

RECOGNITION OF A LEUKEMIA-RELATED ANTIGEN BY
AN ANTIIDIOTYPIC ANTISERUM TO AN ANTI-gp70
MONOCLONAL ANTIBODY

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Spontaneous T cell leukemias in AKR and HRS/J mice produce recombinant retroviruses that contain no known oncogene sequences (1), yet cause leukemia even when injected into mice with a low incidence of the disease (2). The transforming mechanism of these viruses is unknown, but an important early event in the process is the binding of the viral envelope glycoprotein, gp70, to receptors of the target cell (3-7). This interaction most likely permits entry of the virus into the cell. Binding characteristics of retroviruses have been demonstrated in several models (3-5, 8-10) and candidate receptor molecules have been isolated (11-16).

Since each cloned leukemogenic recombinant retrovirus from AKR and HRS/J mice has a unique gp70 (2, 17, 18), the corresponding gp70 receptors may possess individual binding specificities. It is therefore possible that a given thymic leukemia arises from a single target cell whose receptor binds specifically to the unique gp70 of a particular recombinant virus. The demonstration that a cloned leukemogenic virus induces T cell leukemias with uniform surface phenotypes in HRS/J mice (19) is consistent with this idea. Moreover, McGrath and colleagues (20, 21) have described a thymic leukemia that binds preferentially to the retrovirus it produces. They suggest that the retroviral receptor of the T cell is actually an antigen-binding receptor and that leukemogenesis involves an immunoproliferative response to the novel glycoprotein (22).

In the present experiments we attempted to identify leukemia cell membrane structures with specificity for recombinant gp70. The method we used was based on the hypothesis that the virus-binding sites of retrovirus receptors share structural elements with the antigen-binding sites of anti-gp70 antibodies. Receptors that share binding structures with antibodies against hormonal (23), drug (24), neurotransmitter (25), and viral (26-28) antigens have been described. In principle, therefore, an antiidiotypic antibody against an anti-gp70 antibody could bind to cellular receptors for retroviral gp70. Here we report the recognition of a structure on the surface of murine leukemia cells by an antiidiotypic antiserum against an anti-retroviral gp70 monoclonal antibody. This idiotypic-like determinant was expressed on the surface of several murine thymic leukemias

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as well as on leukemias of B cell and erythroid lineage, but not on normal lymphoid cells.

Materials and Methods

Experimental Animals. CBA/J, DBA/2J, BALB/c, and AKR/J mice were obtained from The Jackson Laboratory, Bar Harbor, ME and were bred in our own animal facility. NFS/N mice were a gift from the National Institutes of Health, Bethesda, MD. NZW rabbits were obtained from Pine Acres Rabbitry, West Brattleboro, VT.

Viruses. Ecotropic, xenotropic, and polytropic retroviruses were isolated from HRS/J mice as previously described (29) and cloned by limiting dilution. The three polytropic viruses were termed P1, P2, and P5. Viruses were maintained on either mink lung epithelium (CCL64) or NIH-3T3 fibroblasts. Purification of viruses was accomplished by sucrose density gradient centrifugation (30) or Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden) column chromatography (31). Titers of infectious polytropic viruses were determined by an immunofluorescent focus-forming assay on CCL64 cells (32).

Antisera. Goat antisera prepared against purified AKR gp70 and Tween-ether-disrupted Moloney virus were obtained from Dr. J. Cole, National Cancer Institute, Bethesda, MD.

Cell Lines and Tumors. Cell lines derived from 14 spontaneous AKR thymic leukemias were a gift from Dr. Hiroshi Hiai, Aichi Cancer Center Research Institute, Nagoya, Japan. Cloned cell lines from a Friend virus complex-induced erythroleukemia (CL85) and an Abelson virus-induced pre-B cell leukemia (298-18) were provided by Dr. Stuart Levy and Dr. Naomi Rosenberg, Tufts Cancer Research Center, Boston, MA. All cell lines were maintained in Dulbecco's minimal essential media (DMEM)¹ supplemented with 10% fetal calf serum (FCS). The pre-B cell medium also contained 5×10^{-5} M 2-mercaptoethanol. Thymic leukemias induced by P1 and P2 (termed P1 Thy and P2 Thy) were obtained from 8-12-mo-old CBA/J mice that were injected at birth with stocks of these cloned recombinant viruses (2). The leukemias were propagated in vivo by intraperitoneal injection of 10^6 tumor cells into newborn CBA/J mice. The NS-1 murine myeloma cell line was used for hybridizations. It was maintained in DMEM with 15% FCS and was treated twice with 8-azaguanine before use as a fusion partner.

Preparation of an Anti-gp70 Monoclonal Antibody. 3-mo-old NFS/N mice were immunized by intraperitoneal injection with 10^7 P1 virus-infected NIH-3T3 cells emulsified in complete Freund's adjuvant (Difco Laboratories, Detroit, MI). Two subsequent immunizations with the cells in incomplete adjuvant were performed at 2-wk intervals. The mice were boosted with 0.1 ml of concentrated virus (10^6 plaque-forming units/ml) 3 d before the hybridization. The hybridization was performed at room temperature according to the technique of Kohler and Milstein (33) with minor modifications.

Identification of Anti-Polytropic Virus 1 (Anti-P1) Monoclonal Antibody. An enzyme-linked immunosorbent assay (ELISA) was used to detect antiviral antibodies. Immulon I plates (Dynatech Laboratories, Inc., Alexandria, VA) were coated with 100 μ l of a 10 μ g/ml solution of purified virus in phosphate-buffered saline (PBS), 0.02% sodium azide, pH 7.2 (PBS-A), and incubated overnight at 4°C. The buffer was removed and the wells were blocked for 2 h at 37°C by the addition of 200 μ l of 5% bovine serum albumin in PBS (BSA-PBS-A). The BSA-PBS-A was replaced by 100 μ l of hybridoma supernatant and the plates were incubated for 2 h at 37°C. The supernatants were removed and the wells were washed three times with 1% BSA-PBS-A. Then, 100 μ l of a 1:1500 dilution of alkaline phosphate-conjugated goat anti-mouse IgG/IgM (Sigma Chemical Corp., St. Louis, MO), diluted in 1% BSA-PBS-A was added to each well and the plates were

¹ *Abbreviations used in this paper:* BSA-PBS-A, PBS containing bovine serum albumin and sodium azide; DMEM, Dulbecco's minimum essential medium; ELISA, enzyme-linked immunosorbent assay; FACS, fluorescence-activated cell sorter; FCS-PBS-A, PBA containing fetal calf serum and sodium azide; NP-40, Nonidet P-40; PBS-A, phosphate-buffered saline containing sodium azide, P1 Thy or P2 Thy, P1 or P2 virus-induced thymic leukemia cells; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; RIA, radioimmunoassay.

incubated overnight at 4°C. The wells were then washed four times with 1% BSA-PBS-A and 100 μ l of substrate solution (20 ml 0.05 M NaHCO₃, pH 9.5, 0.02 M MgCl₂, and four substrate tablets [Sigma Chemical Co.]) was added to each well. The optical density (A₄₀₅) was read on an ELISA spectrophotometer (Dynatech Laboratories, Inc.).

Antiidiotypic Antisera. New Zealand White rabbits were immunized subcutaneously with an initial injection of 500 μ g of anti-P1 gp70 monoclonal antibody (referred to as 1416) in complete Freund's adjuvant and subsequently boosted twice at 2-wk intervals with 500 μ g of same antibody in an emulsion of incomplete adjuvant. The sera obtained were extensively absorbed on pooled mouse Ig (Cappel Laboratories, Cochranville, PA), followed by mouse IgG2a and IgG2b coupled to Sepharose 4B (Pharmacia Fine Chemicals). When no further protein could be eluted from these immunoabsorbents with 0.5 N acetic acid, the flow-through was adsorbed to and eluted from 5 mg of affinity-purified 1416 coupled to Sepharose 4B. The eluate was concentrated, dialyzed against PBS-A, and stored at 4°C until used.

