Induction of Retinoic Acid-binding Protein in Normal and Malignant Human Myeloid Cells by Retinoic Acid in Acute Promyelocytic Leukemia Patients

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ABSTRACT

Retinoic acid has striking effects on development and cell differentiation. Its biological effect is a highly regulated process that is controlled by specific proteins. In the nucleus, different retinoic acid receptors have been identified and their genes cloned. In the cytosol, retinoid binding proteins, cellular retinoic acid-binding protein and cellular retinol-binding protein, have been correlated with normal and malignant tissue differentiation. Recently, differentiation therapy of acute promyelocytic leukemias (AML3 subtype) with all-trans-retinoic acid has been shown to be an efficient alternative to chemotherapy. The retinoic acid receptor α gene has been shown to be specifically rearranged in AML3. Through the t(15;17) translocation, the molecular basis of the effect to reverse the leukemic phenotype of all-trans-retinoic acid is not yet elucidated. To further study retinoic acid efficacy in AML3 leukemia, retinoic acid-binding proteins were studied in the cytosol extracts of hematopoietic cells. No retinoic acid binding activity was detected in normal or malignant hematopoietic cells whether sensitive or not to retinoic acid. However, detectable binding to a cytosolic protein corresponding to cellular retinoic acid-binding protein (Mr, 15,000, Kd 3 nM) was observed in the bone marrow cells of AML3 patients undergoing all-trans-retinoic acid therapy. We suggest that both the induction and subsequent presence of cellular retinoic acid-binding protein may influence the therapeutic efficacy of retinoic acid and must be taken into account when studying its effect in acute promyelocytic patients.

INTRODUCTION

Retinoic acid, a natural derivative of vitamin A, plays an important role in the growth, differentiation, and development of known normal tissues (see Refs. 1–3 for review). Direct antiproliferative effects of retinoic acid have been demonstrated in various malignant cells such as F9 teratocarcinoma cells and HL-60 or U-937 human myeloid leukemia cells (4, 5). Growth inhibition by retinoic acid is sometimes associated with a commitment to differentiation and reversion of the malignant phenotype as observed in F9 and human leukemia cells (6–9). How retinoic acid exerts its biological effects in various cell systems remains unclear. There is now evidence that two distinct classes of proteins directly interact with retinoic acid: one recently cloned group of nuclear receptors identified as members of the thyroid/steroid receptors family (see Ref. 10 for review) and one previously known group of cytoplasmic proteins (11).

The nuclear receptors, RARα, RARβ, RARγ, and RXRs, are proteins with the same general structure which act as trans-regulating proteins of target genes (12–17). The cytoplasmic proteins, CRBP and CRABP, despite considerable structural homology, differ from each other by their retinoid ligand-binding specificities and lack of immunological cross-reactivity (18, 19). A close correlation between a differential retinoic acid receptor expression, CRABP detection, and retinoic acid concentration is now well documented in embryogenesis (20), thus confirming the role of retinoic acid and its binding proteins in tissue development. In the chick embryo limb bud, a distinct pattern of CRABP distribution and retinoic acid concentration gradient has been reported to determine the limb anteroposterior orientation (21, 22). In adult skin differentiation, a similar gradient distribution of CRABP is noted (23). CRABP, originally detected and identified in chick embryo skin (24), has been found to be present in most of the retinoid-responsive epithelial tissues of the rat, mouse, and chick embryo (25, 26), as well as in various experimental and metastatic tumors (27). In F9 teratocarcinoma cells, striking correlations are noted between the presence of CRABP and its responsiveness to retinoids (6). It was thus subsequently suggested that the biological effects of retinoic acid were mediated through this cytosolic protein, a hypothesis supported by the detection of the CRABP-retinoic acid complex in the nucleus (28). Like the H770 protein (29), CRABP would transport its ligand to the nucleus, bringing retinoic acid in contact with its nuclear receptors. However, exceptions to this rule were found in other retinoic acid-responsive cells and in the fact that biologically active analogues of retinoic acid do not bind to CRABP (30). In HL-60 and U-937 myelomonocytic leukemia cell lines and in AML3 cells which differentiate in the presence of retinoic acid, CRABP levels were not detected (5, 31). The recent striking efficacy of all-trans-retinoic acid in vitro and in vivo in acute promyelocytic leukemic cells (32–36) has prompted more research on the mechanism of action of retinoic acid. This subtype of myeloid leukemia, AML3 [according to the French-American-British classification (37)], represents a clonal expansion of malignant myeloid cells blocked at a specific stage of differentiation resulting in a high proportion of leukemic promyelocytes. It is strongly associated with a specific cytogenetic abnormality, a reciprocal chromosomal translocation between chromosomes 15 and 17 (38). We and others have observed that in this leukemia one allele of the retinoic acid receptor α is rearranged through the specific t(15;17) translocation (39–42), suggesting that the altered receptor may block granulocytic differentiation and participate in the leukemogenic steps of this leukemia (43, 44), leaving the molecular action of retinoic acid in these cells unexplained.

