Expression of Murine Leukemia Virus Structural Antigens on the Surface of Chemically Induced Murine Sarcomas

(radioimmunoassay/humoral cytotoxicity/membrane immunofluorescence/viral antigens/tumor antigens)

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ABSTRACT Cultured cells of different chemically-induced C57BL/6N murine sarcomas produced variable amounts of infectious murine leukemia virus (MuLV) and contained proportional amounts of MuLV structural components as determined by radioimmunoassay. Monospecific antisera directed against the major MuLV glycoprotein (gp11), the major internal antigen (p30), and the ribonucleoprotein (p10) were capable of mediating tumor cell lysis in the presence of complement, suggesting that these viral structural components were localized at least in part to the cell surface. Membrane immunofluorescence studies with MuLV p30 antisera confirmed surface localization. Addition of MuLV p30 polypeptide to normal cells and tumor cells enhanced the cytotoxicity of MuLV p30 antisera. Studies are presented which suggest that the presence of MuLV structural components on cell surfaces can be independent of virus production and cellular transformation.

Genetic material of murine leukemia virus (MuLV) is contained in the DNA of most mouse cells. In vivo activation of endogenous MuLV occurs spontaneously in strains with a high incidence of leukemia or occurs following exposure to chemical or physical carcinogens in strains with a low incidence of spontaneous leukemia. Similar virus activation can occur in cultured murine cells spontaneously or following addition of DNA base analogues (for review see refs. 1 and 2).

The biological significance of endogenous MuLV genetic information is under study. Our effort has been concerned with changes in cell surface membranes. In addition to virus-induced neoantigens (3), and possibly expression of fetal antigens (4), it has been reported that a major internal MuLV polypeptide (designated p30) is also expressed on the surface of cells that replicate activated MuLV (5). The mechanism whereby this polypeptide appears on the cell surface and its significance are not clear.

Recent work in this and other laboratories suggests that antigenic similarities exist between different chemically-induced syngeneic murine tumors (6–8). Such similarities might be due to common tumor associated surface antigens, reexpression of fetal antigens, or common antigens arising from activation of endogenous viruses. Since chemical carcinogens can activate MuLV, it was important to determine if our chemically induced tumors expressed MuLV structural antigens on their surface membranes and if these antigens participated in the host's immunological response to the tumors.

MATERIALS AND METHODS

Cultured Cell Lines. Six C57BL/6N murine sarcomas (designated MCA-4,5,6,10,11, and 18) induced by 3-methylcholanthrene (MCA) and normal 18-day-old C57BL/6N embryonic fibroblasts (NEF) were explanted into cell culture. S*L-m3 transformed cells (9,10) and the nontransformed spontaneous revertant designated mSR (11) were maintained as continuous culture lines.

Eve cells, derived from MuLV (Friend) infected STU mice, were kindly provided by Professor Werner Schäfer, Max-Planck-Institut, Tübingen, Germany.

BALB/3T3 embryonic fibroblasts (12) and a Kirsten murine sarcoma virus transformed BALB/3T3 line designated K234 (13) were generously provided by Dr. Stuart Aaronson, Hazleton Laboratories, Virginia.

Viruses Assays. Aliquots of media from 24-hr incubations of 75% confluent monolayer cell cultures were assayed for standard strains of MuLV in the cloned FG-10 subline of murine S*L- cells (9,14). The presence of xenotropic MuLV (15) was determined by the technique of Fischinger et al. (16).

Isolation of Viral Structural Polypeptides. The isolation of internal polypeptides of Friend MuLV, Rickard feline leukemia virus (FeLV), and avian myeloblastosis virus (AMV) has been described (17). Friend MuLV surface glycoprotein (gp71) was obtained from W. Schäfer (18).

Antisera. Rabbit antisera to MuLV polypeptides (p10, p12, and p30) and to FeLV p30 were prepared (17). MuLV glycoprotein gp71 antisera was kindly provided by Professor W. Schäfer. Murine MCA-10 tumor antisera was obtained from mice 10 days after removal of transplanted, growing tumors. Control serum was obtained from age-matched C57BL/6N mice.

Absorption of Antisera. Nonspecific reactivity of rabbit sera with murine cells was removed by absorption with Fischer 344 rat fibroblasts which did not contain MuLV or its structural antigens. In addition, MuLV and FeLV p30 antisera and preimmune rabbit serum were absorbed with purified MuLV and FeLV p30 polypeptides, AMV p27 polypeptide, and MCA-10 tumor cells. Normal and anti-MCA-10 murine serum were absorbed with MuLV p30 polypeptide or MCA-10 tumor cells.

