Molecular Biological Characterization of a Highly Leukaemogenic Virus Isolated from the Mouse. IV. Viral Proteins


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SUMMARY

The proteins of a highly leukaemogenic murine virus (DMBA-LV) endogenous to the CFW/D mouse have been characterized. This virus does not contain ecotropic, xenotropic or polytropic type C retroviruses capable of replicating in established tissue culture cell lines. Analysis of the viral proteins indicated that proteins characteristic of both type C and type B retroviruses were present. All the proteins characteristic of a competent, mature type B retrovirus, such as the murine mammary tumour virus, were present in the virions and were expressed at high levels in a virus-producing thymic lymphoma cell line. Type C internal structural proteins were detected in the virus and cells producing virus. Type C envelope protein was detected at very low levels in both the virions and the virus-producing tumour cell lines. Chymotryptic polypeptide profiles of this gp70 indicated similarity with the envelope glycoproteins characteristic of xenotropic and recombinant type C retroviruses.

INTRODUCTION

A highly leukaemogenic virus (DMBA-LV) was isolated from a thymic lymphoma induced by 7,12-dimethylbenz[a]anthracene (DMBA) in CFW/D mice (Ball & McCarter, 1971, 1979). This virus is produced by DMBA-LV-induced tumours and does not contain any detectable xenotropic or ecotropic type C viruses that can grow in established tissue culture cell lines. The leukaemogenic titre of DMBA-LV was calculated to be a minimum of 1 × 10^6 to 5 × 10^6 infectious units per ml (Ball, 1979). The average latent period to death with thymic lymphoma was 63 days.

A preliminary characterization of the virus (Ball, 1979) indicated that while it had a buoyant density and RNA genome size characteristic of type C virions, the viral proteins were of size classes distinct from those of type C viruses. Furthermore, electron microscopical studies indicated that there were abundant virions with the morphology of intracytoplasmic A particles in both the cultured tumour cells and purified virions. However, budding or mature type C particles were very rare, and in fact were not significantly different from the numbers detectable in normal thymus tissue of newborn CFW/D mice (Frei et al., 1973).

There have been numerous reports of the presence of intracytoplasmic A particles in leukaemic cells (de Harven & Friend, 1960; Tanaka et al., 1972; Hilgers et al., 1973; Vaidya et al., 1980). In addition, the presence of mouse mammary tumour virus (MMTV) antigens on normal lymphoid cells has also been reported (Gillette et al., 1974; Charyulu et al., 1979). In certain studies (Nusse et al., 1979; Vaidya et al., 1980) the accumulation of intracytoplasmic A particles in lymphoid tumours has been attributed to a defect in the processing of the envelope precursor into mature envelope glycoproteins.

In a preceding paper (Ball et al., 1983) we showed, using molecular probes, that DMBA-LV consisted of two unrelated retrovirus genomes: an intact type B retrovirus highly related to the
exogenous milk-borne MMTV isolate and a biologically defective type C genome. In this paper we report that DMBA-LV contained all the proteins corresponding to those characteristic of type B retroviruses and that the processing of these proteins in cells producing the virus proceeds normally. DMBA-LV also contained low amounts of type C retrovirus gag gene products and very low levels of type C env gp70.

METHODS

Virus and cells. The source of all DMBA-LV was from tissue culture media (Eagle's MEM plus 10% heat-inactivated foetal calf serum) in which thymic lymphoma cells (485-2, 485-10 or 485-41) were growing. The origin and properties of these cell lines have been described (Ball, 1979; Ball & McCarter, 1979). The DMT-10 cell line which produces a non-infectious type C retrovirus has been described (Ball et al., 1983). The 3T3 FL cells chronically infected with Moloney murine leukaemia virus (MoMuLV/ICB) and the Hix cell line chronically infected with a recombinant type C retrovirus were kindly provided by Dr P. J. Fischinger. The GR mammary tumour cell line was kindly provided by Dr V. Morris. The C3H-derived MMTV-producing cell line (mm5mt) has been previously described (Arthur et al., 1978). Both the latter cell lines were stimulated with 10^{-6} M-dexamethasone for 12 h prior to labelling with [35S]methionine.

