5q – Chromosome in Acute Leukemia With Lymphoid Morphology and Expression of Myeloid Membrane Determinants

By Oskar A. Haas, Peter Bettelheim, Waltraud Schmidmeier, Helmut Gadner, Heinz Ludwig, Otto Majdic, and Ursula Schulz

We present three patients, two children and one adult, with an unusual type of acute leukemia. Whereas the blast cells showed lymphoid morphology with correlating cytochemical staining, immunological phenotyping exhibited a pure myeloid in one patient and a biphenotypic membrane marker profile in the other two patients. Cytogenetic studies revealed a 5q- chromosome as a common marker and additional individual changes. Two of the patients who were treated according to acute lymphoblastic leukemia (ALL) therapy protocols died without remission five and four weeks after diagnosis, respectively. Despite relapsing several times, another patient survived for over eight years. These three patients seem to represent one new subgroup of leukemias that can only be distinguished from typical ALL by both determination of cell surface markers and cytogenetic analysis.

THE USE OF monoclonal antibodies against cell surface antigens has proven to be of great value in distinguishing various functional and/or differentiation stages of normal as well as malignant cells. According to the expression of antigen patterns, even morphologically uniform blast cell populations can now be subdivided into clinically relevant subgroups.

With the application of the double fluorescence technique, it is furthermore possible to distinguish between leukemias with two distinct subpopulations (“double leukemias”) and leukemias with two distinct markers, eg, myeloid and lymphatic, on the same cell population (“biphenotypic leukemias”).

On the other hand, several consistent chromosomal abnormalities have been identified during the past decade. These are also specifically associated with particular subtypes of various hematological malignancies. Only a few attempts have been made so far to combine both diagnostic tools in order to correlate changes of the genetic material with the expression of various immunologic differentiation markers of malignant cell populations. Such expanded diagnostic approaches may be very useful, as has been demonstrated on a subgroup of acute leukemias associated with a 4;11 rearrangement.

We have studied three patients with morphologically lymphoid leukemias: one with a pure myeloid and two with mixed lymphatic/myeloid marker expression and a 5q- chromosome—a marker generally associated with myeloid disorders.

MATERIALS AND METHODS

Patients

Patient H.W. has been described in detail elsewhere. In brief, the 6-year-old boy was first admitted to the hospital in May 1978, four years after acute lymphoblastic leukemia (ALL) had been diagnosed (WBC 2.1 × 10⁹/L, platelets 27 × 10⁹/L, no hepatosplenomegaly). On treatment following the Memphis protocol VII, he had achieved complete remission. On admittance, the boy presented with a hematological relapse (20% lymphoid blast cells in bone marrow). Induction therapy according to a modified Memphis protocol VIII yielded complete remission. Despite intensive maintenance therapy, the patient relapsed again in December 1979. For the following 20 months, maintenance therapy was continued, and the patient stayed in excellent physical condition despite blast cell counts in bone marrow (BM) being constantly elevated between 10% and 30%. In October 1981, the differential cell count showed 50% of blast cells in the peripheral blood (PB). The BM smear revealed 94% blast cells. Following a change of therapy (BFM-ALL 76 protocol, modified induction therapy with VM26 and cytosine arabinoside7), the patient again achieved complete remission. However, a further relapse occurred ten months later. At this time, the disease could no longer be controlled and the patient died eight years and three months after diagnosis of ALL.

Patient C.M., a 5-year-old Yugoslavian girl, was admitted to hospital in May 1979 because of thrombocytopenic purpura. Hematologic data on admission were as follows: hemoglobin 13.5 g/dL, WBC 124 × 10⁹/L (with 96% of blast cells), platelet count 24 × 10⁹/L. The BM smear also showed 95% blast cells. On physical examination, neither hepatosplenomegaly, lymph node enlargement, nor skeletal or CNS involvement was found. Treatment according to a modified BFM-ALL 76 protocol (including cytosine arabinoside, prednisolone, asparaginase, 6-mercaptopurine, and cyclophosphamide), the patient again achieved complete remission. However, a further relapse occurred five weeks later. At this time, the disease could no longer be controlled and the patient died due to septicemia five weeks after diagnosis.

Patient T.A., a 72-year-old woman, had hepatosplenomegaly (both 3 cm below costal margin) and the following hematological data at diagnosis: hemoglobin 8.5 g/dL, WBC 8.2 × 10⁹/L (with 79% blast cells), platelets 11 × 10⁹/L. The BM smear revealed 97% blast cells. Treatment was started according to the CALGB 7612 protocol with prednisolone, vincristine, and Adriamycin, which reduced the WBC count without affecting the percentage of blast cells. Cytostatic therapy was discontinued because the patient...
developed cerebral hemorrhage. Despite intensive supportive care, she died four weeks after diagnosis.