Radioimmunoassays of MuLV Polypeptides. Radioimmunoassays were performed as previously described (19,20). The 50% endpoint serum titer for competition was determined by direct double antibody radioimmunoassay (19). Cells were sonicated in 0.5 M NaCl with 1% Triton-X. Aliquots were used in competition for antibody with labeled MuLV polypeptides. Results were expressed on the basis of similar com-

Abbreviations: AMV, avian myeloblastosis virus; FeLV, feline leukemia virus—Rickard strain; FI, fluorescent index; FIU, focus inducing unit; MCA, 3-methylcholanthrene; MuLV, murine leukemia virus—Friend strain; NEF, normal embryonic fibroblasts.
petition by measured amounts of unlabeled polypeptides. The test could detect about 2 ng of polypeptide.

Membrane Immunofluorescence. Indirect membrane immunofluorescence assays were done with mechanically dispersed suspensions of viable cells in microtiter plates (21, 22). Cells were examined with a Zeiss Universal microscope under darkfield ultraviolet illumination with BG38 exciter, KP500 interference, and 47 barrier filters. A fluorescence index (FI) for undiluted antiserum was calculated as follows:

$$FI = 100 \times \frac{(A - B)}{B}$$

where A is the percentage of non-fluorescing cells with preimmune serum and B is the percentage of non-fluorescing cells with antiserum. All FI ≥ 30 were significant when based on the percentage of non-fluorescing cells with preimmune serum (82 ± 12%) observed in 42 consecutive determinations.

Humoral Cytotoxicity Assay. Details of the humoral cytotoxicity assay in which 125I-labeled 5-iodo-2'-deoxyuridine (Amersham/Searle) is used as a target cell label have been described (23). The cytotoxicity of immune serum relative to normal serum against various target cells was calculated as follows:

$$\text{Percent Cytotoxicity} = 100 \times \frac{\text{cpm obtained with normal serum} - \text{cpm obtained with immune serum}}{\text{cpm obtained with normal serum}}$$

All cytotoxicities ≥ 20% were statistically significant by the Student t test.

RESULTS

MuLV p30 Antigen and Infectious MuLV in MCA-Induced Tumors. The presence of MuLV p30 in chemically-induced tumor, infected cells, and uninfected cells was determined by double antibody radioimmunoprecipitation. The values for the six MCA-induced tumors (Table 1) ranged from low levels (MCA-4 and MCA-18) to amounts similar to those contained in the virus producing Evefline cells (MCA-6 and MCA-10). Normal mouse embryo fibroblasts (NEF) demonstrated barely detectable levels of p30. High infectious virus production was associated with p30 expression but no production, even of xenotropic virus, was demonstrable in lines with low to barely detectable p30 (Table 1).

Cell Lysis in the Presence of Specific MuLV p30 Antiserum. To determine whether MuLV p30 expression might result in cell lysis in the presence of specific antiserum, we assayed microcytotoxicity by using MuLV p30 rabbit antiserum with the six MCA-induced tumor lines and NEF. Complement dependent cytotoxicity occurred with five of the six tumors, whereas, MCA-18 and NEF cells, with low to barely detectable MuLV p30, were not lysed. Comparison of Fig. 1 and Table 1 indicates that cytotoxicity correlated with the radioimmunoassays of p30.

Localization of MuLV p30 on the Cell Surface. Tumor cell lysis by MuLV p30 antiserum suggested that at least part of the MuLV p30 present was accessible to antibody and presumably on the cell surface. To substantiate this, indirect fluorescent antibody assays were performed using MuLV p30 antiserum and fluorescein-labeled anti-rabbit IgG.

Table 1. Presence of MuLV p30 in MCA-induced murine sarcomas, NEF, and Evefline cells and the production of infectious MuLV

<table>
<thead>
<tr>
<th>Cell line</th>
<th>MuLV p30 (ng per 10⁶ cells)*</th>
<th>Mouse tropic MuLV (F/U/10⁶ cells per 24 hr)†</th>
<th>Xenotropic MuLV (F/U/10⁶ cells per 24 hr)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCA-4</td>
<td>5</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>MCA-5</td>
<td>70</td>
<td>1 × 10⁴</td>
<td>&lt;1</td>
</tr>
<tr>
<td>MCA-6</td>
<td>90</td>
<td>2 × 10⁴</td>
<td>&lt;1</td>
</tr>
<tr>
<td>MCA-10</td>
<td>150</td>
<td>8 × 10⁴</td>
<td>&lt;1</td>
</tr>
<tr>
<td>MCA-11</td>
<td>10</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>MCA-18</td>
<td>5</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>NEF</td>
<td>~2</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Evefline</td>
<td>180</td>
<td>6 × 10⁴</td>
<td>NT</td>
</tr>
</tbody>
</table>

