RELATIONSHIPS BETWEEN MEMBRANE ANTIGENS OF HUMAN LEUKEMIC CELLS AND ONCOGENIC RNA VIRUS STRUCTURAL COMPONENTS*

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During the past several years, data has been steadily increasing which establishes a relationship between mammalian RNA tumor virus constituents and similar molecules in human tumors. Thus far most of the evidence is based on DNA-RNA hybridization studies and reverse transcriptase activity (1), although there have been reports establishing some serological relationships between antigens in human cancers, particularly human leukemias, and those of RNA tumor viruses or their transformed cells (2–4).

Most of what is known about mammalian oncogenic RNA virus antigens comes from studies with the major internal protein (p30)[1] and the primary surface glycoprotein (gp71) of the viruses (5). These molecules from all well studied mammalian viruses including those of primates, contain multiple antigenic determinants (6). Both p30 and gp71 molecules are found at the surface of cells which harbor the virus genome (7, 8). The expression of some of the determinants of these molecules on the cell surface can be noncoordinate and independent of virus elaboration or cell transformation (8, 9) and appears to be subjected to genetic control by the host (10–12). Antisera to purified gp71 and p30 are cytotoxic for some animal cells which express these antigens on their surface (8).

The serological specificity of human leukemia-associated antigens detected by certain cytotoxic nonhuman primate antisera to human leukemia cells have

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[1] *Abbreviations used in this paper:* ALL, acute lymphocytic leukemia; AML, acute myelocytic leukemia; AMML, acute myelomonocytic leukemia; AMV, avian myeloblastosis virus; CGL, chronic granulocytic leukemia; CLL, chronic lymphocytic leukemia; DOC, deoxycholate, FCS, fetal calf serum; FeLV, feline leukemia virus (Rickard); FLV, Friend murine leukemia virus; GLV, Gross murine leukemia virus; gp, glycoprotein; HBSS, Hanks' balanced salt solution; HRBC, human erythrocytes; HWBC, human leukocytes; MMTV, murine mammary tumor virus; p, protein; RLV, Rauscher murine leukemia virus; SDS, sodium dodecyl sulfate; STU, Schäfer Tübingen mouse strain.

been previously described (13, 14) and will not be detailed here. However, in order to clarify the serological data in this report, a brief summary of the specificity of these reagents follows. Primate antisera to cells from chronic lymphocytic leukemia (CLL) patients detect membrane antigens which are specific for cells from CLL patients and which cross-react with cells from acute lymphoblastic leukemia (ALL). These antisera do not react with cells from myeloid leukemia patients. In contrast, primate antisera to cells from myeloid leukemia patients can be shown to detect antigens on cells of some patients with myeloid leukemia which are not present on cells of patients with ALL or CLL. There are several myeloid leukemia-associated antigens detected by the various nonhuman primate antisera. Moreover, not all of the patients with acute myelocytic leukemia (AML) and chronic granulocytic (myeloid) leukemia (CGL) have cells which react with each of the simian antisera to myeloid leukemia cells. Absorption studies of the antisera with individual AML and CGL cell donors indicate that these antisera can detect antigens which are cross-reactive between AML and CGL cells and may also detect antigens which are unique for cells of certain AML and CGL donors (14).

The studies described below utilized, in addition to the primate antisera to human leukemia-associated antigens, a variety of well studied oncogenic RNA virus structural components and their corresponding antisera. The data reported establishes a complex antigenic relationship between certain membrane-associated antigens of human myeloid leukemia cells and the virion molecules gp71 and p30 from Friend murine leukemia virus (FLV).

Materials and Methods

Viruses. FLV was grown in a continuous cell culture line (Eveline) originally obtained by infection of Schäfer Tübingen mouse strain (STU) embryos with FLV (15). Animal passaged FLV was kindly provided by Dr. C. Friend, Mt. Sinai School of Medicine, New York and passed in our laboratory in DBA/2J mice. Rauscher murine leukemia virus (RLV) and Gross (GLV) murine leukemia virus were a gift from R. Gilden, Flow Laboratories, Inc., Bethesda, Md. The Rickard strain of feline leukemia virus (FeLV) was a gift from F. de Noronha, Cornell University Medical College, New York. Avian myeloblastosis virus (AMV) was harvested from the plasma of leukemic chickens. Murine mammary tumor virus (MMTV) purified from RIII mouse milk was provided by W. Parks, NCI, Bethesda, Md. All virus preparations were purified extensively by density gradient centrifugation.

Isolation of Viral Structural Polypeptides. The isolation of the p30 polypeptide (30,000 mol wt) and other polypeptides of FLV and FeLV has been described (16). The major FLV surface glycoprotein of 71,000 mol wt (gp71) was also obtained from virus grown in Eveline cells and was prepared as described by Moennig et al. (17). AMV polypeptides were isolated in an analogous manner (18). AMV gp85 (glycoprotein of 85,000 mol wt) was purified from disrupted AMV as recently described (19). For complete nomenclature of these components see August et al. (6).

The major MMTV glycoprotein (gp52) was isolated from MMTV with 0.3% deoxycholate (DOC), 0.1 M NaCl, and 0.01 M Tris, pH 8. Sodium dodecyl sulfate (SDS) gel electrophoresis of this preparation indicated six components in major amounts having mol wt of 10,000, 12,000, 14,000, 27,000, 36,000, 52,000, and 65,000 daltons. Minor amounts of proteins were observed above 65,000 daltons and at 42,000 and 48,000 daltons. After dialysis to remove DOC, this material was reacted with concanavalin A covalently linked to agarose and washed with diluted salt solution. Subsequent elution with methyl mannoside gave a preparation containing gp36 and gp52. This material, when passed through Sephadex G75, yielded gp52 and gp36 as separable entities. Antisera to gp52 were prepared in rabbits as described previously (20).

