COMMON CELL SURFACE ANTIGEN ASSOCIATED WITH MAMMALIAN C-TYPE RNA VIRUSES*

CELL MEMBRANE-BOUND gs ANTIGEN

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The antigenic analysis of the structural proteins specified by C-type RNA viruses has been advanced in recent years. The presence of an interspecies cross-reacting antigenic determinant associated with an intraspecies antigenic determinant of the structural proteins of mammalian C-type RNA viruses was first described by Geering et al. (1, 2). The interspecies cross-reacting determinant of mammalian C-type RNA viruses has become increasingly important for the search and the analysis of C-type RNA viruses in other species, especially in man. The protein containing these antigenic determinants has been purified in several laboratories and shown to be a major internal polypeptide of mammalian C-type RNA viruses with a mol wt of about 30,000 daltons (3–6), being termed p 30 by the consensus of participants in an oncogenic RNA virus meeting held in June, 1973, at Memorial Sloan-Kettering Cancer Center, New York. The antigens of p 30 protein have been termed gs-1,[1] intraspecies (species specific) determinant, and gs-3, interspecies cross-reacting determinant (1, 2). gs-1 and gs-3 were proved to be on the same molecule of p 30 protein (7–9). Two other interspecies cross-reacting antigenic determinants of mammalian C-type RNA viruses have been demonstrated recently: (*a*) RNA-dependent DNA polymerase of mammalian C-type RNA viruses (10, 11), and (*b*) the viral envelope glycoprotein component with a mol wt of 69,000–71,000 daltons (12, 13).

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[1] *Abbreviations used in this paper:* FeLV, feline leukemia virus; gs, group specific; gs-1, intraspecies (species specific) determinant; gs-3, interspecies cross-reacting determinant; IFA, immunofluorescence absorption test; MuLV, murine leukemia virus; PBS, phosphate-buffered isotonic saline; RRTC, epitheloid cell line derived from rat leukemia induced by Rauscher strain of MuLV; RSV-2, Rous sarcoma virus; SSV, simian sarcoma virus; ST-FSV, Snyder-Theilen strain of feline sarcoma virus.

In recent work undertaken in this laboratory an antibody which recognizes a common cell surface antigen associated with murine and feline C-type RNA viruses was demonstrated by the use of rabbit antiether disrupted feline leukemia virus (FeLV) antiserum and the indirect membrane immunofluorescence method (14). We now present data which establish that the common cell surface antigen is membrane-bound group-specific (gs) antigen bearing the identical gs determinant known to be shared by mammalian C-type RNA viruses. The possible biological properties of membrane-bound gs antigen are also discussed.

Materials and Methods

Mice.—AKR/J and C57BL/6J mice were purchased from Jackson Laboratories, Bar Harbor, Maine. These mice were maintained in a specific pathogen-free environment.

Leukemias and Tumors.—Mice bearing spontaneous or induced, autologous or transplantable, leukemias and tumors were available in our laboratory or supplied by Dr. L. J. Old (Sloan-Kettering Institute for Cancer Research, New York). Specimens of feline and canine lymphomas were obtained from fresh autopsy materials. Fresh specimens of human leukemias, sarcomas, and other neoplasms were obtained from the surgical and medical services of Memorial Hospital, Sloan-Kettering Institute for Cancer Research, New York, as they became available through standard therapeutic and diagnostic procedures.

Tissue Culture Lines.—Cultures of chicken, hamster, rat, feline, canine, bovine, porcine, and human cells with or without infection by avian and feline C-type RNA viruses, and woolly monkey tumor cells producing woolly monkey sarcoma virus were obtained from Dr. P. S. Sarma (National Cancer Institute, Bethesda, Md., under contract NIH-No.1-43254 of the Virus Cancer Program). Cultures of an epitheloid cell line derived from a rat leukemia (RRTC) induced by Rauscher strain of murine leukemia virus (MuLV), and of cat fibroblasts infected with FeLV were from our laboratory (4, 15).

Subviral Proteins of C-Type RNA Viruses.—

Crude viral proteins of mammalian C-type RNA viruses: Crude viral proteins were prepared from concentrated suspensions of Rauscher strain of MuLV, Rickard strain of FeLV, and SSV-1 strain of simian sarcoma virus (SSV). The virus suspensions were prepared from isolated viruses recovered from the media of cultured cells by zonal ultracentrifugation followed by pelletization, and final suspensions contained more than 10^{10} virus particles/ml. FeLV was derived from Rickard-F-422 cat thymus leukemia line and SSV-1 was derived from SSV-1-HF infected marmoset tumor cell line (16). The virus suspensions were supplied through the courtesy of Dr. J. Gruber (National Cancer Institute, Bethesda, Md.). Each virus suspension was frozen-thawed twice and sonicated for 60 s in an ice bath. The clear supernate was recovered by centrifugation at 10,000 rpm for 30 min and was used as crude viral proteins of C-type RNA viruses.

Subviral fractions of MuLV and FeLV isolated by gel filtration in guanidine hydrochloride (GuHCl): Six subviral proteins were isolated and prepared by preparative gel filtration in GuHCl from Rauscher leukemia virus and FeLV derived from our own culture lines according to the method described by Nowinski et al. (4). Pellets of virus previously banded by density gradient centrifugation on a sucrose gradient (15–60%) were suspended in saturated GuHCl, 2% mercaptoethanol, and 10% sucrose, pH 8.6. Samples were heated at 100°C for 3 min and were then layered upon an agarose column (Bio Gel A-5 mm 200–400 mesh, Bio-Rad Laboratories, Richmond, Calif.) Column buffer was 6 M GuHCl with 0.01 M dithiothreitol, pH 6.5. Column was run at room temperature at a flow rate of 1.5 ml/h. Six peak fractions of C-type viral protein were dialyzed free from GuHCl and dithiothreitol and stored at −70°C until use.

p 30 protein of MuLV and FeLV purified by isoelectric focusing: Isoelectric-focused p 30 protein of MuLV and FeLV were kindly supplied by Doctors R. V. Gilden and S. Oroszlan

(Flow Laboratories, Inc., Rockville, Md.). The method of preparation was fully described elsewhere (5, 9).

