Study of the Expression of a Glycoprotein of Retroviral Origin in the Plasma of Patients With Hematologic Disorders and in the Plasma of Normal Individuals

By P.C. Jacquemin, P. Strijckmans, and L. Thiry

A 74,000 molecular weight glycoprotein was purified from the plasma of a patient with chronic myelogenous leukemia in blast crisis. Monoclonal antibodies were produced in the mouse and used to characterize this protein. It was shown to contain p15E antigenic determinants and portions of a reverse transcriptase. The level of this protein was found to be elevated in leukemic patients with high white blood cell counts and also in some patients with other hematopoietic disorders as compared to the level measured in normal individuals. The level of the protein was strongly reduced in acute leukemia patients after intense chemotherapy treatment. We tentatively conclude that this protein is of endogenous retroviral origin and perhaps regulates hematopoietic tissues.

IN SEARCH FOR an etiologic agent, human myeloid leukemic cells have been investigated for the expression of type C virus proteins. Data have been published showing the presence of cytoplasmic reverse transcriptase (RT) in some cases of leukemia. The RT that was partially purified from the cells of acute myelogenous leukemia (AML) patients was shown to be related to primate retroviruses [Simian sarcoma virus (SiSV) and gibbon ape leukemia virus (GaLV)]. A similar enzymatic activity was reported in leukemic spleens. We previously reported the presence of surface immunoglobulins with anti-RT activity on the membrane of blood cells. Antibodies purified from the surface of cells from patients in chronic myelogenous leukemia (CML) in blast crisis (BC) neutralized specifically the reverse transcriptase of Feline leukemia virus (FeLV), whereas antibodies purified from the surface of chronic phase CML cells neutralized various isolates of GaLV-RT. The antibodies eluted from the membrane of acute myelogenous leukemia (AML) cells preferentially reacted with SiSV-RT. In normal individuals, antibodies were also isolated from the membranes of blood cells that were shown to neutralize the RT from GaLV,

The antigen was prepared as follows. Large quantities of plasma were obtained from patients with chronic myelogenous leukemia (CML) blast crisis in relapse (patient I, Table 2). The plasma was stored at −70 °C until use. Forty milliliters of plasma was combined with 0.5% Triton X-100, 0.1 mmol/L dithiothreitol (DTT), 0.3 mol/L NaCl, 0.05 mol/L Tris-HCl, pH 7.9, 0.2 mmol/L phenyl methyl-sulfonylfluoride (PMSF), and chromatographed on a 10-mL column of DEAE-agarose to remove nucleic acids. After a threefold dilution, the flow-through of the column was incubated for ten minutes with 50 μmol/L polyguanilic acid [poly(G)] (P.L. Biochemical, Milwaukee) with 0.5 mmol/L MnCl2 and applied on a DEAE-agarose column. After extensive washing, 2.7 mg of specifically

From the Institut Pasteur du Brabant, Department of Virology, and the Institut Borda, Brussels.

Supported in part by the Fondation Huguet, and by funds partly provided by the International Cancer Research data bank program of the National Cancer Institute, National Institute of Health (USA), under contract NOI-CO-65341 (ICRETT) and partly by the International Union Against Cancer.

Submitted Oct 17, 1983; accepted April 28, 1984.

Address reprint requests to Dr P.C. Jacquemin, Institut Pasteur, Virology, rue England, 642, 1180 Brussels, Belgium.

© 1984 by Grune & Stratton, Inc.

0006-4971/84/6404-0012/$03.00/0
bound proteins were eluted at 0.3 mol/L NaCl. The crude antigen was iodinated by the chloramine T method.  

Radioimmunoprecipitation Conditions  
The labeled antigen (crude antigen, 5 x 10^6 cpm; purified antigen, 10^6 cpm) was incubated overnight at 4°C with different hyperimmune sera (5 µL) and tissue culture supernatants of hybridomas (200 µL) in 50 mmol/L Tris-HCl, pH 7.9, 0.2 mol/L NaCl, 2% Triton X-100, 1% bovine serum albumin (BSA), and 1 mmol/L PMSF (buffer A). The immunocomplexes were precipitated with 250 µL of 1% (wt/vol) Staphylococcus aureus (Cowan strain) solution of protein A-Sepharose. A first precipitation was done with protein A-Sepharose to remove residual IgG and immune complexes. The immunoprecipitates were washed with 1 mL of buffer A and with 2 mL of the same buffer without bovine serum albumin. In the competition radioimmunoprecipitations, an amount of antibody capable of precipitating 30% of the labeled antigen was preincubated four hours at 0°C with the competitors and then left overnight with the labeled antigen at 0°C in the conditions described above.