Solubilization of Leukemia Antigens with Sodium DOC. Leukemic cells from spleen or peripheral blood were washed three times with saline at 800 *g* and stored at −70°C. The cell pellets (40–200 ml) were homogenized in a VirTis apparatus (Model 45; VirTis Co., Inc., Gardiner, N. Y.) for 10 min at 4°C in the presence of 100 μg/ml DNase (Sigma Chemical Co., St. Louis, Mo.). The material was diluted fivefold with 0.02 M Tris·HCl, 0.2 M NaCl, pH 8.5, and DOC was added to a final concentration of 0.5%. This suspension was then placed in a Branson sonifier (Bransonic 220; Branson Instruments Co., Stanford, Conn.) for 10 min at 37°C and centrifuged at 40,000 *g* for 120 min in a Beckman L3-50 ultracentrifuge (Beckman Instruments, Inc., Fullerton, Calif.). The supernate containing the antigenic activity was dialyzed against distilled water, concentrated, and resolubilized in 0.1% Triton X-100. The material was then partially purified by gel filtration on Sephadex G200 in the presence of 0.05% DOC in 0.02 M Tris·HCl, and 0.2 M NaCl, pH 8.0.

Antiserum to Human Leukemia Antigens. The nonhuman primate antisera to human leukemia cell antigens used in this study have been listed and the specificity described in detail in earlier reports (13, 14). These sera include a chimpanzee (*Pan-troglodytes*) antiserum to cells from a CLL donor (C1), a chimpanzee antiserum to cells from an AML patient (C2) and a monkey (*Macaca-speciosa*) antiserum to cells from a CGL donor (M3). These antisera were heat inactivated at 56°C and then absorbed with human erythrocytes (HRBC) and peripheral blood leukocytes (HWBC) from normal donors until they failed to react with lymphocytes from a panel of HL-A-typed normal donors. Such absorbed antisera did not react with cells from any of the normal donors tested but were cytotoxic to cells from leukemia patients.

Antisera to Viruses. Information on the antisera to RNA tumor viruses or their purified antigens which were used in this report are described in Table I.

Absorption of Antisera to Oncornaviruses. The rabbit or goat antisera to the RNA tumor virus antigens were heat inactivated at 56°C for 30 min and subsequently absorbed with HRBC from blood group A and B donors until they no longer agglutinated these cells. Such HRBC-absorbed antisera did not react with lymphocytes prepared from peripheral blood of normal volunteers. Portions of the antisera absorbed with normal HRBC were then absorbed with either HWBC from normal adult donors, mouse spleen cells, mouse embryo cells, or cells from individual leukemic patients at a concentration of 2×10^8 cells/ml of serum. Other samples of the HRBC-absorbed antisera were absorbed with either human platelets devoid of other peripheral blood cells or peripheral blood lymphocytes or neutrophils. For the preparation of platelets, blood collected in EDTA was centrifuged at 800 *g* and the supernate recentrifuged at 1,000 *g* for 10 min to remove erythrocytes and leukocytes. The platelet-rich supernate was then centrifuged at 4,000 *g* for 20 min at 4°C. The platelets were thrice washed in isotonic saline before being used for absorption. Purified lymphocytes and neutrophils were prepared from heparinized peripheral blood of normal donors by gradient centrifugation. Erythrocytes were sedimented with 5% dextran in the presence of carbonyl iron and the leukocyte-rich supernate was centrifuged over a Ficoll-Hypaque gradient (21). The interphase enriched in lymphocytes (>95%) was washed thrice with saline at 800 *g* and the cell concentration adjusted to the desired level. The leukocytes that passed through the Ficoll-Hypaque gradient were predominately neutrophils (>90%). These cells were then thrice washed with saline and used for absorption. The numbers or volumes of cells used for absorption are given in Tables III and IV. The cells were incubated with antiserum at room temperature for 30 min, centrifuged at 800 *g* for cells, 4,000 *g* for platelets, and the absorption repeated.

Absorption of the antisera with RNA tumor viruses (partially disrupted by freezing and thawing) or gp71 and p30 antigens was performed as follows: Antiserum (nine volumes) was incubated overnight at 4°C with one volume of antigen (protein concentrations of viruses or antigens ranged from 0.1 to 1 mg/ml). The mixture was then centrifuged at 100,000 *g* for 2 h and the supernate used for serological testing.

Target Cells for Cytotoxicity Test. The method of preparation of lymphocytes or leukemic cell suspensions for cytotoxicity assays has been detailed earlier (14). In short, the erythrocytes were sedimented with Plasmagel (HTI Corp., Associated Biomedic Systems, Buffalo, N. Y.) and the supernate centrifuged at 800 *g* for 10 min. The leukocytes suspended in plasma were added to a nylon column and incubated for 20 min at 37°C. The cells were eluted from the column with warm Hanks' balanced salt solution (HBSS). Tris buffer (0.17 M, pH 7.2) was then added to lyse the remaining erythrocytes in the nonadherent cell population. Leukemic blasts of all morphological

types and immature cells of the granulocytic series were eluted from the nylon columns under these conditions. The cells were adjusted to a concentration of 4×10^6/ml in HBSS for use in the microcytotoxicity test. Unless specifically classified as cells from a remission patient, all leukemic test cells were from patients in relapse or the untreated stage of their disease.

