RAPID COMMUNICATION

Molecular Evaluation of Response to All-Trans-Retinoic Acid Therapy in Patients With Acute Promyelocytic Leukemia

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The advent of retinoic acid (RA) in the treatment of acute promyelocytic leukemia (APL) has led to a high frequency of short-lasting complete remissions (CR). We studied the response to RA by molecularly analyzing the RA receptor (RARα) gene, which has recently been shown to be rearranged in all APLs. Southern blot analysis demonstrated that the RARα rearrangements persisted in the APL samples containing maturing myeloid cells 2 to 3 weeks after the start of RA treatment, but disappeared after 5 to 8 weeks, when the patients achieved CR. Our investigations provide clear evidence that CR occurs at molecular level and that there is reconstitution of an apparently normal, nonclonal hematopoiesis. Further, it shows that RA acts by triggering differentiation rather than by exerting a cytotoxic effect on the leukemic clone.

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ACUTE PROMYEOLOCYTIC LEUKEMIA (APL) is a well defined subtype of acute myelogenous leukemia (M3 of the French-American-British [FAB] classification) with peculiar clinical and biologic characteristics. These characteristics include the frequent association of a life-threatening coagulopathy and the presence of a consistent and specific cytogenetic abnormality, t(15;17) (q21;q11-22) in the blasts, which is also found in the “microgranular” variant form (M3v).1,4 Clinical trials have demonstrated that the vitamin A derivative all-trans retinoic acid (RA) is singularly efficacious in inducing complete remission (CR) in APL patients. The absence of the coagulopathy and the morphologic finding of maturing elements in the bone marrow (BM) and peripheral blood (PB) during RA treatment both indicate that the response is obtained without the imposition of hypoplasia and suggest that a differentiative mechanism is involved.5,6

In the last few months, we and others have provided conclusive evidence that the APL chromosome translocation breakpoint is consistently located within the RA receptor α (RARα) gene locus.7,10 By producing genomic probes that specifically recognize the APL chromosome 15 and 17 breakpoints, we have demonstrated that 100% of APLs, including the M3v and cases with apparently normal karyotypes, display abnormal hybridization fragments at the DNA analysis.11 Because these features disappear during the remission phases in patients treated with conventional chemotherapy, they may be regarded as sensitive and specific tumor markers of leukemic promyelocytes.13 This study provides molecular evidence of CR in APL patients treated with RA and that RA activity on leukemic blasts is differentiative rather than cytotoxic.

PATIENTS AND METHODS

Five adult APL patients were entered in the study during the period of April to November 1990. Table 1 gives their clinical and biologic characteristics. All patients were diagnosed and treated at a single institution (Hematology, Dept. of Human Biopathology, University “La Sapienza,” Rome). Morphologic and immunophenotypic characterization showed hypergranular BM blasts (FAB M3) that were HLA/DR negative, CD9 and CD13 positive in all cases.13,15 Cytogenetic analysis of the three cases where metaphases were available showed the typical t(15;17) in two cases and a normal 46,XX karyotype in the other. After informed consent had been obtained, 45 to 50 mg/m²/d all-trans RA (Istituto delle Vitamine, Segrate, Italy) was administered as induction therapy. Two patients greater than 60 years old were treated at onset of disease; the other three received the same treatment at the time of first relapse. Four patients were hospitalized until CR had been obtained. The fifth was treated as an outpatient and was checked twice weekly for white blood cell (WBC) count and coagulation parameters.

Molecular analyses were performed on BM and PB samples collected at diagnosis, between the treatment days 14 to 16, and at confirmation of CR, which was defined as the absence of leukemic promyelocytes in morphologically normal BM and PB. High molecular weight DNAs were extracted from fresh BM mononuclear cells recovered after Ficoll-Hypaque density gradient centrifugation, and PB granulocytes purified from erythrocytes by dextran sedimentation. Following digestion with EcoRI or HindIII, DNAs were separated by electrophoresis on 0.8% agarose gels, blotted, and hybridized according to standard procedures. A 650-bp EcoRI-HindIII fragment derived from a genomic library obtained from the DNA of an APL patient14 was labeled with 32P by random priming and used to probe filters. We have previously shown that this probe (designated H18) identifies abnormal DNA fragments in 80% of APL cases.11 A partial restriction map of the RARα locus 5' portion is reported in Fig 1, together with location of the genomic H18 probe. After washing at appropriate stringency, filters were exposed at −80°C for 48 to 72 hours using intensifying screens. DNA lanes corresponding to morphologically evaluated remission phases were overloaded in cases 2, 3, and 5 (up to 20 μg) with the aim of detecting residual disease.

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Table 1. Clinical and Biologic Characteristics of APL Patients

<table>
<thead>
<tr>
<th>Pt</th>
<th>Age/ Sex</th>
<th>Disease Status</th>
<th>Karyotype</th>
<th>WBC $\times 10^9$/L</th>
<th>Hb g/dL</th>
<th>Pts $\times 10^9$/L</th>
<th>% BM Blasts</th>
<th>Coagulopathy*</th>
<th>Therapy</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>67/F</td>
<td>Onset</td>
<td>t(15;17)</td>
<td>1.9</td>
<td>8.3</td>
<td>54</td>
<td>70</td>
<td>Yes</td>
<td>All-t-RA</td>
<td>NE†</td>
</tr>
<tr>
<td>2</td>
<td>63/F</td>
<td>Onset</td>
<td>NA</td>
<td>0.8</td>
<td>9.6</td>
<td>9.0</td>
<td>92</td>
<td>Yes</td>
<td>All-t-RA</td>
<td>CR</td>
</tr>
<tr>
<td>3</td>
<td>18/F</td>
<td>Relapse</td>
<td>46,XX</td>
<td>1.7</td>
<td>12.9</td>
<td>177</td>
<td>80</td>
<td>No</td>
<td>All-t-RA</td>
<td>CR</td>
</tr>
<tr>
<td>4</td>
<td>51/F</td>
<td>Relapse</td>
<td>t(15;17)</td>
<td>2.4</td>
<td>14.9</td>
<td>140</td>
<td>70</td>
<td>No</td>
<td>All-t-RA</td>
<td>CR</td>
</tr>
<tr>
<td>5</td>
<td>39/M</td>
<td>Relapse</td>
<td>NA</td>
<td>1.9</td>
<td>14.9</td>
<td>79</td>
<td>76</td>
<td>No</td>
<td>All-t-RA</td>
<td>CR</td>
</tr>
</tbody>
</table>

