Early T Cell Differentiated Chronic Myeloid Leukemia Blast Crisis With Rearrangement of the Breakpoint Cluster Region but Not of the T Cell Receptor β Chain Genes


Early T cell differentiation is described in a case of Philadelphia chromosome-positive chronic myeloid leukemia (CML) in blast crisis, supporting multi-lineage differentiation potential of CML precursor cells. In the absence of myeloid markers, strong positivity for terminal deoxynucleotidyl transferase (TdT) and reactivity with T cell antibody 3A1, but lack of more mature T cell antigens, provided evidence for immature T cell differentiation. Molecular analysis of the breakpoint cluster region (bcr) in chromosome 22 revealed a rearrangement and thus confirmed the CML origin of the early T cell blasts. T cell receptor β chain sequences were found in germline configuration and therefore suggest a very immature stage of T cell differentiation in the CML blasts.

© 1987 by Grune & Stratton, Inc.

MATERIAL AND METHODS

Immunocytological analysis. Peripheral blood mononuclear cells (PBMCs) were obtained by Ficoll-Metrizoate density-gradient centrifugation, and staining procedures were used as previously described. Monoclonal antibodies used included OKT3, OKT4, and OK1.

Cytogenetical studies confirmed the diagnosis of CML by typical Philadelphia translocation in accordance with 18 U.S.C. §1734 solely to lend official status to the present paper and to distinguish those from the Philadelphia chromosome-positive acute lymphoblastic leukemias (ALL).

Chromosomal analysis. Slides were prepared from 24-hour cultures made from a frozen stock of PBMCs. Conventional chromosomal analysis was performed using quinacrine fluorescence banding techniques for identification of chromosomes, and classification was made according to the Paris nomenclature.

DNA analysis. DNAs were extracted from mononuclear cells after Ficoll-Hypaque centrifugation by standard techniques. Fifteen micrograms of DNA was digested with appropriate restriction enzymes (Boehringer, Mannheim, FRG), electrophoresed on a 0.7% agarose gel, blotted and hybridized to 3' and 5' bcr sequences, a

From the Institute of Clinical Immunology, the Department of Medicine, and Institute of Medical Genetics, University of Erlangen-Nürnberg, Erlangen; the Hof Medical Center, Hof; and the Department of Pediatrics II, University of Ulm, FRG.

Submitted July 29, 1986; accepted November 1, 1986.

Supported by grants from the Deutsche Forschungsgemeinschaft.

Address reprint requests to Dr Martin Gramatzki, Institute of Clinical Immunology, University Hospital, Krankenhausstrasse 12, 8520 Erlangen, FRG.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. §1734 solely to indicate this fact.

© 1987 by Grune & Stratton, Inc.

0006-4971/87/6904-0018$3.00/0

Fig 1. Leukemic blasts in the peripheral blood of patient E.S.
Table 1. Phenotypic Analysis of the Patient's Blood Mononuclear Cells Using Monoclonal Antibodies