Antisera.—The following antisera were used for the present study.

Rabbit anti-FeLV: Rabbit anti-FeLV antiserum was produced by immunizing rabbits with ether-disrupted FeLV. The purified FeLV banded by density gradient centrifugation on a sucrose gradient was disrupted with ether, mixed with Freund's adjuvant, and injected subcutaneously at several sites (17).

Rabbit anti-FeLVp 30: Rabbit anti-FeLVp 30 antiserum was produced by immunizing rabbits with FeLVp 30, the third protein fraction eluted from GuHCl-disrupted FeLV by gel filtration and known to be gs protein. The immunizing schedule was the same as described above.

Rabbit and anti-MuLVp 30: Rabbit anti-MuLVp 30 antiserum was produced in rabbits with MuLVp 30 prepared from GuHCl-disrupted Rauscher leukemia virus by gel filtration. The immunizing schedule was described above.

All antisera were heat inactivated at 56°C for 30 min and were stored at −70°C until use. The unabsorbed antisera contained antibodies against normal cellular components of mouse tissues. The antisera were absorbed in vitro with C57BL/6J spleen and thymus cells in order to remove these antibodies. Procedures of preliminary absorption were fully described in a previous paper (14). The antisera were titrated against viable E♂G2 cells by the indirect membrane immunofluorescence test by doubling dilutions, and the fluorescent end point (50% fluorescent cells) was determined.

Membrane Immunofluorescence Test.—The indirect immunofluorescence test with viable cells was carried out as described by Aoki et al. (18) with slight modification. The standard target cells were transplanted leukemia E♂G2 originally induced in C57BL/6 mouse by passage A Gross virus (19). Suspensions of viable E♂G2 cells were prepared from the spleens of C57BL/6J mice bearing transplants of E♂G2 as described previously (20). A mixture of 50 μl of antiserum at a certain dilution and 25 μl of cell suspension (4×10^7/ml) was incubated at 4°C for 30 min with occasional shaking. The cells were then washed twice with medium 199 (Microbiological Associates, Inc., Bethesda, Md.) in the cold and suspended in 50 μl of fluorescein-conjugated goat antiserum to rabbit 7S γ-globulin (Hyland Div., Travenol Laboratories, Los Angeles, Calif.) at a dilution of 1:15. After incubation for 30 min at 4°C, the cells were finally washed twice with medium 199, suspended in 50% glycerol in 0.01 M phosphate-buffered (pH 8.0) isotonic saline (PBS), and examined under a cover slip with a Zeiss fluorescence microscope (exciter filter BG12, barrier filters 50 and 44, and dark field condenser) (Carl Zeiss, Inc., New York). Negative controls were either medium 199 alone or normal rabbit serum and both were virtually unstained.

Immunofluorescence Absorption Test (IFA).—Washed cells and tissue homogenates to be tested were transferred into small glass tubes (6 × 50 mm) and centrifuged for 10 min at 2,000 rpm. Equal volumes of washed cells, tissue homogenates, or protein preparations and the antiserum diluted two tubes below the fluorescent end point (50% fluorescent cells) were incubated for 60 min at 4°C with constant shaking. After centrifugation for 15 min at 2,000 rpm, the supernatant fluids at 1:1 and 1:2 dilutions were tested for the residual antibody activity on viable E♂G2 cells by the indirect membrane immunofluorescence test. Preparation of cells and tissue homogenates were described (20).

The Indirect Immunofluorescence Test on Cryostat Sections.—In order to demonstrate gs antigen in the cytoplasm of cells, the indirect immunofluorescence test was performed on cryostat sections of C57BL/6J mouse spleens bearing E♂G2 transplant. The sections were air dried, fixed in acetone for 30 min, and washed twice in PBS (pH 7.6). The sections were incubated at room temperature for 35 min with rabbit anti-MuLVp 30 and FeLVp 30 antisera, followed by washing with PBS and treatment with fluorescein-conjugated goat antiserum to rabbit 7S γ-globulin diluted 1:30. The fluorescent antibody was previously absorbed with mouse γ-globulin (Miles Laboratories, Inc., Miles Research Div., Kankakee, Ill.).

Immunoelectron Microscopy.—10 × 10^6 washed viable cells were incubated for 30 min in 0.2 ml of a 1:5 dilution of rabbit anti-FeLVp 30 antiserum. After two washings, the cells were resuspended in 0.2 ml of ferritin-conjugated sheep antibody to rabbit γ-globulin. After further incubation for 30 min, the cells were washed twice. The final pellets were covered with 2 ml of 2% glutaraldehyde for 40 min. After postfixation for 60 min in 1% osmium tetroxide, the pellets were left overnight in 2% uranyl acetate. The next morning, they were dehydrated progressively in 70, 95, and 100% ethanol. All procedures were carried out at 4°C except for dehydration in 100% ethanol at room temperature. They were embedded in Epon (Shell Chemical Co., New York) as usual. Thin sections were cut on a Sorvall MT-2 ultramicrotome (Ivan Sorvall, Inc., Norwalk, Conn.) 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 (Siemens Corp., Medical Industrial Div., Iselin, N. J.). Ferritin-conjugated sheep antibody to rabbit γ-globulin was a generous gift of Dr. K. C. Hsu (Columbia University, New York) and was extensively absorbed with C57BL/6J spleen and thymus cells, and E♂G2 cells.