Gel Electrophoresis  
The washed precipitates were suspended in electrophoresis sample buffer and boiled for two minutes. The bacterial absorbant or the protein A-Sepharose were removed by centrifugation. These supernatants were analyzed in 10% polyacrylamide gels. 12 Immunoprecipitates were also dissociated in 8 mol/L urea and submitted to isoelectric focusing. 13

The pH gradient ran from pH 3 to pH 7.5; cold BSA was included in the gel as an internal marker. The second dimension used was a 10% polyacrylamide gel, iodine-125 labeled proteins on the gel were detected by autoradiography using Kodak-X-omat XR-5 film (Rochester, NY) or by dividing the gel into 1-mm slices and counting the radioactivity in a gamma counter. The scanning pattern of autoradiograms was also measured at 500 nm with a Beckman spectrophotometer.

Hyperimmune Sera Used in Immunoprecipitation  
The sera used were hyperimmune sera raised against purified viral proteins obtained from Dr R.C. Gallo (NIH, Bethesda, MD). These were: a rabbit serum raised against pure Rauscher murine leukemia virus (R-MuLV) RT, which neutralized most actively R-MuLV-RT but also to some extent the other type C virus RTs, including baboon endogenous virus (BaEV)-RT but not avian myeloblastosis virus (AMV)-RT, and which did not precipitate 121-labeled pure R-MuLV p30 nor glycoprotein 70 (gp70); rabbit antisera raised against SiSV-RT, RD-114-RT, and BaEV-RT, which showed more virus specificity than the anti-MuLV-RT serum; 13 a rabbit antiserum raised against pure R-MuLV p30 and specific for p30; a rabbit antiserum raised against R-MuLV gp70 and specific for gp70; 16 a goat antiserum raised against SiSV specific for p30 and a goat antiserum against SiSV gp70 specific for gp70; goat serum raised against HTLV p24 and a monoclonal antibody against HTLV p19. Natural cat sera with anti-FelV-RT activity; 17 and monoclonal antibody against MuLV p15E (19F8). 18 These were obtained from Dr M. Essex (Harvard School of Medicine, Boston). Rabbit sera raised against guanidine-hydrochloride-purified FelV p15E and against FeLV core p15 were obtained from Dr W. Hardy (Sloan Kettering Memorial Institute, New York).

Production of Monoclonal Antibodies  
The monoclonal antibodies were prepared as follows. BALB/c mice were immunized subcutaneously at weekly intervals with 10 µg of crude preparation of antigen, prepared as described above in complete Freund's adjuvant for the first two weeks, then without adjuvant for two months. Four weeks later, the sera of the mice were tested in a solid-phase binding assay, 16 and the mouse with the highest titer serum was boosted intravenously with 10 µg of antigen three days before the fusion with SP/2 myeloma cells. The nucleated spleen cells were fused with 2 x 10^5 SP/2 drug-marked nonimmunoglobulin-secreting myeloma cells. 18 The cells were then resuspended in 0.1 mmol/L hypoxanthine, 1 mmol/L methotrexate, and 32 mmol/L thymidine in conditioned medium (DMEM supplemented with 30% fetal calf serum precipitated overnight at 37°C with 10% of DBA/2 mice). Cells were plated into 96-well Falcon plates. Macrophagic colonies were transferred to 24-well Limbro plates and assayed four to eight days later. Cells producing monoclonal antibodies with activity against the antigen were cloned by the limited dilution technique and injected intraperitoneally into BALB/c mice pretreated with 0.5 mL of pristane to obtain ascites. The monoclonal antibodies obtained were all recognized by the staphylococcal protein A and were of the IgG1 subclass. They were referred to as: 10EC, 3DI, 6GD, 5GD, 11EH, 8CE, 5DD, 11CE, and 9BC.

Affinity Purification on a Monoclonal Antibody Column  
A specific monoclonal antibody (9BC) obtained in large quantity as ascites was purified on a column of protein A-Sepharose and then immobilized at 1 mg/mL of packed gel on Cyanogen bromide-activated Sepharose. Thirty milligrams of partially purified antigen, as described above, was applied to a 2-mL column three times. After extensive washing in buffer A without BSA and with 2 mol/L NaCl, the antigen was eluted with 0.2 mol/L NH4OH; 19 1.4 mg of purified antigen was obtained.