Radioimmunoassay (RIA). Two types of RIA were used: (a) an indirect RIA measured 1416 idiotype binding to the antiidiotype, and (b) a competitive RIA measured Ig or virus inhibition of 1416 idiotype binding to the antiidiotype. For both types of RIA, polystyrene tubes (12 \times 75 mm) were coated with 250 ng of the antiidiotype in 100 μ l of PBS-A, and incubated overnight at 4°C. The tubes were then blocked with 5% BSA-PBS-A for 2 h at 37°C. For the indirect RIA, this step was followed by the addition of 100 μ l of the idiotype (1416) or other Ig diluted in 1% BSA-PBS-A. The tubes were incubated for 6 h at 4°C, washed three times with 1% BSA-PBS-A, and 100 μ l of ¹²⁵I-labeled rabbit anti-mouse Ig (10⁵ cpm) diluted in 1% BSA-PBS-A was added. After an overnight incubation at 4°C, the tubes were washed four times and counted in an Auto Gamma Counter 4000 (Beckman Instruments, Inc., Fullerton, CA). All determinations were performed in triplicate. For the competitive RIA, two methods were used. In experiments to determine Ig inhibition of idiotype binding to the antiidiotype, 50 μ l of ¹²⁵I-labeled 1416, in a concentration previously determined to be limiting for binding to the antiidiotype, was added to 50 μ l of Ig. This mixture was immediately added to antiidiotype-coated tubes that were prepared and blocked as stated above. After overnight incubation at 4°C, the tubes were washed four times with 1% BSA-PBS-A and counted. For assays to evaluate virus inhibition of idiotype binding to antiidiotype, 100 ng of 1416 in 50 μ l 1% BSA-PBS-A was preincubated with 50 μ l of purified virus for 2 h at 4°C. The antibody-virus mixture was then added to antiidiotype-coated tubes that were prepared and blocked as before. After incubation for 6 h at 4°C, the tubes were washed three times with 1% BSA-PBS-A, and 10⁵ cpm of ¹²⁵I-labeled rabbit anti-mouse Ig was added. The tubes were then incubated overnight at 4°C, washed four times, and counted.

Immune Precipitation Assays. Anti-P1 virus monoclonal antibodies were screened for binding to viral gp70 by immune precipitation with both radiolabeled purified virus preparations and membrane lysates from virus-infected cells. For immune precipitation with viral proteins, 25 μ g of purified virus solubilized in PBS containing 0.5% Nonidet P-40 (NP-40) was radiolabeled with 0.6 mCi Na¹²⁵I (Amersham Corp., Arlington Heights, IL) using Iodobeads (Pierce Chemical Co., Rockford, IL) according to the method of Markwell (34). The solubilized virus preparation was precleared twice with normal mouse sera preadsorbed to Staph A (IgG-sorb; The Enzyme Center, Inc., Boston, MA) and once with Staph A alone. 10 μ g of affinity-purified antiviral monoclonal antibody was incubated overnight at 4°C with 2 \times 10⁶ cpm of the labeled virus lysate in a final volume of 100 μ l. 80 μ l of a 10% Staph A suspension was then added for 1 h, followed by four pellet washes with 0.1% BSA-PBS-A containing 0.5% NP-40. Immune precipitates were then eluted from the Staph A by incubation at 100°C for 5 min in sample buffer (0.06 M Tris-HCl, 10% glycerol, 3% sodium dodecyl sulfate (SDS), 0.02% bromphenol blue, with or without 5% 2-mercaptoethanol).

For immune precipitation with cell membrane lysates, 2 \times 10⁷ cells were radiolabeled with 1.0 mCi Na¹²⁵I by the lactoperoxidase technique (35). Cells were washed four times before and immediately after labeling with ice-cold PBS, and then lysed in 300 μ l of 0.01 M Tris-HCl, 0.15 M NaCl, 0.5% NP-40, 2 mM phenylmethylsulfonyl fluoride, pH 7.0,

for ½ h on ice. The lysate was twice centrifuged at 13,000 rpm for 45 min at 4°C in an Eppendorf centrifuge (Brinkmann Instruments Co., Westbury, NY) to remove cellular debris. The remaining supernatant was precleared and immune precipitated as above. When the antiidiotypic serum was used to immune-precipitate radiolabeled membrane lysates, preimmune rabbit serum and rabbit anti-mouse Ig were substituted for normal mouse serum in the preclearing procedure.

Polyacrylamide Gel Electrophoresis (PAGE). Immediately after elution from the Staph A, the immune precipitates were analyzed by PAGE in 12.5% acrylamide slab gels, according to the method of Laemmli (36). Gels were run at 150 V (constant voltage) at room temperature. Autoradiography was enhanced by the use of x-ray intensifying screens at -70°C.

Virus Protein Blotting. 10 µg of purified P1 virus was added to 40 µl of sample buffer (with 5% 2-mercaptoethanol) and heated for 5 min at 100°C. The sample was then applied to a 12.5% acrylamide slab gel and electrophoresis was performed as above. Protein blotting was performed according to the method of Towbin et al. (37) for 4 h at 250 mA onto 0.45-µm pore nitrocellulose paper (Schleicher & Schuell, Inc., Keene, NH). The paper was blocked with 3% BSA-PBS-A/0.5% NP-40 for 2 h at 4°C and then soaked in hybridoma supernatant overnight at 4°C, with slow, constant rotation. After six washes with 1% BSA-PBS-A/0.5% NP-40, the paper was soaked for 3 h at 4°C in a solution of ¹²⁵I-rabbit anti-mouse Ig (10⁵ cpm/ml). The paper was then washed eight times as above, air dried, and autoradiographed for 24 h at -70°C with an x-ray intensifying screen.

Immunofluorescent Flow Cytometry. Murine leukemia cell lines and tumors were stained in suspension for analysis with a fluorescence-activated cell sorter (FACS IV; B-D Automated Immunochemistry, Becton, Dickinson & Co., Salt Lake City, UT). Cells were washed twice with cold PBS, pH 7.2, containing 0.5% heat-inactivated FCS and 0.02% sodium azide (FCS-PBS-A), and assayed for viability by trypan blue exclusion. Only cell suspensions demonstrating >90% viability were used. 5 µg anti-P1 gp70 monoclonal antibody or 2.5 µg antiidiotype diluted in 50 µl of FCS-PBS-A were added to pellets containing 10⁶ cells. The pellets were resuspended and incubated on ice for 30 min. Cells were washed once with cold FCS-PBS-A and incubated as before with 50 µl of a 1:30 dilution of fluorescein isothiocyanate-conjugated goat antiserum to mouse IgG (Meloy Laboratories, Inc., Springfield, VA) or rabbit IgG (Cappel Laboratories). The cell pellets were then washed once again with FCS-PBS-A and fixed in 1% formaldehyde before FACS analysis.

Results

Characterization of a Monoclonal Antibody to P1 gp70. Previous work (2, 17, 18, 38, 39) has shown that recombination within the envelope (*env*) region of the retroviral genome is responsible for the expression of novel gp70s in spontaneously generated leukemogenic viruses. Distinct gp70s have been demonstrated in three recombinant leukemogenic retroviruses (P1, P2, and P5) and in two nonleukemogenic viruses (ecotropic and xenotropic) isolated from HRS/J mice (2). Hence, a panel of these five purified viruses was used in a screening assay to detect monoclonal antibodies with specificity for the leukemogenic virus P1. Of all the antiretroviral monoclonal antibodies that were obtained after immunizing NFS/N mice with P1 virus-infected cells, one of them (1416) was found to bind to P1 virus but not to the P2, P5, ecotropic, and xenotropic isolates (Fig. 1).