Radioactive labelling of virus and cells. Thymic lymphoma cells were labelled with [35S]methionine to obtain labelled virus, 10^6 to 2 x 10^6 cells were washed in methionine-free medium [Dulbecco's modified Eagle's MEM (Flow Laboratories)] containing 1% dialysed foetal calf serum and resuspended at 1 x 10^7 cells/ml in methionine-free medium in a small spinner flask. The cells were then incubated at 34 °C for 30 min to deplete the methionine pool before addition of 0.5 mCi [35S]methionine (600 Ci/mol). After 4 h, cells were diluted to 2 x 10^6 cells/ml with complete Eagle's MEM plus 10% foetal calf serum and incubated for another 8 h. At this time the cells were collected and the medium harvested. The cells were resuspended again at 2 x 10^6 cells/ml and further harvests were taken at 24 and 36 h post-labelling. Labelled virus was purified as described earlier and only virus banding at 1792 was detected by a binding assay with 125I-labelled Protein A (IPA) from S. aureus.

Sources of antisera for immunoprecipitations. A monospecific, broadly reacting interspecies Rauscher type C p30 goat antiserum and a monospecific gp70 antiserum raised against a MoMuLV polytropic recombinant virus were obtained from Dr S. Ruscetti (Ruscetti et al., 1978). This latter antiserum specifically recognizes polytropic murine leukaemia virus (MuLV) gp70 env proteins. A broadly reacting interspecies type C gp70 goat antiserum prepared from Friend MuLV (Fischinger et al., 1976) was kindly provided by Dr P. J. Fischinger.

Polyvalent antiserum directed against the MMTV(C3H) proteins p28 and gp52 and the monoclonal antibody to MMTV gp36 have been described previously (Massey et al., 1980). In addition three other monoclonal antibodies to MMTV gp36 were kindly provided by Dr P. Hand (Colcher et al., 1981).

Antiserum against DMBA-LV was prepared from rabbits immunized with DMBA-LV (J. K. Ball, unpublished results).

Formalin-fixed Staphylococcus aureus was kindly supplied by Drs S. Ruscetti, M. Weeks and W. G. Robey. Immunoprecipitations and SDS-polyacrylamide gel electrophoresis. Immunoprecipitations and SDS-PAGE were performed according to the procedures of Ruscetti et al. (1978, 1979). Radioactive proteins in the gels were visualized by fluorography using the method of Bonner & Laskey (1974). The molecular sizes (in kd) noted in each of the figures were determined by comparison with the standard markers: lysozyme (14.4), soybean trypsin inhibitor (21.5), carbonic anhydrase (31.0), ovalbumin (45.0), bovine serum albumin (66.2), phosphorylase B (92.5), β-galactosidase (116.25) and myosin (200.0) as supplied by Bio-Rad.