Source of Viruses and Reverse Transcriptase Assays  
Rauscher MuLV was grown in JLSV 10 cells. GaLV was grown in UCD 144 cells. Partially purified reverse transcriptases were used. The assays were performed in 0.05 mol/L Tris-HCl buffer, pH 7.8, containing 140 µmol/L H-dTTP (3.6 Ci/mmol), 130 µmol/L dATP, 50 µg/mL DT 12-18 poly A, 5 mmol/L DTT, 0.5 mmol/L MnCl2 for R-MuLV-RT, FeLV-RT, GalV-RT, and 5 mmol/L MgCl2 for AMV-RT. 5 Also introduced into the assay were 0.5 µg and dilutions of the affinity-purified antigen.

RESULTS  
Purification of an Antigen With pl5E and Reverse Transcriptase Antigenic Determinants and Production of Specific Monoclonal Antibodies  
Plasma from a CML patient in relapse following treatment for blast crisis (patient 1, Table 2) was chromatographed to purify a protein with affinity for poly (G) in the presence of MnCl2, as described for primate leukemia virus RTs. 9 The partially purified preparation that eluted from the column at 0.3 mol/L NaCl still contained several proteins, as shown after iodination by the chloramine T method (Fig 1B, lane 6, and Fig 2A). The iodinated preparation was characterized by precipitation with a battery of antisera directed against purified viral proteins (Fig 1A, Table 1). A 74-kD protein was precipitated by a rabbit anti-
MuLV-RT antiserum, a rabbit anti-FeLV p15E antiserum, and a goat anti-SiSV p30 antiserum, but not by a serum raised against human albumin, by a normal rabbit serum (Fig 1A), by normal goat, cat, gibbon, and mouse sera, by antisera raised against R-MuLV gp70, SiSV gp70, R-MuLV p30, FeLV p15, or by antisera against human α1-glycoproteins and HLA antigens (Bw17, Bw4, A2). RT determinants were detected on the 74-kD protein only by a cross-reactive anti-MuLV-RT antiserum, which did not precipitate 125I-labeled R-MuLV gp70 nor R-MuLV p30 under conditions described earlier, but were not detected by type-specific antisera raised against BaEV, RD 114, SiSV, or FeLV RTs. A hyperimmune serum specific for HTLV p24 and a monoclonal antibody specific for HTLV p19 did not recognize the 74-kD protein nor did monoclonal antibodies directed against BaEV gp70, BaEV p15, MuLV gp70, MuLV p15, and MuLV p30 (Table 1).

To obtain monoclonal antibodies, BALB/c mice were immunized with the preparation described above, and the nucleated spleen cells were later fused with a
Table 1. Summary of the Antibody Results Against the 74-kD Glycoprotein

<table>
<thead>
<tr>
<th>Source of Antibodies</th>
<th>Poly (G) + Immuno-affinity Purified</th>
<th>Poly (G) Purified</th>
</tr>
</thead>
</table>
| Normal sera: rabbit, goat, mouse, cat, gibbon, human | -/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/...
these viruses (data not shown for R-MuLV). We deduced from these competition data that the anti-RT antibody most likely recognized group-specific determinants on the 74-kD proteins that were present in MuLV, GaLV, and FeLV RTs. The iodinated antigen, which was purified on a monoclonal antibody column, still actively bound to DEAE-agarose in the presence of poly(G) and MnCl₂, and 50% of it could still be eluted with 0.3 mol/L NaCl and the rest at higher salt concentrations (data not shown). This antigen, which by itself did not have any enzymatic activity, nevertheless was capable of completely inhibiting an RT assay of FeLV, MuLV, and GaLV RTs (Fig 5). This was most likely due to competition between the antigen and the enzymes for the template primer. We verified that the purified antigen had no proteolytic activity and that no nucleolytic activity was detectable on 32P-labeled RNA and DNA (data not shown).

Detection of a Similar Protein in Normal Human Plasma and Not in the Plasma of Other Normal Animal Species

The presence of a competing protein was investigated in different plasma and sera with the use of a competition radioimmunoassay using the specific type of competition of precipitation was also obtained with partially purified R-MuLV-RT (data not shown), by virus cores prepared from GaLV grown in gibbon T cells (UCD 144) (Fig 4), and by virus cores prepared from R-MuLV (data not shown). Very little competition was noted with the supernatants from the cores of

