Deposition of Retrovirus Associated Antigens (p30 and gp70) on Cell Membranes of Feline and Murine Leukaemia Virus Infected Cells

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SUMMARY

A quantitative estimation of retrovirus associated cell membrane antigens of murine and feline cells infected with their respective type C leukosis virus is presented. Using a radio-immune assay with three broadly reactive antisera, the minimum estimated number of retrovirus associated antigenic determinants on YAC [Moloney leukaemia virus (MuLV) infected murine] and FL-74 [feline leukaemia virus (FeLV) infected feline] cells was $1.3 \times 10^6$ and $1.6 \times 10^6$ determinants per cell respectively. The virus structural proteins p27-30 and gp70 were detected by three component specific antisera on murine and feline cell surfaces in amounts which varied between cell isolates. MuLV infected cells produced as many as $1.9 \times 10^5$ p30 antigenic determinants and $7.5 \times 10^5$ gp70 determinants on infected cells. FeLV infected cells (FL-74) expressed $5.6 \times 10^5$ p27 and $7.5 \times 10^5$ gp70 antigenic determinants per single cell surface. The major core protein (p27-30) and the major envelope glycoprotein (gp70) antigens are sufficiently physically separated on cell surfaces so that binding of either of the membrane antigens with component specific antibodies does not interfere with binding of antibodies specific for the other. Despite the expression of interspecies determinants for p30, gp70, and other retrovirus associated antigens detected by antibody procedures, interspecies determinants of cell mediated immunity could not be demonstrated in immune mice bearing Moloney sarcoma virus (MSV) induced tumours. Furthermore, xenogeneic immunization of mice with FL-74 cells failed to protect mice against the growth of MSV induced lymphoma or sarcoma.

INTRODUCTION

The immunological determinants expressed by the structural proteins of type C RNA viruses have been the object of intensive study. Murine virion structural proteins, p30, gp70 and virus reverse transcriptase have been shown to possess type-specific antigenic
determinants, group-specific determinants which are shared with type C virus induced proteins which infect the same species, and interspecies determinants which cross react with similar proteins of type C viruses which infect widely divergent species (Scolnick et al. 1972; Strand & August, 1973, 1974, 1975; Fleissner et al. 1974; Lilly & Steeves, 1974; Steeves, Strand & August, 1974; Stephenson, Tronick & Aaronson, 1974; Parks et al. 1975). The expression of virus structural protein determinants on the membranes of infected cells has biological significance from at least four perspectives: first, shared antigenicity as a measure of genetic and evolutionary homology between type C viruses within a species and between species; second, the orientation of these antigens on cell surfaces with respect to budding viruses and to accessibility of virion antigens to extracellular antibodies; third, the stage in retrovirus polyprotein processing at which surface associated antigens exist; and fourth, the relationship of virus protein determinants to tumour immunity. In this communication we present quantitative estimations of type, group and interspecies determinants of virus proteins on the cell membranes of murine and feline cells infected with type C virus. These data are related to virus cell surface antigens involved in cell mediated immunity and in tumour rejection.

METHODS

**Animals.** Male A/J and BALB/c mice, 6 to 12 weeks of age, obtained from the inbred stocks at the National Institutes of Health or the Jackson Laboratory (Bar Harbor, Maine), were used in these experiments.

**Cell lines.** Basc-2 is a normal mouse fibroblast cell line derived by subcutaneous implantation of nylon gauze in adult BALB/c mice. Ten days later the gauze was removed and the adhering cells were detached with trypsin and planted in tissue culture. These cells failed to produce type C viruses detectable by electron microscopy or by assay for culture fluid associated reverse transcriptase (O'Brien, Simonson & Boone, 1976a).

RAG cells are tissue culture derivatives of a transplantable renal adenocarcinoma of BALB/c mice (Klebe, Chen & Ruddle, 1970). These cells produce an endogenous B-tropic type C virus (O'Brien et al. 1976a) with RNA-dependent DNA polymerase activity of 20 to 40 pmol $^{3}$H-TTP/ml culture fluid.

A-g cells are 8-azaguanine resistant L cell derivatives (Littlefield, 1964) which produce an N-tropic murine C-type virus at approximately the same polymerase levels as RAG cells (Fenyo, Nazerian & Klein, 1974).

YAC lymphoma was produced by injection of newborn A/J mice with Moloney virus (Fenyo et al. 1968). YAC lymphoma cells were carried in ascites fluid by continuous in vivo passage. YAC cells produce high levels of retrovirus particles detected by electron microscopy and by reverse transcriptase assay (100 to 200 pmol/10$^6$ cells).

The LSTRA lymphoid leukaemia line was originally induced in BALB/c mice by murine leukaemia virus (Moloney) and the ascites tumour serially transplanted (Glynn, Halpern & Fefer, 1969). This line is also a producer of Moloney leukaemia virus.