* Measured by competition radioimmunoprecipitation after cell sonication. Cells were collected from 75% confluent monolayer cultures by brief trypsinization.
† The aliquots of media after 24 hr of incubation of 75% confluent monolayer cell cultures were assayed for infectious tropic MuLV by the technique of Bassin et al. (14).
‡ Media aliquots as above were assayed for xenotropic MuLV using the technique of Fischinger et al. (16).
NT, not tested.

Significant membrane immunofluorescence occurred with MuLV-producing Evefline cells (FI = 83) and MCA-10 cells (FI = 75), but not with MCA-18 or NEF (Fig. 2). Patterns of fluorescent-staining included concentric ring fluorescence, partial ring staining, and patch formation (Fig. 3). Yoshiki et al. (5) observed capping in their studies and suggested that MuLV p30 was an integral membrane component. Although we observed cap formation, the amount and degree was unremarkable under routine conditions or when reactions were carried out at 4° with subsequent warming to 37°.

Presence of Other MuLV Structural Proteins on the Cell Surface. MCA-10 tumor cells were found by radioimmunoprecipitation to contain other MuLV structural polypeptides (p10, p12, and p15) and the major surface glycoprotein (gp71) (Table 2). Further, MCA-10 cells were significantly lysed by gp71 antiserum and to a lesser extent by p10 antiserum suggesting that these polypeptides, like p30, were associated with the cell surface.

In contrast, NEF cells were found to have only barely detectable levels of p10, p12, p15, and p30 by radioimmuno-
assay, but contained appreciable amounts of gp71 and were very sensitive to gp71 antiserum (Table 2). This suggests that gp71, a portion thereof, or a very similar molecule, can be expressed in the absence of other MuLV polypeptides.

Specificity of MuLV p30 Antiserum. The specificity of membrane immunofluorescence for MuLV p30 was shown by reduction of the FI for MCA-10 cells and MuLV p30 antiserum from 65 to 18 after four sequential absorptions of antiserum with 320 ng of MuLV p30 but not with the immunologically distinct AMV p27 (FI = 65). Furthermore, membrane-staining was abolished after absorption of MuLV p30 antiserum with 10^4 MCA-10 cells but not with 10^7 NEF cells.

Cytotoxicity of MuLV p30 antiserum diluted 1:200 against MCA-5, MCA-6, and MCA-10 was completely absorbed by MuLV p30 (Table 3). In contrast, no absorption of activity against MCA-10 cells was observed with AMV p27 (not shown). Absorption with MCA-10 tumor cells removed all cytotoxic activity against MCA-6 and MCA-10 cells.

Intraspecies and Interspecies Antigenic Determinants in Cytotoxicity of MuLV p30 Antiserum. Antiserum against FeLV p30, which shares only interspecies antigens with MuLV p30, demonstrated low but significant cytotoxicity against MCA-6 and MCA-10 cells (37% and 28% respectively at 1:100 serum dilution) but not against MCA-11, MCA-18, or NEF. Absorption of MuLV p30 antiserum diluted 1:200 with purified FeLV p30 only partially removed cytotoxicity against MCA-6 and MCA-10 (Table 3). This is consistent with the relative proportions of interspecies and interspecies antigenic determinants of MuLV p30 (24). These findings suggested that both determinants of MuLV p30 were expressed by MCA-6 and MCA-10 cells and indicated that each determinant was accessible to antibody. The same conclusion was reached by Yoshiki et al. (5).

Mechanism Whereby Internal MuLV Structural Components Appear on the Cell Surface. The presence of MuLV structural polypeptides on murine cell surfaces might be related to cellular transformation, viral morphogenesis, to passive settlement on cell surfaces of free MuLV polypeptides from disrupted virus particles or cells, or expression of MuLV genetic information not necessarily associated with virus morphogenesis.