![Fig 3. Characterization of the purified antigen. (Top) Electrophoretic profile of 125I-labeled antigen, purified on a column of monoclonal antibody (9BC) coupled to Sepharose and iodinated using the chloramine T procedure. Electrophoresis was performed in the presence of sodium dodecyl sulfate on 10% polyacrylamide gels, according to the method of Laemmli. The gel was divided into 1-mm slices, and the radioactivity was determined by a gamma counter. The standards used for molecular weight calibration were phosphorylase B (Ph, 94,000), bovine serum albumin (BSA, 68,000), ovalbumin (OV, 45,000) and carbonic anhydrase (CA 30,000). (Bottom) Immunoprecipitation of 125I-labeled antigen, purified on a column of monoclonal antibody. The ability of the different sera and supernatants from hybridoma cells to precipitate the antigen was determined by incubating 10,000 cpm of the labeled probe with serial fivefold dilutions of each one, as described in Materials and Methods. The radioactivity was measured in the pellets and was expressed as the percentage of total 125I counts per minute precipitated at each dilution. (A) Antisera tested included: (●) anti-FeLV p15E, (●) anti-MuLV RT, (●) anti-MuLV p30, and (●) normal serum. (B) The supernatants of hybridoma tested included: (●) 9BC, (●) 19F8 anti-MuLV p15E, (●) 6GD, (●) 5GD, and (●) the conditioned medium used to grow the hybrids.

![Fig 4. Scanning pattern of precipitated purified antigen separated by slab electrophoresis and visualized by autoradiography. A quantity of 10,000 cpm of 125I-labeled purified antigen was used in each immunoprecipitation and an amount of serum capable of precipitating 30% of it. Curve A shows precipitation of the antigen by 0.5 μL of normal rabbit serum, and curves B to G show precipitation of the antigen by 0.5 μL of rabbit serum anti-MuLV-RT. Curves B and E represent the precipitate in absence of competitor; curve C represents the precipitate obtained in presence of FeLV-RT (a quantity of enzyme capable of incorporating 4 nmoI 3H-dTMP into acid-insoluble precipitate in 30 minutes at 30 °C, as described in Materials and Methods); curve D the precipitate obtained in presence of R-MuLV p30 (300 ng); curve F the precipitate obtained in presence of 100 μg of GaLV cores; and curve G the precipitate obtained in presence of 100 μg of the supernatant of GaLV cores. The molecular weight of the protein peak is 74 kD.}
ENDOGENOUS RETROVIRUS PROTEIN IN HUMANS

obtained with 0.03 \( \mu \)L of these normal human sera. goat, horse, fetal calf, gibbon, and cat did not prevent amounts of purified antigen from CML plasma. AMV-RT, GaLV-RT, purified from normal human plasma (patient 32, Table to the protein from the CML plasma was partially than serum. A protein with antigenic properties similar normal human sera were able to compete very effi-

petant 9BC was competed for by increasing amounts of various sera. The sera used were (N) normal mouse serum, (G) normal goat serum, (R) normal rabbit serum, (O) normal cat serum, (G) normal gibbon serum, (H) normal horse serum, and (C) fetal calf serum.

The sera used were normal rabbit, mouse, goat, horse, fetal calf, gibbon, and cat did not prevent the precipitation of the labeled antigen even at 8 \( \mu \)L, which was the lowest dilution used. On the other hand, normal human sera were able to compete very efficiently for the precipitation, and 50% competition was obtained with 0.03 \( \mu \)L of these normal human sera. Plasma was usually found to contain 10% more antigen than serum. A protein with antigenic properties similar to the protein from the CML plasma was partially purified from normal human plasma (patient 32, Table 2) using the same procedure. After iodination, this partially purified protein was shown to be precipitated by a specific monoclonal antibody (9BC, Fig 7, lane 5), by a rabbit anti-FeLV p1SE, and by rabbit anti-MuLV-RT antisera (Fig 7, lanes 7 and 9). In all these immunoprecipitations, some immunoglobulin heavy and light chains also coprecipitated with the specifi-

cally formed immune complexes. This Ig coprecipitation was lessened when the specific antibodies used were covalently linked to Sepharose (Fig 7, lanes 1 to 5). This protein, purified from normal individuals, was found to have an isoelectric point similar to the one isolated from the CML patient by electrophoresis on two-dimensional gels (data not shown).

