**Common Cell-Surface Antigen Associated with Murine and Feline C-Type RNA Leukemia Viruses**

*(membrane immunofluorescence/rabbit anti-FeLV/EαG2)*

**TAKASHI YOSHIKI*, ROBERT C. MELLORS*, AND WILLIAM D. HARDY, JR.†**

*The Hospital for Special Surgery. Affiliated with The New York Hospital–Cornell University Medical College and the Department of Pathology, Cornell University Medical College, New York, N.Y., and †The Division of Immunology, Sloan-Kettering Institute for Cancer Research, New York, N.Y.*

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**ABSTRACT**  A new common cell-surface antigen associated with murine and feline C-type RNA leukemia viruses was demonstrated by the use of rabbit antiserum against feline leukemia virus and the indirect membrane immunofluorescence test. Common cell-surface antigen was found in all leukemias of all strains of mice tested, in normal lymphoid tissues of Gross-positive (high incidence of leukemia) mouse strains AKR, AKR-H-2*, C57, and NZB, in cultured rat fibroblasts infected with Rauscher virus, in cultured feline fibroblasts infected with feline leukemia virus, and in spontaneous feline lymphosarcoma. The antigen was not demonstrable in normal adult and fetal tissues of Gross-negative mouse strains or in tissues and cultured fibroblasts derived from normal rats and normal cats. The immunoferritin study of murine leukemia cells revealed that the antigen was located on the cell surface in discrete areas; budding and C-type RNA viral envelope was not labeled as antigen site. The distribution of common cell-surface antigen on murine and feline leukemias, as well as on normal lymphoid tissues of Gross-positive mouse strains, indicates the presence of an antigen distinct from any cell-surface antigen hitherto shown to be associated with, or specified by, mammalian C-type RNA viruses.

The study of the antigens associated with murine C-type RNA leukemia viruses has advanced considerably in recent years (1–12). In this communication we describe a new common cell-surface antigen associated with murine and feline C-type RNA leukemia viruses and detected by rabbit antiserum against FeLV (feline leukemia virus) and the indirect membrane immunofluorescence test.

**MATERIALS AND METHODS**

*Mice.* The following strains were used: AKR/J and C57BL/6J mice from Jackson Laboratories, Bar Harbor, Me.; NZB mice from our colonies; other strains listed in Table 2 kindly provided by Dr. Lloyd J. Old (Sloan–Kettering Institute for Cancer Research, New York).

Leukemias. Leukemias and tumors used are listed in Table 3. The origin and nature are briefly described in the text.

**Antiserum.** Rabbit anti-FeLV antiserum was produced by immunization of rabbits with ether-disrupted feline leukemia virus (FeLV). The virus was derived from feline fibroblasts grown in tissue culture and infected with FeLV (30). The virus was purified by density gradient centrifugation on a potassium tartrate gradient. The purified virus was disrupted with ether, mixed with an equal volume of Freund's adjuvant, and injected subcutaneously at several sites. Rabbits were inoculated at least four times at about monthly intervals. The antiserum was stored at −70°. The antiserum was heat-inactivated at 56° for 30 min before use.

**Preliminary Absorption Procedures.** The unabsorbed rabbit anti-FeLV antiserum contained antibodies against normal cellular components of mouse tissues. This antiserum was absorbed as follows in order to remove these antibodies:

(a) In Vitro Absorption. Spleen cells from C57BL/6J mice were washed twice with medium 199. One volume of the washed spleen cells and 3 volumes of antiserum diluted 1:5 were mixed and incubated for 1 hr at 4° with constant shaking. After centrifugation, the recovered antiserum was reabsorbed in a similar manner with washed thymus cells from C57BL/6J mice.

(b) In Vivo Absorption. 1 ml of undiluted antiserum was injected intraperitoneally in a 2-month-old C57BL/6J mouse. The antiserum was recovered the next morning by bleeding from the periorbital sinus.