Morphological classification and cytochemical reactivity was determined on both PB and BM smears in all three cases. Immunologic and cytogenetic studies were performed on the same specimen of isolated BM cells obtained at diagnosis from patients C.M. and T.A. and, obtained during the third relapse from patient H.W. Furthermore, the above-mentioned studies were repeated on cryopreserved samples of the same material from patient C.M.

**Morphology**

Morphological diagnosis was based on the French-American-British (FAB) classification.

**Cytochemistry**

Cytochemical stainings were performed according to standard methods and included myeloperoxidase (MPO), periodic-acid Schiff (PAS), acid phosphatase (ACP), and o-naphthyl acetate esterase (ANAE) staining.

**Immunologic Phenotyping**

The binding of various antibodies to isolated mononuclear cells was assessed, as previously described, by indirect immunofluorescence with fluoresceinated goat F(ab')2 anti-mouse IgG and IgM antibodies. Double fluorescence staining was performed as described. Fluorescence reactivity of the cells was evaluated using a Leitz microscope with incident illumination, equipped for dual wavelength method.

Terminal deoxynucleotidyl transferase (TdT) was determined biochemically and by immunofluorescence technique as described previously. Biochemical values below 0.03 U/10⁶ peripheral mononuclear blood cells and 0.1 U/10⁶ bone marrow cells were considered negative.

**Cytogenetic Analysis**

Chromosome preparations from PB and/or BM as well as G-banding with trypsin were performed according to standard methods described previously. At least 25 metaphases were examined in each specimen of nonstimulated cultures of PB and/or BM from each case.

**RESULTS**

**Morphological Characteristics**

Blast cells showed lymphoblastic morphology in all three patients. They were classified as L1 in patients H.W. and T.A. and as L2 in patient C.M.

**Cytochemical Staining**

Cytochemical reactions were in accordance with the morphological appearance. MPO and ANAE were negative in all three patients. The blast cells from patient H.W. were positive for PAS, and those of patient C.M. were strongly focal positive for ACP (Table 1).

**Immunologic Phenotyping**

Data on the expression of various determinants on blast cells are summarized in Table 2.

<table>
<thead>
<tr>
<th>Patients</th>
<th>H.W.</th>
<th>C.M.</th>
<th>T.A.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>♂</td>
<td>♀</td>
<td>♀</td>
</tr>
<tr>
<td>Age at diagnosis (yr)</td>
<td>2½</td>
<td>5</td>
<td>72</td>
</tr>
<tr>
<td>Survival time</td>
<td>8½ yr</td>
<td>5 wk</td>
<td>4 wk</td>
</tr>
<tr>
<td>Morphology (FAB)</td>
<td>L1</td>
<td>L2</td>
<td>L1</td>
</tr>
<tr>
<td>Cytochemistry</td>
<td>MPO</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td></td>
<td>PAS</td>
<td>Pos</td>
<td>Neg</td>
</tr>
<tr>
<td></td>
<td>ACP</td>
<td>Neg</td>
<td>Pos</td>
</tr>
<tr>
<td></td>
<td>ANAE</td>
<td>Neg</td>
<td>Neg</td>
</tr>
</tbody>
</table>

Blast cells of patient C.M. were first phenotyped on admittance to the St Anna Children’s Hospital in May 1979 with heteroantisera. They reacted with anti-Ia and rabbit anti-human polyclonal T cell antiserum. On the other hand, they were negative for s/cyA, common ALL antigen (CALLA)-heteroantiserum, TdT, and did not form E-rosettes with sheep erythrocytes. Although double fluorescence staining was not performed in this case, there was neither morphological appearance of blast cells. No lymphatic membrane marker profile on the blast cells. No lymphatic markers were present on the cell surface. However, 50% of blast cells reacted strongly with TdT antiserum. Although double fluorescence staining was not performed in this case, there was neither morphological nor immunological evidence of two distinct blast cell populations. There was a clear overlap in the percentage of reactivity between myeloid marker MCS 2 and TdT.

**Karyotype Analysis**

Although cytogenetic analysis revealed various abnormalities, a common marker, a 5q- chromosome was detected in all three patients.