The Level of the 74-kD Protein Was Found to be Elevated in Plasma of Patients With Hematopoietic Disorders and Was Lowered in Plasma of Patients After Intensive Chemotherapy

The level of the protein was measured in a competition RIA, as described before in Fig 6. Ten samples of cord blood obtained at birth were assayed and the mean level of protein measured in these was 23 \( \mu \)g/mL, which was a value very similar to the one found in ten normal adult individuals tested (25.5 \( \mu \)g/mL) (Table 2). The same measures were performed in the sera or plasma of patients with hematopoietic disorders (CML patients, including chronic phase and blast crisis patients; patients with myeloproliferative syndromes were included in this group, as well as patients with bone marrow aplasia and a few other cases) and correlated to their individual leukocyte counts (see Table 2). In CML patients (Nos. 1, 2, 8, 10, and 11) with high white blood cell counts, a high level of antigen was measured, i.e., 351, 100, 100, 200, and 234 \( \mu \)g/mL, respectively. Patients 3, 4, 5, and 12, with lower WBC counts, had an intermediate level or high level of antigen in their plasma, while patient 13, with myelofibrosis, had a normal level. In this last patient, even though the WBC count was high, the total blood cell count in the body was most likely normal, as the bone marrow was invaded by fibrotic tissue. In patient 15, suffering from AML and having a high WBC, the level of antigen detected was elevated (80 \( \mu \)g/mL).

Patients 6 and 7 in CML blast crisis, patient 14 with AML, and patient 18 with ALL, who had received intensive courses of chemotherapy to treat an acute phase of their disease, had levels of antigen measured that were much lower than the levels in normal individuals. A highly interesting group was composed of patients with bone marrow aplasia either of unknown etiology (aplastic anemia in patients 23 and 24) or of known etiology (eg, patient 9, who received an excessive dose of busulfan in treatment of a chronic phase CML, and patient 26, treated with gold salts for rheumatoid arthritis), where a high level of antigen was detected in the plasma, ranging from 90 to 200 \( \mu \)g/mL. Patient 25, treated for congenital neutropenia, also had a fivefold increase of the level of antigen. Similarly, in patient 16, suffering from smoldering leukemia, a high level of the 74-kD glycoprotein
Table 2. Level of 74-kD Glycoprotein in Various Patients

