Trisomy 4 Identifies a Subset of Acute Nonlymphocytic Leukemias

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We present four patients with acute nonlymphocytic leukemia, one M2 and three M4 French-American-British (FAB) types and one patient with refractory anemia with excess of blasts in transformation who at diagnosis had trisomy of chromosome 4 as the primary karyotypic anomaly. This chromosome anomaly probably defines a previously undescribed subset of acute nonlymphoid leukemias. Hematologic characteristics commonly found in these patients were dysplastic features of all bone marrow lineages, suggesting that trisomy 4-associated disorders involve the early myeloid hematopoietic stem cell.

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CYTOGENETIC studies have shown that some acquired chromosomal abnormalities are characteristically associated with morphologically defined types of acute nonlymphocytic leukemia (ANLL). For example, a t(8;21) is typically associated with an M2 leukemia with granulated myeloblasts; a (15;17) is specific for both the granular and the hypogranular form of the promyelocytic M3 leukemia; a structural anomaly of chromosome 16 involving bands p13 and q22 identifies an M4 type of acute leukemia with bone marrow eosinophilia; and structural rearrangements of the long arm of chromosome 11 (11q) are nonrandomly associated with acute monocytic M5 leukemia. In this paper we describe four patients with ANLL and one patient with a myelodysplastic syndrome in transformation, all associated with a trisomy of chromosome 4. The available evidence suggests that the target cell in these trisomy 4–associated disorders may be the early myeloid hematopoietic stem cell capable of differentiating along either the monocytic or the granulocytic lineage.

MATERIALS AND METHODS

Patients. Five patients with trisomy 4 were identified among a total number of 700 patients with ANLL and refractory anemia with excess blasts (RAEB) who were referred for chromosome investigation to the Centre for Human Genetics, University of Leuven, Belgium, between 1973 and 1984.

Patient N.V.C., a 17-year-old female student, was admitted for the first time in April 1974 because of persistent fever (38.5°C), gingivitis, and otitis. Hematologic data were as follows: hemoglobin level, 10.4 g/dL; red blood cell (RBC) count, 3.2 × 10¹²/L; WBC count, 33.7 × 10⁹/L, with 52% blasts, 45% promyelocytes, 1% segmented neutrophils, and 2% lymphocytes. Bone marrow smears showed 43% blast cells. Treatment consisted of cytosine arabinoside and 6-mercaptopurine. The patient died 9 months after diagnosis.

Patient H.B., a 64-year-old female white-collar worker, was referred for the first time in October 1978 because of weight loss, sore throat, and fever. Physical examination revealed an enlargement of the lymph nodes in the cervical region and diffuse petechiae on the legs. There was no hepatosplenomegaly. Hematologic data were as follows: hemoglobin level, 9 g/dL; platelet count, 17 × 10⁹/L; and WBC count, 248 × 10⁹/L, with 76% blasts, 2% promyelocytes, 7% lymphocytes, and 15% monocytes. A myelogram showed 89% blast cells, 9% promonocytes, and 2% monocytes. The serum lysozyme level was 16 μg/mL (NV, 0 to 20) and the urine lysozyme level, 20 μg/mL (normal values [NV], 0 to 5). Therapy consisted of cytosine arabinoside, Adriamycin (Adria Laboratories, Columbus, Ohio) and 6-thioguanine. Remission was not obtained, and the patient died of broncho-pneumonia 2 months after diagnosis.

Patient A.Z., a 56-year-old housewife, was referred for the first time in June 1978 because of weight loss, sore throat, and fever. Physical examination revealed an enlargement of the lymph nodes in the cervical region and diffuse petechiae on the legs. There was no hepatosplenomegaly. Hematologic data were as follows: hemoglobin level, 7.6 g/dL; RBC count, 2.16 × 10¹²/L; and WBC count, 4.7 × 10⁹/L, with 13% blasts, 4% band neutrophils, 13% segmented neutrophils, 1% basophils, 64% lymphocytes, and 5% monocytes. The platelet count was 31 × 10⁹/L. A bone marrow aspirate and bone biopsy specimen were hypercellular with a massive infiltration by blast cells. A myelogram showed 87% blasts, 2% promyelocytes, 2% myelocytes, 3% band neutrophils, 3% segmented neutrophils, 1% eosinophils, and 2% lymphocytes. Remission was obtained using a therapeutic regimen with cytosine arabinoside and daunomycin. The patient died in aplasia during consolidation therapy.