RESULTS

Demonstration of Common Cell Surface Antigen Associated with Mammalian C-Type RNA Viruses in the Tissue-Cultured Cells Infected or Transformed by C-Type RNA Viruses.—In the previous communication (14), a new common cell surface antigen associated with murine and feline C-type RNA viruses was demonstrated in all leukemias of mice tested: in normal lymphoid tissues of Gross-positive (high incidence of leukemia) mouse strains AKR, AKR/*H-2*^{b}, C58, and NZB; in cultured rat fibroblasts infected with MuLV; in cultured feline fibroblasts infected with FeLV; and in spontaneous feline lymphosarcoma by the IFA with rabbit anti-FeLV antiserum as described in Materials and Methods. We extended the investigation with various cultured cells infected or transformed by C-type RNA viruses and examined for the expression of common cell surface antigen as determined by the IFA with rabbit anti-FeLV antiserum (Table I). Antiserum diluted to 1:20, two tubes below end point (50% fluorescent-positive cells), was absorbed with the cultured cells listed in Table I, and the residual antibody activity of the recovered serum was titrated both undiluted and at 1:2 dilution against viable E♂G2 cells by the indirect immunofluorescence test. Embryo feline fibroblasts infected with various subgroups of FeLV or transformed by Snyder-Theilen strain of feline sarcoma virus (ST-FSV), feline fibroblasts infected with FeLV (our own strain), and RRTC cells absorbed the antibody activity from the antiserum. Hamster, canine, porcine, bovine, and human embryo cells infected or transformed by ST-FSV equally absorbed the antibody activity. No trace of the antigen was demonstrable in comparable homologous normal cells, chicken cells transformed by Rous sarcoma virus (RSV-2), or woolly monkey tumor cells producing woolly monkey sarcoma virus.

Search for Common Cell Surface Antigen in Various Mammalian Leukemias and Tumors.—Neoplastic tissues of mouse, cat, dog, and human were examined for the expression of common cell surface antigen by the IFA with rabbit anti-FeLV antiserum (Table II). Common cell surface antigen was demonstrable

TABLE I

Demonstration of Common Cell Surface Antigen in Cultured Cells Infected or Transformed by Mammalian C-Type RNA Viruses

Cells used for absorption	Residual antibody activity* Dilution of absorbed serum 1:1	1:2	Result of absorption
	%	%	
Uninfected feline embryo fibroblasts	73	65	−
Feline embryo fibroblasts infected with FeLV, subgroup A‡	<10	<10	+
Feline embryo fibroblasts infected with FeLV, subgroup B	<10	NT§	+
Feline embryo fibroblasts infected with FeLV, subgroup C	39	NT	+
Feline embryo fibroblasts transformed by ST-FSV¶	<10	<10	+
Feline fibroblasts infected with our own FeLV	<10	<10	+
—	80	71	
Uninfected canine embryo fibroblasts	75	NT	−
Canine embryo fibroblasts transformed by ST-FSV	<10	NT	+
Uninfected whole porcine embryo cells	NT	52	−
Whole porcine embryo cells transformed by ST-FSV	<10	<10	+
Uninfected whole bovine embryo cells	72	55	−
Whole bovine embryo cells transformed by ST-FSV	NT	<10	+
Uninfected whole human embryo cells	NT	54	−
Whole human embryo cells infected with ST-FSV	NT	<10	+
—	84	79	
Uninfected chicken cells	81	NT	−
Chicken cells transformed by RSV-2	79	NT	−
Hamster cells transformed by ST-FSV	19	<10	+
—	86	77	
Uninfected rat fibroblasts	65	NT	−
RRTC cells producing Rauscher virus	15	<10	+
—	76	49	
Woolly monkey cells producing WMSV¶	75	69	−
—	87	76	

* % fluorescent-positive cells.

‡ FeLV, feline leukemia virus (see reference 31).

§ NT, not tested.

¶ WMSV, woolly monkey sarcoma virus.

in all murine leukemias, spontaneous or induced, and all spontaneous cat lymphosarcomas. Two plasma cell tumors and a mammary tumor of mice and normal lymphoid tissues of cat were negative for the expression of the antigen. The common cell surface antigen was not found in any canine or human naturally occurring lymphoid or nonlymphoid tumors.

TABLE II

Occurrence of Common Cell Surface Antigen in Tumor Cells of Various Species

Species	Tumor	Result‡
Mouse*	Leukemia, spontaneous	9/9
	Leukemia, induced	5/5
	Mammary tumor	0/1
	Myeloma	0/2
Cat	Spontaneous lymphosarcoma	4/4
	Normal lymphoid tissues	0/4
Dog	Spontaneous lymphosarcoma	0/1
Human	Chronic lymphatic leukemia	0/7
	Acute monocytic leukemia	0/2
	Hodgkins sarcoma	0/6
	Osteogenic sarcoma	0/3
	Carcinoma	0/5

* See reference 14.

‡ Number positive/number examined by IFA.