**Membrane Immunofluorescence Test.** The indirect immunofluorescence test with suspensions of viable cells was done as described (13). The standard target cells were transplanted leukemia EαG2 originally induced in C57BL/6 mouse by passage A Gross virus (1). A mixture of 50 μl of antiserum at a certain dilution and 25 μl of well-washed cell suspension (4 × 10⁶/ml) was incubated at room temperature for 20 min. The cells were then washed twice with medium 199 in the cold (4°) and resuspended in 50 μl of fluorescein-conjugated goat antiserum to rabbit 7S gammaglobulin (Hyland Lab., Los Angeles, Calif.) diluted 1:15. After incubation for 20 min at room temperature (25°), the cells were washed twice with medium 199, suspended in 50% glycerol in phosphate-buffered (pH 8.0) isotonic saline, and examined under a cover slip with a Zeiss dark-field fluorescence microscope (filters BG 12, 50, 44). Negative controls with medium 199 alone or normal rabbit serum were virtually unstained.

The antibody titer was determined by diluting antiserum to an end point of 50% fluorescent cells.

**Absorption Test.** Equal portions of washed packed cells or tissue homogenates and the antiserum diluted two tubes below
the fluorescent end point were incubated for 2 hr at 4° with constant shaking. After centrifugation for 15 min at 2000 rpm (550 × g), the supernatant fluids diluted 1:1 and 1:2 were tested for residual antibody activity on viable E°C G2 cells by membrane immunofluorescence test. Preparation of cells and tissue homogenates for absorption was fully described elsewhere (14).

**Indirect Immunoferritin Technique.** The surface location of the antigen was determined by indirect immunoferritin technique (15) with slight modifications. 1 ml of ferritin-conjugated IgG fraction from goat antiserum against rabbit gammaglobulin (Cappel Lab., Downington, Pa.) was absorbed with an equal volume of packed viable E°C G2 cells and 20 mg of mouse gammaglobulin (Miles Labs., Inc., Kanakee, Ill.) polymer before use. 10 × 10⁶ washed viable cells were incubated for 30 min in antiserum absorbed in vivo diluted 1:4. After two washings, the cells were resuspended in the absorbed ferritin-conjugated antibody against rabbit gammaglobulin.

**Table 1. Representation of common cell-surface antigen on mouse cells and tissues determined by absorption test.**

<table>
<thead>
<tr>
<th>Cells and tissues</th>
<th>Dilution of absorbed serum</th>
<th>Result of absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1:1</td>
<td>1:2</td>
</tr>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spleen AKR/J</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Lymph node</td>
<td>16</td>
<td>5</td>
</tr>
<tr>
<td>Thymus</td>
<td>44</td>
<td>10</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Liver</td>
<td>59</td>
<td>37</td>
</tr>
<tr>
<td>Kidney</td>
<td>58</td>
<td>35</td>
</tr>
<tr>
<td>Brain</td>
<td>62</td>
<td>33</td>
</tr>
<tr>
<td>E+G2f</td>
<td>61</td>
<td>37</td>
</tr>
<tr>
<td>2-month-old C57BL/6J</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>58</td>
<td>35</td>
</tr>
<tr>
<td>Thymus</td>
<td>63</td>
<td>36</td>
</tr>
<tr>
<td>E+G2f</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>None</td>
<td>74</td>
<td>57</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>85</td>
<td>67</td>
</tr>
<tr>
<td>Lymph node</td>
<td>NT</td>
<td>70</td>
</tr>
<tr>
<td>Thymus</td>
<td>86</td>
<td>66</td>
</tr>
<tr>
<td>Bone Marrow</td>
<td>85</td>
<td>67</td>
</tr>
<tr>
<td>Liver</td>
<td>83</td>
<td>66</td>
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<tr>
<td>Kidney</td>
<td>86</td>
<td>62</td>
</tr>
<tr>
<td>Brain</td>
<td>86</td>
<td>65</td>
</tr>
<tr>
<td>E+G2f</td>
<td>83</td>
<td>64</td>
</tr>
</tbody>
</table>

2-month-old AKR/J |
| Spleen           | 33  | 11  | + |
| E+G2f            | 0   | 0   | + |
| None             | 92  | 83  | + |

*Antiserum diluted two tubes below end point (50% fluorescence positive cells) absorbed with tissues listed, and residual antibody activity was titrated undiluted and at 1:2 dilution of recovered serum against E+G2 cells by membrane fluorescence test.

† % fluorescent cells.

‡ E+G2 cells were used as positive control.

After further incubation for 30 min, the cells were finally washed twice. The reaction was done entirely in the cold (4°) to avoid cap formation and possible endocytosis. Negative controls were either E+G2 cells treated with normal rabbit serum absorbed in vivo in C57BL/6J mice or C57BL/6J spleen cells treated with rabbit anti-FeLV antiserum; the negative controls were virtually unlabeled.

