Chronic Leukemia With a Hybrid Surface Phenotype (T Lymphocytic/Myelomonocytic): Leukemic Cells Displaying Natural Killer Activity and Antibody-Dependent Cellular Cytotoxicity

By Günter Schlimok, Eckhard Thiel, Ernst Peter Rieber, Dieter Huhn, Helmut Feucht, Jürgen Lohmeyer, and Gert Riethmüller

SUBSETS OF thymus-derived lymphocytes (T cells) play an important role in regulating the immune response (helper T cells, suppressor T cells) and as effector cells in various immune functions. In addition, cells forming rosettes with sheep erythrocytes and reacting with some heteroantibodies against human T cells can mediate part of the natural killer activity (NKA) and reactivity in antibody-dependent cellular cytotoxicity (ADCC). In a minority of patients suffering from chronic lymphoproliferative diseases, the neoplastic cells are of T-cell origin. In contrast to the numerous reports about cell surface markers, there are only sporadic functional studies of the proliferating cells in these diseases. Such investigations, however, are of great interest because in some cases the neoplastic cells display functions their nonmalignant counterparts are programmed for. So far, helper activity has been reported for Sézary cells, suppressor activity for T-cell leukemias, and antibody-dependent cellular cytotoxicity for chronic lymphoproliferative diseases originating from T cells with receptors for the Fc portion of IgG (T cells). In this article, the unusual clinic features of a patient with a chronic leukemia of T-lymphocytic origin and the morphological and functional properties of the leukemic cells are described. In addition to T-cell determinants, the leukemic cells expressed antigens associated with cells of the myelomonocytic differentiation lineage. The differentiation of normal B lymphocytes into immunoglobulin-secreting cells was neither increased nor suppressed by the malignant cells. In contrast, these Fc-IgG receptor bearing cells were potent effectors both in NK and ADCC. Furthermore, monoclonal antibodies raised against the T-CLL cells could be used to delineate lymphocyte subsets in normal individuals that contain cytotoxic and suppressor T cells as well as lymphocytes active in NK and ADCC.

CASE REPORT

A 71-yr-old man was transferred to the clinic with the diagnosis of a chronic lymphocytic leukemia. On admission he suffered from fatigue and weight loss. We found a distinct splenomegaly without hepatomegaly, but no enlargement of the lymph nodes and no erythroderma. An increased susceptibility to infections was not noted. The patient showed a leukocytosis of 484,000/cu mm due to a lymphocytosis of 99%. Platelets were decreased to 79,000/cu mm. Red blood cell count was normal (erythrocytes 4.8 × 10^12/liter; Hb 15.6 g/100 ml). Bone marrow aspirates revealed a dense infiltration with atypical large lymphoid cells displacing normal blood cell precursors. Polyclonal immunoglobulins in the serum were elevated—IgG 2200 mg/100 ml, IgA 520 mg/100 ml, IgM 135 mg/100 ml, IgD 0.4 U/ml, IgE 67 U/liter (normal range 10–150); monoclonal immunoglobulins were not detected in serum or urine. Autoantibodies (rheumatoid factor; antibodies to native DNA, smooth muscles, mitochondria; direct and indirect Coombs test) could not be found; serum complement was in the normal range. The patient was discharged without specific therapy. Increasing fatigue and violent pains in the left upper abdomen due to massive splenomegaly caused a readmission 8 mo later. The hematologic findings were unchanged. We now began a cautious irradiation of the spleen (single dose 25 R, 3 irradiations per week).

Under this therapy, the leukocytes decreased rapidly and the splenomegaly was notably reduced. Despite stopping the irradiation therapy (total dose 125 R), the leukocytes continued to decrease to a white cell count of 15,200/cu mm. Five days after the last irradiation, the patient developed hematuria and profuse gastrointestinal blood loss and hemorrhageal skin lesions. Laboratory investigations revealed typical signs of disseminated intravascular coagulation. There was no evidence for septicemia. In spite of intensive therapy, the patient died. Autopsy was refused by the next of kin.

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MATERIALS AND METHODS

Cytochemistry, Electron Microscopy

The following cytochemical reactions were performed: acid phosphatase (APh), acid esterase (ANAE), periodic acid Schiff (PAS), naphthol-AS-acetate-esterase (NAS), peroxidase (POX). Peripheral blood cells were investigated by electron microscopy after fixation with glutaraldehyde, embedding in Maraglas, and staining with lead hydroxide and uranyl acetate.