Patient J.M., a 15-year-old male student, was observed for the first time in October 1984 because of weakness. Physical examination revealed hepatosplenomegaly. Hematologic data were as follows: hemoglobin level, 7.6 g/dL; RBC count, 2.16 × 10¹²/L; and WBC count, 4.7 × 10⁹/L, with 13% blasts, 4% band neutrophils, 13% segmented neutrophils, 1% basophils, 64% lymphocytes, and 5% monocytes. The platelet count was 31 × 10⁹/L. A bone marrow aspirate and bone biopsy specimen were hypercellular with a massive infiltration by blast cells. A myelogram showed 87% blasts, 2% promyelocytes, 2% myelocytes, 3% band neutrophils, 3% segmented neutrophils, 1% eosinophils, and 2% lymphocytes. Remission was obtained using a therapeutic regimen with cytosine arabinoside and daunomycin. The patient died in aplasia during consolidation therapy.

Patient P.B., a 58-year-old housewife, was first observed in July 1983. On admission she showed splenomegaly and the following hematologic data: hemoglobin level, 8 g/dL; platelet count, 199 × 10⁹/L; and WBC count, 3.6 × 10⁹/L, with 32% neutrophils, 2% eosinophils, 1% basophils, 53% lymphocytes, and 12% lymphocyte-like unclassifiable elements. Bone biopsy findings showed focal infiltration by small round elements with a high nuclear cytoplasmic ratio. The disease remained stationary until October 1984 when leukocytosis (WBC count, 15.8 × 10⁹/L) and thrombocytopenia (platelet count, 85 × 10⁹/L) were noted. The peripheral blood differential count consisted of 20% neutrophils, 10% monocytes, 48% lymphocytes, and 22% blast cells. Bone marrow smears showed 28% blasts. Treatment with 6-mercaptopurine was started, but it was discontinued after 1 month because severe leukopenia and septicemia developed. The last follow-up in May 1985 showed a patient in good general condition and the following hematologic data: 9.9 g/dL of WBC, 18% neutrophils, 7% monocytes, WBC count, 18.7 × 10⁹/L, with 32% neutrophils, 2% eosinophils, 1% basophils, 12% lymphocytes, and 57% blasts. The platelet level was 54 × 10⁹/L. A myelogram showed 6% promonocytes, 6% monocytes, 51% blast cells, 4% promyelocytes, 5% myelocytes, 2% metamyelocytes, 8% neutrophils, 1% basophils, 4% pronormoblasts, 9% normoblasts, and 4% lymphocytes. Remission was obtained after two cycles of treatment with cytosine arabinoside and daunomycin. In September 1984 the patient was readmitted with icterus and hepatomegaly because of non-A, non-B hepatitis. No signs of leukemia were found upon examination of the peripheral blood and bone marrow. At the last hematologic follow-up in May 1985 she was still in complete remission.

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Table 1. Clinical Data of the Patients and Morphologic, Cytotoxic, and Antigenic Characteristics of Their Blast Cells at the Time of Chromosome Investigation