Cytotoxicity and Cytotoxicity Inhibition Assays. A two stage microcytotoxicity technique was used (14). 1 μl of antiserum (diluted in HBSS) was mixed with 0.5 μl of cells (4×10^6/ml) in Falcon microtest plates (no. 3034; Falcon Plastics, Div. of BioQuest, Oxnard,Calif.). The mixture was incubated for 35 min at room temperature, rabbit complement (C) (5 μl) was added and the plates incubated at 37°C for another 35 min. Thereafter, 5 μl of 5% eosin and 2 μl of 30% buffered formalin were added and the cell death was assessed by phase-contrast microscopy. Cell lysis of 30% or greater above normal serum controls was considered a positive reaction. When the number of cells lysed in the C controls was 30% or greater, rabbit C absorbed with leukemic cells (14) was substituted for the regular rabbit C in the cytotoxicity test.

Competing antigenic activity in solubilized antigen and virus preparations was determined by inhibition of cytotoxic antisera to human leukemia-associated antigens or the antisera to oncornavirus antigens. Serial dilutions of the antigen preparations (0.5 μl) were incubated with 1 μl of antiserum for 1 h at 37°C. The dilution of antiserum used was determined on the day of testing by end-point titration of the particular antiserum with a given target cell. The highest dilution of antiserum giving target cell lysis from 60 to 90% was used. After the 1-h incubation with antiserum, 0.5 μl of target cells was added and the remainder of the test was performed as described for direct cytotoxicity. Competing antigen activity of a given preparation represented the amount of antigen required for a 50% reduction in the cytotoxic activity of the test antiserum. In each experiment, controls for the anticomplementarity of the antigen preparation were included.

Results

During the course of studies on the isolation and characterization of human leukemia-associated membrane antigens, a partially purified antigen fraction was obtained by DOC extraction of human leukemic cells which blocked not only the cytotoxic reactions of the primate antisera with human leukemic cell antigens, but also had activity by competition radioimmunoassay for the interspecies determinant of FLV gp71 (22). The nature of the competing activity will be reported in a separate publication. This finding, however, prompted us to test various antisera to FLV or FLV structural components and antisera to other RNA tumor viruses for cytotoxic activity for normal and leukemic human cells.

Cytotoxic Reactivity of Antisera to RNA Tumor Viruses and Viral Antigens with Human Cells. The antiserum panel described in Table I was absorbed with HRBC and tested by microcytotoxicity with human normal and leukemic test cells. Of the antisera described in Table I only those to FLV and FLV gp71 reacted with human leukemic cells (Table II). Antisera to FeLV and primate RNA tumor viruses not listed in Table I were also studied. The cytotoxic reactivity of these antisera with human leukemic cells will be presented in a separate publication. The goat antiserum to FLV was cytotoxic for cells from all CGL, AML, and acute myelomonocytic leukemia (AMML) patients tested and reacted with cells from three of five ALL patients. The antiserum failed to react with cells from CLL patients, normal donors, or AML and CGL patients in clinical remission. The remission patients had no leukemic blast cells in the bone marrow or peripheral blood by morphological criteria and both bone marrow and peripheral blood cells were nonreactive with the simian antisera detecting human leukemia-associated antigens. The goat and rabbit antisera to FLV gp71 were cytotoxic for cells from most or all CGL patients and for cells

TABLE I
Oncornavirus Antiserum Panel Studied by Cytotoxicity

Antiserum designation	Immunizing antigen	Species immunized	Properties
G-anti-RLV gp71*	RLV gp69/71 of RLV produced by $JLSV_5$ mouse cells	Goat	No cross-reactivity with other RLV structural proteins (34)
R-anti-AMV	Tween ether disrupted AMV	Rabbit	Reacts with most of the virion structural components (unpublished)
R-anti-AMV gp85	AMV gp85	Rabbit	No cross-reactivity with other AMV structural proteins (19)
R-anti-AMV p27	AMV p27	Rabbit	No cross-reactivity with other AMV structural proteins (18)
R-anti-MMTV gp52	gp52 (isolated from mouse mammary tumor virus from RIII milk)	Rabbit	No reactivity with C-type virus glycoproteins. Stains budding MMTV by immunoelectron microscopy (unpublished)
R-anti-STU	STU tissue culture cells from which Eveline cells were derived	Rabbit	No reactivity with virus or purified FLV antigens (22)
R-anti-FCS	FCS	Rabbit	Good precipitating antibodies to FCS (22)
G-anti-FLV	Tween ether degraded FLV from Eveline cells	Goat	Virus specific. Reacts preferentially with p30 and gp71. Serum was absorbed with normal mouse antigens and FCS (36)
G-anti-FLV gp71	gp71 of FLV from Eveline cells	Goat	No cross-reactivity with other FLV structural components (22)
R-anti-FLV gp71	gp71 of FLV from Eveline cells	Rabbit	No cross-reactivity with other FLV structural components (22)
R-anti-FLV p30	p30 of FLV from Eveline cells	Rabbit	No cross-reactivity with other FLV structural components (16)
R-anti-FLV p15(E)‡	p15(E) of FLV from Eveline cells	Rabbit	No cross-reactivity with other FLV structural components (35)
R-anti-FLV p13§	p13 and p15(E) from Eveline cells	Rabbit	Reacts with both p13 and p15(E) (unpublished)
R-anti-RLV	SDS-treated RLV from mouse plasma	Rabbit	Reacts weakly with several MuLV structural components but strongly with p30(36)

* Obtained from T. August, Albert Einstein College of Medicine, Bronx, N. Y.
‡ p15(E) is a distinct component present on the surface of the virus (37).
§ p13 is a new antigen recently isolated from FLV. Its properties are currently being studied.