Solid-phase radioimmunoassays for detection of MMTV gp36. Binding of monoclonal antibodies to retroviruses was detected by a binding assay with 125I-labelled Protein A (IPA) from S. aureus. Virus concentration was adjusted to 1 μg/50 μl and 50 μl was added to each well of a 96-well plate. The plates were incubated overnight to allow adsorption of the virus to the plastic. Unbound sites in the wells of the plate were blocked from further non-specific protein adsorption by a 2 h incubation with 250 μl of 5% bovine serum albumin in phosphate-buffered saline pH 7.2. The monoclonal antibodies were diluted in phosphate-buffered saline (pH 7.2) and 50 μl of each dilution was added to a well containing immobilized virus. After a 1 h incubation at 37 °C, unbound immunoglobulins were removed from the wells by washing three times with phosphate-buffered saline containing 1% bovine serum albumin. Radiolabelled Protein A (10^5 c.p.m.) was added to each well to detect the bound immunoglobulin. After 30 min incubation, the residual unbound IPA was removed by washing and the positive
Purification of type C envelope glycoproteins. Envelope glycoproteins were isolated from cell culture supernatants by immunoaffinity chromatography. To do this, the IgG fraction of a broadly reactive antiserum to Friend MuLV gp70 (Schwarz et al., 1979) or ascites fluid containing the monoclonal antibody P2G6 (Massey & Schochetman, 1981) were isolated by (NH₄)₂SO₄ precipitation (Campbell et al., 1975). Cell culture fluids containing the glycoproteins were adjusted to 1 M-KCl, 1% Triton X-100 and 0.01 M-Tris-HCl pH 8.5 in order to dissociate the envelope gp70 from virions: following chromatography, the column was washed with 2.5 M-MgCl₂ and the envelope glycoproteins were then eluted with 4 M-MgCl₂ (Thiel et al., 1978). The glycoprotein fraction was extensively dialysed against phosphate-buffered saline and then radiiodinated with Na¹²⁵¹ (Amersham) using the chloramine-T method (Elder et al., 1977). Radiodinated gp70 was then immunoprecipitated with Friend MuLV antiserum and the gp52 by an anti-MMTV(C3H) gp52 serum and S. aureus (Richert et al., 1979). Electrophoresis was carried out on a 10% acrylamide gel with SDS. The gels were dried and the glycoproteins localized by autoradiography with SB X-ray film. Since the culture fluid from 485-10 cells contained both gp52 and gp70 it was found that the culture fluid from these cells could first be chromatographed on an anti-gp52 column and the effluent chromatographed on an anti-gp70 column.

Oligopeptide analysis of gp70. By using the autoradiogram to locate the type C gp70 on the dried gel, the appropriate region was cut out, rehydrated and digested with 0.1 mg α-chymotrypsin (Worthington) in 0.1 ml of 0.05 M-NH₄HCO₃ and processed as described previously (Elder et al., 1977; Green et al., 1980). This digest was then analysed by electrophoretic chromatography on thin-layer cellulose plates (acetic acid:formic acid:water, 15:5:80) for the first dimension and by ascending chromatography (butanol: pyridine: acetic acid, 32.5:25.5:20) for the second dimension as described by Elder et al. (1977) and Green et al. (1980). The second dimension solvent system also contained 6% (w/v) 2,5-diphenyloxazole to enhance autoradiography using SB X-ray film.

RESULTS

Antigenic relatedness of DMBA-LV to type B and C retroviruses

Immunoprecipitations were conducted using antisera specifically directed against total and individual proteins of type B and C retroviruses and DMBA-LV. For these experiments [³⁵S]methionine-labelled DMBA-LV was obtained from three different isolates (tumour cell lines 485-2, 485-10 and 485-41) and [³⁵S]methionine-labelled MMTV(GR) was obtained from a GR mammary tumour cell line.

As seen in Fig. 1, rabbit antiserum directed against either MMTV(C3H) or DMBA-LV virions specifically immunoprecipitated viral proteins from all three DMBA-LV preparations as well as from MMTV(GR) virions. MMTV is known to have two envelope glycoproteins, gp52 and gp36. DMBA-LV from the three different tumour cell lines contained varying amounts of a diffuse protein of molecular weight 52K (Fig. 1, lanes 3 to 8). A similar protein was also detected in MMTV(GR) (Fig. 1, lanes 1 and 2). A 52K protein was also immunoprecipitated by a monospecific antiserum directed against MMTV(C3H) gp52 (Fig. 2a, lower arrow). A 52K protein was also purified by immunoaffinity chromatography using a monoclonal antibody to MMTV(C3H) gp52 (Fig. 3, lane 4). The glycoprotein nature of the 52K protein was demonstrated by its positive periodate-Schiff (PAS) staining (data not shown).

Varying amounts of a 34K to 37K protein were also detected (Fig. 1, all lanes as indicated by dashes). A protein of this size range also stained with PAS (data not shown). Since gag precursor proteins also exist in the 34K to 37K size range, further confirmation that DMBA-LV did have a gp36 was obtained. Solid-phase radioimmunoassays were performed using monoclonal antibodies to MMTV gp36. As shown in Table 1, DMBA-LV produced by 485-10 cells contained a gp36 protein which behaves, in the binding assay for monoclonal antibodies, much like the MMTV(C3H) gp36 except that antibody M2.1 may be able to discriminate DMBA-LV from MMTV(C3H).