The MSC cell line was established from fibrosarcomas induced in BALB/c mice with Moloney sarcoma virus (Massicot, Woods & Chirigos, 1971).

The CRFK cell line was derived from a normal adult male feline kidney and is non-virus producing. The line was obtained from the Naval Biochemical Research Laboratories, Oakland, California (Crandell, Fabricant & Nelson-Rees, 1973).

FL-74 (also called FLA) is a feline lymphoid leukaemia cell line established in static suspension culture from a solid filtrate of a leukaemic cat's kidney (Theilen et al. 1969). These cultures are infected with and continually produce the FL-74 strain of FeLV detect-
Retrovirus associated cell membrane antigens

able by electron microscopy and reverse transcriptase measurements (100 to 200 pmol/10^6 cells; Sarma & Log, 1973; Sarma et al. 1975; O'Brien & Boone, 1977).

Antisera used

Bovine anti-feline sarcoma virus (FeSV). Bovine anti-FeSV was prepared by infecting a portion of thymic tissue from a young steer with FeSV (ST strain) and inoculating the cells back into the donor. The serum was a gift from Dr T. Kawakami.

Goat anti-FeLV-p27. Goat anti-p27 was prepared by immunizing goats with FeLV-p27 purified by isoelectric focusing. These sera were kindly provided by Drs R. Gilden and S. Oroszlan of Flow Laboratories, Inc. (Oroszlan et al. 1970; Oroszlan, Huebner & Gilden, 1971).

Rat anti-murine sarcoma virus (MSV). Rat anti-MSV was obtained from Fischer rats bearing Moloney sarcoma virus induced tumours.

Goat anti-MuLV. Goat anti-MuLV was prepared by immunizing a goat with tween-ether disrupted Moloney MuLV which had been banded by sucrose density gradient centrifugation.

Goat anti-Rauscher leukaemia virus (RLV)-p30. Goat anti-RLV-p30 was prepared by immunizing goats with Rauscher MuLV-p30 purified by gel filtration in 6 M-guanidine hydrochloride.

Goat anti-RLV-gp70. Goat anti-RLV-gp70 was prepared by immunizing goats with Rauscher MuLV-gp70 purified by gel filtration in 6 M-guanidine hydrochloride.

Each of the antisera against murine viruses and their proteins were kindly provided by Dr Roger Wilsnack of Huntingdon Research Laboratories. IgG fractions from each serum were prepared by precipitation with 33% ammonium sulphate. Two of the IgG preparations (anti-RLV-p30 and rat anti-MSV) were further purified by passage through Sephadex G-150. Binding data obtained with chromatographed IgG were indistinguishable from those obtained with crude ammonium sulphate fractions of the same antisera. Virus proteins (MuLV-p30 and gp70) gave single bands on acrylamide gels and did not cross react in competition radio-immune assays.

Paired label assay for cell surface antigens. Cell surface antigens were quantitatively measured by the paired label assay (Boone, Irving & Rubinstein, 1971; Boone, Gordin & Kawakami, 1973; O'Brien et al. 1976b). Immune and normal IgG from the same animal species were purified and labelled catalytically with 131I and 125I, respectively. A paired label mixture (PLM) consisting of equimolar amounts of normal and immune IgG was absorbed with 10^7 cultured mosquito cells/mg of IgG for 2 h at room temperature to remove non-specific serum binding proteins, and with virus negative cat (CRFK) or mouse (Basc-2) cells to remove species-specific antigens. Target cells were incubated in 1 ml of an appropriate PLM for 30 min at 25°C. The cells were washed three times and counted in dual channels in a gamma spectrometer. Using previously determined specific radioactivities (ct/min/mg protein), the amount (ng) of antibody binding per cell was determined as the difference between immune and normal IgG binding. Normal bovine, goat and rat sera were purchased from Grand Island Biological Company (Grand Island, New York) and tested for reactivity to type C virus proteins before use. The use of labelled normal IgG in the reaction mixture provides an internal control for the specificity of antibody binding.

Radioisotopic foot pad assay. The detection of cell mediated immunity was measured using a radioisotopic foot pad assay (Paranjpe & Boone, 1972; Davis & Boone, 1974). The procedure involves the simultaneous inoculation of antigen in the mouse foot pad and 125I-labelled serum albumin into the peritoneal space. A positive immune reaction results in
accumulation of circulating $^{125}$I-labelled serum albumin in the inflamed spaces in the inoculated foot. Control levels of radioactivity are obtained in the untreated contralateral foot pad. MSV(M) was obtained from tumour extracts of BALB/c mice containing a mixture of Moloney sarcoma virus and leukaemia virus. MSV(M) was provided by the virus cancer program of the National Cancer Institute. Immune and normal mice were inoculated with test cells (antigen) in the foot pad. Immediately thereafter, $^{125}$I-labelled human serum albumin was injected intraperitoneally. Twenty-four hours later the inoculated foot pad and the untreated contralateral control foot pad were cut off and counted in a gamma spectrometer. Results were expressed as the ‘foot count ratio’: ct/min of test foot divided by ct/min of control foot. Specificity of this assay for cellular immunity has been demonstrated previously (Paranjpe & Boone, 1972; Davis & Boone, 1975).