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/Sex</th>
<th>Date</th>
<th>FAB Type</th>
<th>HB (g/dL)</th>
<th>Platelets (x 10^9/L)</th>
<th>WBC (x 10^9/L)</th>
<th>Cytochemistry*</th>
<th>Immunophenotype†</th>
<th>Survival From Diagnosis (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N.V.C.</td>
<td>17/F</td>
<td>April 1974</td>
<td>M2</td>
<td>10.4</td>
<td>90</td>
<td>33.7</td>
<td>BM 43, 55, ND, ND, ND, ND, ND</td>
<td>TdT-neg, BA-2-pos, OKT10-pos, OKT3-neg, Slg-neg</td>
<td>ARA-C 6-TG, 7, died</td>
</tr>
<tr>
<td>H.B.</td>
<td>64/F</td>
<td>June 1984</td>
<td>M4</td>
<td>11.7</td>
<td>54</td>
<td>18.7</td>
<td>BM 51, 4, ND, 40, 50, ND, ND</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>A.Z.</td>
<td>56/F</td>
<td>June 1978</td>
<td>M4</td>
<td>9</td>
<td>17</td>
<td>248</td>
<td>BM 89, 9, 0, 0, 10, 49, ND</td>
<td>—</td>
<td>ARA-C 6-TG, 12. +</td>
</tr>
<tr>
<td>J.M.</td>
<td>15/M</td>
<td>Oct 1984</td>
<td>M4</td>
<td>7.6</td>
<td>31</td>
<td>4.7</td>
<td>BM 87, 2, 0, 40, ND, 30, ND</td>
<td>TdT-neg, OKla1-pos, OKM5-pos</td>
<td>ARA-C 6-TG, 1.5, died</td>
</tr>
<tr>
<td>P.B.</td>
<td>58/F</td>
<td>Oct 1984</td>
<td>RAEBt</td>
<td>8</td>
<td>199</td>
<td>15.8</td>
<td>PB 22 — 0, ND, 8, ND, 2</td>
<td>TdT neg, BA-1-neg, B1-neg, J5-neg, OKT3-neg, OKla1-pos, OKT9-pos, OKT10-pos</td>
<td>ARA-C 6-MP, 6-MP, 22. +</td>
</tr>
</tbody>
</table>

Abbreviations: BM, bone marrow; PB, peripheral blood; RAEBt, refractory anemia with excess of blasts in transformation; ARA-C, cytosine arabinoside; 6-TG, 6-thioguanine; Adria, Adriamycin; Dauno, daunomycin; 6-MP, 6-mercaptopurine; ND, not determined.

*Numbers indicate positive cells among blast cells.
†Reactivity of monoclonal antibodies: TdT, lymphoid progenitor cells; BA-1, pre-B cells; B cells, granulocytes; BA-2, pre-B and B cells; vast majority of non-T acute lymphocytic leukemia (ALL), some cases of nonlymphoid leukemias; J5, common ALL antigen; OKla1, HLA-DR antigens, B cells, macrophages, activated T cells; OKT3, mature T cells; OKT9, transferrin receptor, proliferating cells, including stem cells and activated lymphocytes; OKT10, early stem cells, activated lymphocytes; OKM5, monocytes and platelets.
hemoglobin, 157 x 10^9/L platelets, and 9.4 x 10^9/L WBC with 19% blast cells.

**Morphologic and cytochemical studies.** May-Grünwald-Giemsa (MGG)-stained slides of bone marrow and peripheral blood were reviewed retrospectively in all the cases using the criteria of the French-American-British (FAB) classification for acute leukemias and myelodysplastic syndromes.67 Cytochemical studies included assays for periodic acid–Schiff stain (PAS), Sudan black B (SBB), peroxidase (POX), alpha-naphthyl-acetate-esterase (ANAE), and alpha-naphthyl-butyrate esterase (ANBE) and were performed following the usual criteria.7

**Immunologic marker studies.** Surface markers were detected by an indirect immunofluorescence technique using fluorescein isothiocyanate (FITC)-conjugated goat antirabbit IgG (Ortho Pharmaceutical Corp, Raritan, NJ) as the second antibody. Cell surface immunoglobulins were tested by direct fluorescence with FITC-conjugated antibodies.

Monoclonal antibodies OKIa1, OKT3, OKT9, OKT10, OKM5 (Ortho Pharmaceuticals), BA-1, BA-2 (Hybritech, San Diego), B1, and J5 (Coulter Immunology, Hialeah, Fla) were obtained commercially. Reactivity is reported in Table I. Terminal deoxynucleotidyl transferase (TdT) activity was determined by immunofluorescence using rabbit antitNF1 TdT serum and FITC-conjugated goat antirabbit serum (Bethesda Research Laboratories, Md).

**Cytogenetic studies.** Chromosome analysis was performed at diagnosis on all patients. Metaphase chromosome preparations were obtained from bone marrow and/or peripheral blood cells after 48-hour stimulated cultures. A reverse banding with acridine orange was used. Karyotypes were arranged according to the International System for Human Cytogenetic Nomenclature.18

### RESULTS

**Morphologic, cytochemical, and immunologic studies.** The age, sex, presenting symptoms, WBC count, hemoglobin level, platelet count, cytochemical properties and immunophenotype of blast cells, type of treatment, and survival from diagnosis of the five patients with trisomy 4 are listed in Table I.

