a possible relationship between their phenotypical and genotypical properties, a serological characterization of their proteins was undertaken by means of interference, neutralization and type-specific radioimmunological experiments. In addition, these viruses were compared on the basis of the electrophoretic mobility of their proteins. They were also compared with the prototype endogenous N-ecotropic (BL6/Ni) and xenotropic (T530/X) retroviruses of the C57BL/6 mouse as well as the AKR-MuLV and the RadLV/VL-3 (thymotropic leukaemogenic retrovirus involved in radio-induced lymphoma). With respect to the p12, T1223/B and T128/B viruses were of xenotropic type as in RadLV/VL-3, whereas T98/B p12 displayed ecotropic and xenotropic type-specific antigenic determinants. The gpT1 of all B-ecotropic virus isolates were indistinguishable and of the AKR-MuLV type. This latter result was further supported by interference and neutralization experiments. This supports the view that the B-ecotropic virus isolates originated by recombination (one or two events) between N-ecotropic and xenotropic endogenous retroviruses. The RadLV/VL-3 possesses a unique envelope recombinant glycoprotein of which the antigenicity was not observed in the RadLV-Rs complex. Thus, it may be assumed that the leukaemogenic components of RadLV and RadLV-Rs arose by different recombinational mechanisms.

INTRODUCTION

Radiation leukaemia virus, Rs strain (RadLV-Rs), is a virus complex derived by cell-free passage from a radiation-induced thymoma of the C57BL/6 mouse (Latarjet & Duplan, 1962). When injected into 30-day-old C57BL/6 mice it produces lymphoreticulosarcomas localized in the spleen and lymph nodes, and 100% of the animals die within 100 days (Mistry & Duplan, 1973; Legrand et al., 1981; Lonai & Haran-Ghera, 1980). A permanent cell line (13-3C) was derived from a RadLV-Rs-induced tumour; this 13-3C line produced a virus complex, 13C, which displayed the same tumourigenic effect as RadLV-Rs (Mamoun et al., 1978). Due to an excess of virus pseudotypes, repeated attempts to isolate and clone a leukaemogenic virus component of 13C were unsuccessful, although B-ecotropic and
xenotropic viruses were regularly detected. However, B-ecotropic and xenotropic viruses could be recovered from RadLV-Rs-induced tumours and several clones of B-ecotropic viruses were isolated (B. Guillemain et al., unpublished results). Three of them, T1223/B, T98/B and T128/B, are the object of the present report. They were shown to be polymorphic in terms of XC cell fusion activity. Indeed, cells infected with T1223/B or T98/B induced XC syncytia of small size within 72 h, whereas in the same condition T128/B induced extensive polykaryocytosis within a few hours. In addition, in contrast to RadLV-Rs, the three virus clones produced lymphomas of different types and localizations with a latent period averaging 500 days (E. Legrand et al., unpublished results). It may therefore be concluded that all three B-ecotropic isolates, although associated in the RadLV-Rs complex, do not represent the rapid leukaemia-inducing agent. B-ecotropic retroviruses of this type have been shown not to be endogenous to the C57BL/6 mouse (Benade & Ihle, 1980), but to be recombinants between N-ecotropic and xenotropic viruses. In view of the polymorphic XC activity and of the different pathogenic effects, the structural proteins p12 and gp71 of the three above-mentioned clones were characterized by type-specific radioimmunoassays in comparison with those of the endogenous retroviruses of the C57BL/6 mouse and with the virus prototype AKR-MuLV and the thymotropic leukaemogenic RadLV/VL-3.