This monoclonal antibody was further characterized by SDS-PAGE of immunoprecipitates of lysates from radiolabeled purified virus, virus-induced thymic leukemia cells, and normal CBA/J thymocytes (Fig. 2). A 70,000 (70 K) mol wt protein was immunoprecipitated from P1 by 1416, but not from P2 or ecotropic

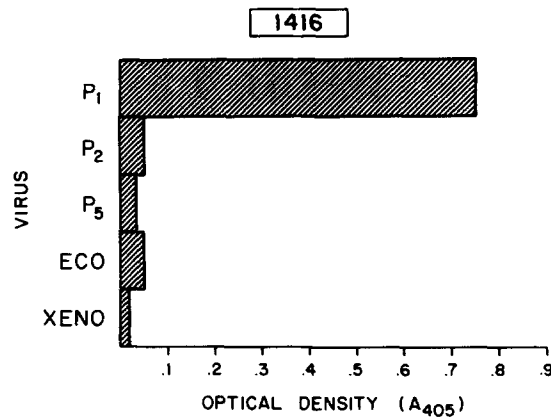


FIGURE 1. Binding of monoclonal antibody 1416 to different viruses. 1 μ g of purified virus was used to coat microtiter wells in polystyrene plates and an ELISA was performed. The antibody bound only to the P₁ virus.

viral lysates (Fig. 2, *a-c*). Fig. 2*d* shows the proteins that were immunoprecipitated from purified P₁ virus with goat anti-gp70 antiserum; the most prominent band corresponds to gp70. An 85 K mol wt protein was immunoprecipitated by 1416 from the cell membranes of P₁ virus-induced thymic leukemia cells (P₁ Thy), but not from P₂ Thy or from CBA/J thymocytes (Fig. 2, *e-g*). A protein of this molecular weight has been shown by others to represent either a precursor to gp70 or a non-ionic detergent-induced, disulfide-linked complex between gp70 and the other viral envelope protein, P15E (40). Western blot analysis of proteins from purified P₁ virus confirmed that the epitope recognized by 1416 is present on the 70 K mol wt molecule (Fig. 2*h*). Fig. 2*i* demonstrates that the P₂ virus does not contain a similar epitope.

Characterization of an Antiidiotypic Antiserum Against Monoclonal Antibody 1416. An antiserum to antibody 1416 was raised by immunization of a rabbit. After extensive absorption (see Materials and Methods), the antiserum from this animal was found to recognize an idiotypic determinant of 1416. Fig. 3*A* illustrates that the antiidiotypic antiserum did not bind to normal pooled mouse Ig, normal NFS mouse serum, or to the myeloma protein MOPC-195, which has the same isotype (IgG2b) as 1416. Complete inhibition of 1416 binding to the antiidiotypic antiserum was achieved with 1416 but not with MOPC-195, pooled mouse Ig, or NFS mouse serum, which contains Ig with the same allotypic determinants as 1416 (Fig. 3*B*).

The P₁ virus specifically inhibited the binding of 1416 to the antiidiotypic (Fig. 4). In the presence of P₁ virus, the idiotype-antiidiotypic interaction was completely inhibited when we used a protein concentration of virus 100-fold greater than that of the idiotype. Identical inhibition was seen when purified P₁ gp70 was used as the inhibitor in 50-fold less quantities than the whole virus (data not shown). The specificity of the inhibition was shown by the failure of purified P₂ virus to inhibit the reaction.

Binding of the Antiidiotypic to Different Murine Leukemias. Immunofluorescence flow cytometry was used to assay the antiidiotypic serum for its ability to bind to leukemia cells. We did not observe the anticipated pattern of binding. Even

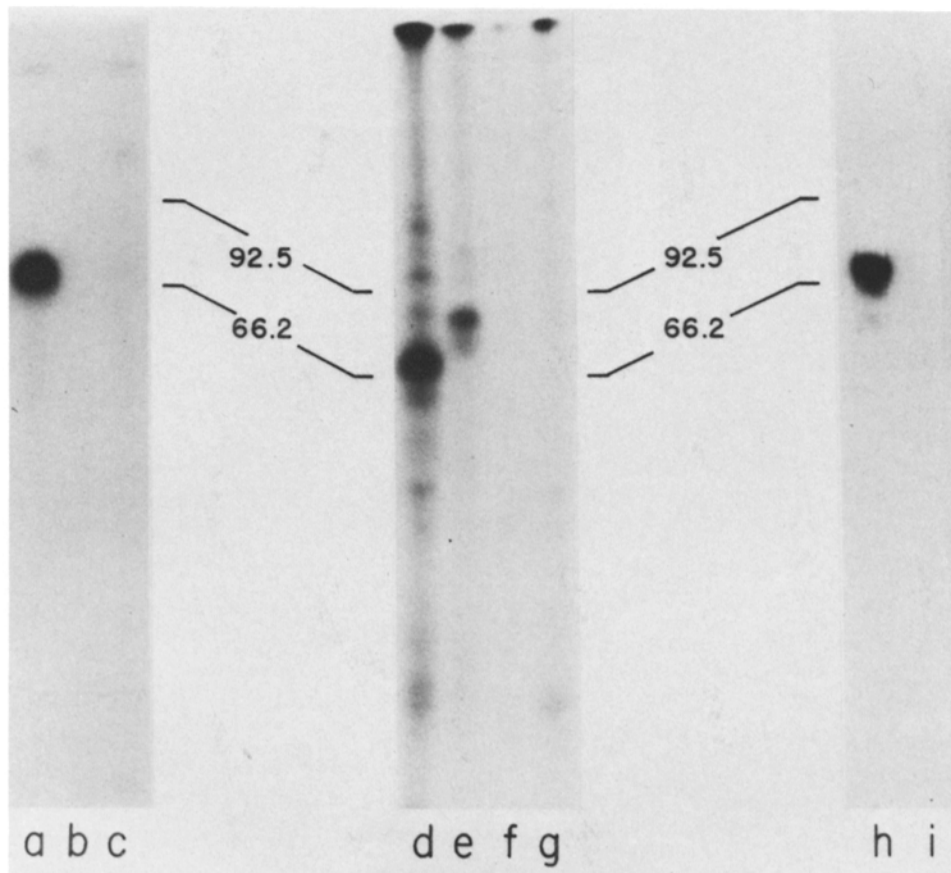


FIGURE 2. Binding of 1416 to retroviruses and leukemic cells. NP-40 lysates of purified, radiolabeled virus were immunoprecipitated with 1416 and analyzed by SDS-PAGE under reducing conditions. (a) P1 virus; (b) P2 virus; (c) ecotropic virus. NP-40 lysates of surface-iodinated leukemic or normal thymocytes were immunoprecipitated with 1416 and analyzed by SDS-PAGE under reducing conditions. (e) P1 Thy; (f) P2 Thy; (g) normal CBA/J thymocytes. (d) Radiolabeled P1 virus that was immunoprecipitated with goat anti-Moloney virus antiserum. (h and i) Western blots of unlabeled purified P1 virus (h) and P2 virus (i) lysates that were reacted with 1416 and then ^{125}I -labeled rabbit anti-mouse Ig. In this and subsequent gel electrophoreses, the numbers are molecular weight markers ($M_r \times 10^{-3}$).