Demonstration of Common Cell Surface Antigen in Subviral Proteins of MuLV and FeLV Prepared By Gel Filtration in GuHCl and Isoelectric Focusing.— Since the antiserum detecting common cell surface antigen was produced by immunizing rabbits with ether-disrupted FeLV, it is reasonable to suppose that common cell surface antigen is related to, or indentical with, some structural protein of C-type RNA virus. We examined this possibility by using the IFA of rabbit anti-FeLV antiserum with sonicated crude viral proteins of MuLV, FeLV, and SSV; with six isolated subviral proteins of MuLV prepared by gel filtration in GuHCl; and with p 30 protein of MuLV and FeLV purified by isoelectric focusing. The residual antibody activity of the above was tested on E♂G2 cells by the indirect membrane immunofluorescence test (Table III). Common cell surface antigen was demonstrable equally in crude viral proteins of MuLV and FeLV; in contrast, crude viral protein of SSV did not show demonstrable absorption. Of six subviral proteins of MuLV prepared by gel filtration in GuHCl, only p 30 protein, the third protein fraction eluted and the fraction known to contain gs-1 and -3 antigenic determinants, completely absorbed the antibody activity from the antiserum. The other five protein fractions were virtually negative for the absorption except gp 45 (void volume fraction) which showed weak absorption. These results were confirmed by the IFA with purified MuLVp 30 and FeLVp 30 protein prepared by isoelectric focusing; purified gs antigen of both MuLV and FeLV equally absorbed the antibody activity against common cell surface antigen from rabbit anti-FeLV antiserum. These findings strongly indicate that common cell surface antigen

TABLE III

Demonstration of Common Cell Surface Antigen in Structural Proteins of Mammalian C-Type RNA Viruses

Antigens used for absorption	Residual antibody activity*		Result of absorption
	Dilution of absorbed serum		
	1:1	1:2	
Exp. 1: Crude viral proteins			
Sonicated MuLV	<10%	<10%	+
Sonicated FeLV	17	<10	+
Sonicated SSV	73	66	−
—	76	69	
Exp. 2: Subviral proteins of MuLV prepared by gel filtration			
gp 45 (50 μg)§	71	58‡	−(±)
gp 70 (40 μg)	78	65	−
p 30 (20 μg)	<10	<10	+
p 15 (30 μg)	72	62	−
p 12 (20 μg)	77	61	−
p 10 (30 μg)	76	63	−
—	79	65	
Exp. 3: gs antigen purified by isoelectric focusing			
MuLVp 30 (20 μg)	<10	<10	+
FeLVp 30 (20 μg)	10	<10	+
—	91	85	

* % fluorescent positive cells.

‡ Weak fluorescence reaction.

§ Parentheses indicates μg protein used for absorption of 50 μl of antiserum. Rabbit anti-FeLV antiserum two tubes below end point (50% fluorescent-positive cells) were absorbed with viral proteins at 4°C overnight, and residual antibody activity was titrated at 1:1 and 1:2 dilution of recovered serum against E♂G2 cells by the membrane immunofluorescence test.

is indeed related to a known viral structural component of mammalian C-type RNA viruses and identical with gs-3 antigen known to be shared by mammalian C-type RNA viruses.

Indirect Membrane Immunofluorescence Reaction with Rabbit Anti-FeLVp 30 Antiserum.—To confirm these results, the rabbit antiserum against purified p 30 protein of FeLV which contains only specific antibodies to gs antigen was examined by the indirect membrane immunofluorescence test to determine whether it contained interspecies cross-reacting antibody reacting with viable E♂G2 cells. After in vitro absorption with C57BL/6J tissues as described in the Materials and Methods, the antiserum reacted with the cell surface of E♂G2. The fluorescence end point (50% fluorescent-positive cells) was at a

serum dilution of 1:80, which closely parallels the titer found against acetone-fixed E♂G2 cells.

The Specificity of the Indirect Membrane Immunofluorescence Reaction of E♂G2 Cells with Rabbit Anti-FeLVp 30 Antiserum.—In order to demonstrate the specificity of the antibody, the antiserum diluted two tubes below the fluorescence end point was absorbed with various tissues, cultured cells, and purified subviral proteins of mammalian C-type RNA viruses listed in Table IV. The absorbed antiserum was tested for the residual antibody activity against viable E♂G2 cells by the indirect membrane immunofluorescence test. Lymphoid tissues of AKR/J mice absorbed the antibody activity, whereas no absorption (except sometimes weak absorption with bone marrow and spleen cells) was observed with comparable C57BL/6J tissues. The antigen was demonstrated in all mouse leukemias so far assayed but was not demonstrable in a transplantable myeloma. Cultured feline fibroblasts infected with FeLV and RRTC cells producing Rauscher virus equally absorbed the antibody activity. By the method employed, no trace of the antigen was demonstrable in comparable homologous cells or in woolly monkey tumor cells producing woolly monkey sarcoma virus. Crude viral proteins of MuLV and FeLV equally absorbed the antibody activity, whereas those of SSV did not. p 30 protein of MuLV isolated by gel filtration in GuHCl strongly absorbed the antibody activity, whereas gp 45 protein of MuLV did not. Isoelectric-focused p 30 protein of MuLV and FeLV absorbed antibody activity from rabbit anti-FeLVp 30 antiserum. It was concluded that the antibody specificity of rabbit anti-FeLV and rabbit anti-FeLVp 30 antiserum against viable E♂G2 cells was virtually the same and, again, that the antigen demonstrated on the cell surface by rabbit anti-FeLVp 30 antiserum was gs-3 determinant of mammalian C-type RNA viruses.