METHODS

Cells and viruses. Cells were grown in RPMI 1629 medium supplemented with 10% heat-inactivated foetal calf serum, penicillin (100 units/ml) and streptomycin (100 µg/ml). A number of cell lines were used in our study which can be classified according to their ability to be infected and to support the growth of ecotropic or xenotropic murine retroviruses. With respect to ecotropic viruses (Pincus et al., 1971), mouse cells with the Fv-1<sup>nm</sup> genotype preferentially support infection of N-ecotropic viruses, mouse cells with the Fv-1<sup>b+</sup> genotype preferentially support infection of B-ecotropic viruses, while cells which lack the Fv-1 gene are equally permissive to both types of viruses. The following normal mouse cell lines with different alleles at the Fv-1 locus were used: 3T3 (random-bred Swiss) established by Todaro & Green (1963), TAC with Fv-1<sup>bb</sup> genotype, NIH/3T3 (Jainchill et al., 1969) with Fv-1<sup>mm</sup> genotype, 3T3-FL (Bassin et al., 1970) apparently lacking Fv-1 gene (Fv-1<sup>-</sup>). TAC was established in our laboratory as a permanent cell line and was derived from the thymus of a normal young adult C57BL/6 mouse. TAC cells grow as monolayers with an epithelioid morphology; they do not harbour the typical markers of T lymphocytes, i.e. θ antigen and terminal deoxynucleotidyl transferase activity. For xenotropic murine retroviruses which are restricted to heterologous cells, a normal rabbit corneal cell line, SIRC (Lieber et al., 1974), was used. The cell clone, 13-3C, derived from a RadLV-Rs-induced spleen tumour, produces a mixture of B-ecotropic and xenotropic viruses (13C) displaying the same pathogenicity as the original in vivo-maintained RadLV-Rs (Mamoun et al., 1978). Different virus assays (see below) were carried out on XC (Svoboda, 1961) and FG10 S<sup>+</sup>L<sup>-</sup> (Fischinger et al., 1972) cells.

The virus sources were supernatant fluids of cultured infected cells. They included the endogenous C57BL N-ecotropic virus BL6/Ni (isolated in our laboratory after iododeoxyuridine activation of C57BL/6 mouse embryo fibroblasts) produced by NIH/3T3 cells, the AKR-MuLV endogenous N-ecotropic virus produced by AKR embryo fibroblasts (Hartley et al., 1970) or by 3T3-FL cells. The B-ecotropic T1223/B, T98/B and T128/B viruses were recovered from RadLV-Rs-induced tumours. T1223/B was cloned after serial virus passages at limiting dilutions; T98/B and T128/B were cloned by infecting cell monolayers at such an m.o.i. as to obtain a successful infection in one out of ten monolayers. Later, they were produced on chronically infected TAC cells. The C57BL/6 endogenous xenotropic T530/X was harvested from infected SIRC cells. The RadLV-Rs originated from
Proteins of RadLV-Rs-associated viruses

13-3C cells. The RadLV/VL-3 was produced by the BL/VL-3 cell line of thymic lymphoma 
(Declève et al., 1978). The xenotropic Balb:virus-2 (Stephenson et al., 1974) was kindly 
supplied by Dr S. A. Aaronson (NCI, Bethesda, Md., U.S.A.).

Virus assays. The focus induction assay in sarcoma-positive leukaemia-negative FG10 
S^+L^- cells was used to detect and quantify (Bassin et al., 1971) the mouse sarcoma virus 
(MSV) helper activity of MuLVs. MSV pseudotypes produced by infecting FG10 S^+L^- cells 
were assayed on normal cells (Guillemain et al., 1980). The XC cell fusion assay in mixed 
cultures (Guillemain et al., 1980) was used for the detection of MuLV-infected cells.

Interference tests. Cells infected with various virus isolates were superinfected with the 
MSV pseudotype of the virus to be tested at multiplicities of 6 × 10^{-4} to 6 × 10^{-2} f.f.u./cell. 
Focus formation, indicative of a lack of interference, was recorded 5 to 7 days post-infection 
and after one passage of the cultures. In case the tested virus had a different host range, 
dually permissive 3T3-FL cells were used.

Sera. Neutralization experiments and competitive radioimmunoassays (cRIAs) were 
carried out with antiserum raised in rabbit by repeated injections of gradient-purified viruses. 
The anti-AKR-MuLV p12 and anti-RadLV/VL-3 were kindly supplied by Dr J. N. Ihle 
(NCI, Frederick, Md., U.S.A.).

Neutralization tests. Virus preparations were incubated with serial \log_{10} dilutions of rabbit 
anti-T1223/B virus. The permissive cells were infected with neutralized viruses and assayed 
as syncytia-inducing centre units (SIU). The neutralizing activity of the serum was measured 
by the number of fold reduction of SIU of the virus preparation.

SDS–polyacrylamide gel electrophoresis (SDS–PAGE). Fifty μg of gradient-purified 
viruses were treated with SDS (0.5 %) and β-mercaptoethanol (0.14 M) at 100 °C for 2 min. 
The preparations were then adjusted to 10% glycerol, layered on top of 10% polyacrylamide 
gels and separation of the proteins was achieved according to Weber & Osborn (1969). The 
gels were stained with Coomassie Brilliant Blue.