A much smaller amount of C-type viral proteins were also detected in DMBA-LV. A p31 protein was detected in DMBA-LV preparations from both 485-10 and 485-41 tumour cell lines when immunoprecipitated with anti-DMBA(LV) serum (Fig. 1, lanes 3 and 7 as indicated by the dots) but not by anti-MMTV(C3H) serum. This 31K protein appears to be the major core
Fig. 1. Radioimmunoprecipitations of [35S]methionine-labelled MMTV(GR) and three separate isolates of DMBA-LV. Odd-numbered lanes were precipitated with anti-DMBA-LV and even-numbered lanes precipitated with anti-MMTV(C3H). Lanes 1 and 2 contained MMTV(GR); DMBA-LV from lymphoma cell lines 485-10 (lanes 3 and 4), 485-2 (lanes 5 and 6) and 485-41 (lanes 7 and 8) are shown. Viral proteins were separated on a 12% SDS-polyacrylamide gel.

protein of the type C virus in DMBA-LV since it is also immunoprecipitated by a monospecific type C p30 antiserum (Fig. 2, lanes 3, lower arrow).

Using conventional methods to detect the major type C envelope glycoprotein (gp70) in DMBA-LV yielded negative results when virus from 2 \times 10^8 cells was labelled with [35S]methionine and isolated from 50 ml of tissue culture medium (Fig. 2a, lane 3 and 2b, lane 2). Two different antisera were employed in these experiments. The first (Fig. 2a, lane 3) was an antiserum directed against determinants of recombinant gp70, and the second (Fig. 2b, lane 2) was against the ecotropic Friend gp70. However, when more sensitive procedures were used as demonstrated by the data shown in Fig. 3, type C gp70 could be detected in DMBA-LV produced from 1-8 \times 10^{10} to 2-4 \times 10^{10} 485-10 cells (31). Lane 1 of Fig. 3 shows the amount of gp70 recovered from 300 ml of supernatant from confluent monolayers of MoMuLV/ICB chronically infected 3T3 FL cells. In lane 2 is shown the amount of gp70 recovered from 31 of supernatant from DMT-10 cells which produce a biologically non-infectious type C retrovirus (Ball et al., 1983). Clearly, the presence of very low levels of type C gp70 in DMBA-LV in this experiment explains why we were unable to detect type C gp70 following [35S]methionine labelling of virus from low numbers (2 \times 10^8) of 485-10 cells. In lane 4 is shown the amount of gp52 recovered from the same 31 of supernatant of 485-10 cells from which the type C gp70 was recovered. The gp52 was removed by passage through the anti-gp52 immunoaffinity column prior to the isolation of the type C gp70.

In order to confirm that this low radioactive signal in Fig. 3, lane 3 was indeed gp70, 201 of DMBA-LV supernatant from the 485-10 tumour cell line was concentrated by banding in
Fig. 2. Radioimmunoprecipitation of $^{35}$S-labelled DMBA-LV with type B and type C antisera. (a) Lane 1 contains non-immunoprecipitated, sucrose density-banded DMBA-LV and lanes 2 and 3 contain the same virus preparation immunoprecipitated with anti-MMTV gp52 and a monospecific antiserum against recombinant gp70 respectively. (b) $[^{35}$S]Methionine-labelled DMBA-LV immunoprecipitated with anti-Friend gp70 (lane 2) serum, interspecies p30 (lane 3) serum and a mixture of non-immune normal rabbit and normal goat sera (lane 1).