though the idiotype (1416) bound to P1 and not to P2 virus (Fig. 1), and to P1 Thy and not to P2 Thy (Fig. 5B), the antiidiotype bound to both P1 Thy and P2 Thy (Fig. 5A). Possible explanations for the results with the antiidiotypic serum include: (a) the presence of two or more antibody populations with different antigen specificities in the antiidiotypic serum; (b) binding to virus-encoded determinants present on the surface of both leukemias; and (c) recognition of a determinant that is shared by both P1 Thy and P2 Thy. The first possibility is unlikely because the antiidiotype was extensively absorbed with mouse Ig and then affinity purified on a 1416-Sepharose column (see Materials and Methods). Separate absorptions of the antiidiotype with four different leukemia cell lines greatly reduced its reactivity with P1 Thy (Fig. 6A); moreover,

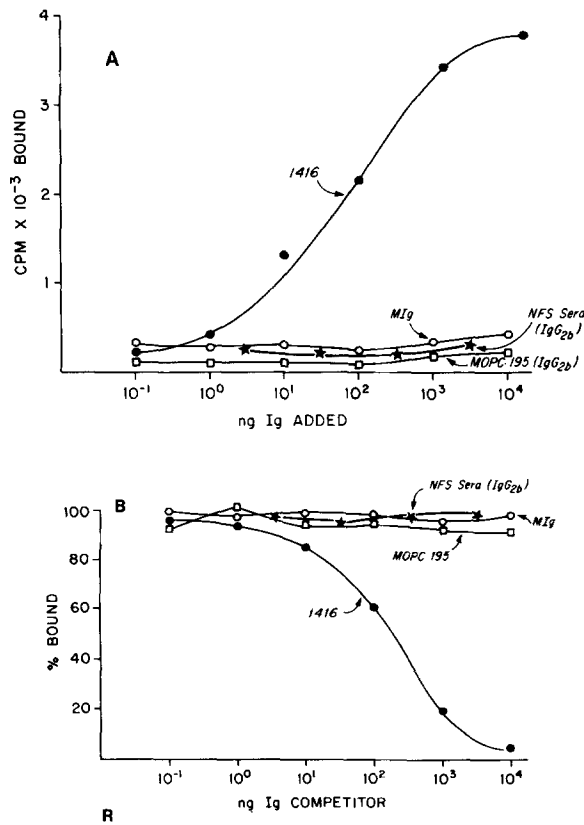


FIGURE 3. Binding of the monoclonal anti-gp70 antibody 1416 to the antiidiotype. (A) Increasing amounts of 1416 or other mouse Ig were incubated in antiidiotype-coated polystyrene tubes. Bound Ig was detected with ¹²⁵I-labeled rabbit anti-mouse Ig. For the NFS serum, the concentrations represent calculated amounts of the IgG2b isotype. (B) Increasing amounts of inhibitor proteins were mixed with 60 ng of ¹²⁵I-labeled 1416 (sp act, 5 × 10³ cpm/ng) and the mixture was added to antiidiotype-coated polystyrene tubes. The percentage of bound 1416 is represented on the ordinate.

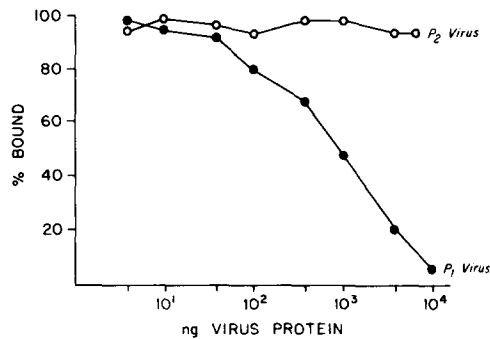


FIGURE 4. Inhibition of the reaction of 1416 with the antiidiotype by virus. Increasing amounts of purified virus were incubated for 2 h at 4°C with 100 ng of 1416. The virus-1416 mixture was then added to antiidiotype-coated tubes and the amount of bound 1416 was detected with ¹²⁵I-labeled rabbit anti-mouse Ig.

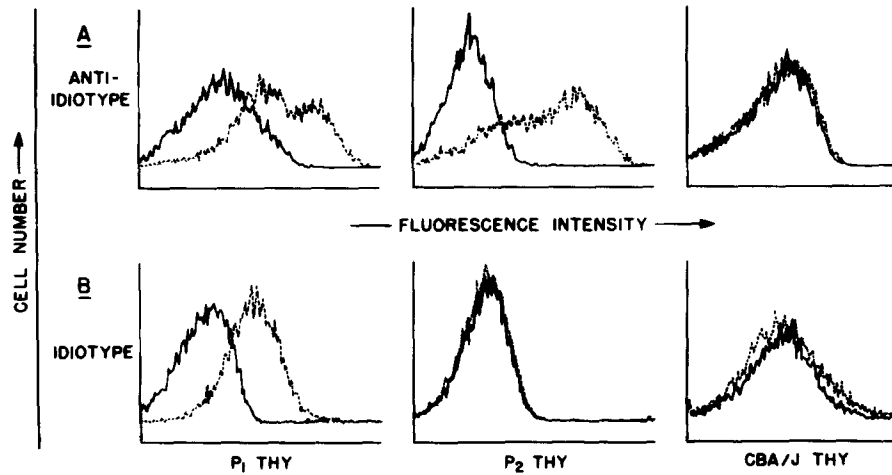


FIGURE 5. Fluorescence flow cytometry of the binding of antiidiotype and 1416 to normal and leukemic thymocytes. (A) Indirect immunofluorescence of 20,000 cells stained with 2.5 μg of affinity-purified antiidiotype (---) or 2.5 μg of affinity-purified control rabbit Ig (—). (B) Indirect immunofluorescence of 20,000 cells stained with 5 μg of affinity-purified 1416 (---) or 2.5 μg of affinity-purified MOPC-195 (—). Note that the antiidiotype reacted with P2 Thy, whereas 1416 did not (compare with Fig. 2, *e* and *f*). Neither the antiidiotype nor 1416 reacted with normal thymocytes (compare with Fig. 2 *g* and Fig. 7 *b*).

antiidiotype that was absorbed with P1 Thy cells lost its ability to bind to the 1416 idiotype, whereas absorption with normal CBA/J thymocytes did not affect its ability to bind to the 1416 idiotype (Fig. 6B). The second possibility was excluded by immunoprecipitation studies as shown below.

The third possibility was explored by testing the reactivity of the antiidiotype with a panel of different murine leukemia cell lines. 14 spontaneous thymus leukemias from different AKR/J mice, one Abelson virus-induced "pre-B" cell leukemia (298-18), and one Friend virus complex-induced erythroleukemia (CL-85) were examined for antiidiotype binding by flow cytometry. Results of these experiments (Table I, column A) showed that the antiidiotype bound to several leukemia cell lines, including both T cell and non-T cell leukemias. A control preparation of normal rabbit Ig demonstrated no binding. Normal lymphoid cells from the murine strains in which these leukemias were induced did not bind to the antiidiotype. Normal CBA/J thymocytes that were stimulated for either 48 or 96 h with concanavalin A ($1 \mu\text{g}/(10^6 \text{ cells} \cdot \text{ml})$) also failed to bind to the antiidiotype, indicating that the idiotype-like structure was not a proliferation antigen.