**Electron Microscopy.** Cell pellets were covered with 2 ml of 2% glutaraldehyde for 40 min at 0°. After postfixation for 60 min in cold 1% osmium tetroxide, they were dehydrated in alcohol and embedded in Epon. Thin sections were cut on a Sorval MT-2 ultramicrotome with a diamond knife, stained with 0.5% uranyl acetate in alcoholic solution and lead citrate, and examined with a Siemens Elmiskop IA electron microscope.

**RESULTS**

**Indirect Membrane Immunofluorescence Test with Rabbit Anti-FeLV Antiserum.** After being absorbed in vivo or in vitro rabbit anti-FeLV antiserum was found to contain antibody that reacted with the cell surface of viable E+G2 and K36 leukemia cells of mice but not with C1498, EL4, RADA1, MOPC-104E, and SMT-1 cells by the indirect membrane immunofluorescence test. The antibody titer against viable E+G2 cells was 1:64 with the antiserum absorbed in vivo and 1:80 with the antiserum absorbed in vitro. Fig. 1 shows the titration curve against E+G2 and other leukemia and tumor cells for the antiserum absorbed in vitro. The membrane immunofluorescence pattern of the labeled E+G2 cells was mostly patchy in appearance when the reaction was done at room temperature (Fig. 2).

**Tissue Representation and Strain Distribution in Mice of the Antigen Detected with Rabbit Anti-FeLV Antiserum.** In order to demonstrate the specificity of the antibody, the antiserum was absorbed with various tissues obtained from 2-month-old AKR/J and C57BL/6J mice (Table 1), and the absorbed antiserum was tested for residual antibody activity against viable E+G2 cells by the indirect membrane immunofluorescence test. Bone marrow and lymphoid tissues of AKR/J mice absorbed the antibody activity from the antiserum, whereas no absorption was observed with C57BL/6J tissues. A summary of the distribution of the antigen in strains of mice is given in

*Strain distribution was determined by absorption test with pooled spleen cells as antigen source.*
TABLE 3. Distribution of common cell-surface antigen on mouse leukemia cells and tumor cells

<table>
<thead>
<tr>
<th>Tumors tested</th>
<th>Origin and nature of leukemias and tumors</th>
<th>Result of absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td>Transplantable leukemia induced by dimethylbenzanthracene. GCSA-</td>
<td>+</td>
</tr>
<tr>
<td>EL 4</td>
<td>Transplantable leukemia induced by x-irradiation. GCSA-</td>
<td>+</td>
</tr>
<tr>
<td>EoG2</td>
<td>Transplantable leukemia induced by Passage A Gross virus. GCSA+</td>
<td>+</td>
</tr>
<tr>
<td>C1498</td>
<td>Transplantable myeloid leukemia. GCSA+</td>
<td>+</td>
</tr>
<tr>
<td>A</td>
<td>Transplantable leukemia induced by x-irradiation. GCSA-</td>
<td>-</td>
</tr>
<tr>
<td>RADA 1</td>
<td>Transplantable spontaneous leukemia. GCSA-</td>
<td>+</td>
</tr>
<tr>
<td>ASL 1</td>
<td>Transplantable leukemia induced by x-irradiation. GCSA+</td>
<td>-</td>
</tr>
<tr>
<td>Balb/c</td>
<td>Transplantable myeloma producing µδ and λ. GCSA-</td>
<td>-</td>
</tr>
<tr>
<td>MOPC-104E</td>
<td>Transplantable leukemia induced by x-irradiation. GCSA+</td>
<td>+</td>
</tr>
<tr>
<td>BALB c’ RL1</td>
<td>Transplantable leukemia induced by x-irradiation. GCSA+</td>
<td>+</td>
</tr>
<tr>
<td>AKR</td>
<td>Transplantable spontaneous leukemia. GCSA+</td>
<td>+</td>
</tr>
<tr>
<td>K 36</td>
<td>Transplantable spontaneous leukemia. GCSA+</td>
<td>+</td>
</tr>
<tr>
<td>SL-1</td>
<td>Transplantable spontaneous leukemia. GCSA+</td>
<td>+</td>
</tr>
<tr>
<td>SL-2</td>
<td>Transplantable spontaneous leukemia. GCSA+</td>
<td>+</td>
</tr>
<tr>
<td>SL-3</td>
<td>Transplantable spontaneous leukemia. GCSA+</td>
<td>+</td>
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<tr>
<td>SL-4</td>
<td>Transplantable spontaneous leukemia. GCSA+</td>
<td>+</td>
</tr>
<tr>
<td>SL-5</td>
<td>Transplantable spontaneous leukemia. GCSA+</td>
<td>+</td>
</tr>
<tr>
<td>C3H/An</td>
<td>Transplantable mammary tumor. GCSA-</td>
<td></td>
</tr>
<tr>
<td>SMT-1</td>
<td>Transplantable mammary tumor. GCSA-</td>
<td>-</td>
</tr>
</tbody>
</table>