Patient N.C.V. was classified as FAB M2 on the basis of the presence of maturation of the bone marrow cells, including granulated myeloblasts, Auer’s rods, and a high number of promyelocytes. Pelger-Huët–like anomaly, megaloblastic features of the red cell series, and a very few, abnormal megakaryocytes were noted.

Patient H.B. was classified at diagnosis as M4. MGG-stained slides of bone marrow showed maturation of the myeloid blasts and a few elements with single Auer’s rods. Nuclear abnormalities with bilobated or clover nuclei were prominent in the blast elements. Bone marrow smears showed 30% of the blast cells positive for ANAE. The more differentiated granulocytic forms showed a Pelger-Huët–like anomaly as well as hypersegmentation. A few eosinobasophilic elements were also observed. The red cell series was megaloblastic, and several binucleated forms were present. Megakaryocytes were abnormal with monolobulated nuclei and cytoplasmic basophilia. Blast cells in this case reacted with the BA-2 monoclonal antibody. All other lymphoid markers, however, were negative, including TdT, T3 antigen, and surface immunoglobulins.

In patient A.Z., bone marrow was classified as M4 and showed abnormal maturation of the myeloid series with hypogranulation. Pelger-Huët–like anomaly was present. Several blast cells showed scanty cytoplasm and sometimes folded nuclei. Most of these elements (49%) showed ANBE activity partially inhibited by NaF. Megaloblastosis of red cell series, hypolobulation of megakaryocytes, and a few eosinobasophils were associated morphologic anomalies.

Patient J.M. showed a typical FAB M4. ANBE activity was present in 30% of the immature elements. Morphologic anomalies, ie, megaloblastosis of the red cell series, dysmegakaryopoiesis, Pelger-Huët–like anomalies, and eosinobasophilic elements were consistently present in this case.

In patient P.B., the diagnosis was RAEBt in Oct 1984 when cytogenetic studies were performed. Morphologically, two types of blast cells were identified: small blastic elements with scanty cytoplasm, diffuse nuclear chromatin, and one or sometimes two nucleoli; and blasts with folded monocytoid nuclei. There was abnormal maturation of the myeloid series with a Pelger-Huët–like anomaly. Dysmegakaryopoiesis was present with both monolobulated and apparently polynucleated forms. The red cell series was megaloblastic. Iron staining did not show ringed sideroblasts. Plasma cells and eosinobasophils were noted. The myeloid nature of the disorder in this patient was confirmed by positivity of the SBB stain in more than 5% of the blast cells. Moreover, immunophenotype studies revealed a negativity of the antibodies reacting with lymphoid antigens, ie, TdT, BA-1, B1, J5, and OKT3.

**Cytogenetic studies.** The karyotypic investigations of the five patients at diagnosis and during the course of the disease are summarized in Table 2. Figure 1 shows the trisomy 4 in a partial karyotype of the five patients. In patients 1, 2, and 4, the predominant clone with trisomy 4 was associated with normal karyotypes at the time of diagnosis. In patient 1, no mitoses were obtained at the time of a possible hematologic remission, but trisomy 4 was again present in 80% of the bone marrow metaphases during the terminal phase of the disease. Patient 3 showed trisomy 4 as sole anomaly in 23 out of 25 metaphases. In this patient, however, also one subclone with an additional trisomy 7 was identified. All five metaphases obtained from the peripheral blood of patient 5 showed a 47, XX, +4 karyotype.

