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, strain San Francisco (SF), grown in human lymphoid cells. These data indicated that molecules closely related to different viral RTs were expressed on the membrane of myeloid cells, and that specific antibodies were made against these proteins. Recently, the sequences coding for an endogenous human RT were found using cloned DNA probes. This prompted us to further investigate for the relevance of an RT molecule in leukemic patients and in normal individuals.

The identification and isolation of the membrane-associated RT from fresh leukemic and normal myeloid cells appeared very difficult. Therefore, we looked for the presence of free molecules displaying some RT properties in the plasma of a CML patient in blast crisis in relapse. In this patient, no antibodies with anti-RT activity were detected in the plasma nor isolated from the relapse blast cells. A glycoprotein with p15E antigenic determinants and some RT properties was purified with the help of a specific monoclonal antibody. This protein was also shown to be present in normal individuals. A radioimmunoassay was developed with the purified antigen and a monoclonal antibody that allowed us to study the level of expression of this protein in various hematologic situations. From these studies, we deduced that the glycoprotein that we purified was associated with the proliferation of hematopoietic tissues.

MATERIALS AND METHODS

Source of Plasma and Antigen Purification

Blood from normal individuals and individuals with various hematopoietic disorders (data on the diagnosis and white blood cell counts are displayed in Table 2) were obtained fresh in heparin and the plasma separated by centrifugation at 1,000 g for ten minutes. Cord blood samples were obtained from full-term placenta.

The antigen was prepared as follows. Large quantities of plasma were obtained in heparin by repeated plasmapheres of a patient in CML blast crisis in relapse (patient 1, 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 polyglycinic 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...
bound proteins were eluted at 0.3 mol/L NaCl. The crude antigen was iodinated by the chloramine T method.\textsuperscript{16}

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 \mu L) and tissue culture supernatants of hybridomas (200 \mu L) 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 \mu L of 1% (wt/vol) \textit{Staphylococcus aureus} (Cowan strain) prepared as described earlier,\textsuperscript{12} or with 250 \mu L of a 10% (wt/vol) 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 radioimmunounassays, 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.\textsuperscript{12} Immunoprecipitates were also dissociated in 8 mol/L urea and submitted to isoelectric focusing.\textsuperscript{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 autoradiography films 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 \textsuperscript{12}I-labeled pure R-MuLV p30 nor glycoprotein 70 (gp70); rabbit antisera raised against (antibody against MuLV p15E (19F8)). These were obtained from Dr M. Essex (Harvard School of Medicine, Boston). Rabbit sera raised against HTLV-1 and HIV-1 were used as positive controls.

Production of Monoclonal Antibodies

The monoclonal antibodies were prepared as follows. BALB/c mice were immunized subcutaneously at weekly intervals with 10 \mu g 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,\textsuperscript{14} and the mouse with the highest titer serum was boosted intravenously with 10 \mu g 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.\textsuperscript{15,16} 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 preincubated overnight at 37°C with 10/\mu L thyosomes of DBA/2 mice). Cells were plated into 96-well Falcon plates. Macroscopic 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 NH_4OH;\textsuperscript{17} 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 mmol/L \textit{H}-dTTP (3.6 Ci/mol), 130 mmol/L dATP, 50 \mu g/mL DT 12-18 poly A, 5 mmol/L DTT, 0.5 mmol/L MnCl\textsubscript{2} for R-MuLV-RT, FeLV-RT, GalV-RT, and 5 mmol/L MgCl\textsubscript{2} for AMV-RT.\textsuperscript{9} Also introduced into the assay were 0.5 \mu g and dilutions of the affinity-purified antigen.