sucrose using a continuous flow ultracentrifuge. This concentrate was then passed over a gp70 immunoaffinity column, eluted and iodinated. After further purification, the iodinated gp70 was digested with chymotrypsin. The gp70 proteins from MoMuLV/ICB (100 ml) and the Moloney recombinant Hix MuLV (1 l) were also purified, iodinated and digested with chymotrypsin. The $^{125}$I-labelled chymotryptic peptides were subjected to two-dimensional analysis to generate chymotryptic maps of the three viral isolates. Fig. 4 shows that the gp70 from DMBA-LV (panel a) was indeed a type C viral gp70 by virtue of the fact that its chymotryptic map very closely resembled those of the recombinant Hix MuLV (panel b) and the ecotropic MoMuLV/ICB (panel c). The DMBA-LV gp70 chymotryptic map appears to be more similar to that of Hix MuLV, which acquired endogenous type C nucleotide sequences as a consequence of recombination (Fischinger et al., 1983). This suggests that the type C virus in DMBA-LV was derived from one of the endogenous proviruses present in the genome of the CFW/D mouse.

No antigenic relationship between DMBA-LV proteins and intercisternal A particles could be detected (data not shown) using an intercisternal A particle antiserum (kindly provided by Dr E. Kuff).
Fig. 3. SDS-PAGE of iodinated gp70 and gp52. The supernatants from 100 ml of 3T3 FL cells producing MoMuLV (lane 1), 31 of DMT-10 cells (lane 2) and 31 of 485-10 cells (lanes 3 and 4) were passed over the appropriate immunoaffinity columns as described. The purified gp70 preparations were iodinated, and then immunoprecipitated with the appropriate antisera. Electrophoresis was carried out in a 10% SDS-polyacrylamide gel. The molecular weights noted were determined from the protein markers described in Methods.

Table 1. Reactivity of MMTV gp36 monoclonal antibodies to DMBA-LV

<table>
<thead>
<tr>
<th>Monoclonal antibody</th>
<th>P2H2</th>
<th>M1.1</th>
<th>M2.1</th>
<th>M3.1</th>
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<tbody>
<tr>
<td>MMTV(C3H)</td>
<td>1·2 × 10^5*</td>
<td>1 × 10^3</td>
<td>1 × 10^4</td>
<td>1 × 10^4</td>
</tr>
<tr>
<td>MMTV(C3Hf)</td>
<td>3·6 × 10^5</td>
<td>10</td>
<td>10</td>
<td>1 × 10^2</td>
</tr>
<tr>
<td>MMTV(GR)</td>
<td>4 × 10^4</td>
<td>1 × 10^3</td>
<td>1 × 10^3</td>
<td>1 × 10^3</td>
</tr>
<tr>
<td>DMBA-LV(485-10)</td>
<td>1·4 × 10^4</td>
<td>ND†</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>DMBA-LV(485-2)</td>
<td>1·4 × 10^4</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>MuLV(DMT-10)</td>
<td>ND†</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
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* Reciprocal of the highest dilution giving 50% of maximum binding in a solid-phase assay.
† ND. Not determined.
‡ Background.
Fig. 4. Chymotryptic maps of \( ^{32} \)P-labelled, purified type C gp70 from tissue culture supernatants of (a) 485-10 tumour cells, (b) mink lung cells producing Hix MuLV and (c) 3T3 FL cells producing MolMuLV/C.B.
Expression of type B retrovirus proteins in lymphoma cell lines

The 485-10 tumour cell line was pulse-labelled with $^{35}$S-methionine for 10 min and the label chased with normal medium for 1 h. Immunoprecipitation of the cell-free extracts with antiserum directed against MMTV(C3H) p28 detected the major $gag$ precursor proteins. The Pr110 precursor was chased in both 485-10 and 485-41 cells (Fig. 5, lanes 5 to 8). The main $gag$ precursor in MMTV-producing cells is a Pr76$^{ag}$ polyprotein as shown in Fig. 5, lanes 1 and 2. However, in 485-10 and 485-41 cells this protein reproducibly migrated as Pr85$^{ag}$ (lanes 5 to 8). The reason for this higher molecular weight is not clear, but may represent a strain difference. The Pr85$^{ag}$ protein was chased and a p37, which is a known $gag$ cleavage product (Dickson & Atterwill, 1979), appeared to accumulate (lanes 5 to 8), while no p28 was detected (Fig. 5, lanes 6 and 8). The p37 protein probably represents the $gag$ gene cleavage product which has been found in fairly large amounts in intracytoplasmic type A particles (Tanaka, 1977) and these have been shown to accumulate in 485-10 cells (Ball & McCarter, 1979). The failure to detect any p28 in the pulse-chase experiment (Fig. 5) suggests that the precursors must be processed very rapidly as the virions bud from the cell surface. The rapid processing of MMTV precursor proteins has also been reported in T-cell lymphomas by Vaidya et al. (1980).