**Studies on tumour immunity.** Male A/J mice were inoculated with $5 \times 10^6$ irradiated YAC, FL-74 or CRFK cells weekly for 3 weeks. Nine days following the last immunization, the mice were challenged with $5 \times 10^4$ viable YAC cells subcutaneously. Twenty days later tumour incidence was determined. The tumours were then surgically removed and their weight determined.

BALB/c mice were immunized intraperitoneally three times at weekly intervals with $10^6$ mitomycin C (20 μg/ml)-treated MSC, FL-74 or CRFK cells. Two weeks after final immunization, animals were injected with $10^7$ f.f.u of MSV in the hind leg. Animals were observed twice weekly and tumour incidence determined.

**RESULTS**

Detection of MuLV and FeLV associated cell surface antigens by the paired label radio-assay

A group of six antisera (described in Methods) were selected for quantitative measurement of the expression of virus structural proteins and virus induced antigens of the cell surface of murine cells infected with MuLV and feline cells infected with FeLV. Three of the sera, bovine anti-FeSV, rat anti-MSV, and goat anti-MuLV were broadly reactive. The three component specific sera were selected from a number of lots of serum preparations on the basis of high titre and lack of specificities to virus proteins other than the indicated component (see below).

The number of antigenic determinants detected by each antiserum on a tested cell was determined from the results of an immune serum titration of a constant cell number in a fixed volume (Fig. 1a, b). Since antibody binding to cell surface antigens conforms to the mass action law, a linear equation was derived which permitted the determination of the maximum amount of antibody molecules which bound per million cells (termed $\beta$ value) (Boone et al. 1971; O'Brien et al. 1976b). By plotting the ng immune IgG bound versus the ng antibody binding/10^6 cells for each of the test systems are tabulated in Table I. The broadly reacting anti-MuLV sera bound to YAC cells in high amounts (325 ng/10^6 cells), as did the monospecific goat anti-gp70 (77 ng/10^6 cells), whereas only small amounts of bound anti-RLV-p30 (14 ng/10^6 cells) were detectable. The broadly reacting bovine anti-FeSV and the anti-FeLV-p27 also bound to FL-74 cells in high concentrations, 400 and 140 ng/10^6 cells respectively. None of the sera reacted significantly with the virus negative control cell lines.
Retrovirus associated cell membrane antigens

Fig. 1. (a) Immune serum titration of FL-74 (○) and YAC (○) cells. Cells (4 \times 10^6) were incubated with increasing concentrations of rat anti-MSV IgG in a paired label mixture in a volume of 1 ml for 30 min, washed and counted as described in Methods. (b) Plot of data from (a) as the ng antibody bound/µg IgG/ml v. ng IgG bound per unit volume (see text). For example, YAC cells bind an extrapolated maximum of 520 ng rat anti-MSV globulin per 4 \times 10^6 cells or 130 ng/10^6 cells. This represents (130 \times 10^{-6} \times 6.023 \times 10^{23})/(1.5 \times 10^{5} \times 10^9) = 5.2 \times 10^{-9} IgG molecules bound per YAC cell. The number of antigenic sites per cell is therefore the same as or up to twice this figure depending upon whether IgG binds mono- or bivalently to the cell surface. In at least one case (anti-RLV-p30 bound to YAC cells, see text), this number approximates bivalent binding since equal mg quantities of IgG and FAB fragments bind equivalently to the same cell (S. O'Brien, unpublished observations). (○) FL-74 cells; (○) YAC cells. (c) Cell titration of FL-74 (FLA) (○), YAC (○), Basc-2 and CRFK (FCO-121) (○). Increasing cell numbers were incubated with a constant PLM concentration (50 µg goat anti FeLV-p27/ml) for 30 min, washed and counted as described in Methods. In regions of antibody excess, the slope of the curve provides an estimate of the binding of antibody on a per 10^6 cell basis (Boone et al. 1971; O'Brien et al. 1976b) for the indicated IgG concentration in the reaction mixture. For example, the goat anti-FeLV-p27 binds 50 ng IgG/10^6 FL-74 cells and 1.4 ng/10^6 YAC cells, while the control cell lines had negligible binding.

