Distinct Subtype Within the Spectrum of Hairy Cell Leukemia

By J. Jansen, H. R. E. Schuit, G. M. Th. Schreuder, H. P. Muller, and C. J. L. M. Meijer

Most cases of hairy cell leukemia represent malignancies of B cells. However, recent findings suggest that there is a spectrum of functional capacities within the entity hairy cell leukemia. Two patients with hairy cell leukemia, whose malignant cells in the peripheral blood showed both T- and B-cell features, are reported. The malignant cells of the spleens showed only B-cell characteristics. The hairy cells of both patients did not adhere to glass and lacked the Ia antigen. Both patients showed pronounced polyclonal hypergammaglobulinemia and developed frank leukemic blood pictures after splenectomy. Within the spectrum of hairy cell leukemia, these two cases probably represent a distinct subtype.

A B-lymphocytic origin of the malignant cell of hairy cell leukemia (HCL; leukemic reticuloendotheliosis) becomes more and more probable. Nevertheless, the hairy cells (HC) behave rather unusually and resist a clear classification. Not only have many authors claimed phagocytic capacities for these cells, but there is almost certainly a spectrum of functional capacities of HC. Various surface immunoglobulin patterns, various degrees of adherence to glass surfaces, and various degrees of phagocytosis have been described in some large series.

We report two cases of HCL in which the HC in the peripheral blood exhibited both T-cell and B-cell features (T⁺/B⁻). These cells lacked some of the characteristics seen in other cases of HCL, for instance, adherence to glass and the presence of the Ia antigen. Both patients had pronounced polyclonal hypergammaglobulinemia and developed frank leukemic blood pictures after splenectomy. The spleens of both patients contained only HC with B-cell features.

MATERIALS AND METHODS

Patients

Patient De

De, a male aged 66 yr, presented with a 6-mo history of general weakness and weight loss. Hepatomegaly and splenomegaly (2 cm and 4 cm under the respective costal margins) were found, but no lymphadenopathy. Pancytopenia (Hb 11.2 g/dl; WBC 2900/cu mm with 22% neutrophils, <1% monocytes, and 60% hairy cells; platelets 30,000/cu mm) and polyclonal hypergammaglobulinemia (31 g/liter, with a particularly increased IgG fraction) were present. The HC of the peripheral blood showed tartrate-resistant acid phosphatase activity. Attempts to aspirate bone marrow failed repeatedly. Bone marrow biopsy disclosed diffuse proliferation of HC and an increased amount of reticulin fibers.
When the pancytopenia progressed, splenectomy was performed (spleen weight: 400 g) 7 mo after diagnosis. No hematologic recovery was observed, but the number of HC transiently increased to 30,000/cu mm. The patient had repeated infections and died from septicemia 8 mo after the operation. Permission for autopsy was not granted.

The characteristics of the HC of the spleen of this patient have been described elsewhere.3

Patient RW

RW, a female aged 31 yr, had a 6-mo history of increased menstrual bleeding and a recent episode of pneumonia. Discrete cervical lymphadenopathy and an 8-cm palpable spleen were found. Laboratory studies showed Hb 9.9 g/dl; WBC 7200/cu mm with 7% neutrophils, <1% monocytes, and 60% HC; platelets 35,000/cu mm, and polyclonal hypergammaglobulinemia of 18 g/liter (IgG 21 g/liter, IgA 2.3 g/liter, and IgM 2.1 g/liter). The HC showed tartrate-resistant acid phosphatase activity. Bone marrow aspiration and biopsy showed diffuse infiltration with HC and increased reticulin fibers.

Splenectomy (spleen weight: 1900 g) resulted in an increase of Hb (13.6 g/liter), neutrophils (2700/cu mm), and platelets (110,000/cu mm). The HC increased to 18,000/cu mm. The patient is in good health 10 mo after the operation.

The peripheral blood films and spleen sections of both patients have been reviewed by Dr. I. Katayama (Worcester, Mass.) who confirmed the diagnosis "leukemic reticuloendotheliosis" in both cases.

Techniques

All techniques used have been described in detail in a previous paper3 and will therefore only be mentioned briefly.

Cell Separation

The Ficoll-Isopaque technique6 was used to prepare suspensions of mononuclear cells from the peripheral blood. For bone marrow and spleen suspensions, this separation procedure was omitted.

Immunofluorescence

Cytoplasmic immunofluorescence was performed on washed and fixed cells on slides. Membrane fluorescence was performed with washed cells ("unfixed"), cells incubated for 1 hr at 37°C in RPMI 1640 with 10% fetal calf serum ("incubated"), and in particular, with cells fixed for 10 min at room temperature with 0.04% formaldehyde.