To approach the mechanism of action of all-trans-retinoic acid efficacy in AML3 patients, we undertook to evaluate the role of cytoplasmic binding proteins in these cells. Although, we confirm the absence of detectable levels of retinoid acid-binding proteins on normal and leukemic myeloid cells, we report measurable concentrations of a protein which has the characteristics of CRABP in the bone marrow cells of AML3 patients treated with all-trans-retinoic acid. These results suggest that the induction of determinant parameters of intranuclear concentration of retinoic acid, such as CRABP, must be
taken into account when monitoring the efficacy of retinoic acid therapy in these patients.

MATERIALS AND METHODS

Materials. All-trans-retinoic acid and all-trans-retinol were obtained from Aldrich; all-trans-[3H]retinoic acid (2035 GBq/mmole) and all-trans-[3H]retinol (8769 GBq/mmole) were from Du Pont/New England Nuclear. All-trans-retinoic acid was prepared in absolute ethanol as a stock solution at 10⁻² M and stored in the dark at −80°C.

Myeloid Cell Preparation, Culture, and Differentiation Assay. The mouse fibroblast cell line, 3T6 (gift from Dr. Yaniv, Institut Pasteur, Paris, France) was grown in Dulbecco’s modified Eagle’s medium supplemented with 7% heat-inactivated fetal calf serum from Gibco. Human myeloblastic leukemia HL-60 cells (gift from Dr. Breitman, National Cancer Institute, Bethesda, MD), U-937 cells (gift from Dr. Rosenfeld, Villejuif, France), and human promyelocytic leukemia NB-4 cells (gift from Dr. Lanotte, Institut d’Hematologie, Hopital Saint-Louis, Paris, France) were grown in RPMI 1640 supplemented with 15% fetal calf serum, l-glutamine (2 mM), penicillin (100 IU/ml), and streptomycin (1%). All cultures were incubated at 37°C in a 5% CO₂ and in a humidified atmosphere. Normal peripheral blood lymphocytes were isolated from heparinized normal blood samples after Ficoll-Hypaque density gradient and adherence of monocytes to the plastic surface. Polymorphonuclear cells were isolated in the remaining pellet by density gradient with a concentrated glucose solution (5 g/liter glucose and 5 g/liter gelatin) and resuspended in culture medium. Bone marrow or blood samples from 15 patients with acute myeloblastic leukemia were collected in heparinized tubes. Mononuclear cells were removed, and the leukemic cell fraction was obtained and cultured as already described for 5 days in the presence or absence of 10⁻⁸ M all-trans-retinoic acid (8). The extent of differentiation by retinoic acid was assessed by morphology and the generation of superoxide anion O₂⁻ production was visualized by the nitroblue tetrazolium reduction assay as previously described (8).

Preparation of Cytosolic Extracts. Fresh human leukemic cells, myeloid human leukemia HL-60, U-937, and NB-4 cell lines, 3T6 mouse cell line, and normal blood cells were washed once in medium without fetal calf serum and in cold sodium phosphate buffer before protein extraction. Cells were homogenized at full speed three times for 20 seconds in ice-cold extraction buffer (100 mM Tris-50 mM NaCl-5 mM EDTA-2 mM diithiothreitol, pH 7.4) using a tissue homogenizer Polytron (Kinematica GmbH, Luzern, Switzerland). Supernatants were obtained by centrifugation at 4°C and 100,000 × g for 60 min to remove insoluble material. Protein concentrations were estimated with a Pierce protein assay kit with bovine albumin as standard.