Interspecies cross reactivity on infected cell surfaces was detected with anti-MuLV sera (on FL-74 cells) and to a lesser extent with the anti-FeSV sera (on YAC cells). The anti-RLV-gp70 reacted strongly with both YAC and FL-74 cell surfaces. Specific binding of goat anti-RLV-gp70 to YAC cells was eliminated by pre-absorption of the PLM with FL-74 cells, but not by absorption with virus negative mouse or cat cells (data not shown). The interspecies p27-30 determinants detected by anti-RLV-p30 on the FL-74 surface represented only 6% of the total p27 accessible to the binding of the group-specific anti-FeLV-p27 serum. Another mouse cell (RAG) which produces an endogenous B-tropic type C virus (O'Brien et al. 1976a) bound three times more anti-RLV-p30 than did YAC cells. These RAG cells produced only about 20% of the virus levels (assayed by RNA-dependent DNA synthesis)
Table 1. Maximum ng immune IgG bound/10^6 cells/ml (β values)* in immune serum titrations

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>FL-74</th>
<th>YAC</th>
<th>Basc-2</th>
<th>CRFK</th>
<th>RAG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat MSV</td>
<td>45 (1.8)</td>
<td>130 (5.2)</td>
<td>1 (0.04)</td>
<td>0.0 (0.0)</td>
<td>-</td>
</tr>
<tr>
<td>Goat MuLV</td>
<td>45 (1.8)</td>
<td>325 (13.0)</td>
<td>0.0 (0.04)</td>
<td>0.0 (0.0)</td>
<td>-</td>
</tr>
<tr>
<td>Goat RLV-p30</td>
<td>9 (0.36)</td>
<td>14 (0.56)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>47.3 (1.9)</td>
</tr>
<tr>
<td>Goat RLV-gp70</td>
<td>187 (7.5)</td>
<td>77 (3.1)</td>
<td>0.0 (0.0)</td>
<td>0.0 (0.0)</td>
<td>-</td>
</tr>
<tr>
<td>Bovine FeSV</td>
<td>400 (16.1)</td>
<td>≥ 10 (0.4)</td>
<td>1.1 (0.04)</td>
<td>0.0 (0.0)</td>
<td>-</td>
</tr>
<tr>
<td>Goat FeLV-p27</td>
<td>140 (5.6)</td>
<td>13.7 (0.53)</td>
<td>0.1 (0.004)</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

* Determined from immune serum titrations as illustrated in Fig. 1 (a, b). β values are expressed in ng/10^6 cells. Numbers in parentheses are number of IgG molecules bound per cell × 10^-5. Values are averages of two to five titrations.

† Control values of 0.0 represent experimental situations where the 125I-labelled non-immune serum has a slightly greater affinity for the target cell than does the 125I-labelled antiserum. This variation is non-specific and varies with the cell and the serum. This variation also occurs in favour of the antiserum. Since the maximum deviation in the control cell below 0 was approximately −2 ng, we arbitrarily discount any binding below 2 ng as being specific.

‡ This value represents a minimum value since the titration remained in extreme antigen excess.

Table 2. Immune IgG bound (ng)/10^6 cells/ml in cell titrations*

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>YAC</th>
<th>RAG</th>
<th>A-9</th>
<th>Basc-2</th>
<th>FL-74</th>
<th>CRFK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat MSV</td>
<td>18</td>
<td>80</td>
<td>5.1</td>
<td>0.0</td>
<td>6.6</td>
<td>0.0</td>
</tr>
<tr>
<td>Goat MuLV</td>
<td>26.5</td>
<td>370</td>
<td>31</td>
<td>-</td>
<td>8.5</td>
<td>0.0</td>
</tr>
<tr>
<td>Goat RLV-p30</td>
<td>11</td>
<td>42.4</td>
<td>14.5</td>
<td>0.02</td>
<td>1.3</td>
<td>0.0</td>
</tr>
<tr>
<td>Goat RLV-gp70</td>
<td>19</td>
<td>15</td>
<td>23</td>
<td>0.6</td>
<td>15</td>
<td>1.0</td>
</tr>
<tr>
<td>Bovine FeSV</td>
<td>1.6</td>
<td>8.9</td>
<td>6.9</td>
<td>-</td>
<td>94.7</td>
<td>0.0</td>
</tr>
<tr>
<td>Goat FeLV-p27</td>
<td>1.4</td>
<td>43</td>
<td>36</td>
<td>0.5</td>
<td>50</td>
<td>0.5</td>
</tr>
</tbody>
</table>

* Cell titrations were carried out at an immune IgG concentration of 50 μg/ml as described in Methods. Values are averages of two to six titrations. Each result was determined as illustrated in Fig. 1 (c).

The above data were verified using cell titration studies (Fig. 1 c). In these experiments, immune IgG protein concentration (50 μg/ml) of each of the six antisera was held constant while cell concentrations were varied. The initial slope of a plot of ng IgG bound vs cell concentration provides an estimate of the maximum IgG bound/10^6 cells at the stated immune serum concentration (O'Brien et al. 1976a, b). The number obtained by this plot may be lower than the β value obtained from immune serum titrations by a constant factor that depends on the antibody concentration and the equilibrium constant of the reaction between surface sites and antibodies. Since the antibodies tested may be multivalent and therefore elicit multiple antibody specificities, the results of cell titrations (Table 2) are best interpreted as sensitive qualitative estimates of antigen expression. In addition to confirming the conclusions derived from Table 1, the cell titration experiments detected strong interspecies p30 determinants on the murine RAG and A-9 cell surfaces. The detection of interspecies determinants of FeLV-p27 on FL-74 cells is evident from the immune serum titration (Table 1), although the cell titration was not sensitive enough to reveal them.