**DISCUSSION**

To the best of our knowledge, trisomy 4 as the primary karyotypic anomaly has never been described in human hematologic malignancies. A few cases of ANLL, namely, one M2 and five M5 FAB types have been described in which trisomy 4 was part of a complex abnormal karyotype with both numerical and structural anomalies of other chromosomes.19-22

The present study documents for the first time the existence of a unique hematologic disorder associated with a trisomy of chromosome 4. This hematologic disorder is nonlymphoid in nature as demonstrated on the basis of morphology, cytochemistry, and monoclonal antibody reactivity of the blast cells. Furthermore, the fact that the acute leukemias in this study showed either granulocytic or monocytic differentiation, ie, M2 and M4 FAB types, indicates
TRISOMY 4 AND ANLL

Table 2. Cytogenetic Investigations in Five Patients With ANLL and Trisomy 4

<table>
<thead>
<tr>
<th>Patient</th>
<th>Status of Disease</th>
<th>Source of Cells</th>
<th>Culture (h)</th>
<th>Abnormal Karyotypes</th>
<th>Number of Normal Karyotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>N.C.V.</td>
<td>diagnosis</td>
<td>BM 48</td>
<td>47, XX +4 (16/24)</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>remission</td>
<td>PB 48</td>
<td>47, XX, +4 (3/17)</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>relapse</td>
<td>BM 48</td>
<td>no mitoses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H.B.</td>
<td>diagnosis</td>
<td>PB 48</td>
<td>47, XX, +4 (20/25)</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>remission</td>
<td>BM 48</td>
<td>47, XX, +4 (14/15)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>A.Z.</td>
<td>diagnosis</td>
<td>BM 48</td>
<td>47, XX, +4 (1/17)</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>47, XX, +4 (23/25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>48, XX, +4, +7 (2/25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J.M.</td>
<td>diagnosis</td>
<td>BM 48</td>
<td>47, XY, +4 (8/13)</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>P.B.</td>
<td>diagnosis</td>
<td>PB 48</td>
<td>47, XX, +4 (5/5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figures in parentheses are the number of karyotypes over the total number of metaphases studied.

that at least the common granulomonocytic progenitor cell was involved. Yet, from this study two lines of evidence suggest that an early myeloid precursor, possibly the myeloid hematopoietic stem cell, multipotent for erythrocytes, platelets, granulocytes, and monocytes, was the primary site of lesion for the trisomy 4–associated disorders. First, in all our cases of ANLL, namely, one M2 and three M4, the erythrocytic and megakaryocytic cells showed a marked dysplasia consistent with the involvement of both these series in the neoplastic process. Fialkow and colleagues, using G6PD isoenzyme studies, have demonstrated that ANLLs with such morphologic features arose from a pluripotent myeloid cell. Furthermore, the last patient of our series showed a myelodysplastic syndrome that is a disorder of a pluripotent stem cell according to both morphologic and enzymatic studies. Trisomy 4 thus probably arises in a myeloid totipotent stem cell that maintains the capacity of differentiating along the granulocytic and monocytic pathways. Dysplasia of the three bone marrow cell lines is often observed in cases of ANLL or myelodysplastic syndrome arising in patients who have a history of occupational exposure to toxics or prior cytotoxic therapy. Our five patients with trisomy 4, however, were not professionally exposed to toxics. Moreover, in all our cases, cytogenetic studies were performed before any chemotherapy.

Finally, on the basis of our experience and previously reported large series of ANLL patients, trisomy 4 associated disorders appear to be uncommon. In our cases, however, the trisomy 4 was found after 48 hours of culture, which is rarely used in cytogenetic laboratories. This culture method might be especially suitable in determining the trisomy 4 anomaly.

The significance of a trisomy of chromosome 4 for the biology of the malignant clone is unclear at present. Trisomy may be associated with quantitative changes in the production of specific gene products. A gene dosage effect has been described in some disorders associated with trisomy 8 and increased levels of the enzyme glutathione reductase, the gene of which is located on the short arm of chromosome 8. A similar example was recently given by Koprowski et al who demonstrated that the epidermal growth factor receptor, coded for by chromosome 7, was expressed by only melanoma cells with a trisomy 7. Therefore, one may speculate that trisomy 4 is involved in the abnormal proliferation of the neoplastic clone through an increased production of one or more factor(s) that are controlled by genes on this chromosome. Two genes involved in the proliferation and self-perpetuation of malignant cells have been mapped on chromosome 4, one coding for the T cell growth factor, the other for the epidermal growth factor. It remains to be tested whether trisomy 4 confers a proliferating advantage to the malignant cells by amplification of one or more of these growth factor(s).
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Trisomy 4 identifies a subset of acute nonlymphocytic leukemias

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