Anti-MMTV(C3H) gp52 immunoprecipitated the Pr77$^{env}$ polyprotein contained in extracts of both 485-10 and 485-41 cells (Fig. 6, lanes 3 to 6). Some gp52 was also detected in both the pulse and chase lanes, with some accumulation during the chase time. After a 3 h chase the Pr77$^{env}$ had disappeared (data not shown).

Expression of type C retrovirus proteins in tumour cell lines

Experiments were performed to examine the expression of the type C component of DMBA-LV in 485-10 cells. Cells were pulse-labelled with $^{35}$S-methionine for 20 min then chased with medium containing no label for 4 h. The cell extracts were immunoprecipitated with the type C interspecies p30 antiserum. The results, shown in Fig. 7, indicated that the low level of Pr65$^{ag}$ which was present in 485-10 cells (lane 1) had disappeared following the 4 h chase period (lane 2). During this same chase interval a p31 protein appeared (lane 2).

The thymic lymphoma cell lines 485-10 and 485-41 were examined for the presence of envelope precursor proteins using a monospecific type C gp70 antiserum. As shown in Fig. 8,
Fig. 6. Radioimmunoprecipitations using an anti-MMTV(C3H) gp52 in a \([^{35}S]\)methionine pulse-chase experiment. Lanes 1 and 2 contain extracts from mm5mt cells stimulated with dexamethasone. DMBA-LV-producing lymphoma cell extracts were from 485-10 cells (lanes 3, 4, 7 and 8) and 485-41 cells (lanes 5 and 6). The odd-numbered lanes are extracts from a 10 min pulse and the even-numbered lanes are extracts prepared following a 1 h chase. Lanes 1, 2, 3, 4, 5 and 6 were immunoprecipitated with anti-MMTV(C3H) gp52 and lanes 7 and 8 with normal rabbit serum. The normal serum controls for lanes 1 and 2 are lanes 3 and 4 of Fig. 4. The molecular weights indicated (arrows) are based on molecular weight markers described in Methods.

Fig. 7. Pulse-chase experiment of \([^{35}S]\)methionine-labelled type C proteins in 485-10 cells. These cells were pulse-labelled for 20 min with \([^{35}S]\)methionine and then chased for 4 h. Cell-free extracts were prepared from \(1 \times 10^6\) cells after the 20 min pulse (odd-numbered lanes) and after the 4 h chase (even-numbered lanes). The 485-10 cell extracts were immunoprecipitated with a monospecific anti-p30 (lanes 1 and 2) and normal rabbit serum (lanes 3 and 4). The molecular weights indicated (arrows) were determined using the molecular weight markers described in Methods.
extracts from $1 \times 10^8$ cells from each cell line, labelled for 2 h with $[35S]$methionine, contained some Pr80env and lesser amounts of gp70 (Fig. 8, lanes 2 and 3). The Pr80env was slightly smaller in size than the Pr85env in $5 \times 10^6$ cells chronically infected with Hix MuLV (lane 1, arrow) which served as a positive control for the presence of type C Pr85env. The results of these experiments again indicate that the very low level of gp70 present in virions produced by DMBA-LV cells is reflected by similarly low levels of both gp70 and its precursor in extracts from virus-producing cells.