Demonstration of gs-1 Determinant of MuLV on the Surface of Cells.—Since Gilden et al. and Oroszlan et al. (7–9) presented evidence that gs-1 and gs-3 antigenic determinants are present on the same protein molecule, it was reasonable in our work to ask whether gs-1, the intraspecies (species specific) antigenic determinant of mammalian C-type RNA viruses, is also present on the surface of cells. We examined this possibility by using rabbit anti-MuLVp 30 antiserum which contained specific antibody to gs-1 determinant of MuLV. Viable E♂G2 cells were used as target cells in the indirect membrane immunofluorescence test. After in vitro absorption with C57BL/6J tissues, the antiserum was found to contain antibody that reacted with viable E♂G2 cells by the membrane immunofluorescence test. The fluorescence end point was at a serum dilution of 1:640, in close parallel with the titer against acetone-fixed E♂G2 cells. The specificity of the antiserum determined by the IFA is summarized in Table V. The lymphoid tissues of AKR/J mice absorbed the antibody activity, whereas the tissues of C57BL/6J mice did not, although bone marrow cells and spleen cells of C57BL/6J mice occasionally showed weak

TABLE IV

Specificity of Cell Surface Antigen Demonstrated with Rabbit Anti-FeLV p 30 Antiserum

Antigens used for absorption	Residual antibody activity*		Result of absorption
	Dilution of absorbed serum		
	1:1	1:2	
	%	%	
AKR/J (2-mo old)			
Spleen	17	11	+
Thymus	35	21	+
Lymph node	24	NT	+
Bone marrow	<10	<10	+
Liver	80	69	−
Kidney	76	61	−
Brain	78	63	−
Erythrocytes	80	NT	−
—	86	77	
C57BL/6J(2-mo old)			
Spleen	81	64	−
Thymus	78	NT	−
Lymph node	82	NT	−
Bone marrow	61	43	±
Liver	80	62	−
Kidney	83	65	−
Brain	80	65	−
Erythrocytes	84	67	−
—	87	74	−
Tumors‡			
C57BL/6 E♂G2	<10	<10	+
EL4	13	<10	+
C1498	48	35	+
Balb/c BALB♂RL1	<10	<10	+
MOPC-70A	76	65	−
AKR K36	<10	<10	+
SL	<10	<10	+
—	84	68	
Cultured cells			
Uninfected rat fibroblasts	80	75	−
RRTC cells producing Rauscher virus	39	15	+
Uninfected feline fibroblasts	81	77	−
Feline fibroblasts infected with FeLV	<10	NT	+
Woolly monkey cells producing WMSV	80	74	−
—	93	79	
Viral proteins			
Sonicated MuLV	<10	<10	+
Sonicated FeLV	24	15	+
Sonicated SSV	87	76	−
—	88	78	

TABLE IV—*Continued*

Antigens used for absorption	Residual antibody activity*		Result of absorption
	Dilution of absorbed serum		
	1:1	1:2	
	%	%	
Subviral proteins of MuLV prepared by gel filtration			
gp 45 (15 μg)§	71	57	−
p 30 (15 μg)	<10	<10	+
—	71	65	
gs antigen purified by isoelectric focusing			
MuLVp 30 (20 μg)	<10	<10	+
FeLVp 30 (20 μg)	16	<10	+
—	90	86	

Rabbit anti-FeLVp 30 antiserum was used at a serum dilution of 1:20.

* % fluorescent-positive cells.

‡ see reference (14).

§ Parentheses indicates μg protein used for absorption.

absorption. RRTC cells producing Rauscher virus did absorb the antibody activity. The antigen was not demonstrable in normal rat fibroblasts, in feline fibroblasts with or without infection by FeLV, or in woolly monkey tumor cells producing woolly monkey sarcoma virus. Crude viral proteins of MuLV absorbed the antibody activity, whereas neither those of FeLV nor of SSV did so. Of six subviral proteins of MuLV prepared by gel filtration in GuHCl, only p 30 protein completely absorbed the antibody activity. The other five fractions were virtually negative for the absorption. These results were confirmed by the absorption test with p 30 protein of MuLV purified by isoelectric focusing which completely absorbed the antibody activity from rabbit anti-MuLVp 30 antiserum, whereas purified p 30 protein of FeLV similarly prepared by isoelectric focusing did not absorb the antibody activity. These absorption data are consistent with the hypothesis that gs-1 antigenic determinant of MuLV is present on the surface of cells. It is to be noted that the fluorescence end point against viable E♂G2 cells for the three gs-typing sera described above closely paralleled that against acetone-fixed E♂G2 cells. These collective observations virtually excluded the possibility that minor contaminants in p 30 protein fraction had induced the antibody reacting against common cell surface antigen. The antigen being detected on the cell surface with rabbit anti-FeLV, rabbit anti-FeLVp 30, and rabbit anti-MuLVp 30 antiserum was concluded to be, and was given the designation, membrane-bound gs antigen.

Location of Membrane-Bound gs Antigen.—In our previous report, the location of common cell surface antigen was demonstrated solely on the cell surface

TABLE V

Demonstration of gs-1 Antigen of MuLV on the Surface of Cells

Antigens used for absorption	Residual antibody activity*		Result of absorption
	Dilution of absorbed serum		
	1:1	1:2	
AKR/J (2-mo old)			
Spleen	<10%	NT	+
Thymus	28	13	+
Lymph node	NT	<10	+
Bone marrow	<10	<10	+
Liver	73	56	−
Kidney	70	NT	−
Brain	73	58	−
Erythrocytes	71	57	−
—	80	62	
C57BL/6J(2-mo old)			
Spleen	82	75	−
Thymus	83	NT	−
Lymph node	80	NT	−
Bone marrow	82	73	−
Brain	81	70	−
—	86	79	
Cultured cells			
Uninfected rat fibroblasts	83	NT	−
RRTC cells producing Rauscher virus	13	NT	+
Uninfected feline fibroblasts	82	74	−
Feline fibroblasts infected with FeLV	NT	71	−
Woolly monkey cells producing WMSV	76	69	−
—	88	77	
Viral proteins			
Sonicated MuLV	<10	<10	+
Sonicated FeLV	77	73	−
Sonicated SSV	76	71	−
—	79	72	
Subviral proteins of MuLV prepared by gel filtration			
gp 45 (50 μg)	77	70	−
gp 70 (40 μg)	75	69	−
p 30 (20 μg)	<10	<10	+
p 15 (30 μg)	79	69	−
p 12 (20 μg)	78	66	−
p 10 (30 μg)	79	67	−
—	78	67	
gs antigen purified by isoelectric focusing			
MuLVp 30 (20 μg)	<10	<10	+
FeLVp 30 (20 μg)	79	71	−
—	84	73	

Rabbit anti-MuLVp 30 antiserum was used at a serum dilution of 1:160.