The idiotype (1416) also bound to some of these leukemia cell lines (Table I, column B). Immunoprecipitation and SDS-PAGE analysis of [^{35}S]methionine-labeled AKR leukemia cell lysates confirmed that the structure recognized by 1416 was an 85 K mol wt protein (data not shown). Thus, an epitope common to the gp70 precursor of several of these independently derived tumors was also expressed on P1 Thy. Yet, as seen with P2 Thy, AKR 21, AKR 71, and pre-B 298-18, the lack of expression of this epitope (Table I, column B) did not preclude expression of the structure that crossreacted with the anti-gp70 idiotypic

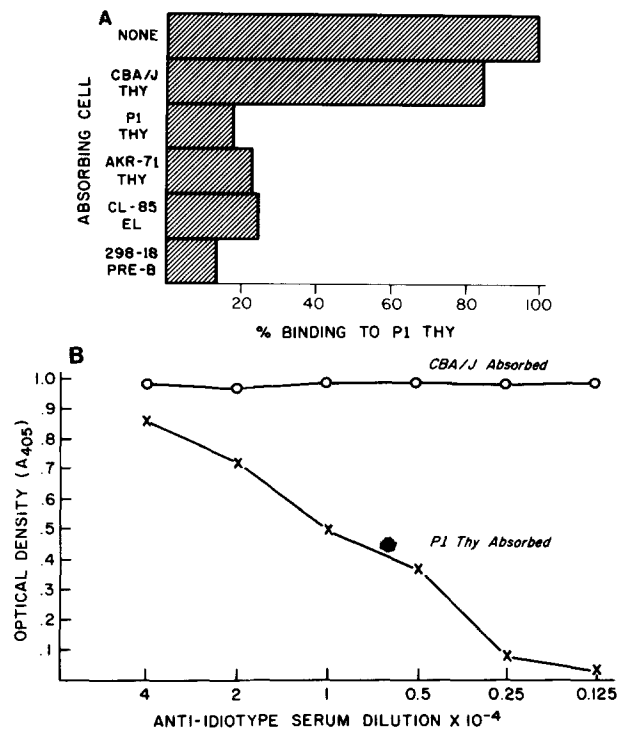


FIGURE 6. Binding of absorbed antiidiotypic antiserum to P1 Thy and 1416. (A) 50 μ l of antiidiotypic antiserum was absorbed three times with 20×10^6 normal or leukemic cells and then tested for binding to P1 Thy. Binding was detected with 125 I-labeled goat anti-rabbit Ig. Unabsorbed antiidiotypic reactivity with P1 Thy was assigned 100% binding. (B) Identical absorptions of the antiidiotypic serum were performed with either normal CBA/J thymocytes or P1 Thy, followed by a test for binding to 1416.

determinant (Table I, column A). Therefore, the anti-gp70 antibody (1416) and the antiidiotypic reacted with different determinants on the leukemia cell surface.

Immunoprecipitation of the Idiotypic-like Structure from Cell Membranes. The structure(s) recognized by the anti-(anti-gp70) idiotypic serum was further analyzed by immunoprecipitation of membrane lysates from surface-radiolabeled leukemia cells. All lysates were precleared twice with a preimmune rabbit serum-Staph A complex, once with a rabbit anti-mouse Ig/Staph A complex and once with Staph A alone. The immunoprecipitates were analyzed by SDS-PAGE under reducing conditions. Fig. 7A demonstrates that the antiidiotypic failed to immunoprecipitate proteins from normal CBA/J thymocyte membranes (lane b), whereas anti-Moloney virus antiserum immunoprecipitated several proteins (lane a). These proteins most likely represent endogenous ecotropic *gag* and *env* gene products. The anti-Moloney virus antiserum also immunoprecipitated several proteins from P1 Thy membranes (Fig. 7B, lane c) that are probably gene products of endogenous ecotropic or xenotropic viruses as well as the P1 virus. Neither rabbit anti-mouse Ig (Fig. 7B, lane b) nor preimmune rabbit serum (lane a) immunoprecipitated any proteins from the P1 Thy membranes. The

TABLE I
Binding of Antiidiotype and Idiotype to Normal and Leukemic Cells

Source of cell	Fluorescence*	
	A. Antiidiotype	B. Idiotype
Normal (4 wk old)		
CBA/J thymus	3	0
CBA/J thymus (Con A stim.) [‡]	3	0
CBA/J lymph node	1	0
CBA/J spleen	3	0
BALB/c thymus	0	0
BALB/c lymph node	1	0
BALB/c spleen	0	0
DBA/2J thymus	0	0
DBA/2J lymph node	1	0
DBA/2J spleen	1	0
AKR thymus	2	0
Leukemic		
P1 Thy	18	26
P2 Thy	32	0
Pre-B 298-18	51	0
Erythroleukemia CL85	35	0
AKR-18	14	10
AKR-21	41	0
AKR-29	8	20
AKR-52	2	10
AKR-65T	9	11
AKR-65I	50	0
AKR-67	34	0
AKR-69	24	5
AKR-70	11	22
AKR-71	66	0
AKR-75	15	9
AKR-79	7	16
AKR-89	10	0
AKR-92	40	1

* All values represent Δ MCF (median channel fluorescence of test antibody - median channel fluorescence of control antibody).

[‡] Normal CBA/J thymocytes stimulated in vitro with concanavalin A ($1 \mu\text{g}/10^6$ cells/ml) for 48 h.

absence of bands in Fig. 7B, lane *b* verifies the completeness of the preclearing procedure.

Fig. 7C demonstrates that the antiidiotype did not react with cell-bound Ig or virus-encoded proteins. Labeled P1 Thy membrane lysates precleared as above were further precleared three times with the goat anti-Moloney virus antiserum and then immunoprecipitated with the antiidiotypic serum. The completeness of the viral protein preclearing procedure was verified by the absence of bands in Fig. 7C, lane *b*, where the precleared lysate was immunoprecipitated with anti-

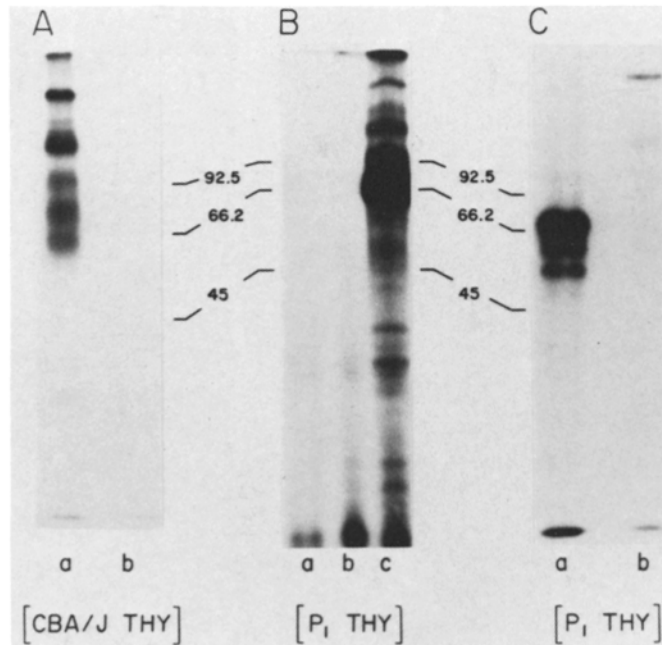


FIGURE 7. SDS-PAGE analysis of cell surface proteins from normal and leukemic CBA/J thymocytes. Cells were surface iodinated and lysed in a buffer containing 0.5% NP-40. (A) Thymocyte membrane proteins from normal 4-wk-old CBA/J mice that were immunoprecipitated with the antiidiotype (lane *b*) or goat anti-Moloney virus antiserum (lane *a*). (B) P₁ thymic leukemia cell membrane proteins that were precleared with normal rabbit serum and rabbit anti-mouse Ig and then immunoprecipitated with preimmune rabbit serum (lane *a*), rabbit anti-mouse Ig (lane *b*), or goat anti-Moloney virus antiserum (lane *c*). (C) P₁ thymic leukemia cell membrane proteins that were further precleared with goat anti-Moloney virus antiserum and then immunoprecipitated with the antiidiotypic antiserum (lane *a*) or the goat anti-Moloney virus antiserum (lane *b*).

Moloney virus antiserum. By contrast, three bands with molecular weights of 70, 63, and 55 K were found in the precleared lysates of P₁ Thy cells that were immunoprecipitated with the antiidiotype (Fig. 7C, lane *a*). Experiments with lysates from surface-labeled P₂ Thy cells yielded identical results (data not shown).