The cell titrations with the goat anti-RLV-gp70 revealed significant reactivity on virus infected feline and murine cells. Only RAG cells failed to bind anti-gp70 despite the higher
Table 3. Absorption of virus antisera with virus components

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Target cell</th>
<th>Absorbent</th>
<th>% Unabsorbed antibody binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goat FeLV-p27</td>
<td>FL-74</td>
<td>FeLV-p27</td>
<td>≤ 15‡</td>
</tr>
<tr>
<td>Bovine FeSV</td>
<td>FL-74</td>
<td>FeLV-p27</td>
<td>60</td>
</tr>
<tr>
<td>Bovine FeSV</td>
<td>FL-74</td>
<td>intact FeLV†</td>
<td>30</td>
</tr>
<tr>
<td>Bovine FeSV</td>
<td>FL-74</td>
<td>sonicated FeLV†</td>
<td>≤ 5‡</td>
</tr>
<tr>
<td>Goat RLV-p30</td>
<td>YAC</td>
<td>MuLV-p30</td>
<td>≤ 17‡</td>
</tr>
<tr>
<td>Goat RLV-p30</td>
<td>RAG</td>
<td>MuLV-p30</td>
<td>≤ 35‡</td>
</tr>
<tr>
<td>Goat RLV-p30</td>
<td>YAC</td>
<td>MuLV-p70</td>
<td>100</td>
</tr>
<tr>
<td>Goat RLV-gp70</td>
<td>YAC</td>
<td>MuLV-gp70</td>
<td>120</td>
</tr>
<tr>
<td>Goat RLV-gp70</td>
<td>A-9</td>
<td>MuLV-gp70</td>
<td>200</td>
</tr>
<tr>
<td>Goat RLV-gp70</td>
<td>LSTRA</td>
<td>MuLV-gp70</td>
<td>400</td>
</tr>
<tr>
<td>Rat MSV</td>
<td>YAC</td>
<td>MuLV-p30</td>
<td>100</td>
</tr>
<tr>
<td>Rat MSV</td>
<td>FL-74</td>
<td>MuLV-p30</td>
<td>100</td>
</tr>
<tr>
<td>Goat RLV-gp70</td>
<td>YAC</td>
<td>sonicated YAC MuLV</td>
<td>≤ 12</td>
</tr>
<tr>
<td>Goat RLV-gp70</td>
<td>YAC</td>
<td>sonicated A-9 MuLV</td>
<td>≤ 12</td>
</tr>
<tr>
<td>Goat RLV-gp70</td>
<td>YAC</td>
<td>intact YAC MuLV</td>
<td>≤ 14</td>
</tr>
</tbody>
</table>

* Immune IgG (25 μg) in a paired label mix was incubated with increasing amounts of the absorbing virus component (up to 10 μg p30, p27 or gp70) in 0.7 ml PBS at 4°C. After 4 h, 0.1 ml foetal bovine serum and 2 × 10⁵ target cells were added to the mixture and tested for cell membrane binding as described in Methods. Values are plateau levels of maximum absorption.

‡ Purified FeLV was obtained from high speed pellets of filtered (Millipore 0.45 μm) culture fluid from 23 h log phase FL-74 cultures. Virus pellets from 3 × 10⁶ FL-74 cells were suspended in 0.7 ml PBS to which was added 0.2 ml of anti-FeSV PLM.

‡ These values were the lowest observed but the absorption curves were still declining at the maximum concentration of absorbent.

levels of p30 detected on this same cell (Table 2). Since the anti-gp70 sera bound both virus infected mouse cells and FL-74 cells, the RAG cell must lack both group and interspecies gp70 determinants on their cell surface. The possibility that group-specific region(s) of cell membrane bound gp70 were relatively inaccessible has been suggested (Obata et al. 1975; Tung et al. 1975). The binding of anti-gp70 to A-9 cells in this study suggested that the A-9 virus contains gp70 determinants that are antigenically related to RLV-type gp70. Immunological relatedness between the A-9 virus and Moloney virus has been demonstrated by immunofluorescence with anti-Moloney leukaemia virus (Fenyo, Nordenskjold & Klein, 1971; Fenyo et al. 1973). In the absence of type-specific antisera for the RAG and A-9 virus glycoproteins, we can only state that both YAC and A-9 cells express a gp70 antigen on their cell membranes which is detectable by anti-RLV-gp70. This antigen is either absent or inaccessible to antibody on the RAG cell surface.