Despite frequent cytogenetic investigations, we were
Table 2. Reactivity Pattern of Blast Cells From Patients H.W., CM, and TA With Monoclonal Antibodies

<table>
<thead>
<tr>
<th>Clone Designation</th>
<th>Specificity</th>
<th>H.W.</th>
<th>C.M.</th>
<th>T.A.</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIL-A1</td>
<td>Common ALL-antigen</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Knapp et al22</td>
</tr>
<tr>
<td>V1B-C5</td>
<td>(pre-B) cells, granulocytes</td>
<td>85</td>
<td>0</td>
<td>0</td>
<td>Majdic et al23</td>
</tr>
<tr>
<td>BA-1</td>
<td>(pre-B) cells, granulocytes</td>
<td>90</td>
<td>NT</td>
<td>NT</td>
<td>Abramson et al24</td>
</tr>
<tr>
<td>anti-μ (s/cy)</td>
<td>IgM</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Lansdorp (unpublished observations)</td>
</tr>
<tr>
<td>Leu 4</td>
<td>Peripheral T cells</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Ledbetter et al25</td>
</tr>
<tr>
<td>Leu 3</td>
<td>T Helper cells</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Evans et al26</td>
</tr>
<tr>
<td>Leu 2</td>
<td>T Suppressor cells</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Evans et al26</td>
</tr>
<tr>
<td>OKT 11</td>
<td>T cells (E⁻)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Kung et al27</td>
</tr>
<tr>
<td>OKT 6</td>
<td>Corticohymocytes</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Reinhertz et al28</td>
</tr>
<tr>
<td>WT-1</td>
<td>Thymocytes, peripheral T cells, some myeloid blasts</td>
<td>NT</td>
<td>0</td>
<td>10</td>
<td>Vodinelich et al29</td>
</tr>
<tr>
<td>VIM-D5</td>
<td>Myeloid cells, monoblasts</td>
<td>90</td>
<td>0</td>
<td>0</td>
<td>Majdic et al20</td>
</tr>
<tr>
<td>VIM-2</td>
<td>Myeloid cells, monocytes</td>
<td>0</td>
<td>25</td>
<td>15</td>
<td>Majdic et al31</td>
</tr>
<tr>
<td>MCS 2</td>
<td>Myeloid cells, monocytes</td>
<td>0</td>
<td>80</td>
<td>90</td>
<td>Minowada et al32</td>
</tr>
<tr>
<td>VIM-12</td>
<td>Monocytes, granulocytes, O lymphocytes (OKM-equivalent)</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>Majdic (unpublished observations)</td>
</tr>
<tr>
<td>63D3</td>
<td>Monocytes, granulocytes</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>Ugolini et al33</td>
</tr>
<tr>
<td>C17-27</td>
<td>Platelet glycoprotein illia</td>
<td>NT</td>
<td>0</td>
<td>0</td>
<td>Tetteroo et al34</td>
</tr>
<tr>
<td>J15</td>
<td>Platelet glycoprotein Illa/Illa complex</td>
<td>0</td>
<td>NT</td>
<td>NT</td>
<td>Vainchenker et al35</td>
</tr>
<tr>
<td>VIE-G4</td>
<td>Glycoprophin A</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Liszka et al36</td>
</tr>
<tr>
<td>VID-1</td>
<td>HLA-DR</td>
<td>50</td>
<td>40</td>
<td>90</td>
<td>Majdic (unpublished observations)</td>
</tr>
<tr>
<td>TdT (IFLO)</td>
<td>Early lymphoid cells</td>
<td>90</td>
<td>0</td>
<td>50</td>
<td>Bollium37</td>
</tr>
<tr>
<td>TdT (Biochem)</td>
<td></td>
<td>2.09*</td>
<td>0</td>
<td>NT</td>
<td>Modak et al38</td>
</tr>
</tbody>
</table>

NT, not tested.
*Units/10⁶ bone marrow cells.

only successful in patient H.W. during his third relapse. A pseudotriploid karyotype with an average of 63 chromosomes was found in 30% of investigated metaphases. The karyotype change was caused mainly by triplication of various chromosomes. Structural rearrangements were rare in this case and included an abnormal chromosome 1 (duplication of region q11-q25), two marker chromosomes and one to two additional 5q- chromosomes, which were the product of a deletion: del(5)(q21) (Fig 1).

The 5q- chromosome probably resulted from a translocation 5:6 in patient C.M. The translocation occurred together with loss of one chromosome 22 in all analyzed mitoses/karyotype: 45XX,t(5;6)(q22;p21?),-22/ (Fig 2).