Incubation with Retinoids and Retinol. An aliquot containing 300 µg protein from the cytosolic fraction was incubated with 300 nM of either all-trans-[3H]retinoic acid or all-trans-[3H]retinol in the presence or absence of a 200-fold excess of unlabeled retinoic acid or retinol, in a final volume of 100 µl. After 16 h at 4°C in the dark, the Tritiated CRABP-retinoic acid or tritiated CRBP-retinol complex was separated by polyacrylamide gel electrophoresis.

Slab Polyacrylamide Gel Electrophoresis. Incubated cytosolic extracts were subjected to vertical slab gel polyacrylamide electrophoresis under dim light at 14°C as described by Siegenthaler and Saurat (46) with slight modifications of the method. Briefly, sample volumes of 70 µl of cytosolic fraction extracts were applied to each well, and electrophoresis was carried out for 4 h at 40 mA. The different gel fractions were hydrolyzed as described previously (46). Radioactivity was determined and specific all-trans-retinoic acid binding was calculated as the difference between the total binding and nonspecific binding.

Saturation Binding and Scatchard Plot Analysis. Saturation experiments were carried out to measure the apparent Kd of the cytoplasmic retinoic acid-binding protein of the NB-4 cell line. Equilibrium binding affinities were measured by performing binding assays of cytosolic extracts with increasing amounts of [3H]retinoic acid (0.1-100 nM) in the presence or absence of a 200-fold excess of retinoic acid. After incubation, as described above, the extracts were then subjected to vertical slab gel polyacrylamide electrophoresis. At each retinoid concentration, the number of retinoid molecules bound was determined and Scatchard plot analysis performed.

Gel Filtration Assays. Cytosolic fractions were incubated with [3H]retinoic acid (60 nM). After incubation, the CRABP-retinoic acid complex was separated by a single-step gel filtration on a Sephadex G-25 column (1 × 80 cm) equilibrated with an extraction buffer containing 10 mM sodium azide. Molecular weight calibration was achieved using calibrated markers. Elution of the binding protein was followed by monitoring of the absorbance at 280 nm, and the radioactivity recovered in each fraction was determined.

Analysis of CRABP and CRBP Gene Expression. To study the expression of the CRABP and CRBP genes in 3T6, HL-60, and NB-4 cells, Northern blot analyses were performed from pellets of 10⁶ cells. The total RNA was isolated by the guanidium isothiocyanate-cesium chloride (4 M) centrifugation method, and poly(A⁺) mRNA was obtained as described previously (47). Total RNA (15 µg/lane) or poly(A⁺) mRNA (5 or 20 µg/lane) was fractionated by electrophoresis on a 1% agarose-2.2 M formaldehyde gel after denaturation in formamide loading buffer. After electrophoresis, RNA fragments were transferred onto a nitrocellulose filter (Hybond C, Amersham). Hybridizations of the blots were done as previously described (39). The cDNA probes used were a total 452-base pair cDNA-CRABP (EcoRl-BamHI) probe and a 590-base pair cDNA-CRBP (PstI-BamHI) probe, both subcloned in pGEM3 plasmids. The CRABP and CRBP cDNA probes were gifts from Dr. Nilsson (Huddinge, Sweden). A rat glyceraldehyde-3-phosphate dehydrogenase probe was used to rehybridize the filters and normalize RNA expression.

RESULTS

Detection of Cellular Retinoic Acid-binding Activity in Hematopoietic Cytosol Extracts of AML3 Patients Treated with All-trans-Retinoic Acid. The presence of cellular retinoic acid binding can be detected in high-speed supernatants of homogenized tissues by a variety of means, such as centrifugation on a sucrose gradient (23, 24, 48), gel filtration chromatography and slab electrophoresis (24, 46), and immunological detection (48). We have chosen electrophoresis to isolate cellular retinoic acid-binding protein from cellular retinol-binding protein because both have a different migration position.

The cytosol extracts of hematopoietic cells from 15 patients (1 AML1, 13 AML3, 1 AML5) were thus analyzed. Of these AML3 patients, 9 were tested before treatment with all-trans-retinoic acid, 9 during, and 1 after withdrawal (Table 1).