Purification of virus proteins. The p12 and gp71 virus proteins of T1223/B and T98/B 
viruses were purified according to Strand & August (1976). The purified AKR MuLV p12 
and RadLV/VL-3 gp71 were a gift of Dr J. N. Ihle. They were iodinated (^{125}I) by the 
chloramine-T method (Greenwood et al., 1963) for p12 and by the iodogen method (Devare 
& Stephenson, 1977) for gp71.

Competitive radioimmunoassays. cRIAs were performed as described previously 
(Mamoun et al., 1981) for p12 and with a slight modification of the buffer for gp71 (0.01 
m-tris–HCl pH 7.9, 0.25 m-NaCl, 1 mm-EDTA, 0.4% Triton X-100, 1% bovine serum albumin).

RESULTS

Interference tests

The virus isolates BL6/Ni, T1223/B, T98/B and T128/B were tested for their reciprocal 
interference ability. Cells chronically infected with different virus isolates were superinfected 
with the MSV pseudotype of the virus strain to be tested. In all instances, no focus formation 
was observed, indicative of complete interference between the different isolates (Table 1).

Neutralization tests

The virus isolates T1223/B, T98/B and T128/B were incubated with serial \log_{10} dilutions 
of a rabbit antiserum against T1223/B virus. Permissive cells were infected with the 
normalized virus preparations and assayed as syncytia-inducing centres. The results shown in 
Table 2 indicate that the antiserum readily neutralized the syncytial activity of all virus
Table 1. Interference pattern of different virus isolates of C57BL/6 mice

<table>
<thead>
<tr>
<th>Challenge MSV pseudotype*</th>
<th>BL6/Ni</th>
<th>T1223/B</th>
<th>T98/B</th>
<th>T128/B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells infected with</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No virus</td>
<td>TT†</td>
<td>TT</td>
<td>TT</td>
<td>TT</td>
</tr>
<tr>
<td>BL6/Ni</td>
<td>-‡</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>T1223/B</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>T98/B</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>T128/B</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AKR-MuLV</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Cells infected with the various virus isolates were superinfected with the MSV pseudotypes at multiplicities of 6 × 10⁻⁴ to 6 × 10⁻² f.f.u./cell.
† TT, Total transformation of the monolayer.
‡ -, Complete interference (absence of focus formation).

Table 2. Neutralizing activity of specific antiserum*

<table>
<thead>
<tr>
<th>Virus tested</th>
<th>Normal serum</th>
<th>Anti T1223/B serum</th>
<th>Fold reduction of SIU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1223/B</td>
<td>3.5 × 10³</td>
<td>0</td>
<td>3.5 × 10¹</td>
</tr>
<tr>
<td>T98/B</td>
<td>1.4 × 10⁴</td>
<td>2.6 × 10²</td>
<td>5.2 × 10²</td>
</tr>
<tr>
<td>T128/B</td>
<td>2.6 × 10⁴</td>
<td>1.6 × 10²</td>
<td>1.4 × 10²</td>
</tr>
</tbody>
</table>

* The virus infectivity was measured as syncytia-inducing units per ml (SIU/ml).

isolates. These results, combined with those of interference, suggest a common antigenicity of their glycoproteins.

**SDS–PAGE**

T1223/B, T98/B and T128/B were further analysed by the SDS–PAGE technique. Control viruses included the AKR-MuLV N-ecotropic and the BL6/Ni endogenous viruses. Fig. 1 shows migration patterns of the proteins of the different viruses which are typical of that of murine type C retroviruses. However, clear differences may be observed for the gp71 and p12 proteins. With respect to gp71, as expected, a faint band is observed for the two N-endogenous viruses and also for T1223/B; in contrast, a much larger amount of that protein is observed for T98/B and T128/B. The migration of p12 clearly differs depending on the virus isolate. This protein is easily distinguished by its pink colouration with Coomassie Brilliant Blue (arrows in Fig. 1). For the two N-ecotropic viruses the order of migration is p30, p15, p12, p10; for the three B-ecotropic viruses this order is p30, p12, p15, p10. Lastly, the p12 of T98/B has an electrophoretic mobility slightly greater than that of T1223/B and T128/B.