Surface Markers

Mononuclear cells were isolated from heparinized peripheral blood by means of Ficoll-Isoopaque density-gradient centrifugation. Adherent cells were removed by adherence to plastic surface. Most of the following membrane markers were examined as described previously [17]. T lymphocytes were detected by rosette formation with untreated (E-rosettes) and AET-pretreated (EAT-rosettes) sheep red blood cells at 4°C and by a heterologous T-cell-specific antiserum (HuTLA) in indirect immunofluorescence using fluorescein-labeled F(ab)2 fragments of a goat anti-rabbit Ig antiserum. In addition, the patient’s cells were analyzed with several commercial monoclonal antibodies of the OK series (Ortho Lab., Raritan, N.J.) using indirect immunofluorescence microscopy with a FITC-labeled F(ab)2 fragment of a goat anti-mouse Ig antiserum (Meloy): OKM1—reacting with most granulocytes, large adherent monocytes, most of the null cell population, and a majority of the T-cell population; OKT3—staining 99% of peripheral T lymphocytes; OKT8—reacting with a cytotoxic/suppressor T-cell population; OTT4—labeling helper T lymphocytes.

In addition, the cells were tested with monoclonal antibodies raised against these leukemic cells that have been shown to allow the distinction of functional human lymphocyte subpopulations: T411—reacting with 99% of peripheral T cells; T811—staining a T-cell subpopulation containing cytotoxic and suppressor T cells as well as a subset of NK and K cells; M522—defining an antigen found on monocytes, granulocytes, 5% of E-rosetting cells, and on virtually all NK cells in peripheral blood.

As a control, the cells were incubated with a number of monoclonal antibodies of the same isotype as the above described antibodies which did not react with the patients’ cells. Ia-like antigen was analyzed with a rabbit antiserum to the p23,30 membrane antigen using indirect immunofluorescence microscopy with fluorescein-labeled F(ab)2 fragments of a goat anti-rabbit Ig antiserum. Membrane immunoglobulins (SMIg) were detected with polyvalent and monospecific antisera (IgM, IgD, IgG, kappa, lambda) by direct immunofluorescence microscopy. Fc receptors for IgG and IgM were detected by means of rosette formation with ox erythrocytes sensitized with the IgG or the IgM fraction of a rabbit anti-ox antisera in subaglutinating concentrations. C3 receptors were studied in a rosette assay using ox red blood cells sensitized with an IgM-rich preparation of a rabbit anti-ox red blood cell serum and fresh AKR/J mouse serum (C3d) or human serum (C3b) as the source of complement. Mouse erythrocyte receptor was detected by rosette formation with untreated mouse erythrocytes (Erosettes).

Functional Studies

Natural killer activity (NKA) was measured in a 4-hr 51Cr release assay against MOLT 4 cells and in a 40-hr 3H-proline release test against melanoma tumor cells as described in detail elsewhere. Antibody-dependent cytotoxicity (ADCC) was assessed in a 12-hr 3H-proline release assay using human melanoma cells sensitized with a human anti-melanoma antiserum as target cells as described. Suppressor cell activity was measured by a coculture technique.

Peripheral mononuclear cells of the patient and peripheral mononuclear cells from controls were cultured together in the presence of pokeweed mitogen (PWM). To evaluate helper cell activity, peripheral mononuclear cells of the patient and isolated B lymphocytes from control donors were cultured together in the presence of pokeweed mitogen. The immunoglobulin-secreting cells were revealed by a reverse plaque technique. Phagocytosis of latex particles after incubation at 37°C in fetal calf serum was studied as described.

Fig. 1. Morphology of leukemia cells. (A) Leukemia cells stained with May-Grunwald-Giemsa (x 1250). (B) Demonstration of acid phosphatase; granular reaction product in a paranuclear distribution (x 1250). (C) Fine structural features of the leukemia cells (x 10,000).
Table 1. Membrane Phenotype of the Mononuclear Cells From Peripheral Blood

<table>
<thead>
<tr>
<th>Reactivity With Antibodies (%)</th>
<th>Anti-Ig† Poly</th>
</tr>
</thead>
<tbody>
<tr>
<td>HUTLA</td>
<td>OKT3</td>
</tr>
<tr>
<td>Patient 95±8</td>
<td>84±7</td>
</tr>
<tr>
<td>Normal† Range</td>
<td>78±8</td>
</tr>
</tbody>
</table>

Rosette Formation (%)

<table>
<thead>
<tr>
<th>E</th>
<th>Fc-IgG</th>
<th>C3b</th>
<th>C3d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 95±9</td>
<td>74±12</td>
<td>5±2</td>
<td>24±7</td>
</tr>
<tr>
<td>Normal</td>
<td>78±8</td>
<td>76±7</td>
<td>45±6</td>
</tr>
</tbody>
</table>

*Simultaneous detection of T811 and M522 antigen by use of FITC-conjugated M522 and TRITC-labeled T811 antibody.
†Beside polyclonal anti-lg reagent light (kappa and lambda) and heavy (G.M.D) chain, specific antisera were used; all antisera gave negative results.
‡Mean values of 20 healthy donors are given ± SD.

