CONCISE REPORT

Richter’s Syndrome With Different Immunoglobulin Light Chains and Different Heavy Chain Gene Rearrangements


In a patient with Richter’s syndrome, the chronic lymphocytic leukemia (CLL) expressed $\lambda$, $\mu$, and $\delta$ immunoglobulin (Ig) chains and the non-Hodgkin lymphoma (NHL) $\kappa$, $\mu$, and $\delta$ Ig chains. The difference in Ig light chain expression suggests that the CLL and NHL are independent malignancies, or that the oncogenic event occurred in a B cell differentiation stage after the heavy chain gene rearrange-

Histiocytic Lymphoma or Hodgkin’s disease supervening in the course of chronic lymphocytic leukemia (CLL) has been described as Richter’s syndrome. A possible genetic relationship between the two malignancies was discussed, but according to morphologic criteria they were supposed to be of different origin. When immunologic marker studies demonstrated that most CLL and histiocytic lymphomas were in fact B cell malignancies, a common clonal origin of the two malignancies in Richter’s syndrome was postulated. Attempts were made to provide evidence for this clonality by characterizing the surface immunoglobulins (Ig). However, the assumption that the presence of the same Ig heavy and light chains is synonymous with clonality is an over-

simplification, since most B-CLL and B-NHL express the $\mu$ heavy chain and since statistically the chance of finding the same Ig light chain in two independent B cell malignancies is more than 50%. Furthermore, it was supposed that additional phenotypic characterization with monoclonal antibodies specific for B cell markers provides conclusive proof. Although such characterization is valuable for the diagnosis of Richter’s syndrome, it merely illustrates the differentiation stage of the tumor cells and not their clonal filiation. Other approaches proposed to prove a common clonal origin are the detection of the same chromosomal aberrations and the demonstration of idiotypic identity of the Ig in both malignancies. However, apart from two incomplete cytogenetic studies, there are no reports demonstrating nonrandom chromosomal aberrations in both the original CLL population at first diagnosis and the NHL cell population at the diagnosis of Richter’s syndrome. Furthermore, the use of antidiotypic antibodies in studies on Richter’s syndrome has not been published so far, probably because the raising and purification of such antibodies is rather cumbersome and time consuming.

Since B cell malignancies of the same clonal origin must have identical Ig gene rearrangements, we performed DNA analysis of the Ig heavy chain gene rearrangements to study clonality in a case of Richter’s syndrome in addition to the immunologic characterization and cytogenetic analysis.

CASE REPORT

The patient, a man aged 88 years, had a 23-year history of CLL and had been treated for 15 years because of marked lymphocytosis, splenomegaly, thrombocytopenia, and anemia. Treatment included irradiation of the spleen in 1967 and oral chlorambucil, 6 to 10 mg daily, intermittently in 1969 and 1973. Progressive anemia responded to prednisone, 10 mg daily. On this treatment the hemoglobin remained normal, with stabilization of moderate spleno-

megaly and slight lymphocytosis until May 1982, when he was hospitalized with progressive general symptoms, abdominal pain, splenomegaly, ascites, pleural effusion, and large abdominal masses on a computed tomographic scan. Laboratory values revealed a hemoglobin of 4.9 mmol/L; platelets 35 x 10^9/L; white blood cells 187 x 10^9/L; of which 95% were small lymphocytes, 1% were blast cells, 4% were granulocytes; many ghost cells; serum bilirubin 13 mmol/L; and serum lactate dehydrogenase 916 U/L. Urea, creati-

nine, alkaline phosphatase, aspartate, alanine, and gamma glutamyl transferases in serum were normal. A bone marrow biopsy showed diffuse infiltration with small lymphocytes and a small sharply demarcated area of blastic cells. In addition, pleural fluid and ascites contained 50% and 95% blastic cells, respectively, which is compat-

ible with a NHL of high grade malignancy (histiocytic NHL, Rappaport classification; centroblastic NHL, Kiel classification). The diagnosis of Richter’s syndrome was made. In the peripheral blood, blastic cells increased to 10%. The patient died of pneumonia in July 1982.

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Autopsy revealed generalized lymphadenopathy, hepatomegaly (2,680 g), splenomegaly (1,200 g) and bronchopneumonia. Bone marrow, spleen, and lymph nodes were diffusely infiltrated with NHL cells. Focal infiltrations of the same cells were also seen in the lungs, pleurae, stomach, kidneys, liver, heart, and brain.