from some patients with AMML. The antisera to FLV gp71 failed to react with cells from normal donors or patients with AML, CLL, ALL, or with CGL and AML patients in remission. The cytotoxic titers of the antisera to FLV and FLV gp71 ranged from 1:4 to 1:16 with target cell lysis from 50–100% depending on

TABLE II
Cytotoxicity Testing of Xenoantisera to RNA Tumor Virus Antigens with Human Cells

Goat or rabbit antisera to RNA tumor virus antigens absorbed with HRBC	Normal donors	Cytotoxicity reactions with cells from leukemia patients					
		CGL	AML	CLL	ALL	AMML	AML or CGL remission
Goat anti-FLV	0/12*	21/21	9/9	0/19	3/5	12/12	0/10
Goat anti-FLV gp71	0/12	19/21	0/9	0/19	0/5	3/12	0/10
Rabbit anti-FLV gp71	0/12	21/21	0/9	0/19	0/5	5/12	0/10
Normal goat and rabbit serum‡	0/12	0/21	0/11	0/19	0/5	0/12	0/10

* Number of donors whose cells gave >20% increased lysis with antiserum over normal serum controls/number of donors tested.

‡ Goat or rabbit antisera to these viruses, cells or antigens were also nonreactive with the above cells. FLV p30, FLV p15, FLV p13, RLV, RLV gp71, MMTV gp52, AMV, AMV gp85, AMV p27, STU cells, and FCS (see Table I).

the donor tested. The other antisera described in Table I, including the antiserum to STU cells from which the FLV-producing Eveline cells were derived, and the rabbit antisera to RLV gp71 or fetal calf serum (FCS), were nonreactive with human normal and leukemic cells by this technique.

The ability of the antisera to FLV gp71 to specifically lyse the cells from human CGL patients suggested an antigenic relationship between CGL cells and FLV gp71. Moreover, the cytotoxic reactivity of the antiserum to FLV with cells from patients with other types of leukemia suggested that this cross-reactivity might be via antibodies to FLV components other than gp71. Data from radioimmunoassay and immunoprecipitation studies (22) with purified FLV antigenic components indicates that most of the antibodies in the FLV antiserum reacted with the p30 internal virion component as well as with the gp71 surface glycoprotein. In order to better define the specificity of the reactions of the FLV and FLV gp71 antisera with human leukemic cells, absorption studies were performed.

Cytotoxic Reactivity of Absorbed FLV gp71 Antiserum with Human CGL Cells. Portions of the rabbit antiserum to FLV gp71 absorbed with HRBC were additionally absorbed either with peripheral blood leukocytes, platelets, lymphocytes, neutrophils, or thymocytes from normal donors, or with leukemic cells from individual CLL, AML, or CGL patients. In addition, samples of the antiserum were also absorbed with various oncornaviruses or their purified antigenic components, C57BL/6 spleen cells or STU embryo cells. The absorptions were performed as described in the Materials and Methods. The absorbed antiserum was then tested by cytotoxicity with cells from CGL patients. The results are summarized in Table III. Peripheral blood leukocytes, purified platelets, and neutrophils from five normal donors as well as cells from two different CGL patients were able to absorb out the cytotoxic activity of the gp71 antiserum. Human peripheral blood lymphocytes, thymocytes, and cells from at

TABLE III
Absorption of Cytotoxic Activity of Rabbit Antiserum to FLV gp71

Antigens which absorbed out cytotoxic activity of anti-FLV gp71 antiserum when tested with CGL cells	Antigens which failed to absorb out cytotoxic activity of anti-FLV gp71 antiserum when tested with CGL cells
Human blood neutrophils (2×10^6/ml)*	HRBC (3 volumes packed cells/1 volume antiserum)‡
Human blood platelets (equal volume packed cells)	Blood lymphocytes (3×10^6/ml)
CGL cells (2×10^6/ml)	Thymocytes (3×10^6/ml)
FLV (0.25 mg/ml)	AML cells (3×10^6/ml)
FLV gp71 (0.06 mg/ml)	CLL cells (3×10^6/ml)
FLV spleen cells (2×10^6/ml)	FLV p30 (0.6 mg/ml)
FLV (animal passaged) (0.1 mg/ml)	MMTV gp52 (1.0 mg/ml)
	AMV gp85 (0.5 mg/ml)
	AMV (1.0 mg/ml)
	GLV (0.9 mg/ml)
	RLV (1.0 mg/ml)
	STU cells (1 volume packed cell homogenate/volume antiserum (22)
	C57BL/6 spleen cells (3×10^6/ml)

* Number in parentheses in this column represents the number of cells or protein concentration of virus or virus antigens which completely blocked the cytotoxic activity of the FLV gp71 antiserum with CGL cells. This does not necessarily represent the minimum cell number or protein concentration which will inhibit.

‡ Number in parentheses in this column represents the maximum number of cells or protein concentration of virus or virus antigens which failed to give 50% reduction in the cytotoxic activity of the FLV gp71 antiserum with CGL cells.

least two different CLL and AML patients did not affect the cytotoxic antibody titer of the serum with target cells from CGL donors. Absorptions have not as yet been done with cells from AMML patients. This suggests that platelets and neutrophils from normal individuals do express an antigenic determinant which cross-reacts with one of the determinants detected by the rabbit antiserum to FLV gp71 (see Discussion). Mouse passaged FLV and spleen cells from FLV-infected mice as well as the FLV and FLV gp71 antigens were able to absorb out the cytotoxic reactivity of the antiserum to FLV gp71 when tested with CGL target cells. In contrast, FLV p30, RLV, GLV, AMV, AMV gp85, and MMTV gp52, as well as embryo cells from STU and C57BL/6 mice did not affect the cytotoxic reactivity of the gp71 antiserum. Quantitative data on the ability of FLV and FLV gp71 antigen preparations to inhibit the cytotoxic reactivity of the FLV gp71 antiserum with cells from a CGL patient are shown in Fig. 1. The FLV gp71 antigen gave complete inhibition at a concentration of 0.06 mg/ml and 50% inhibition at a concentration of 0.03 mg/ml. The FLV preparation also inhibited this antibody target cell combination, but less effectively than gp71, which is consistent with the proportion of gp71 (about 5–10% of total protein) in the virus particle (23). AMV at 1 mg/ml or FLV p30 at 0.6 mg/ml (not plotted in Fig. 1) failed to inhibit the reactivity of the gp71 antiserum with CGL cells. A specificity control for these inhibitions can be seen in Table V where FLV and