Specificity of MuLV and FeLV antisera

Specificity of the test antisera was demonstrated by a quantitative absorption of various labelled antisera with purified structural protein, with intact virus particles, or with disrupted virions prior to the exposure of the PLM to a positive target cell (Table 3). The monospecific antisera were absorbed with proteins prepared by a procedure (isoelectric focusing or gel filtration) different from the procedure used to purify the immunogen used in serum preparation.

Absorption of the respective mono-specific antisera with FeLV-p27 and RLV-p30 caused a large reduction in cell surface binding. There was no reduction of binding of the p30 antiserum by pre-absorption to gp70 or vice-versa (anti-gp70 absorbed with p30).
The absorption of anti-gp70 with purified gp70 resulted in an enhancement of anti-gp70 binding to MuLV infected cells. This finding may be due either to the fact that the gp70 is polyvalent and formed aggregates with antibodies that bound to the cell surface through bivalent antibody linkages, or that some of the purified gp70 became bound to available surface receptors for virus attachment (DeLarco & Todaro, 1976). If gp70 antigenic determinants remain accessible to antibody binding when the glycoprotein is bound to cellular receptors, then an enhancement of antibody binding would be observed. Passive absorption experiments with MuLV-gp70 on virus negative cells in our laboratory (S. J. O'Brien, unpublished observations) and elsewhere (DeLarco & Todaro, 1976) have demonstrated the binding of considerable quantities of antigenically active gp70 on cell surfaces. Two other virus producing cells, A-9 and LSTRA, also exhibit this effect to an even greater extent with purified gp70 and anti-gp70 IgG but not with other absorbants (Table 3). The effect was specific for gp70 since serum proteins or RLV-p30 result in no increase in binding of anti-gp70 IgG.

The bovine FeLV antiserum was only partially reduced in its binding to FL-74 cell surfaces by absorption to FeLV-p27. Absorption of this same antiserum with sonicated FeLV particles completely abolished binding to FL-74 cell surfaces suggesting that the majority of residual antibodies are specific for virion proteins. We cannot, however, exclude any non-virion antigens which might have co-purified in membrane vesicles with FeLV. The MSV antiserum contained no detectable p30 specificities since absorption with purified MuLV-p30 failed to reduce binding to YAC or FL-74 cells.

Antibody blocking experiments as a measure of shared specificities and topographical association of virus antigens

Cells were incubated for 60 min with increasing quantities of an unlabelled antiserum, washed and tested for binding using a PLM of a second antiserum (Table 4). The gp70 and the p30 antisera were effective in blocking themselves but did not reduce each other’s binding to MuLV infected YAC cells. The FeSV antiserum was blocked partially by anti-FeLV-p27, again consistent with the presence of multiple specificities in the FeSV antisera including anti-p27. Binding of the MSV serum to YAC and FL-74 cells was reduced by binding of FeSV serum but not by either the anti-p30 or anti-gp70 sera, suggesting that the majority of specificities of the MSV sera were neither p30 or gp70 specific. The reciprocal experiment, however, revealed that unlabelled MSV serum did partially reduce the binding of labelled anti-p30 and anti-gp70 to YAC cells. This block may reflect proximity of the cell surface p30 and gp70 antigens to a third MSV antigen. When two antigens of different specificities are located in close proximity on the cell surface, the binding of antibody to one can impede the subsequent absorption of antibody by the other (Boyse, Old & Stockert, 1968). This explanation is supported by the paucity of p30 specificities found in the MSV antiserum (Table 3).

Presence of virus antigens in addition to p30 and gp70 on infected cell surfaces

The immune serum titrations, absorption experiments, and antibody competition experiments with the broad specificity antisera suggest that additional virus structural components are also expressed on infected cell surfaces. The maximum binding of anti-FeSV to FL-74 cells in this study was \(1.6 \times 10^6\) binding sites. This estimation represents twice the number of p30 plus gp70 determinants calculated to be on FL-74 cells using the same methodology (Table 1). Similarly, rat anti-MSV serum detected \(5.2 \times 10^5\) determinants per YAC cell, very little of which is detected by the p30 or gp70 sera (Table 1, 3 and 4).
Table 4. Reduction in antibody binding to YAC and FL-74 cell surfaces by pre-incubation of cells with different unlabelled antisera (% antibodies blocked in specific binding to target cell membrane)*