A specific radioactivity peak of the [3H]retinoic acid was detected in the cytosolic extracts obtained from the mononuclear cell fraction of all 8 AML3 patients (patients 2, 3, 6, and 9–13), between days 30 and 90 of treatment with all-trans-retinoic acid (45 mg/m²/day). This peak was not detected early in the treatment (patients 7 and 11). One month after withdrawal of all-trans-retinoic acid therapy, the [3H]retinoic acid-binding protein was still detected in the cytosolic extracts (patient 13); however, it was no longer detected when bone marrow samples were taken 3 or 6 months after withdrawal of therapy in the same patient. In one patient in whom sequential studies were performed, the height of the peak increased with the duration of treatment with all-trans-retinoic acid (Table 1). No radioactivity peak was observed in the AML3 cells of these patients prior to retinoic acid therapy (patients 2–5, 9, and 12–15) or in other myeloid leukemic subtypes (patients 1 and 8), HL-60, U-937 cell lines, or normal myeloid peripheral blood cells (lymphocytes, polymorphonuclear cells, or monocytes).

Characterization of the Retinoic Acid-binding Protein Induced in Hematopoietic Cytosol Extracts of AML3 Patients. In hematopoietic cytosol extracts of AML3 patients, the radioactiv-
CRABP INDUCTION DURING ALL-TRANS-RETINOIC ACID THERAPY

Table 1 CRABP-retinoic acid complex detection in cells from AML patients before and during in vivo all-trans-retinoic acid treatment

<table>
<thead>
<tr>
<th>Patient</th>
<th>AML subtype</th>
<th>Duration (d) of all-trans RA therapy</th>
<th>CRABP (fmol/mg protein)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>AML1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>AML3</td>
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<td>0</td>
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<tr>
<td>3</td>
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<td>7.31</td>
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<tr>
<td>5</td>
<td>AML3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>AML3</td>
<td>60</td>
<td>1.2</td>
</tr>
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<td>7</td>
<td>AML3 V</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>AML5</td>
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<td>1.5</td>
</tr>
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<td>17</td>
<td>AML3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>18</td>
<td>AML3</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* French-American-British morphological classification (37).
* AML3 patients were treated with an oral dose of 45 mg/m²/day all-trans-retinoic acid for 3 months.
* CRABP cell concentration was assumed to be proportional to the specific radioactivity peak area and was estimated by using the NB4 cell line as standard.
* Number of days without retinoic acid after obtaining complete remission.

Equilibrium binding curves of cytosolic extracts incubated with various concentrations of [³H]retinoic acid show the binding of [³H]retinoic acid to CRABP as a function of the retinoic acid concentration. Optimal binding was observed at concentrations >10 nM. Scatchard plot analysis of the data via the least-squares method yielded a linear plot (Fig. 4, inset), indicating a single class of binding sites. The apparent equilibrium Kₐ was calculated to be 3 ± 0.2 nM (mean ± SD, n = 3) in NB-4 cells. NB-4 cells were estimated to contain 1300 ± 100 (n = 3) CRABP molecules/cell corresponding to 41 fmol/mg of cytosolic protein. The median of CRABP level estimated in AML3

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CRABP INDUCTION DURING ALL-TRANS-RETINOIC ACID THERAPY

Fig. 3. Gel filtration assay. Molecular weight of \([^3H]\)retinoic acid binding in cellular protein cytosol was determined by gel filtration on Sephadex G-25. Aliquots of NB-4 cell cytosols were incubated with 60 nm \([^3H]\)retinoic acid (C) and with 200-fold excess of unlabeled retinoic acid (O). The radioactivity in each fraction (0.5 ml) was then determined. On the calibration curve for molecular weight determination, arrows indicate specific molecular weight markers as determined by spectrophotometry \((A_{280}\text{nm}).\) These protein markers include ovalbumin (ovalbumine) \((43,000),\) myoglobin (myoglobin) \((17,500),\) RNase (RNase A) \((13,000),\) and cytochrome C (CytoC) \((12,500).\) Each filtration assay was performed twice.