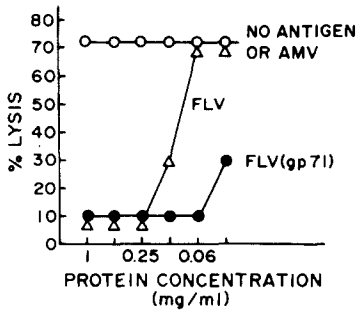

FIG. 1. Inhibition of cytotoxic reactivity of FLV gp71 antiserum with CGL cells. FLV and FLV gp71 inhibited the reaction, whereas AMV did not. FLV p30 at a concentration of 0.6 mg/ml (not plotted) also failed to compete for cytotoxic activity with this antiserum-target cell combination.

FLV gp71 fail to inhibit the reactivity of a chimpanzee antiserum to CLL cells. These antigens were also not inhibitory to the specific cytotoxic activity of HL-A antisera with peripheral blood lymphocytes of normal donors.

Cytotoxic Reactivity of Absorbed FLV Antiserm with Human Leukemia Cells. The goat antiserum to FLV was similarly absorbed with HRBC, blood neutrophils, and platelets from normal donors and with leukemic cells from CLL, CGL, and AML donors. When absorptions were done with human cells at least two different absorbing cell donors were used in each category. The antiserum was also absorbed with FLV gp71 and p30 antigens from FeLV and FLV. In some instances, the antiserum after absorption with either AML or CGL cells was additionally absorbed with FLV gp71, FLV p30, or platelets. The samples of absorbed antiserum were then tested with cells from CGL, AML, or AMML patients (Table IV). The number of patients in each category that were studied varied with each portion of absorbed antiserum. Absorption of the FLV antiserum with CGL cells removed the reactivity for cells from at least 12 other CGL patients tested but left cytotoxic reactivity for cells from 7 AML and 5 AMML patients. Cells from the 2 AML patients used to absorb the FLV antiserum removed reactivity for cells from the 7 AML and 5 AMML patients but left activity for cells from the 10 CGL patients tested. Absorption of the FLV antiserum with blood neutrophils and platelets from normal donors or with FLV gp71 gave the same results as absorption with CGL cells, i.e., reactivity was lost for cells from all CGL donors but was retained for cells from AML and AMML patients. Furthermore, absorption of the FLV antiserum with FLV p30 or FeLV p30 did not affect the reactivity for CGL cells but did remove all reactivity for cells from the AML or AMML patients. When the antiserum to FLV was absorbed with cells from CGL donors plus FLV p30 or when absorption was done with AML cells plus FLV gp71 or human platelets, all cytotoxic reactivity was lost.

The data from Tables III and IV suggest that the antiserum to FLV gp71 is reacting with a FLV gp71-like determinant on CGL cells and on some other myeloid cells including platelets of normal individuals. This antigenic determinant is not present on leukemic cells from patients with CLL, AML, or ALL or

TABLE IV
Results of Absorption of Goat Antiserum to FLV

FLV antiserum absorbed with:	Number of cells or protein concentration of antigen used for absorption	Cytotoxicity reactions with cells from leukemic patients		
		CGL	AML	AMML
HRBC	3 volumes packed, washed cells/1 volume anti-serum	+*	+	+
CGL	2×10^8/ml	−‡	+	+
AML	2×10^8/ml	+	−	−
CLL	3×10^8/ml	+	+	+
Platelets	Equal volume packed cells	−	+	+
Blood neutrophils	3×10^8/ml	−	+	+
FLV gp71	1.0 mg/ml	−	+	+
FLV p30	0.6 mg/ml	+	−	−
FeLV p30	1.0 mg/ml	+	−	−
CGL + FLV gp71	1.0 mg/ml	−	+	+
CGL + FLV p30	1.0 mg/ml	−	−	−
AML + gp71	1.0 mg/ml	−	−	−
AML + platelets	1.0 mg/ml	−	−	−

* +, positive cytotoxic reaction. >50% increased lysis of FLV goat antiserum above normal goat serum controls. Cells from at least three donors with this degree of positive reactivity were tested in each patient category.

‡ −, negative cytotoxic reaction. <10% increased lysis of FLV goat antiserum above normal goat serum controls. Cells from at least five donors were tested in each patient category.

lymphoid cells from normal donors. The antiserum to FLV appears to also detect this gp71-like antigen on CGL cells and in addition, detects another antigen which cross-reacts with FLV p30 and FeLV p30 on cells from AML, AMML, and certain ALL donors. The p30-like antigen on AML and AMML cells detected by the FLV antiserum has not been detected on normal peripheral blood cells.