Abbreviations: NA, not available; NE, not evaluable; CR, complete remission.
*Defined as fibrinogen less than 150 mg/dL and FDP greater than 40 μg/mL.
†Died of myocardial infarction after 20 days from All-t-RA start.

RESULTS

Southern blot analysis of BM samples collected at diagnosis identified RARα rearrangements in all five cases after hybridization with the H18 probe.

Treatment was generally well tolerated and toxicity was limited to mild bone pain and dryness of skin and lips. A significant hyperleukocytosis ($> 20 \times 10^9$/L) was observed in only one case (pt 1), and none of the patients displayed BM hypoplasia or coagulopathy during RA therapy.

Four patients (nos. 2, 3, 4, and 5) obtained CR within 30 to 50 days. The remaining patient (no. 1) died of myocardial infarction with associated hyperleukocytosis on the treatment day 20 and could not be evaluated for the response to RA.

Studies performed on BM specimens taken on the treatment day 14 to 16 documented partial response in three cases (nos. 2, 3, and 4), with a percentage of blasts that varied from 30% to 50%. DNA analysis showed that the rearranged RARα fragments persisted, but at a lower intensity than at diagnosis. Molecular studies performed at the time CR was achieved showed that the abnormal RARα hybridization fragments had totally disappeared (Fig 2, cases 2, 3, and 4).

The blood sample collected on day +15 from patient No. 1 was hyperleukocytic with a WBC 82.3 $\times 10^9$/L and a differential count of 5% promyelocytes, 29% myelocytes, 24% metamyelocytes, 36% neutrophils, and 6% lymphocytes. There was no variation in the intensity of the abnormal rearranged RARα fragment at Southern blot analysis with respect to the diagnostic BM control. The molecular picture remained unchanged in a further BM specimen aspirated on day +18 (Fig 2, case 1).

The intermediate BM control performed on day +15 in patient 5 showed no decrease in the intensity of the rearranged band. The associated picture in the BM was one of maturing cells and dysplastic elements that were not recognizable as typical leukemic promyelocytes. When this patient obtained CR on day +50, molecular analysis showed no abnormal RARα fragments.

DISCUSSION

By molecularly isolating the chromosome 15 and 17 breakpoints from cases of APL with the t(15;17), we and others have defined the architecture of the translocation and demonstrated the location of breakpoints within the RARα locus on chromosome 17 and the MYL transcription unit on chromosome 15. The joining of the residual 15 with the translocated 17 fragment generates a chimeric MYL-RARα gene product. Whatever may be the role of RARα gene rearrangements play in the pathogenesis of APLs, they provide a specific molecular marker for identifying leukemic promyelocytes and monitoring of patient status during the course of the disease.

In the present study we provide molecular evidence of CR in RA-treated APL patients, and show that CR is accompanied by the reconstitution of an apparently normal, nonclonal hematopoiesis. In fact, Southern blot analysis of the four patients who achieved CR, demonstrated that the RARα rearrangements persisted 2 to 3 weeks after the start of RA treatment, but disappeared after 5 to 8 weeks.

In addition, our observations in patients 1 and 5 (persistance of the specific rearrangement pattern in maturing elements) indicate a differentiative effect of RA treatment. In particular, in patient 1 the presence of PB-maturing elements (almost 90% were metamyelocytes, myelocytes, and neutrophils) showing the same intensity RARα rearrangement as BM blasts suggested their origin from leuke-
mic promyelocytes. A similar picture was observed in the intermediate BM control of case 5, in which the persistence of the abnormal band at day +16 was seen in maturing elements and finally disappeared at day +50.

In conclusion, our findings provide further evidence that RA treatment may induce CR in APL, as recently demonstrated by other investigators. Moreover, the results of our molecular analysis suggest a “normal” hematopoietic reconstitution following RA treatment.

Therefore, the possibility of eliminating the leukemic clone of APL (even if temporarily) by maturation and without an ablative approach may further contribute to define this disease as a distinct one within acute leukemias, as recently discussed by Wiernik.

REFERENCES


9. Longo L, Pandolfi PP, Biondi A, Rambaldi A, Mencarelli A, Lo Coco F, Diverio D, Pegoraro L, Avanzi G, Tabilio A, Zangrilli D, Alcalay M, Donti E, Gignani F, Pelicci P-G: Rearrangements of the RARα locus in the five APL patients before and after RA therapy. The numbers above the figures identify each patient. The H18 probe detected RARα rearrangements on EcoRl (pts. 1 and 2) or Hindlll (pts. 3, 4, and 5) digestions. Lanes A, BM controls before treatment. Lanes B, intermediate controls treatment day 14 to 16) performed on BM samples in pts. 2, 3, 4, and 5, and on a hyperleukocytic PB specimen in pt. 1. Lanes C, remission BM specimen in pt. 1. Rearranged bands are indicated by arrows. n = normal placental DNA.


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