* See refs. 1 and 2. GCSA, Gross cell-surface antigen.

Table 2. AKR, AKR/H-2δ, C58, and NZB mice, all of which are Gross-positive strains, were invariably positive for the antigen, while other strains of mice C57BL/6J, 129, DBA/2, Balb/c, A, and C3Hf/Bi were negative for the antigen at the ages tested. The antigen being detected with rabbit anti-FeLV antiserum was given the tentative designation common cell-surface antigen associated with murine and feline C-type RNA viruses.

Distribution of Common Cell-Surface Antigen on Mouse Leukemias and Other Tumors. To examine further the specificity of the antibody against common cell-surface antigen, rabbit anti-FeLV antiserum was absorbed with various mouse leukemia cells known to be positive or negative for Gross cell-surface antigen (Table 3).

Common cell-surface antigen was demonstrated on all mouse leukemias so far assayed by the absorption test, including those negative for the antigen by the indirect membrane immunofluorescence test and also those negative for Gross cell-surface antigen. The antigen was not demonstrable on SMT-1, a spontaneous mammary tumor of C3H/An origin, and MOPC-104E, a transplantable myeloma of Balb/c origin.

Determination of the Specificity of Common Cell-Surface Antigen. To define further the specificity of the antigen, the antiserum was absorbed with various tissues (Table 4). Cultured feline fibroblasts infected with FeLV and cultured rat fibroblasts infected with Rauscher virus equally absorbed the antibody activity, whereas neither normal feline fibroblasts nor rat fibroblasts did so. Fresh autopsy material from feline lymphosarcoma absorbed the antibody activity. No trace of common cell-surface antigen was demonstrable in any tissue of normal rats and cats, nor in thymocytes of mouse-strain 129.

Fetal tissues (late embryos) of AKR/J mice only partially absorbed the antibody activity, while no trace of common cell-surface antigen was demonstrable in comparable fetal tissues of C57BL/6J mice. Purified FeLV and ether-disrupted FeLV prepared by density gradient centrifugation equally absorbed the antibody activity from rabbit anti-FeLV antiserum.

Demonstration of Common Cell-Surface Antigen on the Cell Surface by the Indirect Immunoferritin Technique. Several small discrete areas of attached ferritin were observed on

Fig. 1. Membrane immunofluorescence reaction of rabbit anti-FeLV antiserum against various murine leukemia cells.

Fig. 2. Membrane immunofluorescence pattern of EoG2 leukemia cells given by the reaction with rabbit anti-FeLV antiserum. ×400.
the surface of E5G2 cells that had been incubated with rabbit anti-FeLV antiserum absorbed in vivo in C57BL/6J mice. These ferritin-positive areas were distributed in an irregular fashion over the cell surface, separated by wide negative regions (Fig. 3). In contrast, no ferritin labeling was observed on the C-type RNA viral envelopes thus far examined.

**DISCUSSION**

The indirect membrane immunofluorescence test and absorption analysis with rabbit anti-FeLV antiserum reveal a new common cell surface antigen associated with murine and feline C-type RNA viruses.

The common cell-surface antigen has the following characteristics: (a) The antigen is present in normal lymphoid tissues of mouse strains that have a high incidence of leukemia (Gross-positive strains) (1, 2). (b) The antigen is present in all spontaneous and induced mouse leukemias so far tested. (c) It is also found in spontaneous feline leukemia, feline fibroblasts infected with FeLV, and rat fibroblasts infected with Rauscher virus. (d) It is not present in normal adult and fetal tissues of Gross-negative mouse strains. (e) It is not found in normal tissues of rats and cats. (f) It is located on the cell surface but not on the C-type RNA viral envelope of mouse leukemia cells (E5G2) thus far studied. In recent studies (unpublished), common cell-surface antigen was found in hamster and feline fibroblast cultures transformed by feline sarcoma virus but not in homologous normal cells or in chicken fibroblasts transformed by Rous sarcoma virus.