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Clinical Diagnosis</th>
<th>Age</th>
<th>Sex</th>
<th>WBC Count/µL</th>
<th>µg/mL of 74-kD Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CML BC relapse</td>
<td>77</td>
<td>F</td>
<td>100,000</td>
<td>351</td>
</tr>
<tr>
<td>2</td>
<td>CML BC</td>
<td>68</td>
<td>M</td>
<td>55,000</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>CML BC</td>
<td>37</td>
<td>M</td>
<td>25,300</td>
<td>62</td>
</tr>
<tr>
<td>4</td>
<td>CML BC</td>
<td>53</td>
<td>M</td>
<td>33,000</td>
<td>51</td>
</tr>
<tr>
<td>5</td>
<td>CML BC</td>
<td>50</td>
<td>M</td>
<td>32,000</td>
<td>52</td>
</tr>
<tr>
<td>6</td>
<td>CML BC</td>
<td>25</td>
<td>M</td>
<td>8,200 post-CT</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>CML BC</td>
<td>59</td>
<td>M</td>
<td>4,000 post-CT</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>CML C</td>
<td>45</td>
<td>F</td>
<td>150,000</td>
<td>100</td>
</tr>
<tr>
<td>9</td>
<td>CML C</td>
<td>60</td>
<td>M</td>
<td>Aplasia due to overdose of Busulfan</td>
<td>96</td>
</tr>
<tr>
<td>10</td>
<td>Myeloproliferative syndrome</td>
<td>61</td>
<td>F</td>
<td>78,000</td>
<td>200</td>
</tr>
<tr>
<td>11</td>
<td>Myeloproliferative syndrome</td>
<td>55</td>
<td>F</td>
<td>45,900 (24% blast)</td>
<td>234</td>
</tr>
<tr>
<td>12</td>
<td>Myeloproliferative syndrome</td>
<td>60</td>
<td>M</td>
<td>11,100</td>
<td>100</td>
</tr>
<tr>
<td>13</td>
<td>Myelofibrosis</td>
<td>66</td>
<td>F</td>
<td>50,000</td>
<td>20</td>
</tr>
<tr>
<td>14</td>
<td>AML</td>
<td>44</td>
<td>M</td>
<td>3,600 cytoreduction post-CT</td>
<td>4</td>
</tr>
<tr>
<td>15</td>
<td>AML</td>
<td>—</td>
<td>F</td>
<td>250,000</td>
<td>80</td>
</tr>
<tr>
<td>16</td>
<td>Smoldering leukemia</td>
<td>64</td>
<td>M</td>
<td>Low WBC</td>
<td>92</td>
</tr>
<tr>
<td>17</td>
<td>Acute promyelocytic leukemia in complete remission</td>
<td>14</td>
<td>M</td>
<td>3,400</td>
<td>30</td>
</tr>
<tr>
<td>18</td>
<td>ALL</td>
<td>6</td>
<td>M</td>
<td>5,600 post-CT</td>
<td>4</td>
</tr>
<tr>
<td>19</td>
<td>ALL</td>
<td>75</td>
<td>M</td>
<td>17,600</td>
<td>50</td>
</tr>
<tr>
<td>20</td>
<td>ALL, complete remission after allogeneic bone marrow graft</td>
<td>16</td>
<td>M</td>
<td>2,500</td>
<td>350</td>
</tr>
<tr>
<td>21</td>
<td>Acute erythroblastic leukemia</td>
<td>20</td>
<td>M</td>
<td>154,000 (97% blast)</td>
<td>100</td>
</tr>
<tr>
<td>22</td>
<td>Myelodysplasia</td>
<td>70</td>
<td>M</td>
<td>4,100 (2% blast)</td>
<td>92</td>
</tr>
<tr>
<td>23</td>
<td>Aplastic anemia</td>
<td>11</td>
<td>M</td>
<td>2,600</td>
<td>90</td>
</tr>
<tr>
<td>24</td>
<td>Aplastic anemia</td>
<td>59</td>
<td>M</td>
<td>2,000</td>
<td>100</td>
</tr>
<tr>
<td>25</td>
<td>Congenital neutropenia</td>
<td>16</td>
<td>F</td>
<td>Low granulocytes</td>
<td>125</td>
</tr>
<tr>
<td>26</td>
<td>Rheumatoid arthritis</td>
<td>80</td>
<td>M</td>
<td>Aplasia due to gold salt treatment</td>
<td>200</td>
</tr>
<tr>
<td>27</td>
<td>Myeloma</td>
<td>60</td>
<td>M</td>
<td>3,400</td>
<td>90</td>
</tr>
<tr>
<td>28</td>
<td>Normal</td>
<td>31</td>
<td>F</td>
<td>11,500</td>
<td>15</td>
</tr>
<tr>
<td>29</td>
<td>Normal</td>
<td>40</td>
<td>M</td>
<td>7,400</td>
<td>18</td>
</tr>
<tr>
<td>30</td>
<td>Normal</td>
<td>52</td>
<td>F</td>
<td>13,000</td>
<td>25</td>
</tr>
<tr>
<td>31</td>
<td>Normal</td>
<td>44</td>
<td>F</td>
<td>12,000</td>
<td>31</td>
</tr>
<tr>
<td>32</td>
<td>Normal</td>
<td>41</td>
<td>M</td>
<td>5,000</td>
<td>31</td>
</tr>
<tr>
<td>33</td>
<td>Normal</td>
<td>33</td>
<td>M</td>
<td>10,000</td>
<td>37</td>
</tr>
<tr>
<td>34</td>
<td>Normal</td>
<td>37</td>
<td>F</td>
<td>5,850</td>
<td>22</td>
</tr>
<tr>
<td>35</td>
<td>Normal</td>
<td>44</td>
<td>F</td>
<td>7,800</td>
<td>35</td>
</tr>
<tr>
<td>36</td>
<td>Normal</td>
<td>22</td>
<td>F</td>
<td>6,400</td>
<td>31</td>
</tr>
<tr>
<td>37</td>
<td>Normal</td>
<td>47</td>
<td>M</td>
<td>6,400</td>
<td>10</td>
</tr>
<tr>
<td>28–37</td>
<td>10 Samples</td>
<td></td>
<td></td>
<td>Mean 25.5 ± 10</td>
<td></td>
</tr>
<tr>
<td>38–47</td>
<td>Cord blood 10 samples</td>
<td></td>
<td></td>
<td>23 ± 9</td>
<td></td>
</tr>
</tbody>
</table>

Each individual serum or plasma sample was tested in competition RIA, as described in Fig 6. The amount of competing antigen was determined by comparison to purified antigen introduced in the same assay. The exact amount of purified antigen introduced in the test was determined on Coomassie Blue-stained gel in comparison to known amounts of bovine serum albumin.

CT, chemotherapy.

antigen was detected (92 µg/mL). Other hematopoietic disorders were also tested and found to have a high level of 74-kD antigen (eg, patient 19 with ALL, patient 21 with acute erythroblastic leukemia, patient 27 with a myeloma, and patient 20 in remission with ALL, having received an allogeneic bone marrow graft, where the levels of antigen were, respectively, 50, 100, 90, 350 µg/mL). Patient 17, in complete remission with an acute promyelocytic leukemia, had a normal level of antigen (30 µg/mL).

From these data, we were tempted to deduce that a high level of plasma antigen correlated with a situation of abnormal stimulation of proliferation of hematopoietic tissues and not with the level of WBC in the blood.