Fig. 4. Saturation binding of Scatchard Plot analysis. Increasing concentrations of \([^3H]\)retinoic acid \((0.1-100 \text{nM}; \text{specific activity}, 55 \text{Ci/mmol})\) were incubated with cytosolic extracts of NB-4 cell line in the absence (O) or presence (O) of 200-fold excess of unlabeled retinoic acid. After 16 h of incubation, \([^3H]\)retinoic acid binding was analyzed by polyacrylamide gel electrophoresis. The calculated total binding was analyzed by polyacrylamide gel electrophoresis. The calculated total binding was 5.3 fmol/mg cytosolic protein (minimum, 1.2; maximum, 8) (Table 1).

Absence of Retinoid-binding Protein Detection in AML3 Cells after in Vitro Treatment with All-trans-Retinoic Acid. Cells from human myeloid leukemic cell lines (HL-60, NB-4, U-937) and from fresh human AML cells were incubated for 6 days in suspension culture in the presence of \(10^{-6}\) M all-trans-retinoic acid and induced to differentiate to polymorphonuclear cells for HL-60, NB-4 and AML3 cells and to monocytes for U-937 cells (Table 2). No CRABP-specific radioactive peak was observed after 6 days of incubation in U-937, HL-60, and fresh AML cells. The same quantity of \([^3H]\)retinoic acid-CRABP complex was detected in the NB-4 cell line before and after incubation with \(10^{-6}\) M all-trans-retinoic acid.

Analysis of the CRABP and CRBP Gene Expression in Hematopoietic Cells. RNA isolated from human leukemic HL-60, NB-4 cells, and 3T6 mouse fibroblast cells was analyzed. A 1.1-kilobase transcript of the CRABP gene, similar to that detected in 3T6 cells, was noted in NB4 poly(A+) mRNA extracts (Fig. 5). Poly(A+) mRNA from the HL-60 cell line, in which repeatedly we detected no CRABP protein level, weakly hybridized to the CRABP probe. These data have been confirmed by reverse transcriptase/polymerase chain reaction with specific oligonucleotide sequences of the CRABP human gene (data not shown). The CRBP gene expression was found in only mouse fibroblast 3T6.

DISCUSSION

In the present study, we demonstrate that in the cytosol extracts of bone marrow cells of AML3 patients treated with all-trans-retinoic acid significant levels of a retinoic acid-binding protein were detected. This protein presents major biochemical characteristics of the known cytoplasmic binding proteins CRBP and CRABP (11, 18, 19, 46) (specific binding to retinoic acid and a molecular weight of 14,500). However, distinct electrophoretic migration in the absence of detergent (sodium dodecyl sulfate) and absence of CRBP mRNA expression strongly suggests that the cytoplasmic binding protein induced is CRABP.

Significant CRABP levels were only detected after a prolonged in vivo treatment with all-trans-retinoic acid. A minimal time of treatment was necessary (30 days), and no protein was detectable after 5 days of incubation of leukemic cells with all-trans-retinoic acid treatment was found to be 5.3 fmol/mg cytosolic protein (minimum, 1.2; maximum, 8) (Table 1).

Table 2 Detection of CRABP in human myeloid leukemic cell lines and fresh human myeloid blasts after in vitro incubation with all-trans-retinoic acid during 6 days

<table>
<thead>
<tr>
<th>Human myeloid cells</th>
<th>Control</th>
<th>1 (\mu)M all-trans-retinoic acid</th>
<th>% differentiating cells*</th>
</tr>
</thead>
<tbody>
<tr>
<td>U-937</td>
<td>–</td>
<td>–</td>
<td>94 ± 4</td>
</tr>
<tr>
<td>HL-60</td>
<td>+</td>
<td>+</td>
<td>98 ± 2</td>
</tr>
<tr>
<td>NB-4</td>
<td>+</td>
<td>+</td>
<td>99 ± 4</td>
</tr>
<tr>
<td>AML1(^a) ((n = 2))</td>
<td>+</td>
<td>+</td>
<td>4 ± 4</td>
</tr>
<tr>
<td>AML3(^a) ((n = 8))</td>
<td>+</td>
<td>+</td>
<td>99 ± 9</td>
</tr>
</tbody>
</table>

* Percentage of differentiated cells treated with all-trans-retinoic acid minus the spontaneous differentiation observed in control cells, mean ± SD.

\(^a\) Fresh human myeloid leukemic cells from patients with different AML subtypes (French-American-British classification).