The role of cellular transformation and virus synthesis on cell surface p30 expression was studied in four well characterized cell lines that express MuLV genetic information in various

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**Table 2. Presence of other MuLV structural components in MCA-10 and NEF cells and cytotoxicity of specific antisera**

<table>
<thead>
<tr>
<th>MuLV structural component</th>
<th>MCA-10</th>
<th>NEF</th>
</tr>
</thead>
<tbody>
<tr>
<td>gp71</td>
<td>30</td>
<td>1:333</td>
</tr>
<tr>
<td>p30</td>
<td>150</td>
<td>1:870</td>
</tr>
<tr>
<td>p15</td>
<td>15</td>
<td>NTox</td>
</tr>
<tr>
<td>p12</td>
<td>28</td>
<td>NTox</td>
</tr>
<tr>
<td>p10</td>
<td>20</td>
<td>1:26</td>
</tr>
</tbody>
</table>

* Determined by radioimmunoassay.
† Reported as the highest dilution of specific antiserum directed against MuLV structural component giving 50% cell lysis when compared to similarly diluted normal rabbit serum in microcytotoxicity assays.
NTox, not toxic.

**Table 3. Cytotoxicity of MuLV p30 antiserum against three MCA-induced murine tumor cell lines after absorption with MuLV p30 and FeLV p30 polypeptides and MCA-10 tumor cells**

<table>
<thead>
<tr>
<th>Tumor lines</th>
<th>Absorbed with MuLV p30*</th>
<th>Absorbed with FeLV p30†</th>
<th>Absorbed with MCA-10‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCA-5</td>
<td>77</td>
<td>0</td>
<td>NT</td>
</tr>
<tr>
<td>MCA-6</td>
<td>77</td>
<td>0</td>
<td>57</td>
</tr>
<tr>
<td>MCA-10</td>
<td>71</td>
<td>0</td>
<td>43</td>
</tr>
</tbody>
</table>

* Diluted 1:200.
† 1 cm^2 diluted serum absorbed with 100 ng of p30 polypeptide.
‡ 1 cm^2 diluted serum absorbed with 3 × 10^6 MCA-10 tumor cells.
NT, not tested.
ways (Table 4). As shown in Fig. 4, low dilutions of MuLV p30 antiserum were toxic to the two nontransformed lines (mSR and BALB/3T3) and to one transformed line (S+L-m3) but not to the transformed K234 line.

Our previous findings of p30 antiserum toxicity against nonvirus-producing MCA-induced tumor lines (MCA-4 and MCA-11) as well as virus-producing lines (MCA-5, MCA-6, and MCA-10) suggested that p30 expression was not dependent upon virus synthesis. These findings were supported by p30 antiserum toxicity against mSR and BALB/3T3 cell lines which produced no detectable MuLV. It appears, therefore, that cell surface expression of p30 is poorly related to either cellular transformation or virus synthesis but correlated best to cellular p30 content. This correlation, however, was not quantitative as p30 content by radioimmunoassay did not always agree with observed cytotoxicity of p30 antiserum (Tables 1 and 4, Figs. 1 and 4). Further, MuLV p30 antiserum in our assay showed no cytotoxicity to nonvirus-producing, transformed human cells known to contain the murine sarcoma genome (Hu S+L-) (25) and significant amounts of p30 by radioimmunoassay (80 ng/10⁶ cells).

To determine if MuLV p30 antigen passively associated with cell surfaces, NEF cells, which contained minimal quantities of MuLV p30 and were not lysed by MuLV p30 antiserum, were incubated for 2 hr with MuLV P30 polypeptide, washed, and studied for sensitivity to MuLV p30 antiserum in cytotoxicity assays. Significant enhancement occurred when 0.1–0.8 μg of p30 were added per 10⁶ cells (Fig. 5). This cytotoxicity was abolished by absorption of the antiserum with MuLV p30 polypeptide. Preincubation with larger amounts of MuLV p30 was less effective perhaps due to excess p30 which could compete with cell bound p30 for antibody. Similar enhancement of cytotoxicity by addition of p30 was observed with MCA-18 and MCA-4 tumor cells.

From these experiments, passive absorption of extracellular MuLV p30 onto the cell surface became an important consideration. Although no solubilized MuLV p30 in MCA-10 cell culture supernatants could be detected by radioimmunoassay, this did not rule out the possibility that p30 derived from disrupted virus or from cells rapidly attached to the cell surface. Yet, the cytotoxicity of MuLV p30 antiserum against mSR and BALB/3T3, two nonvirus-producing cell lines (Fig. 4), suggests other mechanisms by which p30 reaches the cell surface. It may be that several mechanisms operate in the various situations studied.