<table>
<thead>
<tr>
<th>Antibody*</th>
<th>Predominant Specificity</th>
<th>Percentage of Positive Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>OKT 3 (CD3)</td>
<td>Mature T cells</td>
<td>3</td>
</tr>
<tr>
<td>OKT 4 (CD4)</td>
<td>Helper T cell subset</td>
<td>0</td>
</tr>
<tr>
<td>Leu-2 (CD8)</td>
<td>Suppressor/cytotoxic T cell subset</td>
<td>0</td>
</tr>
<tr>
<td>3A1 (CD7)</td>
<td>Immature T cells and most mature T cells</td>
<td>60</td>
</tr>
<tr>
<td>TH 69</td>
<td>Immature T cells and subset of mature T cells</td>
<td>54</td>
</tr>
<tr>
<td>B4 (CD19)</td>
<td>B cells, pre-B cells</td>
<td>0</td>
</tr>
<tr>
<td>B1 (CD20)</td>
<td>B lymphocytes</td>
<td>0</td>
</tr>
<tr>
<td>BA-1 (CD24)</td>
<td>B lymphocytes, immature B cells</td>
<td>10</td>
</tr>
<tr>
<td>BA-2 (CD9)</td>
<td>Immature B cells, lymphoid precursors</td>
<td>1</td>
</tr>
<tr>
<td>24.1 (CD10)</td>
<td>Common-ALL antigen</td>
<td>0</td>
</tr>
<tr>
<td>Mo-S39 (CDw14)</td>
<td>Monocytes</td>
<td>1</td>
</tr>
<tr>
<td>Leu-M1 (CD15)</td>
<td>Myeloid precursors, monocytes, granulocytes</td>
<td>14</td>
</tr>
<tr>
<td>Mo-U28 (CDw13)</td>
<td>AML cells</td>
<td>10</td>
</tr>
<tr>
<td>My-7 (CDw13)</td>
<td>AML cells</td>
<td>8</td>
</tr>
<tr>
<td>TC 25</td>
<td>Immature T cell subset and CML blast crisis cells</td>
<td>50</td>
</tr>
<tr>
<td>OKI-1</td>
<td>Ia (HLA-DR) bearing cells</td>
<td>1</td>
</tr>
</tbody>
</table>

ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; CML, chronic myeloid leukemia.

*CD classification in parentheses.

TCR β chain probe26 and an Ig G1 probe (kindly provided by Dr Leder). After hybridization, the filters were washed under stringent conditions and exposed to XAR-5 film (Kodak) using Dupont Lightning-Plus intensifying screens for 14 hours at -70°C.

Case report. A 53-year-old woman had a 6-month history of mild abdominal pain, weight loss, and low-grade fever. When she was admitted to the hospital, she had massive hepatosplenomegaly and, when examined hematologically had a WBC count of 113,000/μL. Anemia was mild, and her hemoglobin level was 9.8 g/dL.

Thrombocytes were slightly elevated to 350,000/μL. Differential count revealed 79% blasts, 16% granulocytes, and 5% lymphocytes. The bone marrow aspirate demonstrated 90% blast cells of medium to large size with irregular, lobulated nuclear shape.

RESULTS

The patient's blast cells could not be classified into lymphoid or myeloid lineage by morphology alone (Fig 1). Cytotoxic analysis of the blast cells revealed no reactivity in esterase, peroxidase, and periodic-acid Schiff (PAS) stains. TdT positivity, however, could be demonstrated in the indirect immunofluorescent test. Results of immunological analysis using a battery of monoclonal antibodies are shown in Table 1. Strong reactivity occurred with 3A1 (Fig 2), an antibody reacting with most mature and all immature T cells.28 Blast cells reacted also with another anti-T cell antibody, designated TH69, which has specificity similar to that of 3A1 (ref. 21 and Gramatzki, unpublished observations). Early T cell differentiation was further suggested by absence of staining with the more mature anti-T cell antibodies2.14 such as OKT3, OKT4, or Leu-2. Most cells expressed an antigen, detected by TC25, that is expressed only on some early T cell ALL, myelomonocytic and monocytic leukemias, and CML in blast crisis.24 In contrast, <15% of the PBMCs of the patient had myeloid or monocytic antigens on their surface, as determined by antibodies Mo-S39, Leu-M1, Mo-U28, and My-7. Likewise, no evidence for B cell or immature non-T cell lymphoid differentiation was found using reagents B1, B4, BA-1, BA-2, and the anti-common ALL (CALLA) antibody 24.1. Ia-antigens were not detected, a familiar finding on malignant cells differentiated on the early T cell level.

Cytogenetic analysis revealed the presence of a Philadelphia chromosome (Fig 3). To study the Philadelphia translocation molecularly, configuration of the bcr gene residing on

Fig 2. FACS histograms showing reactivity of monoclonal antibodies with the CML blasts. (A) Staining intensity by antibody 3A1. (B) Staining intensity of reagent TH 69. (C) Staining intensity of antibody TC25. Horizontal axis, cell number; vertical axis, relative fluorescence intensity; dark area, background staining.
chromosome 22 was analyzed. Southern blot analysis revealed a rearrangement within bcr (Fig 4).