The cells were incubated with monospecific antisera for 30 min at room temperature. A TRITC-labeled horse anti-human T-cell antisera7 was applied before FITC-labeled Fab' fragments of a goat antiserum directed against the Fab part of human IgG and reactive with all Ig classes and both light chains (Nordic, Tilburg, The Netherlands). The specificity of the rabbit antisera against the various heavy and light chains was tested on bone marrow slides from patients with myeloma and macroglobulinemia. In general, these conjugates were prepared from IgG fractions of the antisera; in case RW, Fab' fragments were also used (anti-IgA and anti-IgG were prepared by Dr. F. Skvaril, Bern, Switzerland; anti-IgM and anti-IgD by Dr. W. Knapp, Vienna, Austria, in collaboration with Dr. J. Rödl of the Institute for Experimental Gerontology, Rijswijk, The Netherlands). Antigen—antibody complexes to detect Fc receptors consisted of rabbit anti-ovalbumin antiserum and ovalbumin labeled with FITC. The rabbit anti-p23,30 antisera8 was kindly contributed by Dr. J. L. Strominger (Boston, Mass.). Reactivity of this antisera with HC was studied in indirect immunofluorescence with goat anti-rabbit antisera labeled with TRITC.

Glass Adherence

Adherence to glass was studied in Leighton tubes with floating coverslips. Cells (1–2 x 10⁶) in 1.5 ml medium 199 with 20% fetal calf serum were incubated for 2–4 hr in 10% CO₂ at 37°C. The adherence was studied by phase-contrast microscopy and also, after the coverslips had been dried and stained, by light microscopy.

Terminal-desoxynucleotidyl-transferase (TdT)

TdT activity was assayed according to McCaffrey et al., as reported elsewhere.11
Receptor Detection

Rosettes EA1G and EA1G-M were prepared by coating sheep erythrocytes (RBC) with the IgG or IgM fraction of rabbit anti-sheep RBC antibodies, and were used for the detection of Fc1G and Fc1M receptors. EA1G-MC rosettes (sheep RBC coated with IgM antibody and mouse complement) were used to detect C3 receptors. Spontaneous rosette formation with neuraminidase-treated sheep RBC was performed according to Weiner et al.2 Mouse rosettes were evoked with O20-mouse RBC. Tissue localization of EA1G- and EA1G-M-adhering cells was done as reported previously.3

RESULTS

The HC of both patients were monoclonal with respect to light chains. The cells showed multiple heavy-chain determinants (Table 1). The simultaneous use of different fluorochromes documented the presence of two heavy chains on the same cells, and indirect evidence (Table 1) showed that, in case RW, three heavy chains must have been present on the HC. Incubation for 1 hr at 37°C in immunoglobulin-free medium did not change the immunoglobulin pattern dramatically, nor were different results obtained when F(ab')2 fragments of the antisera were used. The heavy- and light-chain patterns were highly reproducible on various occasions. The fixation technique with diluted formaldehyde proved to be an attractive procedure, in particular, in one case (De) in which the unfixed cells picked up all kinds of labeled antiserum. In patient RW the fluorescence pattern was already selective in the unfixed state. The results obtained with unfixed cells, cells preincubated in immunoglobulin-free medium, and cells fixed with formaldehyde, are given in Table 2. On three occasions, differences were seen between the unfixed and the fixed cells. The spleen cells showed far less α in the fixed state, and the peripheral blood cells assessed on 11/5/78 and 26/6/78 showed fewer T+/B- cells.

Trypsinization was not applied, because this technique is not routinely used in our laboratories and because the results of this procedure in HCL have been rather contradictory.24

The cells of patient De showed cytoplasmic fluorescence with antisera against μ and λ (Fig. 2); the cells of patient RW did not show cytoplasmic fluorescence.

Most of the HC of both patients evoked EA1G rosettes, which is indicative of the presence of a receptor for the Fc part of IgG. The EA1G rosettes were demonstrable in cell suspensions as well as frozen spleen sections. In both test systems, HC did not show EAC-rosettes.

Table 1. The Hairy Cells of Both Patients Studied for Various Cell Markers

<table>
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<th>Specimen/Date</th>
<th>HC*</th>
<th>cfg</th>
<th>μ</th>
<th>δ</th>
<th>γ</th>
<th>α</th>
<th>κ</th>
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Abbreviations: pb, peripheral blood; sp, spleen; bm, bone marrow; AgAb, FITC-labeled antigen–antibody complexes; Fab, FITC-labeled goat anti-human Fab; T, TRITC-labeled horse anti-human T cell; cfg, cytoplasmic Ig; Em, mouse rosettes; NT, not tested.