Fig. 5. Analysis of CRABP and CRBP gene expression in RNA from myeloid leukemic cells and 3T6 mouse fibroblasts: Poly(A+) RNA (20 \(\mu\)g) from HL-60 \((lane 1)\) and (5 \(\mu\)g) NB-4 \((lane 2)\) and total RNA (15 \(\mu\)g) from 3T6 mouse fibroblasts \((lane 3)\). Blots were rehybridized with a glyceraldehyde-3-phosphate dehydrogenase probe to normalize RNA expression.
trans-retinoic acid whether in vitro or in vivo. This induction may not be specific of hematopoietic cells and may occur in other tissues, a possibility which is now being investigated. Increase of CRABP and not CRBP levels in nonlesional skin of psoriatic patients treated with an ethyl ester of retinoic acid has also been reported (49). These CRABP protein inductions may result from the regulation of the CRABP gene in hematopoietic and skin cells as recently observed in embryonic stem cells (50) or lung cells (51). Direct modulation of the CRABP gene by RA was identified and related to the presence of a retinoic acid-responsive element in the promoter region of the gene (50). CRABP has been described in numerous tissues in adult life and embryogenesis (3, 11, 19-25, 52, 53). The quantity detected is variable between 1 nmol/mg protein in seminal vesicles to 26 fmol/mg in dermis (23, 54). The values we have detected of 41 fmol CRABP/mg protein is closest to that observed in the skin and its $K_d$ of 3 nm is within the lowest limit of the estimated dissociation constants of other tissues (2-400 nm) (11, 23, 48, 55). Although the precise functions of CRABP remain to be elucidated, its differential expression in embryonic and skin differentiation suggest that it plays a role in the effect of RA on differentiation and development. The proposed role of this cytosolic binding protein might be to sequestrate, metabolize retinoic acid, or regulate its access to the nucleus.

All-trans-retinoic acid has been recently established as an alternative therapy in human acute promyelocytic leukemia (32-34). After a median time of 45 days, patients achieve complete remission via differentiation of the leukemic clone (32-36). All-trans-retinoic acid therapy offers multiple advantages in that not only does disseminated intravascular coagulation rapidly regress but most patients have little transfusion or antibiotic requirements and may be treated on an outpatient basis. Consolidation therapy relays, however, on chemotherapy because early relapses are observed when retinoic acid is maintained (32-36, 56). Furthermore, patients who subsequently relapse have always shown resistance to retinoic acid (36, 56, 57). In vitro response of RA in the AML3 cells of these patients is either absent, weaker, or identical with the differentiation induction obtained prior to therapy (56, 57).

Although the mechanisms by which RA triggers differentiation in AML3 patients is not completely understood, it is likely that RA-binding proteins and effective RA concentrations are determinant factors. We and others have demonstrated the presence of an abnormal RARα receptor in these cells resulting from the t(15;17) translocation specifically observed in this leukemia (39-42). By the study reported here, we confirm data (31) showing that no CRABP protein is detected in AML3 cells. Our previous in vitro studies have further stressed the importance of the structure and dose of the retinoid used to achieve optimal differentiation (8) through or strongly related to an increased expression of the normal remaining RARα gene (58). The clinical observation of early relapses with RA maintenance therapy and secondary resistance of patients in relapse questions the effect of long-term RA therapy in these patients. Reported data in these patients is scarce. The discrepancy between in vitro and in vivo effect of RA in these patients suggests that data obtained from the leukemic cells alone are not sufficient to apprehend the inherent mechanism behind the induced resistance. Our own data were confirmed by Warrel et al. (57) and do not show modification of the fusion of the RARα and the promyelocytic genes or normal RARα genes in these cells. An altered metabolization or bioavailability of RA may be suggested by the inability of these patients to achieve effective plasma RA concentrations (57). Recently, Boylan and Gudas (59) showed that transfection of the CRABP gene in F9 teratocarcinoma cells decreases responsiveness of retinoic acid in these cells. Further investigations are required to suggest that CRABP induction in AML3 cells may be in itself responsible for the in vivo secondary resistance.

The novel data presented in this study of the induction of a retinoic acid cytosolic binding protein, CRABP, in normal and malignant cells of AML3 patients after prolonged RA therapy suggest that parameters which regulate RA intranuclear concentrations may be modulated during RA therapy and should be studied and taken into account when evaluating long-term effect of RA in AML3 patients.

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