Because the blasts were typed as early T cell origin, whether the gene for the T cell receptor β chain was already rearranged was of interest. Southern blot analysis, however, showed germline configuration (Fig 5).

To exclude any pre-B cell differentiation on the molecular level, Cμ sequences were used to investigate immunoglobulin heavy chain genes. Not surprisingly, they were found in germline configuration (Fig 6).

DISCUSSION

Because T cell differentiation of blasts in CML is a rare event, it appears important to review the evidence for classification in this case. Absence of esterase and peroxidase staining, and lack of reactivity with several antibodies detecting different myeloid antigens, but presence of TdT, an enzyme marker associated with lymphoid differentiation, was highly suggestive of a lymphoid affiliation of the patient's leukemic cells. Reactivity with T cell antibody 3A1, dissecting immature T cells from pre-B cell forms, and lack of staining with antibodies defining Ia antigens, B cells, or mature T cells, finally established differentiation on the early T cell level. This was confirmed by demonstration of immunoglobulin genes in germline configuration.

Although the clinical situation with massive spleen enlargement and thrombocytosis was already suggestive of CML in blast crisis, Philadelphia chromosome positive ALL, although almost always of non-T non-B cell type, had to be excluded. Recently, molecular techniques have become available which appear to be helpful in this situation. The typical Philadelphia translocation seen in CML patients places the c-abl oncogene adjacent to 5'bcr sequences on chromosome 22q-, whereas 3'bcr sequences are translocated to chromosome 9q+. Because true Ph1-
positive ALL studied so far appeared to be different on the molecular level, the typical c-abl/bcr rearrangement seen in our patient firmly establishes this T cell malignancy as a blast crisis of CML.

Recently, additional insight in T cell malignancies was obtained by studying the gene for the TCR, and most but not all T cell tumors had the receptor genes clonally rearranged. In this case of T cell CML, however, the TCR β gene was in germline configuration, which is in agreement with the immature differentiation level, since the receptor becomes rearranged during T cell maturation.

Very recently, two cases of T cell CML with already rearranged TCR were reported, in one of which typical c-abl/bcr rearrangement was documented as well. The present study is the first report of a T cell CML blast crisis with TCR still in germline configuration, indicating heterogeneity of differentiation stages in T cell CML. This is supported by the few published cases of immunologically characterized T cell CML in which some were immature as well and one apparently was late thymic-differentiated, while Griffin and co-workers reported a T cell CML with a mature phenotype that expressed myeloid antigens.

Our data provide definite evidence for the multilineage differentiation capacity for the CML precursor cell including the T cell lineage. Although T cell CMLs—despite their rarity—phenotypically are heterogeneous, they have certain molecular genetically defined alterations in common, namely a particular rearranged c-abl/bcr region. In turn, although c-abl/bcr rearrangement appears to define CML cells, immunological techniques and additional probes for other genetic sequences such as the TCR or the immunoglobulin genes are required for detailed evaluation of the differentiation stage of CML blasts.

ACKNOWLEDGMENT

We wish to thank I. Nüsslein and K. Kistermann for technical support, Dr P. Rohwer for help in cell sorter analysis, and E. Lathan for expert editorial assistance.

REFERENCES

19. Cuttner J, Seremetis S, Najfeld V, Dimitriu-Bona V, Winter J, Nott C: TdT-positive acute leukemia with monocytoïd charac-
teristics: Clinical, cytochemical, cytogenetic, and immunologic findings. Blood 64:237, 1984
Early T cell differentiated chronic myeloid leukemia blast crisis with rearrangement of the breakpoint cluster region but not of the T cell receptor beta chain genes

M Gramatzki, CR Bartram, D Muller, M Walter, H Tittelbach and JR Kalden