*Percent of mononuclear cells.
The HC of the peripheral blood of both patients also showed T-cell features. In patient De, 50% of the mononuclear cells (87% of which were HC) evoked spontaneous E-rosettes and 40% showed fluorescence with the anti-T-cell antiserum. In patient RW, 60% of the mononuclear cells (65% HC) evoked E-rosettes and 98% reacted with the anti-T-cell antiserum. In both cases, however, the fluorescence pattern was atypical (Fig. 1). The hairy cell nature of the rosetting

![Fig. 1. Patient RW. (A) Phase contrast: two hairy cells and one normal lymphocyte (arrow). (B) FITC-labeled anti-Fab antiserum: the hairy cells show typical fluorescence, the normal lymphocyte is negative. (C) TRITC-labeled anti-T-cell antiserum: the hairy cells show atypical patchy fluorescence, whereas the normal lymphocyte shows typical fluorescence (T cell).](image-url)
cells was identified on cytocentrifuge slides by the presence of tartrate-resistant acid phosphatase (Fig. 3) and by electron microscopy of the rosetting cells. The spleen cells of both patients did not show fluorescence with the anti-T-cell antiserum, nor did they evoke spontaneous E-rosettes. In 15 other cases of HCL, the HC were never found to evoke E-rosettes or to show fluorescence with the anti-T-cell antiserum.

The specific nature of the E-rosettes could only be studied in patient RW. Preincubation with the anti-T-cell antiserum, with sodium azide (10−3M) or cytochalasin B (20 μg/ml) inhibited E-rosette formation; preincubation with anti-Fab at 37°C for 30 min (which resulted in extensive capping) did not inhibit rosette formation. No rosettes were seen after incubation with sheep RBC at 37°C for 1 hr or 24 hr; also, no rosettes were seen with human RBC or ox RBC at 4°C or 37°C. This behavior is in accordance with that of genuine E-rosettes.

The HC of patient De did not show adherence to glass, and the cells of patient RW only showed minimal adherence. The HC of 9 other patients with HCL adhered extensively to glass surfaces.

The spleen cells of patient De and the spleen and peripheral blood cells of patient RW did not show fluorescence with the anti-p23.30 antiserum, which reacts with the Ia antigen on B lymphocytes and reacted with HC from the spleen and peripheral blood of 6 other HCL patients.

Finally, the spleen cells of patient RW did not show any TdT activity.
Most investigators agree that HCL probably represents a neoplasm of lymphocytes,\textsuperscript{1,3,14} As in other lymphoproliferative disorders,\textsuperscript{15} the majority of the cases of HCL seem to derive from B cells\textsuperscript{1,3,14} Recently, however, some cases of T-cell HCL have been documented.\textsuperscript{16,17} The cells of the latter patients evoked E-rosettes, reacted with an anti-T-cell antiserum, did not show surface Ig, and did not produce immunoglobulins in vitro. The clinical symptoms of these patients were unremarkable within the picture of HCL; no thymic mass or skin infiltration was observed.

A few cases of lymphoproliferative disorders in which the malignant cell showed T- and B-cell features simultaneously have been reported.\textsuperscript{18,21} Some of these cases most probably represent B-cell proliferations with antibody activity to sheep RBC\textsuperscript{19} or malignancies of T cells with Fc receptors.\textsuperscript{20,21} Others, however, may represent malignancies of true T' / B' cells.\textsuperscript{18,19} It is interesting that many of the T-cell CLL show a clinical picture similar to that of HCL with splenomegaly without lymphadenopathy and with neutropenia and hypergammaglobulinemia.\textsuperscript{20,22} Furthermore, many of these T cells carry Fc receptors, as HC do.\textsuperscript{20}

Cawley et al.\textsuperscript{23} documented a case of HCL with T/B-cell features. The HC of the peripheral blood and the spleen had monoclonal surface Ig and receptors for the Fc part of IgG and IgM, evoked spontaneous E-rosettes, and reacted with antiserum against T cells and against p29,34 antigens. The specific nature of the E-rosettes was studied extensively.

The HC of the peripheral blood of the two cases reported in this article had monoclonal surface Ig; furthermore, those of patient De also contained intracytoplasmic IgMA. The surface Ig patterns of both patients were the same as those found by our group in other cases of HCL.\textsuperscript{3} The influence of fixation or preincubation in immunoglobulin-free medium was studied in patient RW. Only the spleen cells had far less $\alpha$ after fixation. It is interesting that $\alpha$ and $\mu$ were present on the surface of almost all HC of the bone marrow, but in much smaller amount on the HC of the spleen. Perhaps these cells had lost most of their $\alpha$ and $\mu$ during the passage from the bone marrow to the spleen; only $\gamma$ persisted on the spleen cells.