Common cell-surface antigen is clearly distinguished from other cell-surface antigens associated with murine C-type RNA leukemia viruses. [Gross cell-surface antigen (1–5), FMR (4), and G IX (6, 29)] and from the remaining four mouse antigen systems associated with leukemia [TL (18), ML (17), E (18), and L (19)], whose relations to C-type RNA virus have not been clarified. Common cell-surface antigen is not a fetal antigen (20–22).

Recent work (10, 12, 23–26) has indicated that mammalian

**Table 4. Specificity of common cell-surface antigen tested by absorption with various mammalian cells and tissues**

<table>
<thead>
<tr>
<th>Cells and tissues used for absorption</th>
<th>Result of absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal cat tissues (spleen, thymus, lymph node, bone marrow, liver, brain, erythrocyte, kidney)</td>
<td>+</td>
</tr>
<tr>
<td>Cultured cat fibroblasts (normal)</td>
<td>+</td>
</tr>
<tr>
<td>Feline lymphosarcoma no. 1467 (spleen, bone marrow, lymph node, liver, kidney)</td>
<td>+</td>
</tr>
<tr>
<td>Cultured cat fibroblasts infected with FeLV</td>
<td>+</td>
</tr>
<tr>
<td>Normal rat tissues (spleen, thymus, bone marrow, liver)</td>
<td>+</td>
</tr>
<tr>
<td>Cultured rat fibroblasts (normal)</td>
<td>+</td>
</tr>
<tr>
<td>Cultured rat fibroblasts infected with Rauscher virus</td>
<td>+</td>
</tr>
<tr>
<td>C57BL/6J fetus</td>
<td>+</td>
</tr>
<tr>
<td>AKR/J fetus</td>
<td>±</td>
</tr>
<tr>
<td>129 thymus</td>
<td>±</td>
</tr>
<tr>
<td>Sheep erythrocytes</td>
<td>±</td>
</tr>
<tr>
<td>Intact FeLV</td>
<td>±</td>
</tr>
<tr>
<td>Ether-disrupted FeLV</td>
<td>±</td>
</tr>
</tbody>
</table>

C-type RNA leukemia viruses (of mouse, hamster, cat, and rat) contain at least two antigenic determinants associated with the major internal structural protein (gs antigen): gs-1 designates species-specific determinants and gs-3 designates interspecies crossreactive determinants. The question raised is whether common cell-surface antigen is related to gs-3. Recently we have done the absorption test with six subviral protein components of Rauscher leukemia virus prepared and isolated by gel filtration in guanidine hydrochloride as described by Nowinski et al. (26). Only component 3 (P1), which contains gs antigen, completely absorbed the antibody activity against common cell-surface antigen from our rabbit anti-FeLV antiserum (unpublished). This finding suggests strongly that common cell-surface antigen is closely related to, or identical with, gs antigen.

Contaminating cytoplasmic vesicles are commonly found in viral preparations purified by density gradient centrifugation. The presence of Gross cell-surface antigen in density-purified MuLV has been described (27). It is reasonable to suppose that rabbits immunized with such viral preparations may produce an additional antibody against cell-surface antigens that contaminate the density-purified virus preparation used for immunization. This circumstance may possibly explain our absorption results indicating that both density-purified FeLV, which may contain contamination of common cell-surface antigen, and ether-disrupted FeLV equally absorbed the antibody activity from rabbit anti-FeLV antiserum (Table 4). Another possibility is that the
antigen, although not located on MuLV envelope, is present on FeLV envelope. There is also a possibility that immunized rabbits may produce an antibody against viral-envelope antigens. Since our immunoferritin studies did not reveal labeling of the viral envelope of E6G2 cells, it seems unlikely that common cell-surface antigen is a viral-envelope antigen common to FeLV and MuLV.

Huebner and Todaro (28) have proposed the hypothesis that the cells of many, perhaps all, vertebrates contain C-type RNA viral genetic information, usually in covert form but with inducible expression, and these authors and others have provided supporting evidence for this concept. Common cell-surface antigen is apparently an antigenic expression of this genetic information and possibly indicates incipient sites of virus synthesis.

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