<table>
<thead>
<tr>
<th>Test antiserum</th>
<th>Target cell</th>
<th>MSV</th>
<th>RLV-p30</th>
<th>RLV gp-70</th>
<th>FeSV</th>
<th>FeLV-p27</th>
<th>Homologous non-immune serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat MSV</td>
<td>YAC</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>39</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>FL-74</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>80</td>
<td>32</td>
<td>0</td>
</tr>
<tr>
<td>Goat MuLV-p30</td>
<td>YAC</td>
<td>57</td>
<td>100</td>
<td>≤ 10</td>
<td>–</td>
<td>–</td>
<td>0</td>
</tr>
<tr>
<td>Goat MuLV-gp70</td>
<td>YAC</td>
<td>75</td>
<td>0</td>
<td>100</td>
<td>54</td>
<td>≤ 10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>FL-74</td>
<td>0</td>
<td>≤ 29</td>
<td>100</td>
<td>–</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bovine FeSV</td>
<td>FL-74</td>
<td>25</td>
<td>–</td>
<td>–</td>
<td>100</td>
<td>44</td>
<td>0</td>
</tr>
</tbody>
</table>

* Cells (5 × 10⁶) were incubated in 0.7 ml PBS containing increasing amounts (up to 14 mg) of unlabelled blocking IgG for 60 min at 25 °C. At the end of the incubation period 0.1 ml of heat inactivated bovine serum and 0.2 ml of a PLM mix (20 μg immune IgG) were added to each tube. After 30 min incubation the cells were washed and counted. The μg IgG bound were plotted v. μg unlabelled blocking serum and the maximum reduction in unblocked IgG binding was determined.

Interspecies cross reaction of MSV antigen(s) which induced cell mediated immune response

The ability of interspecies cross reacting virus associated cell membrane antigens to elicit a cellular immune response in vivo was examined by the radioisotopic foot pad assay. BALB/c mice were injected with MSV. Tumour bearing mice were selected as immune target animals at 14 to 16 days following infection, a time empirically determined to be the optimum for MSV cellular immunity. MSV immune mice were challenged in the foot pad with 10⁶ YAC, FL-74, and CRFK cells (Table 5). By this assay no significant cell mediated immune reaction was detected when FeLV infected cat cells and the virus negative CRFK cells were used as antigens. Murine virus infected YAC cells elicited a significant positive reaction.

Tumour associated transplantation antigens

The ability of cat cells infected with FeLV to serve as immunogens against tumour producing doses of Moloney sarcoma virus was examined with a tumorigenicity monitor (Fig. 2). Immunization of BALB/c mice with syngeneic MSC cells infected with MSV was absolutely protective against MSV challenge, but three immunizations of 10⁶ FL-74 cells, like virus negative feline cells, failed to protect the mice from MSV derived tumours.

A second experimental approach to detect interspecies virus associated tumour protection was performed using YAC and FL-74 cells as immunogens while challenging with YAC cells (Table 6). The YAC cell expresses a weak tumour transplantation antigen (Fenyo et al. 1971), which is not effective in preventing tumour growth induced by YAC cells. Immune reaction is, however, reflected in diminution of tumour size by weight (Table 6). The xenogeneic immunization with FL-74 cells failed to reveal any protective interspecies antigens between FeLV and MuLV since the mice immunized with FL-74 cells produced tumours of the same size as the control mice which had been immunized with normal cat cells or saline.
Table 5. Foot pad response of MSV-induced tumour bearing mice to YAC, FL-74 and CRFK cells*

<table>
<thead>
<tr>
<th>Inoculum (10^8 cells)</th>
<th>Status of mice</th>
<th>Foot pad ratio ± s.e.</th>
<th>P value §</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRFK Normal</td>
<td>1.22 ± 0.09 (7)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>CRFK MSV-infected</td>
<td>1.20 ± 0.06 (10)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>FL-74 Normal</td>
<td>1.30 ± 0.11 (10)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>FL-74 MSV-infected</td>
<td>1.47 ± 0.12 (15)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>YAC Normal</td>
<td>1.29 ± 0.05 (15)</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>YAC MSV-infected</td>
<td>1.98 ± 0.14 (15)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mice were inoculated in the foot pad with 10^8 cells on day 14 following infection with MSV.
† Ct/min of injected foot divided by ct/min of control foot.
‡ Numbers in parentheses indicate number of animals tested.
§ Student's t-test; NS = not significant when compared to the respective control.

Table 6. Tumour incidence and tumour weight of YAC-induced tumours in immunized strain A/J mice*

<table>
<thead>
<tr>
<th>Immunizing cell</th>
<th>No. of immunizations</th>
<th>Tumour incidence (%)</th>
<th>Average tumour weight ± s.e.</th>
<th>P value ‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 x 10^5 YAC</td>
<td>3</td>
<td>21/24 (87)</td>
<td>4.1 ± 0.48</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>5 x 10^5 FL-74</td>
<td>3</td>
<td>16/19 (86)</td>
<td>7.1 ± 0.7</td>
<td>NS</td>
</tr>
<tr>
<td>5 x 10^5 CRFK</td>
<td>3</td>
<td>27/27 (100)</td>
<td>7.23 ± 0.27</td>
<td>NS</td>
</tr>
<tr>
<td>PBS</td>
<td>3</td>
<td>30/30 (100)</td>
<td>6.2 ± 0.43</td>
<td></td>
</tr>
</tbody>
</table>

* Male strain A/J mice were inoculated three times with X-irradiated cells at 7 day intervals. Nine days after the third immunization, the mice were challenged with 5 x 10^4 viable YAC tumour cells injected subcutaneously.
‡ Twenty-eight days following challenge, the tumours were surgically dissected and their weight was determined.
§ Student's t-test of mean tumour weights; NS = not significant when compared to non-immune control.