MATERIALS AND METHODS

Cell Samples

For immunologic characterization, cytogenetics, and DNA analysis, cells were collected from the peripheral blood by venipuncture and from ascites by needle aspiration.

Immunologic Characterization

Mononuclear peripheral blood cells were isolated by Ficoll-Hypaque density centrifugation and the ascites cells were centrifuged and washed. To detect surface membrane (Sm) markers, the cell suspensions were incubated with optimally determinations of cytoplasmic Ig (clg) were performed. For the combined SmX/M rosette assay, described.27 Terminal deoxynucleotidyl transferase (2,680 g), splenomegaly and brain. lungs, pleurae, stomach, kidneys, liver, heart, and ascites by needle aspiration.

DNA Analysis

DNA was prepared from frozen aliquots of peripheral blood and ascites cells as described.29 BglII + BstI-, KpnI-, KpnI + EcoRI-, and KpnI + HindIII-digested DNA (10 μg per lane) from human placenta, peripheral blood cells, and ascites cells were electrophoresed on 0.7% agarose gels. HindIII- and HindIII + EcoRI-digested λ-DNA were included as molecular weight markers. After blotting to nitrocellulose, the filters were hybridized to a 2.5 kilobase EcoRI-BglII fragment containing the human J segment of the μ heavy chain gene clone H24.30 Hybridization and washing procedures were carried out according to previously described methods.31 Recombinant plasmids were handled under PZEK 1 containments with Orsat HBO mercury lamps and filter combinations for the visualization of FITC. The microscopes were equipped with phase-contrast facilities.

Cytogenetics

Metaphases of ascites cells were harvested using standard methods, after 20-minutes incubation in colcemid or 24-hour culture without addition of mitogens; part of the cultures were treated with methotrexate.38 Attempts to karyotype the CLL cells were made using peripheral blood cells cultured for 3, 5, and 7 days in the presence of one of the following B cell mitogens: Staphylococcus aureus protein A (Pharmacia, Uppsala, Sweden), Escherichia coli 026:B6 lipopolysaccharide B (Difco Laboratories, Detroit) or pokeweed mitogen (Gibco Laboratories, Grand Island, NY).

Table 1. Immunologic Characterization of the Cells in the Peripheral Blood and Ascites

<table>
<thead>
<tr>
<th>Antibodies/Rosettes</th>
<th>Peripheral Blood Positive Cells (%)</th>
<th>Ascites Positive Cells (%)</th>
<th>Antigen Recognized</th>
<th>Reference</th>
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</thead>
<tbody>
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<td>anti κ (Smκ)*</td>
<td>8</td>
<td>97</td>
<td>κ light chain</td>
<td></td>
</tr>
<tr>
<td>anti κ (Smκ)†</td>
<td>5</td>
<td>94</td>
<td>κ light chain</td>
<td></td>
</tr>
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<td>64§</td>
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<td>34§</td>
<td>94</td>
<td>δ heavy chain</td>
<td></td>
</tr>
<tr>
<td>anti γ (Smγ)†</td>
<td>55§</td>
<td>94§</td>
<td>γ heavy chain</td>
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<td>98</td>
<td>B lymphocyte antigen</td>
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<td>B2#</td>
<td>1</td>
<td>0</td>
<td>B lymphocyte antigen</td>
<td>15</td>
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<td>Leu-10*</td>
<td>64</td>
<td>99</td>
<td>B lymphocyte antigen</td>
<td>16</td>
</tr>
<tr>
<td>Y 29/55**</td>
<td>99</td>
<td>99</td>
<td>B lymphocyte antigen</td>
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<tr>
<td>M rosette</td>
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<td>E rosette</td>
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<tr>
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<td>&lt;1</td>
<td>T1 antigen</td>
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<td>95</td>
<td>99</td>
<td>HLA-DR, nonpolymorph antigen</td>
<td>25</td>
</tr>
</tbody>
</table>

*Monoclonal antibodies, Becton Dickinson, Sunnyvale, Calif.
†Conventional antisera, Kallestad Laboratories, Austin, Tex.
‡Monoclonal antibody, Dr J.J. Haaijman, Rijswijk, The Netherlands.
§Weak expression.
||Bethesda Research Laboratories, Bethesda, Md.
|Dr W. Knapp, Vienna, Austria.
#Coulter Corporation, Hialeah, Fla.
**Drs H.K. Foster and I. Werner, Hoffman-La Roche, Basel, Switzerland.
RESULTS