DISCUSSION

When murine retroviruses have been aetiologically associated with leukaemia induction, both biological and molecular biological studies have implicated the type C MuLVs. For this reason when DMBA-LV was first recovered from a transplanted chemically (DMBA) induced lymphoma, it was assumed to be a standard type C retrovirus (Ball & McCarter, 1971, 1979; Ball, 1979). However, subsequent studies have not entirely supported this assumption. First, while type C MuLVs can induce lymphomas in susceptible rats (Gross, 1970), DMBA-LV does not (J. K. Ball, unpublished data). The second inconsistent piece of evidence is that the protein profile of sucrose density gradient-purified DMBA-LV (not immunoprecipitated with type-specific antisera) is quite unlike that of any known replication-competent murine type C MuLV (Ball & McCarter, 1979). It is, in fact, much more like the protein profile of known type B retroviruses. The last piece of evidence inconsistent with DMBA-LV being a typical type C viral isolate is that the 485-10 cell line contains large numbers of intracytoplasmic type A particles but only rare particles with a morphology characteristic of type C virions. Intracytoplasmic particles have been shown to be precursors to mature type B particles (Tanaka, 1977; Nusse et al., 1979).
Immunological studies of DMBA-LV confirmed the presence of type B retrovirus proteins in DMBA-LV. Mature forms of both gp52 and p28 were detected and MMTV gp36 monoclonal antibodies detected a gp36 in DMBA-LV. Furthermore, since the major envelope and gag viral proteins of type B retroviruses were detected in DMBA-LV it would appear that the type B component of DMBA-LV is an intact normal virion. Also, on the basis of the results of the pulse-chase experiments, the type B retrovirus proteins appeared to be processed normally in DMBA-LV-producing cells. The reason why the gag precursor protein appears to be larger (Pr85\textsuperscript{gag}) in DMBA-LV-producing cells than in cells lines producing mammary tumour-inducing type B retroviruses (Pr76\textsuperscript{gag}) is not known. These findings appear to be in contrast to the results of others who report that in lymphoma cell lines expressing MMTV there was a processing impairment of the synthesis of MMTV precursor proteins to mature envelope glycoproteins (Nusse \textit{et al.}, 1979; Vaidya \textit{et al.}, 1980). This impairment of glycoprotein maturation resulted either in blocking the release of MMTV virions (Nusse \textit{et al.}, 1979), or any MMTV virions that were released were highly deficient in envelope glycoproteins.

On the basis of biological infectivity studies, the type C retrovirus present in DMBA-LV has never been shown to be infectious (Ball & McCarter, 1979). A chemically induced (DMBA) thymic lymphoma from a CFW/D mouse has also been established in culture (designated DMT-10) and these cells produce a type C retrovirus only. This type C retrovirus, like that present in DMBA-LV, is non-infectious \textit{in vitro} and is not leukaemogenic \textit{in vivo} (Ball \textit{et al.}, 1983). Because normal lymphoid tissues of the CFW/D mouse have been shown to express type C retrovirus RNA (Ball \textit{et al.}, 1983) and to contain very low levels of type C virions (Frei \textit{et al.}, 1973) it is apparent that all lymphoid tumours induced in this mouse strain will express and contain type C retroviruses.

It would appear that since CFW/D mice do not contain any detectable endogenous ecotropic retrovirus sequences (J. K. Ball, unpublished data) any type C retroviruses present in tissues or tumours derived from CFW/D mice would not be leukaemogenic. Evidence to support this has come from extensive studies (McCarter \textit{et al.}, 1977; Ball & McCarter, 1979; Ball \textit{et al.}, 1983) in which we have never been able to show that the type C retrovirus present in CFW/D tissues or tumours is infectious or leukaemogenic. The similarity of the chymotryptic map of the DMBA-LV type C gp70 with that of the gp70 of the Hix MuLV which acquired endogenous type C sequences as a result of recombination between MoMuLV and endogenous retrovirus sequences (Fischinger \textit{et al.}, 1983) suggests that the type C retrovirus information present in DMBA-LV was derived from non-ecotropic endogenous proviruses present in the CFW/D mouse genome (J. A. McCarter \textit{et al.}, unpublished results). We conclude that the leukaemogenicity of DMBA-LV is not due to the type C retrovirus information present.

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