* % fluorescent positive cells.

but not on the viral envelope by immunoelectron microscopy (14). We confirmed and extended this observation by using rabbit anti-FeLVp 30 and anti-MuLVp 30 antiserum. Several descrete areas of attached ferritin were observed on the cell surface of E♂G2 that had been incubated with absorbed rabbit anti-FeLVp 30 antiserum diluted to 1:5. In contrast, no ferritin labeling was observed on the viral envelopes of C-type RNA viruses (Fig. 1 *a* and *b*). Cell surface labeling of E♂G2 cells that were similarly treated with rabbit anti-FeLV antiserum was used as positive control (Figure 1 *c*). It is of interest that ferritin labeling was frequently observed near the site of virus budding, although viral envelopes were not labeled. The same pattern of ferritin labeling was observed with rabbit anti-MuLVp 30 antiserum, although the cell surface labeling was usually wider in extent than that noted with rabbit anti-FeLVp 30 antiserum (figure not presented). Negative controls using either medium 199 instead of antiserum or C57BL/6J thymocytes instead of E♂G2 cells as target cells were virtually unlabeled.

Intracytoplasmic Location of gs Antigen.—The intracytoplasmic location of gs antigen was examined on cryostat sections of C57BL/6J mouse spleens bearing E♂G2 transplant by the indirect immunofluorescence test as described in the Materials and Methods. Bright granular fluorescence was observed in the cytoplasm of E♂G2 cells after being incubated with rabbit anti-MuLVp 30 antiserum (Fig. 2 *a*). No nuclear fluorescence was observed. The specificity of the reaction was confirmed by specific absorption with purified MuLVp 30 protein prepared by isoelectric focusing (Fig. 2 *b*). The section treated with PBS instead of antiserum was virtually unstained (Figure 2 *c*). The same pattern of fluorescence was observed with rabbit anti-FeLVp 30 antiserum (not presented). These observations are consistent with the results reported by others (4, 21, 22) that gs antigen is located in the cytoplasm of cells.

Some Biological Properties of Membrane-Bound gs Antigen.—Although membrane-bound gs antigen was detected by the IFA on the thymocytes of AKR/J mice at 2 mo of age, the indirect membrane immunofluorescence test was virtually negative against those thymocytes. It was of interest to examine whether membrane-bound gs antigen increased in amount with aging and particularly with the development of thymic leukemia. The summary of the indirect membrane immunofluorescence test of thymocytes of AKR/J and C57BL/6J mice with rabbit anti-FeLV antiserum is shown in Fig. 3. The thymocytes of AKR/J mice at 2–5 mo of age showed very weak or negative membrane immunofluorescence reaction with rabbit anti-FeLV antiserum. With the development of leukemia, the percent fluorescent-positive cells as well as the intensity of fluorescence were markedly increased. The fluorescent-positive cells were mostly large-size leukemia cells. The thymocytes of C57BL/6J mice were invariably negative for the membrane fluorescence reaction with rabbit anti-FeLV antiserum over the same age-range studied.