In patients suffering from CML, the high level of
ENDOGENOUS RETROVIRUS PROTEIN IN HUMANS

Fig 7. Precipitation of 125I-labeled antigen purified from plasma of a normal individual. The immunoprecipitators were handled as described in Fig 1: lane 1 represents the 125I-labeled antigen used in the following immunoprecipitations. They were performed with the following: lane 2, protein A Sepharose, 200 µL of a 1:10 suspension; lane 3, 200 µL of a 1:10 suspension of human IgG-Sepharose; lane 4, 200 µL of a 1:10 suspension of an irrelevant monoclonal antibody-Sepharose; lane 5, 200 µL of a 1:10 suspension of 9BC IgG-Sepharose; lane 6, 5 µL of normal rabbit serum; lane 7, 5 µL of rabbit anti-MuLV RT serum; lane 8, 5 µL of rabbit anti-MuLV gp 70 serum; lane 9, 5 µL of rabbit anti-FeLV p15E serum; lane 10, no antiserum. MW, the molecular weight markers used were the same as the ones described in Fig 1 (from top: 94,000, 68,000, 45,000, 30,000, and 21,000).

The 74-kD molecule was most likely related to the increased WBC, whereas in the cases of hematopoietic tissue depletion in the bone marrow (patients 9, 23, 24, 25, and 26), it could be the result of the low WBC. In this last situation, it would be associated with the stimulation of bone marrow cell multiplication and the concomitant destruction of these cells in the bone marrow as they proliferate. In patient 20, grafted with allogeneic bone marrow while in remission, the high level of antigen could reflect a response of the lymphoid tissues to the graft. The patients with very low levels of 74-kD molecule, represented by acute phase patients submitted to aggressive chemotherapy, would be unable to maintain a normal level of the 74-kD molecule because the cells responsible for its release were temporarily aspecifically blocked or killed (patients 6, 7, 14, and 18).

DISCUSSION

Our data describe the purification of a 74-kD glycoprotein, related to a retroviral protein, from the plasma of a patient in CML blast crisis (patient 1, Table 2) and the partial purification of a similar protein from the plasma of a normal individual (patient 32, Table 2).

The protein was shown to be composed of p15E antigenic determinants by using a polyclonal anti-p15E antibody and principally a specific anti-p15E monoclonal antibody. The p15E antigenic determinants are known to be conserved among the different retroviruses. RT antigenic determinants were also found to be associated with this protein, but in a less obvious way. The arguments that showed the presence of RT antigenic determinants could be summarized as follows.

1. The 74-kD protein was purified using a very efficient procedure developed to purify mammalian RTs, based on their affinity for poly (G).9

2. In the precipitation of the protein on an antibody affinity column, we took advantage of a previously described procedure of elution of the enzyme at alkaline pH that was known to save the enzyme integrity.21

3. The purified iodinated protein was precipitated by a polyclonal anti-R-MuLV-RT antibody that did not precipitate R-MuLV p30 nor R-MuLV gp70, but was known to be broadly reactive with different mammalian RTs.

4. The precipitation of the purified protein by the anti-RT serum was reduced with partially purified RTs and virus cores from R-MuLV, FeLV, and GaLV viruses, but not by purified R-MuLV p30 nor R-MuLV gp70 (not shown).

5. The purified iodinated antigen still bound to poly (G).9

6. The cold purified antigen competed in an enzymatic assay with active enzymes.

These arguments, relevant to an RT-like protein, did not evidence any type-specific determinant on the RT molecule, but only the presence of a highly conserved template primer binding site and the presence of an RT group-specific determinant common to different mammalian RTs, including MuLV-RT, FeLV-RT, and GaLV-RT.

The indication of the presence of human type-specific determinants associated with this 74-kD molecule was detected with the help of the specific monoclonal antibody, 9BC, that we developed. However, this antibody did not react with any of the viral RTs tested (MuLV, FeLV, SiSV, BaEV), and we could not exclude the possibility that it reacted with some human envelope-specific fragments associated with the molecule, as the protein appeared to be glycosylated. We deduced this from the heterogeneity of the electric charges on the two-dimensional gels that were found in the iodinated molecule and also on the cold protein in Coomassie-stained gels (data not presented) and from the fact that we could also purify the protein partially on a concanavalin A-Sepharose column with other plasma glycoproteins (data not shown). We also provide evidence that a similar protein is present in normal individuals and, as shown above, it was also partially purified. From these data it appeared reasonable to
consider this 74-kD glycoprotein as the expression of endogenous retroviral sequences.