RESULTS

Purification of an Antigen With p15E 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 pol (G) in the presence of MnCl\textsubscript{2}, as described for primate leukemia virus RTs.\textsuperscript{9} The partially purified preparation that eluted from the column at 0.3 mol/L NaCl still contained several proteins, as shown after ioidination 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,22 but were not detected by type-specific antisera raised against BaEV, RD 114, SiSV, or FeLV RTs. A hyperimmune serum specific for HTLV p2431 and a monoclonal antibody specific for HTLV p1924 did not recognize the 74-kD protein nor did monoclonal antibodies directed against BaEV gp70, BaEV p15,25 MuLV gp70,18,26 MuLV p15, and MuLV p3027 (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) Purified</th>
<th>Poly (G) + Immuno-affinity Purified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goat anti-SiSV p30, SiSV gp70</td>
<td>+ / -</td>
<td>- / -</td>
</tr>
<tr>
<td>Goat anti-HTLV p2423</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rabbit anti-MuLV p30, MuLV gp7014</td>
<td>- / -</td>
<td>- / -</td>
</tr>
<tr>
<td>Rabbit anti-FaLV p15E, anti FeLV p15</td>
<td>+ / -</td>
<td>+ / -</td>
</tr>
<tr>
<td>Rabbit anti-RTs of MuLV, BaEV, RD 114, SiSV14.15</td>
<td>+ / - / - / - / - / -</td>
<td>+ / - / - / - / - / -</td>
</tr>
<tr>
<td>Cat anti-FaLV-RT17</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gibbon anti-GaLV RT28</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Human anti-HLA (Bw17, Bw4, A2)</td>
<td>- / -</td>
<td>- / -</td>
</tr>
<tr>
<td>Rabbit anti-human α,-glycoproteins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rabbit anti-human albumin</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Monoclonal antibodies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse anti-MuLV p15E18</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mouse anti-MuLV gp7018.28</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rat anti-MuLV p1517</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rat anti-MuLV p3017</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mouse anti-BaEV gp7023</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mouse anti-BaEV p1525</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mouse anti-HTLV p1914</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Newly developed mouse anti-74-kD glycoprotein</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Plus sign indicates a precipitation of the 125I-labeled 74-kD glycoprotein equal to or higher than ten times the precipitation obtained with the normal sera of the same species of origin.

Nonimmunoglobulin producer myeloma, SP/2.19,20 The supernatants of the hybridomas that grew were tested in a solid-phase radioimmunoassay for production of antibody that recognized the immunizing preparation.18 All the monoclonal IgGs precipitated a 74-kD protein, except a monoclonal Ig (8CE) that recognized mostly a higher molecular weight protein that is still undefined (Fig 1B). Two-dimensional gels run on the immunoprecipitates13 showed that the 74-kD protein recognized by the monoclonal antibodies had the same isoelectric point as the one precipitated by the rabbit anti-MuLV-RT antiserum (Fig. 2) and the one precipitated by the monoclonal antibody anti-p15E (19-F8) (data not shown). The newly developed monoclonal antibodies did not neutralize or bind partially purified RTs from MuLV, SiSV, FeLV, and BaEV, nor did these whole disrupted viruses prevent the precipitation of the labeled antigen by the monoclonal antibodies. The precipitation of the 74-kD antigen by the monoclonal antibody directed against p15E was competed for by whole disrupted MuLV and FeLV (data not shown). The 74 kD column with 0.2 mol/L NH4OH, starting from 30 mg of crude antigen prepared as described before. The electrophoretic profile of the 125I-labeled eluted antigen is shown in Fig. 3 (top panel). The molecular weight was calculated to be 74-kD. Similar results were obtained in three consecutive experiments from three separate plasmaphereses of the same patient. The purified protein was found to be glycosylated, and the final step after iodination, which was used to recover an immunologically active protein, was binding and elution from a lentil lectin Sepharose column. On a two-dimensional gel, the purified iodinated protein appeared as five or six spots of very close isoelectric points, typical of a glycoprotein (Fig 2, C and D). The same picture was obtained with the unlabeled protein after Coomassie Blue staining (data not shown).

The 125I-labeled purified antigen was immunologically in good condition and was precipitated to a high level by the specific monoclonal antibody, by a monoclonal antibody against MuLV p15E (19-F8) (Fig 3B), by a rabbit anti-FeLV p15E antiserum, and by a rabbit anti-MuLV-RT antiserum (Fig 3A, Table 1). The anti-SiSV p30 serum that precipitated the 74-kD protein from a cruder preparation did not precipitate the purified protein anymore. Precipitation of the 125I-labeled protein by a rabbit anti-MuLV-RT antiserum was competed for by partially purified FeLV-RT and very little by pure R-MuLV p30 (Fig 4). The same
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 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).

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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 cytorreduction 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>Myelodyplasia</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>Mean 25.5 ± 10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>38–47</td>
<td>Cord blood 10 samples</td>
<td>23 ± 9</td>
<td></td>
<td></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
Fig 7. Precipitation of 125I-labeled antigen purified from plasma of a normal individual. The immunoprecipitates 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 antisera. 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).

74-kD molecule was measured as 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 purification 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 concanavilin 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.7,8 Analysis of these cloned segments revealed the conservation of Gag and Pol sequences common to the Maloney-MuLV genome.29 These findings, and the more recent description of mRNA transcripts related to full-length endogenous retroviral DNA in human cells,30 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.31,32

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,3,6 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.33,34 This proto-oncogene amplification was shown to be often linked to chromosomal aberrations in different hematologic diseases.35-37 Proto-oncogene expression at the RNA level was also reported in hematopoietic cell lines and in some fresh hematologic cell samples.38,39

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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