Inhibition and Absorption with Oncornavirus Antigens of Nonhuman Primate Antisera to Human Leukemia-Associated Antigens. In order to further establish the relationship between FLV antigens and human leukemic cell membrane antigens, various tumor viruses and their antigens were tested for their ability to inhibit the cytotoxic reactions of simian antisera to human leukemia-associated antigens. The ability of these antisera to define antigens specific for human myeloid or lymphocytic leukemia cells has been referenced and summarized in the introduction to this paper. The technique for performing the cytotoxic inhibition is described in the Materials and Methods and its applicability for detection of subcellular and soluble human leukemia-associated antigens has been previously described (24). Serial dilutions of FLV gp71 and the virus suspensions of FLV, FeLV, AMV, and MMTV (partially disrupted by freezing and thawing) were tested for their capacity to block the cytotoxic reactivity of four different simian antisera with human leukemic target cells. In addition, DOC-extracted antigens from cells of a CGL and CLL patient were

TABLE V
Inhibition of Cytotoxic Activity of Simian Antisera to Human Leukemia-Associated Antigens

Antiserum-target cell combination	Cytotoxic inhibition with antigens from:						
	CGL extract	CLL extract	FLV	FLV gp71	FeLV	AMV	MMTV
Monkey anti-CGL (M3) + CGL cells	+* (0.12)	−‡ (4.0)	+ (0.25)	+ (0.06)	− (1.0)	− (1.0)	− (1.0)
Chimpanzee anti-AML (C2) + CGL	+ (0.12)	− (4.0)	+ (0.25)	+ (0.06)	− (1.0)	− (1.0)	− (1.0)
Chimpanzee anti-AML (C2) + AML cells	+ or − (0.12)	− (4.0)	+ or − (0.25)	− (1.20)	− (1.0)	− (1.0)	− (1.0)
Chimpanzee anti-CLL (C1) + CLL cells	− (1.00)	+ (0.2)	− (1.00)	− (1.20)	− (1.0)	− (1.0)	− (1.0)

* +, significant inhibition. 50% or greater reduction of antibody-target cell lysis. Number in parentheses represents minimum antigen concentration (mg/ml) giving 50% or greater reduction.

‡ −, no inhibition. Less than 50% reduction of antibody-target cell lysis. Number in parentheses represents maximum antigen concentration (mg/ml) tested.

partially purified on a Sephadex G200 column and also included in the study. The result of competition of these antigens with various simian antiserum target cell combinations is given in Table V. It should be emphasized that antisera C2 (chimpanzee anti-AML) and M3 (monkey anti-CGL) are not monospecific (14) and that antigens such as the CGL extract, FLV, FLV gp71, and FLV p30 could give different inhibition results when these antisera are tested with cells from other AML or CGL patients. Absorption studies with cells from individual AML and CGL donors have already demonstrated the oligospecificity of the myeloid leukemia antigens detected by these antisera (14). FLV, FLV gp71, and the detergent-extracted antigens of cells from one CGL donor gave 50% or greater inhibition (at concentrations ranging from 0.06 to 0.25 mg/ml) of the reactions of the simian antisera (M3 and C2) with cells from a CGL patient. However, when antiserum C2 was tested with cells from an AML donor, FLV gp71 failed to inhibit the reaction of this antiserum-target cell combination, whereas the FLV preparation was inhibitory. The FLV and FLV gp71 antigens failed to inhibit the cytotoxic reactivity of the C1 antiserum (anti-CLL) to cells from CLL patients. The C1 antiserum-CLL target cell combination was inhibited only by the detergent extract of CLL cells. None of the other oncornaviruses tested (FeLV, AMV, and MMTV) at the concentrations noted gave significant inhibition of the four simian antiserum-target cell combinations. This data provided preliminary evidence that some of the reactions of the simian antisera to myeloid leukemia antigens might be specifically blocked by FLV or FLV gp71. However, the inhibition of a C-dependent serological reaction of an oligospecific antiserum could be misleading due to a possible anticomplementary effect of antigen-antibody complexes. Therefore, absorption followed by ultracentrifugation (in order to remove the complexes from the antiserum) was done before cytotoxicity testing.

TABLE VI
Absorption of Chimpanzee Antiserum to AML Cells with FLV and FLV gp71

Dilution of chimpanzee antiserum (C2)	Cytotoxicity reactions with cells from individual leukemia patients							
	CGL 1	CGL 2	CGL-blast crisis 1	CGL-blast crisis 2	AML 1	AML 2	AML 3	AML 4
Unabsorbed								
1:2	70*	70	90	80	90	100	70	90
1:4	60	60	90	80	90	100	60	90
1:8	60	50	80	90	90	100	60	90
Absorbed With FLV gp71								
1:2	—‡	—	90	80	90	100	60	90
1:4	—	—	80	70	90	100	70	80
1:8	—	—	80	70	90	100	40	80
Absorbed With FLV								
1:2	—	—	50	50	70	60	—	—
1:4	—	—	50	40	60	40	—	—
1:8	—	—	50	—	40	30	—	—

* Number represents % dead cells when antisera gave >30% increased lysis above normal serum controls.

‡ Represents less than 30% increased lysis above normal serum controls.

Undiluted C2 antiserum was absorbed with FLV and FLV gp71 as described in the Materials and Methods. The unabsorbed and absorbed antiserum was then tested with cells from various CGL and AML patients at dilutions from 1:2 to 1:8. Different results depending on the individual cell tested were noted (Table VI). Absorption with FLV gp71 completely removed the reactivity of antiserum C2 for cells from two different CGL donors and corresponded well with the inhibition data in Table V. The FLV gp71 antigen, however, did not alter the reactivity of this antiserum with cells from four different AML patients and the two CGL patients in blast crisis. Cells from the two CGL patients in blast crisis show a reactivity similar to cells from certain AML donors, instead of reacting like cells from CGL patients not in blast crisis. Absorption with FLV removed the reactivity of the antiserum for cells from the two CGL patients which were also affected by absorption with FLV gp71. However, FLV also removed the cytotoxic reactivity for cells from two AML patients that were unaffected by FLV gp71 absorption. Subsequent to absorption with FLV, a decrease in percent lysis and titer was noted for cells from the two CGL patients in blast crisis and the other two AML patients. This type of absorption pattern was also noted in previous studies when another AML antiserum was absorbed with cells from individual CGL donors and then tested with cells from CGL and AML donors (text Fig. 1 in reference 14). Absorption of antiserum C2 with FLV p30 and other internal antigens is in progress in order to determine whether the effect of FLV absorption and testing with AML donors three and four can be linked to a known FLV structural component such as p30.