**Competitive radioimmunoassays**

The serological characteristics of gradient-purified BL6/Ni, T530/X, T1223/B, T98/B, T128/B and 13-3C viruses were compared by cRIAs; the following viruses, namely, AKR-MuLV, Balb:virus-2 and RadLV/VL-3 were used as prototypes. The results of cRIAs for AKR-MuLV p12 with T1223/B, T98/B, T128/B and BL6/Ni viruses as well as with AKR-MuLV ecotropic prototype are presented in Fig. 2(a). AKR-MuLV and BL6/Ni competed completely with the same slopes as purified AKR-MuLV p12, whereas T1223/B and T128/B did not compete. With T98/B virus, although the slope of the competition curve was almost identical to that of AKR/MuLV p12, quantitative differences suggested that this virus contained an abnormally low amount of p12.
Proteins of RadLV-Rs-associated viruses

Fig. 1. SDS-PAGE of proteins of the following virus isolates: lane 1, AKR-MuLV; lane 2, BL6/Ni; lane 3, T1223/B; lane 4, T128/B; lane 5, T98/B. The position of p12 (arrows) was determined by its pink colouration with Coomassie Brilliant Blue. Numbers indicate mol. wt. × 10⁻³.

The T1223/B virus has been shown to have a p12 identical to that of RadLV/VL-3 virus (T. Astier & J. N. Ihle, unpublished results) which is known to be of the Balb:virus-2 type. cRIAs for T1223/B p12 were performed using T1223/B, T98/B, T128/B, BL6/Ni and T530/X viruses. The results shown in Fig. 2(b) indicate complete competition and a slope identical for all the viruses tested except BL6/Ni for which no competition was observed. The combined results of cRIAs for p12 demonstrate that BL6/Ni p12 has an antigenicity identical to that of AKR-MuLV prototype. T128/B, like T1223/B, has a p12 of xenotropic origin. Finally, the p12 of T98/B virus has antigenic determinants of both AKR-MuLV and xenotropic viruses.

Because RadLV/VL-3 is a prototype thymotropic leukaemogenic type-C virus involved in radio-induced thymomas and because its gp71 is unique, it was of interest to search for the
Fig. 2. Homologous competition radioimmunoassays for p12. (a) Rabbit anti-AKR-MuLV p12 versus 125I-labelled AKR-MuLV p12. ●, Purified AKR-MuLV p12; ○, AKR-MuLV; ▲, BL6/Ni; △, T1223/B; ■, T128/B; □, T98/B. (b) Rabbit anti-T1223/B virus versus 125I-labelled T1223/B p12. ●, Purified T1223/B p12; ○, T1223/B; ▲, T98/B; △, T530/X; ■, T128/B; □, BL6/Ni.

Fig. 3. Homologous competition radioimmunoassays for gp71. (a) Rabbit anti-RadLV/VL-3 gp71 versus 125I-labelled RadLV/VL-3 gp71. ●, Purified RadLV/VL-3 gp71; ○, RadLV/VL-3; ▲, T1223/B; △, AKR-MuLV; □, Balb:virus-2; ■, 13-3C. (b) Rabbit anti-T98/B virus versus 125I-labelled T98/B gp71. ●, Purified T98/B gp71; ○, T98/B; ▲, T128/B; △, T1223/B; ■, T530/X; □, BL6/Ni and AKR-MuLV.

The presence of a virus bearing a related glycoprotein in the RadLV-Rs virus complex. For this, cRIAs for the RadLV/VL-3 gp71 were conducted with the leukaemogenic 13C virus complex produced by cultured RadLV-Rs-induced tumour cells. As shown in Fig. 3(a), no competition was observed with this virus complex. The same occurred with T1223/B virus, and the prototypes AKR-MuLV and Balb:virus-2. Thus, RadLV-Rs does not contain at a detectable level virus particles with gp71 of RadLV/VL-3 type.

The results of assays performed with radioiodinated T98/B gp71 and anti-T98/B virus serum are illustrated in Fig. 3(b). It was observed that apart from T530/X virus, all others,
Proteins of RadLV-Rs-associated viruses

Table 3. Summary of RIA data for virus structural proteins

<table>
<thead>
<tr>
<th>Virus</th>
<th>cRIA (p12)</th>
<th>T1223/B (p12)</th>
<th>T98/B gp71</th>
<th>RadLV/VL-3 gp71</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKR-MuLV</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>BL6/Ni</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>T1223/B</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>T98/B</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>T128/B</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>RadLV/VL-3</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

* XC, Late polykaryocytosis; XCEP, early polykaryocytosis.
† T1223/B p12 is of Balb : virus-2 type.

including the prototype AKR-MuLV ecotropic virus, competed completely with identical slopes. These results allow us to conclude that all C57BL/6 mouse-derived ecotropic virus isolates and AKR-MuLV have a gp71 with common antigenicity.