Three other leukemia cell lines (pre-B 298-18, Friend CL-85, AKR-71) were also surface-labeled with ¹²⁵I and the cell lysates immunoprecipitated as above. Protein bands common to all three leukemias were found in the lanes containing the antiidiotype immune precipitates (Fig. 8); as with the P₁ Thy cells, 70 and 55 K mol wt proteins were also precipitated from these three leukemia cell lysates (Fig. 8, lanes *d*, *h*, *l*) and the 63 K mol wt protein was seen in two of them. A less prominent 25 K mol wt band in these three leukemia lines was not detected in the P₁ Thy immunoprecipitates. Several other bands were noted in Fig. 8, lanes *d*, *h*, and *l*; the possibility that all or some of these were the result of coprecipitation remains to be clarified.

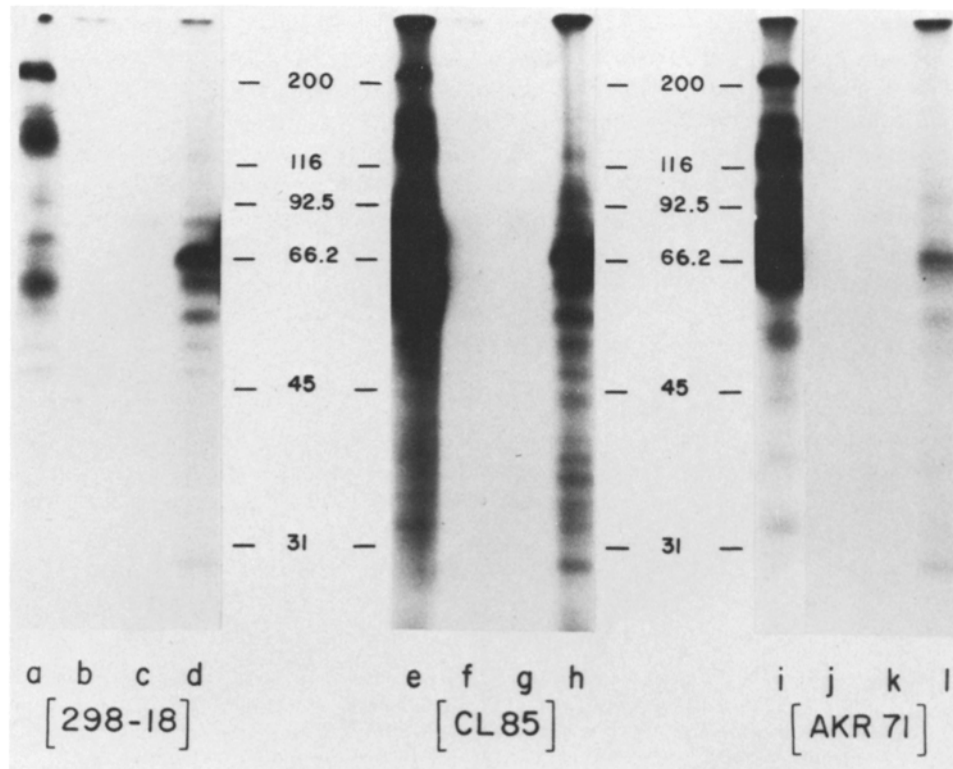


FIGURE 8. SDS-PAGE analysis of cell surface proteins from three unrelated leukemias: 298-18, a pre-B cell leukemia; CL85, an erythroleukemia; and AKR 71, a thymic leukemia. The leukemic cells were surface iodinated and lysed in a buffer containing 0.5% NP-40. Immunoprecipitation was performed with the following reagents: anti-Moloney virus antiserum (*a*, *e*, and *i*), preimmune rabbit serum (*b*, *f*, *j*), rabbit anti-mouse Ig (*c*, *g*, *k*), and the anti-idiotypic serum (*d*, *h*, *l*).

Discussion

Retroviral binding structures on normal murine lymphoid cells have been studied by several investigators. Twardzik et al. (11) demonstrated six proteins with molecular weights ranging from 26 to 170 K that were coprecipitated with Rauscher murine leukemia virus gp70 by treatment of a BALB/c thymocyte membrane-virus mixture with a bifunctional crosslinking chemical and immunoprecipitation with anti-gp70 antiserum. Schaffar-Deshayes et al. (14) found a 190 K protein on BALB/c thymocyte membranes that coprecipitated with the Moloney virus gp70, using an anti-gp70 serum without prior crosslinking. With a similar technique, Robinson et al. (15) detected a 14 K protein on C57BL/6 mouse spleen cell membranes that bound a Friend virus glycoprotein complex (gp85). We have performed similar experiments with thymic leukemias. In our hands, however, pretreatment of cell membranes with a bifunctional crosslinking chemical followed by immunoprecipitation with a monoclonal anti-gp70 antibody only occasionally yielded cellular proteins distinct from endogenous gp85. We also found that without the use of a crosslinking reagent, no proteins coprecipitated with gp85 (unpublished data).

Because of our lack of success with this technique, we used a different approach, based on the idea that cell surface receptors can share binding determinants with anti-ligand antibodies (23–28). In retrovirus-induced murine T cell leukemia, the viral envelope glycoprotein gp70 serves as the ligand to which the target cell receptor binds. Hence, the variable region of an anti-gp70 antibody may resemble the binding region of a retroviral receptor. In principle, therefore, the combining sites of both the receptor and the antibody may be identified by an appropriate antiidiotypic antibody.

A monoclonal antibody that recognized the gp70 of a cloned recombinant leukemogenic polytropic retrovirus termed P1 was the starting point for the present experiments. This antibody (1416) was selected because it failed to react with endogenous ecotropic and xenotropic viruses, and it did not bind to two other recombinant viruses, P2 and P5, with structurally different gp70s (2). In subsequent studies, however, we found that 1416 was reactive with several other virus-induced leukemia cell lines (Table I). Thus, despite its specificity for the gp70 of the P1 virus, this monoclonal antibody crossreacted with other retroviruses, presumably because of shared epitopes in their gp70s.

Antibody 1416 was used to prepare a rabbit antiidiotypic antiserum. This antiserum reacted with structures in or near the antigen-binding site of 1416, as determined by inhibition of the idiotype-antiidiotype reaction by the P1 virus (Fig. 4). The inhibition was specific, as another cloned leukemogenic polytropic retrovirus (P2) did not affect the reaction.

The antiidiotypic reagent failed to react with normal murine lymphoid cells when it was tested by both immunofluorescence and immunoprecipitation methods. Furthermore, mitogenic stimulation of thymocytes from 4-wk-old normal CBA/J mice failed to induce a significant expression of the idiotype-like determinant, suggesting that it is not a widely expressed proliferation antigen. The failure of the antiidiotype to react with normal thymocytes suggests that the idiotype-like structure we found is leukemia specific. Alternatively, the structure might occur on only a few undetectable normal cells that are targets for the retrovirus. After infection and transformation, such cells would increase in number and thus become readily detectable in the leukemia cell population.

The antiidiotypic serum reacted with a structure on the surface of a murine thymic leukemia (P1 Thy) that was induced by the retrovirus (P1) against which the anti-gp70 antibody (1416) was raised. This membrane determinant, however, was also found on several different murine leukemias, irrespective of the serological relatedness of their expressed retroviral glycoproteins to P1 gp70. For example, the leukemia cell lines pre-B 298-18, AKR-651, and AKR-71 reacted strongly with the antiidiotype, but failed to bind to 1416 (Table I).