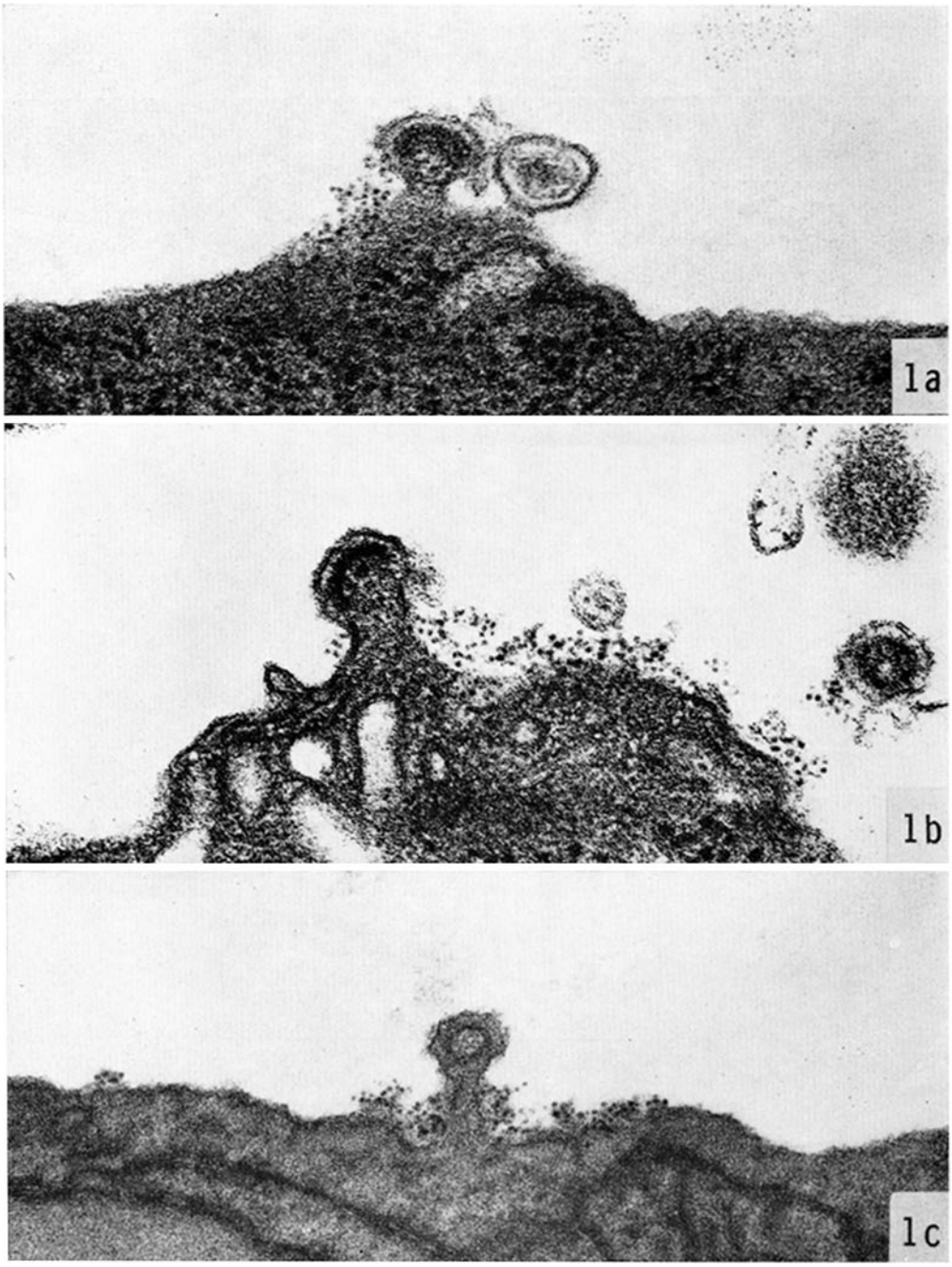

FIG. 1. E♂G2 cells were reacted with rabbit anti-FeLVp 30 antiserum followed by ferritin-labeled sheep antibody to rabbit γ-globulin (*a* and *b*). E♂G2 cells were reacted with rabbit anti-FeLV antiserum and then treated with ferritin-conjugated goat antibody to rabbit γ-globulin (*c*). Several sectors on the cell surface were labeled with ferritin, indicating membrane-bound gs antigen-positive sites, but the envelopes of budding viruses were not labeled at all. *a* and *b*, × 140,000 and *c* × 120,000.

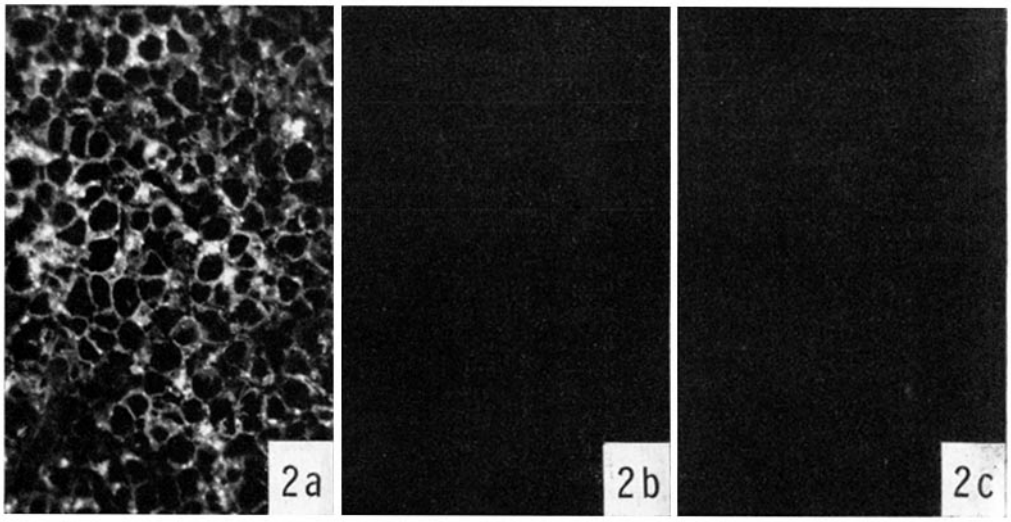

FIG. 2. Frozen section of C57BL/6J mouse spleen bearing E♂G2 transplant was reacted with (*a*) rabbit anti-MuLVp 30, (*b*) rabbit anti-MuLVp 30 antiserum absorbed with purified MuLVp 30 protein, (*c*) PBS, and followed by fluorescein-labeled goat antibody to rabbit γ-globulin. Bright granular fluorescence was observed in the cytoplasm of C57BL/6J mouse spleen bearing E♂G2 transplant but nuclei are not stained at all (*a*). The specific absorption of the fluorescence reaction was observed (*b*). PBS control was virtually unstained (*c*). × 500.

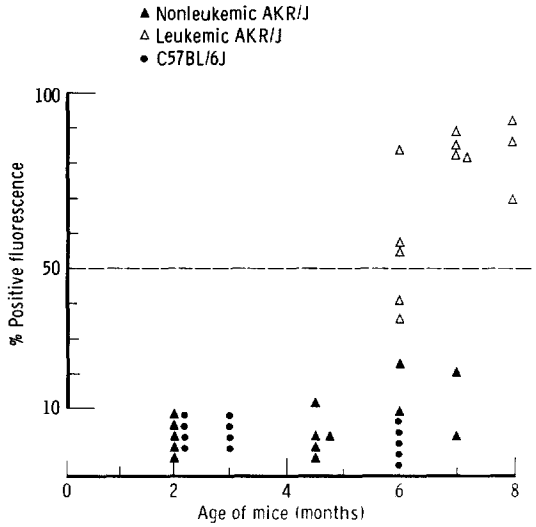

FIG. 3. Indirect membrane immunofluorescence reaction of thymocytes of AKR/J and C57BL/6J mice. Thymocytes of AKR/J and C57BL/6J mice at various ages were reacted with rabbit anti-FeLV antiserum followed by fluorescein-labeled goat antibody to rabbit γ-globulin. Reactions were graded as follows: >50% fluorescent cells, strong positive; 50%–10% fluorescent cells, weak positive; <10% fluorescent cells, negative.