DISCUSSION

The purpose of our investigation was to characterize the structural proteins of the virus populations present in RadLV-Rs-induced tumours. Several B-ecotropic viruses were isolated and cloned (T1223/B, T98/B and T128/B). Their internal p12 protein, as well as the major envelope glycoprotein gp71, were purified and compared by cRIAs with those of the endogenous N-ecotropic virus (BL6/Ni) and xenotropic virus (T530/X) of the C57BL/6 mouse strain. The N-ecotropic endogenous virus of the AKR mouse strain (AKR-MuLV) was used as a reference prototype in these studies. Table 3 summarizes our results.

Using cRIAs, no differences could be detected for the p12 and gp71 proteins of BL6/Ni and AKR-MuLV. With respect to p12, T1223/B and T128/B viruses are of xenotropic (Balb : virus-2) origin and identical to that of RadLV/VL-3. The T98/B isolate displayed two types of antigenicities reassociated in p12, i.e. AKR-MuLV and T1223/B (ecotropic and xenotropic respectively). This finding is in accordance with the intermediate electrophoretic mobility observed for the p12 of T98/B as compared to that of N-endogenous viruses and T1223/B. The gp71 of all B-ecotropic virus isolates were indistinguishable and of the AKR-MuLV type. This finding was further supported by the results of interference and in vitro neutralization experiments; indeed, all N- and B-ecotropic viruses interfered with each other and the same anti-T1223/B serum neutralized efficiently all B-tropic isolates. However, previous experiments (B. Guillemain et al., unpublished results) indicated that T1223/B and T128/B viruses are different in view of their abilities to induce XC cell fusion. Indeed, T1223/B displayed the conventional ‘late’ type polykaryocytosis (72 h) whereas ‘early’ polykaryocytosis (a few hours) was induced by T128/B. Because virus glycoproteins are involved (qualitatively or quantitatively) in MuLV-induced cell fusion one can assume that the two viruses differ in some property not revealed by the cRIAs, interference and neutralization tests. In addition, the electrophoretic patterns of the gp71 of all three virus clones are different. These combined results are indicative of a common origin of our virus isolates although each is unique.

Our results are consistent with those of Declève et al. (1976) and Benade & Ihle (1980) with respect to the recombinational origin of the B-ecotropic viruses isolated from the C57BL/Ka and from the C57BL/6 mouse. Indeed, in view of the xenotropic origin of the p30 sequences related to B tropism (Gautsch et al., 1978) and of genome structure 5' - gag(p15, p12, p30, p10) - pol-env-3' (Barbacid et al., 1975), one can assume that one recombinational event between endogenous N-tropic and xenotropic viruses had to occur either in the p30, in
the p10 or in the pol gene for the generation of T1223/B and T128/B viruses. A second recombination intra-p12 between the two endogenous viruses would explain the emergence of T98/B.

Because both thymotropic RadLV (Lieberman & Kaplan, 1959) and splenotropic RadLV-Rs originated from a radiation-induced thymic lymphoma of C57BL mice, a possible relationship between the two virus strains could be considered. Like RadLV-Rs, RadLV was shown to be a virus complex composed of B-ecotropic, N-ecotropic and xenotropic viruses (Declèeve et al., 1976). The two latter are endogenous non-leukaemogenic viruses. The B-ecotropic viruses have been classified as fibrotropic, not inducing thymomas [BL/Ka(B)], or thymotropic and leukaemogenic (RadLV/VL-3). BL/Ka(B) viruses are recombinants of the same type as T1223/B (p12 of xenotropic type; gp71 of AKR-MuLV type). RadLV/VL-3 possesses a p12 of the same type and a unique envelope recombinant glycoprotein (Declèeve et al., 1978). The search for such an antigenicity in the RadLV-Rs complex gave negative results; thus, it may be concluded that the leukaemogenic components of RadLV and of RadLV-Rs arose by different mechanisms while both presumably originate from the recombined information of the two endogenous viruses of the irradiated host (Benade et al., 1978; Janowski et al., 1978).

We are indebted to Dr J. N. Ihle for supplying several materials used in this study. We greatly acknowledge the technical assistance of Mrs C. Bourget and Mr J. P. Portail and the careful preparation of the manuscript by Mrs N. Jaulin. This work was supported by the Institut National de la Santé et de la Recherche Médicale (CRL 75-5-106-2 and 79-5-002-2) and EURATOM (BIO-D-371-81-F).

REFERENCES


Proteins of RadLV-Rs-associated viruses


(Received 20 November 1981)