The data thus indicate that the membrane structure detected by the antiidiotype is not virally encoded. Additional evidence against its viral origin is the failure of an anti-Moloney virus antiserum (which crossreacted with P1 viral proteins) to eliminate the structure from labeled P1 Thy membrane lysates, whereas the same antiserum removed most virus-encoded proteins from the lysates (Fig. 7C). The determinant is not an artifact caused by adsorption of mouse Ig to the surface of the murine leukemia cells that had been passed *in vivo*; it was found not only on leukemia cells obtained *ex vivo* (P1 Thy, P2 Thy)

but also on leukemia cell lines maintained exclusively *in vitro*. Furthermore, membrane lysates of labeled leukemia cells that were precleared with rabbit anti-mouse Ig still contained the idiotype-bearing structure (Figs. 7C, 8).

If the anti-(anti-gp70) idiotype we describe indeed identifies a retroviral receptor, then receptors for different leukemogenic viruses may share common structures. This possibility is strengthened by the finding that different leukemia cell lines, regardless of their lineage or causative retrovirus, expressed a similar membrane structure. However, since Ig with different binding specificities are known to share idiotypes (41, 42), it is possible that the binding specificities of gp70 receptors may be unique despite their shared reactions with the antiidiotype. The polyclonal antiidiotypic serum used in our experiments increased the likelihood that a family of idiotypic determinants, rather than a single idiotope, was recognized. It is also possible that the antiidiotype was an "internal image" (homobody) (43) of the gp70 of P1, but only to an approximation, so that the specific features of the recombinant gp70 were imprecisely mirrored by the binding site of the antiidiotype. The above factors could explain the broad range of reactivity among the several leukemias examined with the antiidiotype. Hence, the concept that retroviral receptors share common structures and yet possess unique virus-binding specificities is not internally inconsistent.

McGrath et al. (28) have also studied receptors for retroviruses on leukemic cells. They found that the surface IgM of a murine B cell lymphoma bound to a leukemogenic retrovirus, and that antibodies against the idiotype of the surface IgM inhibited its binding to the virus. Fluorescence analysis indicated that the idiotype of the B cell lymphoma was also present on two T cell lymphomas.

Nepom et al. (26) showed that an anti-reovirus hemagglutinin idiotype was shared by lymphoid and nonlymphoid (neural) cells, both of which are targets of the reovirus. In the same system, Kauffman et al. (44) demonstrated that a monoclonal anti-antihemagglutinin idiotypic antibody is capable of blocking virus binding to idiotype-positive cells.

If anti-(anti-gp70) idiotypic antibodies are capable of binding to retroviral receptors *in vivo*, then interference with primary infection, subsequent viral propagation, or inhibition of leukemia cell proliferation may be anticipated. The binding of antiidiotypic antibodies to retroviral receptors might also initiate mitogenic signals similar to those postulated for gp70, and result in lymphoblastic transformation (17, 45), lymphokine sensitivity (46), or both. Inhibition of virus binding to a murine thymoma by monoclonal anti-T cell antibodies has been shown to prevent tumor cell proliferation *in vitro* (47). Those antibodies, however, were not specific for leukemia cells but bound to normal T cell antigens such as Thy-1. The effects of antiidiotypic reagents on these processes may provide insights into the specific events involved in viral leukemogenesis at the level of the cell membrane. Finally, diagnostic application of the kind of antiidiotype we have described, a topic currently under investigation, could lead to a novel way of identifying leukemic cells.

Summary

The possibility that receptors for retroviral gp70 share structural elements with the antigen-binding sites of anti-retroviral gp70 antibodies was investigated.

A monoclonal antibody (1416) was produced that reacted with the gp70 of a cloned recombinant leukemogenic retrovirus, termed P1. An antiidiotypic anti-serum raised to 1416 was tested for its ability to bind to the thymic leukemia induced by P1 (P1 Thy). A membrane structure was identified on the surface of P1 Thy that reacted with the antibody against the idiotypic determinant of 1416. A similar structure was identified on the surface of several different, independently derived murine leukemias of T cell, B cell, and erythroid lineage. The expression of the idiotype-like determinant on these leukemia cells was independent of the serological relatedness of their expressed retroviral envelope glycoproteins to P1 gp70. The determinant recognized by the antiidiotypic was not detected on normal lymphoid cells. The recognition by the anti-(anti-gp70) idiotype of determinants on unrelated murine leukemias suggests that receptors for different leukemogenic viruses may share common structures.

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References

1. Coffin, J. 1982. Structure of the retroviral genome. *In* Molecular Biology of Tumor Viruses: RNA Tumor Viruses. N. Weiss, N. Teich, H. Varmus, and J. Coffin, editors. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 261-368.
2. Green, N., H. Hiai, J. H. Elder, R. S. Schwartz, R. H. Khiroya, C. Y. Thomas, P. N. Tschilis, and J. M. Coffin. 1980. Expression of leukemogenic recombinant viruses associated with a recessive gene in HRS/J mice. *J. Exp. Med.* 152:249.
3. DeLarco, J., and G. J. Todaro. 1976. Membrane receptors for murine leukemia viruses: characterization using the purified viral envelope glycoprotein, gp71. *Cell.* 8:365.
4. Fowler, A. K., D. R. Twardzik, C. D. Reed, O. S. Weislow, and A. Hellman. 1977. Binding characteristics of Rauscher leukemia virus envelope glycoprotein gp71 to murine lymphoid cells. *J. Virol.* 24:729.
5. Choppin, J., L. Schaffar-Deshayes, P. Debre, and J-P. Levy. 1981. Lymphoid cell surface receptor for Moloney leukemia virus envelope glycoprotein gp71. I. Binding characteristics. *J. Immunol.* 126:2347.
6. Rein, A., A. M. Schultz, J. P. Boder, and R. H. Bassin. 1982. Inhibitors of glycosylation reverse retroviral interference. *Virology.* 123:139.
7. Bassin, R. H., S. Ruscetti, I. Ali, D. K. Haapala, and A. Rein. 1982. Normal DBA/2 mouse cells synthesize a glycoprotein which interferes with MCF virus infection. *Virology.* 123:139.
8. Hartley, J. W., and W. P. Rowe. 1976. Naturally occurring murine leukemia viruses in wild mice: characterization of a new "amphotropic" class. *J. Virol.* 19:19.
9. Ganguly, K., V. S., Kalyanaraman, and M. G. Sarngadharan. 1983. Analysis of the interaction between Rauscher murine leukemia virus and murine cell membrane receptor by *in vitro* binding assay. *Cancer Lett.* 18:79.
10. Bishayee, S., M. Strand, and J. T. August. 1978. Cellular membrane receptors for oncovirus envelope glycoprotein: properties of the binding reaction and influence of different reagents on the substrate and the receptors. *Arch. Biochem. Biophys.* 189:161.
11. Twardzik, D. R., A. K., Fowler, O. S. Weislow, G. A. Hegamyer, and A. Hellman.