DISCUSSION

Several observations presented in this paper lead to the conclusion that gs antigen, the major internal structural protein of mammalian C-type RNA viruses, is present on the surface of cells infected or transformed by mammalian C-type viruses. Since we also confirmed that gs antigen is located in the cytoplasm of such cells, in keeping with the observations described by others (4, 21, 22), it is reasonable to conclude that gs antigen is located both in the cytoplasm and on the surface of cells. The biological properties of membrane-bound gs antigen are unknown at the present time. However, there are several possibilities to consider, among them are the following: (*a*) gs antigen may adsorb to the cell surface in a manner similar to that described for other soluble antigens associated with murine C-type RNA viruses; (*b*) gs antigen may remain on or in the cell surface after attachment and entering of cells by C-type RNA viruses; (*c*) gs antigen may appear on the cell surface after being synthesized in the cytoplasm of cells; and (*d*) membrane-bound gs antigen may have some active function relating to virus output from the surface of cells or to some other aspect of the life cycle of the virus.

Stück et al. (23) and Aoki et al. (24) demonstrated by either the cytotoxic or the indirect membrane immunofluorescence test that the soluble antigens associated with murine C-type RNA viruses adsorb on to the cell membrane in vitro, although it is not known whether this phenomenon occurs in vivo. Since gs antigen of C-type RNA viruses has been demonstrated in the serum and the plasma of mice and cats, probably owing to the presence of viremia, membrane-bound gs antigen might derive from the adsorption of gs antigen on the surface of cells. In our recent studies, however, neither C1498 leukemia cells, which can adsorb Gross soluble antigen on their cell surface, nor C57BL/6J thymocytes showed any increased or positive membrane immunofluorescence reaction with gs-typing sera after being incubated in vitro with ether-disrupted MuLV and FeLV which contained large amount of gs antigen. Furthermore, the patchy-ring pattern of the indirect membrane immunofluorescence reaction of E♂G2 cells that had been reacted at 4°C with rabbit anti-FeLV and MuLVp 30 antisera followed by fluoresceinated goat antibody to rabbit 7S γ-globulin became capped in appearance after the cells were incubated at 37°C for 5 min (Yoshiki and Mellors, unpublished observation). These observations indicate that gs antigen is indeed integrated into the molecular arrays of the fluid mosaic of the cell membrane (25).

Miyamoto and Gilden (26) reported in their electron microscopic studies of the entry of MuLV into cultured cells that viral nucleoids sometimes remained on the cell surface after the dissolution of viral envelopes. In our present immunoelectron microscopic studies, viral nucleoid-like materials were not observed at the site of membrane-bound gs antigen labeled by ferritin.

Hilgers et al. (27) reported that the amount of gs antigen in the leukemic thymus of AKR mice was greatly increased in comparison with the nonleukemic thymus of AKR mice. Rowe et al. (28) found that the infectious MuLV titers

in the leukemic thymus of AKR mice were markedly increased over those of nonleukemic mice and suggested that the undifferentiated malignant cells have a greater capacity to produce infectious virus than do normal thymocytes, although a high proportion of cells are infected in both the normal and lymphomatous thymus. Our indirect membrane immunofluorescence reaction of membrane-bound gs antigen on the thymocytes of AKR/J mice showed that percent fluorescent-positive cells of the leukemic thymus were greatly increased compared to those of the nonleukemic thymus. It is to be noted that the fluorescent-positive cells were mainly large-size leukemia cells. In addition, we observed by immunoelectron microscopic studies that the cell surface location of membrane-bound gs antigen frequently was near the site of virus budding, although the viral envelope was not labeled at all. Those observations lead us to propose that membrane-bound gs antigen may have important biological functions relating to virus assembly and maturation near and at the surface of cells or relating to other steps of incipient virus synthesis. Further investigations are necessary to clarify this point.

Parks and Scolnick (29) reported that interspecies gs-3 antigen was found in the primate C-type RNA viruses by radioimmunoassay. However, in order to demonstrate "displacement" of labeled MuLV gs antigen by the primate gs antigen, 10-fold more primate than murine viral protein had to be added to the reaction to give a comparable displacement. Furthermore, under the conditions used primate gs antigen did not displace all of the antibody against murine gs antigen. Recently, Schäfer et al. (30) reported comparable observations in comparative studies using the Ouchterlony test. Our negative absorption data obtained with woolly monkey cells producing woolly monkey sarcoma virus and with crude viral protein of SSV may be conceivably explained either by a lower sensitivity of our assay method employed or by quantitative differences in the amount of antigen used for the absorption test.

Our characterization of membrane-bound gs antigen as a cell membrane-integrated internal protein of mammalian (murine and feline) C-type RNA viruses opens up new possibilities for the study of C-type virus expression and virus-cell relationship.

SUMMARY

The indirect membrane immunofluorescence test and the absorption analysis of rabbit anti-FeLV, rabbit anti-FeLVp 30, and rabbit anti-MuLVp 30 antisera yielded the following conclusions. An antigen shared by mammalian (murine and feline) C-type RNA leukemia and sarcoma viruses was detected on the surface of cells infected or transformed by C-type viruses. The antigen was characterized as membrane-bound gs antigen bearing two determinants, membrane-bound gs-1, intraspecies-specific antigenic determinant, and membrane-bound gs-3, interspecies-specific antigenic determinant. Membrane-bound gs antigen was located on the cell surface, frequently near the site of virus budding but not on the envelope of murine C-type RNA virus.

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