Immunologic Characterization

The results of the immunologic characterization of the peripheral blood and ascites cells are summarized in Table 1. The majority of the peripheral blood cells were small lymphocytes with weak expression of Smδ, Mu, and δ Ig chains, formed M rosettes, were positive for the T1 antigen, reacted with the B cell specific monoclonal antibodies B1, Leu-10 and Y 29/55, and were positive for HLA-DR. The combined Smδ/M rosette assay proved that the Smδ-positive cells in the peripheral blood also formed M rosettes and that the Smδ-negative cells did not (Figs 1 and 2). The ascites cells were large, showed prominent nucleoli, and expressed SmK, δ, and Ig chains. They were also positive for B1, Leu-10, Y 29/55, and HLA-DR but negative for the T1 antigen and did not form M rosettes. Both the peripheral blood cells and the ascites cells were negative for clg. These phenotypes are compatible with a B-CLL and a B-NHL, respectively, expressing the same Ig heavy chain but different Ig light chains.

Cytogenetics

The karyotype of the ascites cells showed a 14q+ marker, whereas the karyotype of the CLL cells could not be established: the peripheral blood cultures failed to produce mitoses. Sixteen metaphases from the ascites cultures were analyzed and showed the same karyotypic changes: 48,XY,1q+,−8,−13,14q+,17q+,17p−,19q+,+Mar1,+Mar2,+2Mar3. The altered chromosomes were interpreted as follows: 1q+ = 1pter−q2::12q13−qter; 14q+ = 14pter−qter::1q2−qter; Mar1 = 13qter−q14::8p12−q24; Mar2 = 13 cen−p12::?; Mar3 = c-like chromosome, possibly 13p+.

DNA Analysis

Southern blotting of BglI1 + BstI1-digested DNA from the peripheral blood and ascites cells revealed that in the B-CLL and B-NHL cells both alleles for the heavy chain gene were rearranged (Fig 3, lanes B and C). Both types of DNA show two strong hybridizing bands of 3.0 and 2.6 kb and of 8.5 and 3.4 kb, respectively, neither of which corresponds with the 4.0 kb germline band of human placenta DNA (Fig 3, lane A). The same phenomenon was also detected in KpnI-, KpnI + EcoR1-, and KpnI + HindIII-digested DNA (not shown). Therefore, the bands in the BglI1 + BstI1 digest are not due to restriction enzyme polymorphisms. The presence of a small percentage (<10%) B-NHL cells in the peripheral blood resulted in faint hybridizing bands of 8.5 and 3.4 kb (Fig 3, lane B). The different localizations of the J-containing bands in the B-CLL and B-NHL DNA indicate that the Ig heavy chains synthesized by the two malignancies are coded for by independently rearranged heavy chain genes.

DISCUSSION

The immunologic characterization of the two malignancies in this patient with Richter's syndrome demonstrated phenotypes that were compatible with a B-CLL and a B-NHL. Since these two neoplasms expressed the same Ig heavy chain isotypes but different Ig light chain isotypes, a hypothetical oncogenic event in a well-defined B cell differentiation stage after the Ig heavy chain gene rearrangement but before the selection of the light chain might have been the common cause for both neoplasms. In that case the two B cell malignancies should have identical Ig heavy chain gene rearrangements. However, DNA analysis by Southern blotting provided evidence that the heavy
chain gene rearrangements were different in the two cell populations, indicating that in this patient the B-NHL cannot be regarded as a progression of the CLL. A possible common oncogenic event in a very early stage of differentiation, before Ig heavy chain gene rearrangements take place, cannot be excluded. However, since patients with CLL have a significantly higher tendency to develop secondary tumors, even if they are not treated for the CLL, the most likely explanation for our findings is that the B-NHL constitutes a second B cell malignancy that arose in a susceptible host.

In conclusion, with regard to the literature and our results, we are still left with the question whether clonality does exist in Richter’s syndrome. Therefore subsequent cases of Richter’s syndrome should also be studied with more specific tools than immunologic characterization, such as cytogenetics and the analysis of the molecular organization of the Ig genes.

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