The HC of the peripheral blood showed T-cell characteristics. They reacted, although atypically, with an anti-T-cell antiserum of documented specificity,\textsuperscript{4} and evoked spontaneous E-rosettes. The specific nature of these sheep RBC rosettes was made likely by the application of conditions known to influence spontaneous E-rosette formation by normal T lymphocytes. Although the presence of an antibody against sheep RBC on the HC cannot be excluded entirely, this possibility seems unlikely, because such an antibody would also have to interfere with the anti-T-cell antiserum. Another explanation of the findings in our patients is T-cell HCL with autoantibodies responsible for the monoclonal surface Ig. It is hard to understand, however, how this could explain the absence of T-cell features on the spleen cells and the coexistence of two populations of cells in the bone marrow and in the peripheral blood of patient RW after splenectomy. Furthermore, an $\alpha\gamma$ nature of such an autoantibody would be very unusual.

A special feature of the cases under discussion, is the discrepancy between the peripheral blood and the spleen. The splenic HC showed only B-cell features, whereas the HC of the peripheral blood were T' / B'. There are several possible explanations for this phenomenon. First, the spleen may have sequestrated the B'
cells, whereas the T⁺/B⁻ cells were not captured. Second, the spleen may have released only cells with T⁺/B⁻ features to the peripheral blood, or third, the spleen may have stripped the T-cell features from the cells. The appearance, after splenectomy in case RW, of HC with only B-cell features in the peripheral blood, supports the hypothesis of selective sequestration. The bone marrow contained, and thus probably produced, both B⁺- and T⁺/B⁻ populations. It is not clear why the T⁺/B⁻ population in the peripheral blood decreased after splenectomy. However, since the patient became frankly leukemic after the operation, the decrease in absolute numbers of T⁺/B⁻ cells is less than suggested by the results in Table 2. Since no bone marrow study was performed before splenectomy, an influence of the operation on the production of T⁺/B⁻ cells cannot be excluded.

The HC of these two patients did not react with the anti-p23,30 antiserum, which detects the La antigen occurring on B lymphocytes, on most of the monocytes, and probably also on immature hematopoietic cells. This antigen has been detected in all cases of HCL studied so far and has also been demonstrated to be really produced by HC. The antigen was also present on the HC of the T⁺/B⁻ cases of Cawley et al.

If a T⁺/B⁻ character of the HC of the patients under discussion is accepted, an attempt might be made to locate the cell in the maturation scheme of lymphocytes. The maturation “arrest” of the cells of other cases of HCL has been suggested to occur in a somewhat later stage than in CLL. The presence of T⁺/B⁻ features, on the other hand, could mean that these cells represent a very early stage of maturation, where the commitment to B or T cell has not yet been completed. In this concept, however, one would expect the cells to be p23,30 positive, as the pre-B cells are, or TdT positive, like the early T cells; the cells of our patients were negative in both tests. More acceptable explanations are that the malignant transformation induces the loss of certain features of normal lymphocytes of this maturation stage and the reappearance of earlier features, or that the cells derive from the normal T⁺/B⁻ lymphocytes present in low percentages in the peripheral blood.

Whatever place these cases occupy in the maturation scheme of the lymphocytes and whatever the origin of the HC may be, the behavior of the cases under discussion differed substantially from that shown by other cases of HCL studied by our group and others (Table 3). The cells not only lacked the p23,30

| Table 3. Features of the Present Patients and 17 Other Cases of Hairy Cell Leukemia Studied by the Same Group |
|--------------------------------------------------|--------|--------|
| Monoclonal surface Ig | 2/2 | 15/17 |
| Cytoplasmic Ig | 1/2 | 2/17 |
| Adherence to glass | −/− | ++/+ (+n = 9) |
| T-cell features | 2/2 | 0/15 |
| Fcγ receptors | 2/2 | 12/12 |
| C3 receptors | 0/2 | 0/12 |
| p23,30 antigen | 0/2 | 0/6 |
| Serum gammaglobulin (g/liter) | 18, 31 | 10.2 (7.0–21.7) (median range, n = 16) |
| Leukemic after splenectomy (hairy cells >20,000/cu mm) | 2/2 | 3/12 |
antigen and showed T\(^+\)/B\(^+\) features, but also adhered poorly to glass. The patients had pronounced hypergammaglobulinemia (31 and 18 g/liter; in 16 other cases the median and range were 10.2 g/liter and 7.0–21.7 g/liter, respectively). After splenectomy, both patients developed frank leukemic pictures with HC counts of 20–30,000/cu mm. Such a leukemic blood picture develops after splenectomy in only about 20%–30% of the cases.

Golomb et al.\(^2\) distinguished various subtypes of HCL on the basis of phagocytosis, surface Ig, and platelet aggregation studies. Since we did not study platelet function and since we believe the HC to be essentially nonphagocytic cells,\(^3\) it is not possible to compare the patients reported here with the subtypes of Golomb et al.\(^2\) However, we agree with these workers that there probably exist several subtypes of HCL, these cases representing only one of these.

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Distinct subtype within the spectrum of hairy cell leukemia

J Jansen, HR Schuit, GM Schreuder, HP Muller and CJ Meijer

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