Thus it appears that FLV and FLV gp71 possess some of the antigenic determinants that are being detected by the simian antisera to human myeloid leukemia antigens. However, since the simian antisera have already been absorbed with human neutrophils and platelets, it seems likely that the determinant on the FLV gp71 molecule reacting in this system is different from that being detected on normal human myeloid cells by the rabbit antiserum to FLV gp71 (see Discussion).

Discussion

This report demonstrates a unique antigenic similarity between certain FLV antigens and membrane-associated antigens on some human myeloid leukemia cells. The serological relationships, however, are complex, which in part may be a reflection of the various types of antigens studied and the immunological perspective of the different species of animals used for antibody production.

The antigenic determinants detected by the cytotoxicity reactions of the FLV gp71 antiserum with human CGL appears to be different from the determinant on FLV gp71 responsible for the inhibition of simian antisera to human myeloid leukemia antigens. The cytotoxic reactivity of the rabbit antiserum to FLV gp71 can be completely absorbed by peripheral blood neutrophils or platelets from normal donors. This reactivity is, therefore, not leukemia specific and probably represents a cross-reaction with a determinant on the FLV gp71 molecule which is also expressed on some normal human myeloid cells. The FLV gp71 determinant being detected in the cytotoxicity assay with human CGL cells is probably different from the usual group-specific or interspecies-specific determinant defined by the FLV gp71 antiserum by radioimmunoassays, immunodiffusion, and C fixation, since an antiserum to the RLV gp71 antigen which shares group- and interspecies-specific determinants with FLV (25) fails to react with human cells. However, Fink et al. (26) have reported a cross-reactivity by immunofluorescence between an antiserum to RLV and bone marrow cells from patients with erythroleukemia, other blood dyscrasias, and nonleukemic malignancies. RLV and GLV also fail to absorb the cytotoxic reactivity of the anti-FLV gp71 antiserum for human CGL cells, suggesting that the cross-reacting determinant detected by this antiserum does not relate to the group or interspecies moiety but may be associated with the type-specific portion of the molecule. However, another possible explanation for these results is that virus preparations such as the RLV and GLV used in this study may have lost a significant portion of their gp71 during purification. This phenomenon has been shown to occur in FLV by Moennig et al. (17). More detailed studies with appropriately absorbed antisera to FLV gp71 will be necessary to determine the molecular nature of the human CGL-FLV gp71 cross-reactivity.

The inhibition by FLV gp71 of the cytotoxicity reaction of the simian antisera to human myeloid leukemia-associated antigens, however, appears to be related to the leukemic process. The primate antisera to human myeloid leukemia antigens have already been absorbed with peripheral blood neutrophils and platelets from normal donors. The number of cells used for this absorption, as noted in other studies, is adequate for the removal of antibodies reactive to determinants present on these normal cells. Therefore, it may be concluded that

the inhibition of the simian antisera by FLV gp71 is related to specific antigens present on myeloid leukemic cell membranes.

The cytotoxic activity of the FLV antiserum with human CGL cells is probably due to antibodies to FLV gp71. This reactivity is lost by absorption with FLV gp71, normal human platelets or cells from CGL donors. Failure of AML cells to absorb out this reactivity indicates the antigen is not present on these cells. In contrast, the cytotoxic activity of the FLV antiserum with AML cells is not affected by absorption with FLV gp71, human peripheral blood neutrophils, platelets, or CGL cells but is removed by absorption with p30 antigens (Table IV). Since both FLV p30 and FeLV p30 absorb the cytotoxic activity of the FLV antiserum for AML cells, it may well be that the cross-reactivity between FLV p30 and AML cells is due to the interspecies-specific determinant. However, direct cytotoxic testing on AML cells with rabbit antisera to p30 did not reveal the expected activity. When tested on FLV-producing murine Eveline cells, the FLV p30 antiserum is cytotoxic, but the end-point titer is considerably lower than that obtained with gp71 antiserum (27). The failure of the FLV p30 antiserum to lyse human cells may reflect the quantitative or spatial distribution of the antigen on the membrane of these cells. It is also possible that the p30 preparation used to elicit the antisera were somehow modified during purification such that antibodies were not produced against the cross-reactive determinant.

The nature of the molecular species on the human leukemic cell surface which cross-reacts with FLV gp71 and p30 is presently under investigation. Attempts to specifically immunoprecipitate similar molecules from antigen-positive human leukemic cells with antisera to FLV gp71 have resulted in multiple molecular species in the immunoprecipitates of which one is a glycoprotein of about 55,000 daltons mol wt. Although this molecular species is not detectable in antigen-negative cells, its relationship to the viral gp71 remains to be established. We have been able to determine, however, that enzymatic removal of a significant portion of the carbohydrate on gp71 (28) does not eliminate its cross-reactivity with human CGL antigens, thereby minimizing the possibility that we are dealing with a spurious cross-reactivity similar to the Forssman antigen.