The identification and molecular cloning of murine leukemia virus-like sequences from human DNA was reported recently. Analysis of these cloned segments revealed the conservation of Gag and Pol sequences common to the Maloney-MuLV genome. These findings, and the more recent description of mRNA transcripts related to full-length endogenous retroviral DNA in human cells, seem to provide a reasonable background to support the hypothesis that the 74-kD glycoprotein represents the expression of some endogenous human viral sequences.

The level of this 74-kD glycoprotein was measured in the plasma of normal individuals and of patients with hematologic disorders. In the blood of normal individuals and in full-term cord blood samples, 25.5 μg/mL and 23 μg/mL of the molecule, respectively, were detected. In the plasma of patients with high WBC, the level of the antigen correlated to some extent with the WBC counts, but when we looked at the plasma of patients with bone marrow aplasia of toxic or unknown origin and at the plasma of a patient with congenital neutropenia, the level of the antigen was also found to be elevated. It was also elevated in the plasma of a patient suffering from multiple myeloma, in the plasma of a patient after bone marrow allograft, and in bone marrow dysplasia, despite the presence of low WBCs in these patients. We were then tempted to assume that the level of the 74-kD molecule reflected more a situation where hematopoietic cells were induced to proliferate than a situation of high blood cell counts. Also, the fact that patients who received an aggressive course of chemotherapy for acute leukemia have a very low level of the antigen, despite a low WBC count, favored this idea. In this last situation, the cells responsible for the production of the 74-kD glycoprotein were most likely blocked or killed specifically by the cytotoxic drugs. To support the theory of an association between the 74-kD glycoprotein and the regulation of hematopoietic cell proliferation, a serial study of the 74-kD glycoprotein levels at various times after administration of chemotherapy will be undertaken.

The glycoprotein that we described here, most likely the product of endogenous human retroviral information, would be associated with the normal growth and regulation of hematopoietic cells. The situation that we are dealing with could be analogous to the "c-sis" situation, which was shown to be highly related to the "platelet-derived growth factor," being most likely a functional product of a human proto-oncogene.

To conciliate the data that we reported here concerning the 74-kD molecule and the previously reported data concerning the membrane-bound antibodies with anti-RT activity, the following hypothesis is proposed. Normal cells would be in a proliferative state as a result of a high plasma level of the 74-kD glycoprotein and would acquire a GaLV SF RT-like antigen on their membrane at a definite stage of differentiation, which would render them able to block the proliferation of their progenitors and lower the plasma level of the 74-kD glycoprotein. CML blast crisis cells, on the other hand, as a result of a high level of 74-kD glycoprotein in the plasma, respond and multiply, but differentiate in an abnormal way and acquire an FeLV-RT-like antigen, instead of a GaLV-RT-like antigen, which cannot block the proliferation of the progenitor cells. In fact, recent publications showed that, in leukemia, the abnormality can be found in the leukemic cells, associated with the abnormal amplification of a proto-oncogene. This proto-oncogene amplification was shown to be often linked to chromosomal aberrations in different hematologic diseases. Proto-oncogene expression at the RNA level was also reported in hematopoietic cell lines and in some fresh hematologic cell samples.

ACKNOWLEDGMENT

We thank Drs R.C. Gallo, M.G. Sarnadharan, C. Saxinger, and M. Robert-Guroff for the generous gift of partially purified reverse transcriptases and antisera against viral proteins. We thank Drs M. Essex and W. Hardy for the generous gift of cat serum with anti-FeLV activity and sera against p15E. We thank Dr J. Portis for the generous gift of monoclonal antibodies against MuLV proteins, and Dr A. Burny for helpful discussion. We also thank Drs De Busscher, Duchateau, and Symann for providing clinical material. P. Dupan provided excellent technical assistance, and we are grateful to J. Herinckx for preparing the manuscript.

REFERENCES

32. Doolittle RF, Hunkapiller NW, Hood LE, Devare SG, Robbins KC, Aaronson SA, Antoniades HN: Simian sarcoma virus onc-gene, V-SIS, is derived from the gene (or genes) encoding a platelet-derived growth factor. Science 221:275, 1983
36. Dalla-Favera R, Bregni M, Erikson J, Patterson D, Gallo R, Croce C: Human c-myc onc gene is located on the region of chromosome 8 that is translocated in Burkitt lymphoma cells. Proc Natl Acad Sci USA 79:7824, 1982
Study of the expression of a glycoprotein of retroviral origin in the plasma of patients with hematologic disorders and in the plasma of normal individuals

PC Jacquemin, P Strijckmans and L Thiry