1979. Cell surface binding proteins for the major envelope glycoprotein of murine leukemia virus. *Proc. Soc. Exp. Biol. Med.* 162:304.
12. Baird, S. M. 1979. Antigenic properties and molecular weights of murine leukemia virus binding proteins. *J. Immunol.* 122:1389.
 13. Landen, B., and C. F. Fox. 1980. Isolation of BPgp70, a fibroblast receptor for the envelope antigen of Rauscher murine leukemia virus. *Proc. Natl. Acad. Sci. USA.* 77:4988.
 14. Schaffar-Deshayes, L., J. Choppin, and J-P. Levy. 1980. Lymphoid cell surface receptor for Moloney leukemia virus envelope glycoprotein gp71. II. Isolation of the receptor. *J. Immunol.* 126:2352.
 15. Robinson, P. J., G. Hunsmann, J. Schneider, and V. Schirmacher. 1980. Possible cell surface receptor for Friend murine leukemia virus isolated with viral envelope glycoprotein complexes. *J. Virol.* 36:291.
 16. Thiry, L., J. Cogniaux-LeClerc, R. Olislager, S. Sprecher-Goldberger, and P. Buekens. 1983. Cell receptors for baboon endogenous virus recognized by monoclonal antibodies. *J. Virol.* 38:697.
 17. Elder, J. H., J. W. Gautsch, F. C. Jensen, R. A. Lerner, J. W. Hartley, and W. P. Rowe. 1977. Biochemical evidence that MCF murine leukemia viruses are envelope (env) gene recombinants. *Proc. Natl. Acad. Sci. USA.* 74:4676.
 18. Rommerlaere, J., D. V. Faller, and N. Hopkins. 1978. Characterization and mapping of RNase T1-resistant oligonucleotides derived from the genomes of Akv and MCF murine leukemia viruses. *Proc. Natl. Acad. Sci. USA.* 75:495.
 19. Zielinski, C. C., S. D. Waksal, L. D. Tempelis, R. H. Khirya, and R. S. Schwartz. 1980. Surface phenotypes in T-cell leukemia are determined by oncogenic retroviruses. *Nature (Lond.)*. 288:489.
 20. McGrath, M. S., M. Lieberman, A. Decleve, H. S. Kaplan, and I. L. Weissman. 1978. The specificity of cell surface virus receptors on RadLV and radiation-induced thymic lymphomas. *J. Virol.* 28:819.
 21. McGrath, M. S., and I. L. Weissman. 1979. AKR leukemogenesis: identification and biological significance of thymic lymphoma receptors for AKR retroviruses. *Cell.* 17:65.
 22. McGrath, M. S., and I. L. Weissman. 1978. A receptor-mediated model of viral leukemogenesis: hypothesis and experiments. *Cold Spring Harbor Symp. Normal Neoplastic Hematopoietic Cell Differentiation.* 577-589.
 23. Sege, K., and P. A. Peterson. 1978. Use of anti-idiotypic antibodies as cell-surface receptor probes. *Proc. Natl. Acad. Sci. USA.* 75:2443.
 24. Schrufer, A. B., P. O. Couraud, C. Andre, B. Vray, and A. Strossberg. 1980. Anti-alprenolol anti-idiotypic antibodies bind to β -adrenergic receptors and modulate catecholamine-sensitive adenylate cyclase. *Proc. Natl. Acad. Sci. USA.* 77:7385.
 25. Wasserman, N. H., A. S. Penn, P. I. Freimuth, N. Treptow, J. Wentzel, W. L. Cleveland, and B. F. Erlanger. 1982. Anti-idiotypic route to anti-acetylcholine receptor and experimental myasthenia gravis. *Proc. Natl. Acad. Sci. USA.* 79:4810.
 26. Nepom, J. T., H. L. Weiner, M. A. Dichter, M. Tardieu, D. R. Spriggs. C. F., Gramm, M. L. Powers, B. N. Fields, and M. I. Greene. 1982. Identification of a hemagglutinin-specific idio type associated with reovirus recognition shared by lymphoid and neural cells. *J. Exp. Med.* 155:155.
 27. Noseworthy, J. H., B. N. Fields, M. A. Dichter, C. Sobotka, E. Pizer, L. L. Perry, J. T. Nepom, and M. I. Greene. 1983. Cell reactions for the mammalian reovirus. I. Syngeneic monoclonal anti-idiotypic antibody identifies a cell surface receptor for reovirus. *J. Immunol.* 131:2533.

28. McGrath, M. S., J. Jerabek, and I. L. Weissman. 1982. Receptor-mediated leukemogenesis: retrovirus receptors on B and T lymphomas share idiotypic determinants. *In* Experimental Hematology Today. S. Baum, G. D. Ledney, and S. Therfelder, editors. S. Karger, Munich. Appendix I.
29. Hiai, H., P. Morrissey, R. Khiroya, and R. S. Schwartz. 1977. Selective expression of xenotropic virus in congenic HRJ/J (hairless) mice. *Nature (Lond.)*. 270:247.
30. Moening, V., H. Frank, G. Housmann, I. Schneider, and W. Schafer. 1974. Properties of mouse leukemia viruses. VII. The major viral glycoprotein of Friend leukemia virus. Isolation and physicochemical properties. *Virology*. 61:100.
31. McGrath, M. S., O. Witte, T. Pincus, and I. L. Weissman. 1978. Retrovirus purification: method which conserves envelope glycoprotein and maximizes infectivity. *J. Virol.* 25:923.
32. Kawashima, K., H. Ikeda, J. W. Hartley, E. Stockert, W. P. Rowe, and L. J. Old. 1976. Changes in expression of murine leukemia virus and production of xenotropic virus in the late preleukemic period in AKR mice. *Proc. Natl. Acad. Sci. USA*. 73:4680.
33. Kohler, G., and C. Milstein. 1975. Continuous cultures of fused cells secreting antibody of defined specificity. *Nature (Lond.)*. 256:495.
34. Markwell, M. A. K. 1982. A new solid-state reagent to iodinate proteins. I. Conditions for the efficient labelling of antiserum. *Anal. Biochem.* 125:427.
35. Kranz, D. M., D. H. Sherman, M. V. Sitkovsky, M. S. Pasternack, and H. N. Eisen. 1984. Immunoprecipitation of cell surface structures of cloned cytotoxic T-lymphocytes by clone-specific antisera. *Proc. Natl. Acad. Sci. USA*. 81:573.
36. Laemmli, U. K. 1970. Cleavage of structural proteins during assembly of the head bacteriophage T4. *Nature (Lond.)*. 227:680.
37. Towbin, H., T. Staehelin, and J. Gordin. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA*. 76:4350.
38. Hartley, J. W., N. K. Walford, L. J. Old, and W. P. Rowe. 1977. A new class of murine leukemia virus associated with development of spontaneous lymphomas. *Proc. Natl. Acad. Sci. USA*. 74:789.
39. Thomas, C. Y., R. Khiroya, R. S. Schwartz, and J. M. Coffin. 1984. Role of recombinant ecotropic and polytropic viruses in the development of spontaneous thymic lymphomas in HRJ/J mice. *J. Virol.* 50:397.
40. Pinter, A., J. Lieman-Hurwitz, and E. Fleissner. 1978. The nature of the association between the murine leukemia virus envelope proteins. *Virology*. 91:345.
41. Oudin, J., and P. A. Cazenave. 1971. Similar idiotypic specificities in immunoglobulin fractions with different antibody functions or even without detectable antibody function. *Proc. Natl. Acad. Sci. USA*. 68:2616.
42. Enghofer, E., C. P. J. Gludemans, and M. J. Bosma. 1979. Immunoglobulins with different specificities have similar idiotypes. *Mol. Immunol.* 16:1103.
43. Bona, C. A. 1984. Parallel sets and the internal image of antigen within the idiotypic network. *Fed. Proc.* 43:2558.
44. Kaufmann, R. S., J. H. Noseworthy, J. T. Nepom, R. Finberg, B. N. Fields, and M. I. Greene. 1983. Cell receptors for the mammalian reovirus. II. Monoclonal anti-idiotypic antibody blocks viral binding to cells. *J. Immunol.* 131:2539.
45. Lee, J. C., and J. N. Ihle. 1981. Chronic immune stimulation is required for Moloney leukemia virus-induced lymphomas. *Nature (Lond.)*. 289:407.

46. Lee, J. C., and J. N. Ihle. 1981. Increased responses to lymphokines are correlated with preleukemia in mice inoculated with Moloney leukemia virus. *Proc. Natl. Acad. Sci. USA.* 78:7712.
47. McGrath, M. S., E. Pillemer, and I. L. Weissman. 1980. Murine leukemogenesis: monoclonal antibodies to T-cell determinants arrest T-lymphoma cell proliferation. *Nature (Lond.)*. 285:259.