We do not feel that the data presented in this report are sufficient to conclude that human CGL or AML are caused by an RNA oncogenic virus. However, it does imply that whatever the mechanism of leukemogenesis in man may be, it is associated with the expression of at least two membrane antigens on myeloid leukemia cells which cross-react with well characterized components (gp71 and and p30) of FLV. There are certain studies which indicate a possible viral association with human leukemia. Gallagher and Gallo (29) reported an isolate from an AML patient with characteristics of primate C-type viruses, and Sherr and Todaro (4) found that cells from some AML patients contain material which cross-reacts with p30 of primate oncornaviruses. The cross-reactivity of p30 and gp71 molecules between murine and certain primate RNA viruses (simian sarcoma and gibbon ape lymphoma) has already been established (25, 30, 31). Moreover, another example of how an endogenous virus gene product of one species may show cross-reactivity with an antigen of oncornaviruses closely associated with a phylogenetically distant species can be seen in the recent work of Lieber et al.

(32). These investigators isolated an endogenous C-type particle from an Asian mouse *Mus-caroli* which shows strong p30 and reverse transcriptase cross-reactivity with type C viruses from the woolly monkey and gibbon ape. This study may also relate to our finding of a cross-reactive gp71 determinant on certain malignant and normal human myeloid cells which is also present on a molecule (FLV gp71) associated with leukemogenesis in the mouse.

In addition, preliminary studies in our laboratory similar to those described in this report suggest that there is another independent relationship between antigens of primate RNA tumor viruses and membrane-associated antigens of CLL, ALL, and AML cells. Our approach has been to test the ability of the primate oncornaviruses and their antigens to inhibit or block the cytotoxic activity of rabbit and simian antisera that are defining human leukemia-associated antigens different from those studied in this report. In animal systems, it is known that different leukemogenic RNA viruses have strain and species specificity, as well as the capacity to induce different forms of leukemia. The species of the host cell infected or transformed by the virus may also influence the cross-reactive antigenicity. For example, Mann et al. (33) studied the cytotoxic reactivity of a rabbit antiserum to a papain digest preparation from the RAJI lymphoblastoid cell line (originally derived from a patient with Burkitt's lymphoma). This antiserum detected a leukemia-associated antigen on human embryonic kidney lines after infection with RLV, Kirsten, or SV-40 virus. However, mouse cell lines infected with RLV and Kirsten virus were nonreactive.

The goat and rabbit antisera to FLV and FLV gp71 are already proving to be valuable diagnostic and prognostic reagents for the detection of human leukemia-associated antigens in clinical studies at Duke University, Durham, N. C. Our rabbit and nonhuman primate antisera to AML and CGL leukemia antigens do not easily distinguish these two types of leukemia. However, the cytotoxic reactions of the rabbit antiserum to FLV gp71 and the goat antiserum to FLV, provides a serological means for making this distinction. One of the major obstacles in the large scale production and distribution of xenoantisera to human leukemia-associated antigens for diagnosis or therapy is that most of the current antisera to human leukemia cells require exhaustive and tedious absorptions with normal human tissue cells before they are rendered leukemia specific. If the antigenic relationship of RNA tumor viruses with human leukemia antigens can be well established and if different specificities based on morphological types of human leukemias are noted, it may be that immunization of animals with the appropriate purified viral protein will result in leukemia-specific antisera that do not require large scale absorptions with normal human tissue cells.

Summary

Leukemic cells from all human chronic granulocytic leukemia (CGL) and some acute myelomonocytic leukemia (AMML) donors are lysed by rabbit antisera to a purified glycoprotein of Friend murine leukemia virus (FLV gp71) in a microcytotoxicity assay. These antisera are not cytotoxic to cells from patients with acute myelocytic leukemia (AML), acute lymphocytic leukemia

(ALL), chronic lymphocytic leukemia (CLL), or to peripheral blood lymphocytes from normal donors. A goat antiserum to gradient purified FLV in addition to reacting with cells from CGL and AMML donors also reacted with cells from AML patients and some ALL donors. However, this antiserum failed to react with cells from CLL patients. Peripheral blood and bone marrow leukocytes prepared from leukemic patients in clinical remission failed to react with antisera to FLV and FLV gp71. Absorption experiments demonstrated that the antigen on CGL cells which is reacting with the antiserum to FLV gp71 is also present on normal human platelets and neutrophils. Similar absorption studies showed that the antigen on AML cells detected by the FLV antiserum is not present on normal leukocytes and platelets and appears to be related to the major internal p30 antigens of mammalian RNA tumor viruses.

Another antigenic relationship between oncornaviruses and membrane antigens of human leukemia cells was shown by the ability of FLV antigens to absorb the cytotoxic reactivity of nonhuman primate antisera detecting human leukemia-associated antigens. FLV and FLV gp71 antigens were able to absorb all cytotoxic activity of monkey and chimpanzee antisera to human myeloid leukemia antigens when these antisera were tested with CGL cells.

These two approaches to an analysis of cross-reactivity indicate that the antigenic determinant(s) detected by the cytotoxic reactions of the FLV gp71 antiserum with human CGL cells is different from the determinant on FLV gp71 which is responsible for the inhibition of the reactivity of simian antisera with CGL cells.

Since the goat and rabbit antisera to FLV and FLV gp71 are able to distinguish AML from CGL cells by direct cytotoxicity testing and absorption, they may be valuable reagents for the serological diagnosis of myeloid leukemia. In addition, since peripheral blood cells from AML and CGL patients in clinical remission were seronegative, the antisera may be valuable as management aids.

The data in this report indicates that whatever the mechanism of leukemogenesis is in man, cells from CGL and AML patients possess certain membrane antigens which cross-react with FLV structural components such as p30 and gp71.

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