Host Response to Cell Surface MuLV p30 Antigen. Since MuLV p30 was on the surface of our MCA-induced murine tumors and could bind antibody with subsequent cell lysis, it became important to determine the immunological response to MuLV p30 in the C57BL/6N mouse. As previously reported, murine antiserum against MCA-10 tumor cells was significantly cytotoxic (69% at 1:16 dilution) against cultured MCA-10 cells (8). In spite of the strong surface expression of MuLV p30 antigen on MCA-10 cells, attempts to demonstrate MuLV p30 antibodies in MCA-10 antiserum by double antibody radioimmunoassay were unsuccessful. Furthermore, although absorption of MCA-10 antiserum with MCA-10 tumor cells completely removed cytotoxicity against MCA-10 cultured cells, absorption with MuLV p30 failed to remove any cytotoxicity. Within the sensitivity limits of our assays, therefore, murine antiserum against syngeneic MCA-10 tumor cells contained no detectable circulating cytotoxic antibody to MuLV p30 antigen. This is consistent with findings of others that, although immunological responses to internal oncorna-virus antigens can occur, free antibody may not be detectable (26).

**DISCUSSION**

This study illustrates that MuLV structural components are present on the surface of various murine cells. The serological

![Graph](attachment:image.png)

**Fig. 4.** Complement dependent cytotoxicity of MuLV p30 antiserum against mSR, S+L-m3, BALB/3T3, and K234 cells. Expression of MuLV genetic information by these cell lines is given in Table 4. Cytotoxicity was unrelated to cellular transformation or virus particle production.

![Graph](attachment:image.png)

**Fig. 5.** Cytotoxicity of MuLV p30 antiserum against NEF cells after preincubation of cells with purified MuLV p30 for 2 hr at 37°. Cells were washed and tested for cell lysis by MuLV p30 antiserum and complement. MuLV p30 antiserum was not cytotoxic to untreated NEF cells (see Fig. 1).
analyses used to investigate these components indicated that in most cases the virion proteins were accessible to antibody and could effect cell lysis in presence of specific antibody and complement.

Since MCA-10 cells synthesized MuLV, it was not surprising to find the major virus surface glycoprotein (gp71) expressed on their cell surface. As demonstrated with avian oncoviruses, the analogous component (gp85) is present on the cell exterior in association with budding virus as well as on other areas of the cell surface (27). A similar situation exists with cells synthesizing murine oncoviruses (W. Schafer, personal communication). The strong expression of gp71 on the surface of NEF was, however, surprising. Additional work in this and other laboratories (R. Lerner and J. T. August, personal communications) suggests that MuLV gp71 is expressed by murine cells harboring the MuLV genome regardless of viral replication, transformation, or expression of other structural viral antigens. In many ways it is analogous to the expression of chicken factor (chf) which can occur in the absence of gs antigens (28). In fact, chf has been shown to be analogous to the avian virus glycoprotein, gp85 (29).

The presence of internal structural virus constituents on the cell surface, such as the major core protein (p30) and possibly the polypeptide closely associated with virus RNA (p10), was unexpected. This may be unique for mammalian oncovirus systems since attempts to detect the corresponding avian constituents have been unsuccessful (30). The mechanism by which these polypeptides arrive at the cell surface is unclear. Although we were able to generate cytotoxicity by addition of purified MuLV p30 to cells, the finding that MuLV p30 antiserum was cytotoxic against nonvirus-producing cell lines suggests that the antigen does not originate exclusively as a consequence of virus production and subsequent disruption. Yet, the possibility remains that MuLV p30 arrives at the cell surface as a result of disruption of other cells. More work needs to be done to relate these observations to the morphogenesis of MuLV.

The demonstration that both interspecies and intraspecies determinants of the surface glycoprotein (gp71) and internal polypeptide (p30) were present on the surface of MCA-induced murine tumor cells, bound antibody, and mediated cell lysis has broad implications. Clearly, such reactivities must be accounted for in studies dealing with tumor antigens or tissue antigens within the mouse species or between different species. Indeed, preliminary studies in our laboratory have shown that cells of a MCA-induced rat sarcoma were lysed by goat antiserum to FeLV.

Although in vitro we were unable to detect MuLV p30 antibody in murine antiserum directed against a syngeneic MCA-induced tumor which expressed surface-bound MuLV p30, this does not rule out the possibility of in vivo interactions. Other structural antigens or virus-induced neopatigens (3) need to be studied in a similar manner. This is particularly important with regard to gp71 and p15 since certain mouse strains contain antibodies against these components (refs. 31 and 32; R. Lerner and T. August, personal communications). The relationship of these components to one another in vivo may have even broader implications and also needs to be examined.

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