RESULTS

Morphological Characterization of the Leukemic Cells

The lymphoid cells appeared larger than normal blood lymphocytes (Fig. 1A). They displayed a homogeneously stained nucleus surrounded by abundant cytoplasm that was slightly basophilic and contained azurophile granules. Activity of acid phosphatase was extraordinarily strong, distributed in a granular pattern (mainly in a paranuclear localization), and was inhibited by tartrate (Fig. 1B). In addition, there were granules of PAS-positive material and granular positivity of acid esterase. In electron microscopy (Fig. 1C), lymphoid cells showed margination of nuclear heterochromatin and distinct nucleoli. The cytoplasmic granules contained a matrix that was either homogeneously electron dense or contained a single darker inclusion.

Surface Phenotype of the Proliferating Cells

Table 1 shows the surface marker analysis of the mononuclear cells isolated from peripheral blood. In indirect immunofluorescence, the leukemic cells were labeled by the heterologous HUTLA antibody as well as by the monoclonal antibodies OKT3, OKT4, OKM1, T411, T811, M522, but not by the antibody OKT4 reacting with helper/inducer T lymphocytes. The reaction with the heterologous antiserum specific for Ia-like antigen was completely negative.

The majority of the cells also formed rosettes with AET-pretreated sheep red blood cells and ox erythrocytes sensitized with rabbit anti-ox IgG. The number of cells bearing receptors for the Fc portion of IgM, for complement (C3b, C3d), and for mouse erythrocytes was very low. Membrane immunoglobulins could not be detected.

Functional Capacities of Leukemic Cells

The patient’s leukemic cells acted as potent effectors both in NK and ADCC using MOLT 4 cells, unsensitized and sensitized melanoma cells as targets (Tables 2 and 3). Since the patient exhibited a polyclonal hypergammaglobulinemia, which is uncommon for a chronic lymphocytic leukemia, the influence of the leukemic cells on the differentiation of normal B lymphocytes was investigated. When the peripheral mononuclear blood cells of the patient were stimulated in vitro with PWM, no Ig-secreting cells were generated (Fig. 2). As can be further seen from Fig. 2, the leukemic cells neither supported nor suppressed the PWM-driven differentiation of B lymphocytes from a normal donor into Ig-secreting cells. As the leukemic cells did react with monoclonal antibodies labeling cells of the myelomonocytic lineage, the phagocytic activity was tested. However, no phagocytosis of latex particles by the neoplastic cells was seen.

DISCUSSION

By clinical criteria, the patient suffered from a chronic leukemia. The marked splenomegaly and the minor involvement of lymph nodes are characteristic symptoms for T-CLL. The course of the disease, however, was rather uncommon for a chronic lymphocytic leukemia. The response to irradiation of the spleen was dramatic. After a total dose of 125 R the

Table 2. Natural Killer Activity of the Leukemic Cells

<table>
<thead>
<tr>
<th>Effector: Target Cell Ratio</th>
<th>4-hr 1Cr Release Test Against MOLT 4</th>
<th>40-hr 3H-Proline Release Test Against Human Melanoma Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>100:1</td>
<td>62±2*</td>
<td>57±0</td>
</tr>
<tr>
<td>50:1</td>
<td>42±1</td>
<td>58±1</td>
</tr>
<tr>
<td>25:1</td>
<td>43±2</td>
<td>29±1</td>
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Percent cytotoxicity ± SD
Table 3. Antibody-Dependent Cellular Cytotoxicity (ADCC) of the Leukemic Cells

<table>
<thead>
<tr>
<th>Effector: Target Cell Ratio</th>
<th>% Cytotoxicity ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>50:1</td>
</tr>
<tr>
<td></td>
<td>25:1</td>
</tr>
<tr>
<td></td>
<td>12:1</td>
</tr>
<tr>
<td>32 ± 2</td>
<td>21 ± 2</td>
</tr>
<tr>
<td>23 ± 3</td>
<td>18 ± 6</td>
</tr>
<tr>
<td>Healthy donor</td>
<td>22 ± 2</td>
</tr>
<tr>
<td>Patient</td>
<td>50:1</td>
</tr>
<tr>
<td></td>
<td>25:1</td>
</tr>
<tr>
<td></td>
<td>12:1</td>
</tr>
<tr>
<td>5 ± 2</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>4 ± 3</td>
<td>3 ± 1</td>
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</table>

spleen shrank to normal size and the leukocytes dropped to 15,200/cu mm. Five days after the last dose of irradiation, the patient died with signs of generalized intravascular coagulation, which was interpreted as a consequence of the massive radiation-induced cell destruction.