All karyotypes from the third patient’s blast cells...
were abnormal. They were pseudodiploid and all had the same rearrangements: a complex translocation, which in our opinion involved both chromosomes 1 and one chromosome 7 (although it cannot be ruled out that the long arm of chromosome 5 also participated), a translocation between chromosome 15 and 21 and a 5q- chromosome caused by an interstitial deletion, which led to the following interpretation of the karyotype: 46XX,t(1;1;7)(p13;pter;p13),t(15;21)(q12; qter),del(5)(q13;q33) (Fig 3).

DISCUSSION

In this study, we report on the presence of a common marker chromosome, a 5q- chromosome, in three patients with morphological ALL and the unexpected expression of myeloid membrane determinants on the blast cells. To our knowledge this constellation has not been reported before.

Immunologic phenotyping revealed in one patient a non-B/non-T marker profile with an atypical expression of the myeloid marker VIM-D5. Of 150 cases of ALL investigated so far, we have only observed two patients with this myeloid marker on their lymphoblastic blast cells.

Despite morphological and cytochemical features that were typical of ALL, we found only myeloid determinants on the cell surface in the second case. A pure myeloid membrane marker profile was also evident in the third patient, although 50% of blast cells were reactive with TdT antiserum.

All three patients had a 5q- chromosome with additional individual changes in their karyotypes; one
of each being hyperdiploid, hypodiploid, and pseudodiploid. In at least one case, the 5q- chromosome was the result of a translocation. There was no evidence of a preleukemic phase or carcinogen exposure prior to development of leukemia. However, the time and sequence of appearance of chromosome changes could not be documented in patient H.W. It may be possible that these karyotype changes only evolved during the prolonged and intensive treatment and, therefore, as in secondary leukemias, could have been therapy-related.

Only a few reports so far have dealt with atypical marker expression on cell surface membrane in acute leukemia. A 5q- chromosome has never been described in such cases. This is also true for TdT-positive acute myelogenous leukemia (AML). In our series of 410 immunologically investigated acute leukemias, we find atypical marker expression in about 10% (P.B., unpublished observations, 1984). Although we have seen both TdT-positive immunologically myeloid leukemias and lymphoblastic leukemias with a myeloid determinant, we have never observed a 5q-chromosome—neither in such cases nor in typical ALL, with the exception of the three presented cases.7,8 This suggests that the 5q-deletion is seen only in a subset of biphenotypic leukemias.

Abnormalities of chromosome 5 have been described in various malignant hematological disorders, mainly in preleukemic syndromes and AML. They are usually the result of a deletion and are rarely the product of a translocation. In certain preleukemic states, an interstitial deletion of the long arm, usually involving band 5q22q23, is often the only abnormality observed. The transformation of such somehow stable and long-lasting conditions into acute leukemia can be accompanied by clonal evolution of the karyotype. In contrast to progression of chronic myelogenous leukemia into blast crisis, however, these changes mainly lead to pseudodiploid or hypodiploid karyotypes. On the other hand, the 5q-anomaly has also been found in AML occurring de novo and, more commonly, in leukemias following carcinogen exposure or occurring secondary to cytotoxic therapy, which might also have been the case in patient H.W.

The 5q- chromosome has only been reported occasionally in ALL, usually in connection with other abnormalities. Diagnosis of these cases was based mainly on morphological and cytochemical criteria. Immunological studies with polyclonal antisera are described only in one patient. As in our patient C.M. (at the first examination), the marker profile was in agreement with the morphological lymphoid features. Using monoclonal antibodies, we could demonstrate, however, that in our case the cells were of pure myeloid origin.

The fact that morphological criteria alone may falsify diagnostic interpretation has been documented in acute leukemia with a 4;11 rearrangement. Until recently it had been considered to be of lymphatic origin because of morphological appearance. Detailed phenotypic studies, however, have shown that they are either of pure myelomonocytic origin or at least can express both myeloid and lymphatic characteristics. Our three cases with the 5q-chromosome demonstrate that morphologically lymphoid blasts with this abnormality may display myeloid determinants. The combination of immunological and cytogenetic investigations can therefore contribute to the demarcation of otherwise indistinguishable forms of acute leukemias. Early recognition of such cases, however, which do not respond satisfactorily to established ALL-therapy regimens, is important in order to adapt therapy. Such a modification of treatment should also take the myeloid character of the cells into consideration.

ACKNOWLEDGMENT

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