Fig. 2. Incidence of tumour induction by MSV in mice immunized with MSC (©), FL-74 (△), CRFK (●) and phosphate-buffered saline (○). BALB/c mice were immunized three times with mitomycin C-treated cells as described in Methods and challenged with 10^7 f.f.u. of MSV in the hind leg.
Retrovirus associated cell membrane antigens

DISCUSSION

This study illustrates that a number of immunologically detectable virion components are expressed on murine and feline cell membranes of type C-virus producing cells. Antigens found on both the major internal structural protein p27-30 and the virus envelope glycoprotein gp70 are found in large amounts on MuLV and FeLV infected cells. Interspecies and intraspecies determinants of these two proteins are detectable by both monospecific and broadly reactive antisera. These results are in agreement with several reports (Yoshiki, Mellors & Hardy, 1973; Grant et al. 1974; Yoshiki et al. 1974; O'Brien et al. 1976a) of p30 on cell surfaces of virus producer and non-producer cells of mice and man.

In several recent reports (Oskarsson et al. 1975; Tung, Yoshiki & Fleissner, 1976; Ledbetter, Nowinski & Emery, 1977) the presence of p30 on MuLV producing cells has been associated with large gag gene polyproteins of mol. wt. 85,000 and 95,000, designated gp85gag and gp95gag. The polyproteins contain not only p30 determinants but also p10 and p12 determinants in the case of gp95gag while gp85gag has only p30 and p12. The polyproteins are glycosylated, and do not chase into the virion particles. It is possible that the cell surface p30 specificities reported in these studies may also reside on unprocessed gag region polyproteins. In light of the finding of correlative appearance of leukaemia and gp85gag/gp95gag on pre-leukaemia AKR thymocytes (Tung et al. 1976), the fluctuating p30 levels observed in the various lines studied here may reflect different stages of leukaemic potential as well as virogene expression.

The finding of gp70 on virus producing cells is predicted since this glycoprotein is the major surface component of budding virions. This glycoprotein is also found on the cell surface of a number of non-virus producing normal mouse tissues (Lerner et al. 1976) and has been identified as the carrier of the G1x type-specific cell surface antigen on G1x+ mouse thymocyte cell surfaces (Obata et al. 1975; Tung et al. 1975).

The amounts of both p30 and gp70 detected on various cell types varied considerably from cell to cell and did not correlate with any other virus antigen or virus production itself. These variations may reflect different levels of passive absorption, divergent cell surface architecture or different turnover of virus antigenic components in various cell types.

A number of additional virion structural proteins have been detected on surfaces of retrovirus infected cells in our laboratory and elsewhere. These include the smaller gag region proteins p10, p12 and p15 and the lower mol. wt. envelope glycoprotein p15e (Grant et al. 1974; Fenyo & Klein, 1976; Lerner et al. 1976; Tung et al. 1976; W. H. Jarrett, personal communication). The feline oncornavirus cell membrane antigen (FOCMA) has also been detected on cell surfaces of feline lymphoid lines including FL-74 (Essex, 1975; Sliski et al. 1977). There is indirect evidence that FOCMA is host specified but virus induced, in an analogous manner to the avian TSSA induced by avian sarcoma virus (Kurth & Bauer, 1973; Essex, 1975). An analogous antigen (MCSA) has been detected on YAC cell surfaces. MCSA does not cross react with any virus structural proteins and its presence on YAC cell surfaces is positively correlated with immunogenicity and tumour rejection sensitivity (Fenyo & Klein, 1976). The relative contribution of each of these virion and non-virion antigens to the total antigen deposition described here by the broad specificity antisera is presently under investigation.

The failure to detect cell mediated immunity against FeLV infected cells in MSV immune mice suggests that the interspecies determinants detected by antibody procedures on FL-74
infected cell surfaces are not of major importance in cell mediated immunogenicity of MSV immune mice. It is difficult to exclude, however, the possibility that a weak immune reaction falls below the sensitivity of the radioisotopic foot pad assay. The inability of FL-74 cells to xenogeneically immunize mice also suggests that the surface associated interspecies antigens are not functionally immunogenic under conditions where syngeneic immunizations are effective. This observation may reflect the failure of ecotropic FeLV to infect and proliferate in heterologous murine cells while Moloney sarcoma and leukaemia viruses are capable of proliferation in the test animal and the subsequent production of additional immunogenic viruses and virion induced antigens.

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REFERENCES


Retrovirus associated cell membrane antigens


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