As to the functional and antigenic characteristics of the malignant cells, two points are noteworthy. First, the isolated cells were active as cytotoxic cells against two different NK-sensitive target cells. The leukemic cells lysed MOLT 4 cells in a 4-hr $^{51}$Cr release test and adherent melanoma cells in a 40-hr $^{3}$H-proline release assay. In addition to their spontaneous lytic activity, the leukemic cells expressed distinct antibody-dependent cytotoxicity in an ADCC assay employing a human antisera as antibody source.

Second, the leukemic cells, though unambiguously classified as thymus-derived lymphocytes because of E-rosette formation and reactivity with T-cell-specific conventional (Hu TLA) and monoclonal antibodies (OKT 3, OKT 8), simultaneously expressed an antigen (OKM 1) associated with cells of the myelomonocytic lineage. The apparent hybrid nature of the leukemic cell was further supported by the finding that by immunization of BALB/c mice with these cells, monoclonal antibodies could be obtained reacting with all peripheral T lymphocytes (T41), with a suppressor/cytotoxic T population (T811), and with cells of the myelomonocytic lineage (M522). Although the monoclonal antibodies OKT8 and T811 are thought to characterize the suppressor/cytotoxic subset of peripheral T cells, the leukemic cells displayed no suppressor activity in PWM-driven differentiation of B lymphocytes. Thus, these antibodies (OKT8, T811) may detect a common antigen shared by different peripheral T-cell subsets mediating suppression, spontaneous or induced cytotoxicity. In accordance with this assumption, Pandolfi et al.11 and Strong et al.12 recently described a CLL of Tγ origin (OKT3', OKT8', OKM1') mediating suppressor but no NK activity.

The key question is whether a normal cell counterpart of the described leukemia cell type does exist or whether the observed phenotype represents just an oddity due to uncontrolled gene expression found in malignant cells. Using light and electron microscopy, a cell phenotype morphologically quite similar to the leukemic cells has been identified as representing most of normal Tγ and "third population" cells. The microscopic and cytochemical (positive for tartrate-sensitive acid phosphatase and sodium-fluoride-resistant acid esterase) appearance of these medium/large-sized lymphocytes with a homogeneously stained nucleus and abundant cytoplasm containing azurophilic granules is strikingly similar to the large granular lymphocytes (LGL) described by Saksela32 and Timonen33 as the main effectors of natural cytotoxicity.

Considerable disagreement still exists about the genealogy of human NK cells, particularly the expression of T-cell antigens. So far, both "third-population" cells,24 bearing no T- or B-cell markers, and cells forming rosettes with sheep erythrocytes and reacting with some heteroantibodies against human T cells2 5,35 were reported to act as NK/ADCC effectors. Recent data, obtained with monoclonal antibodies, suggest that the NK/ADCC effectors in human peripheral blood bear a myelomonocytic antigen (OKM 1), but no T-cell antigen.36 38 Using the monoclonal antibodies raised against the malignant cells, Lohmeyer et al.24 were able to isolate a small subset (4%-5%) of normal peripheral blood mononuclear cells that simultaneously expressed thymic (T41, T811) and myelomonocytic (M522) antigens as well as Fc-receptors. These cells showed a distinctly enriched NK and ADCC activity and were morphologically 90% LGL. Similar results were reported by Fast et al.39 using another set of monoclonal antibodies. These data are in line with our contention that all NK/ADCC effectors bear myelomonocytic antigens, and a subpopulation of these cells also expresses T-cell antigens.24 Further studies will show whether the simultaneous expression of such "discordant" antigens is characteristic for
discrete differentiation stages of NK/ADCC effector cells. Such a view is supported by the finding of Ortaldo et al. that normal large granular lymphocytes (OKM1+) acquire antigens of mature T cells (OKT3) during culture in the presence of T-cell growth factors. Since Fc-IgG receptors are surface structures common to monocytes, NK cells, K cells, and certain T lymphocytes, the question remained whether the OKM1/M522 antibodies recognized a determinant associated with such a functionally defined surface structure. Preliminary data, however, showed no inhibition of Fc-receptor function by monoclonal antibody M522. Nevertheless, their myelomonocytic antigens may still represent a structural correlate to the spontaneous cytotoxic activity found on the malignant cell described here and on a small subset of normal human T lymphocytes. The demonstration of the particular phenotype on malignant as well as on normal blood cells adds weight to the contention that NK activity is exerted by a specialized class of lymphocytes.

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REFERENCES

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