Effect of n-3 and n-6 Fatty Acids on Proliferation and Differentiation of Promyelocytic Leukemic HL-60 Cells


Promyelocytic leukemic HL-60 cells were incubated with different fatty acids. Arachidonic acid (AA; 20:4, n-6) and eicosapentaenoic acid (EPA; 20:5, n-3) were the most potent inhibitors of proliferation in a dose-dependent way. Retinoic acid (RA) was used as a positive control. Inhibitors of cyclooxygenase and lipoxygenase or addition of antioxidants did not influence the effect of EPA or AA on cell proliferation. Increased capacity to generate superoxide anions after phorbol ester treatment and a reduced serglycin messenger RNA level in cells treated with AA or EPA indicated that these fatty acids induced differentiation in HL-60 cells similar to that induced by RA. However, down-regulation of the c-myc mRNA level, also typical for differentiation with RA in HL-60 cells, was not observed in cells incubated with AA or EPA.

STRONG EVIDENCE exists that a high intake of polyunsaturated fatty acids of the n-3 series may have beneficial health effects, particularly with reference to coronary heart disease. 1,3 A series of studies have demonstrated that these fatty acids have many biological effects, ranging from decreasing the levels of serum triacylglycerols 4 and reducing blood pressure 5-6 to modulating symptoms in patients with inflammatory diseases. 7-9 Effects of polyunsaturated fatty acids on tumor development have recently been evaluated in experimental studies. Fatty acids from the n-6 series may enhance tumor growth. 10,11 On the other hand, n-3 fatty acids may inhibit the growth of tumor cells both in vivo and in vitro, 2,13 decrease metastasis 4 and cachexia in animals with tumors, 12 and increase cytotoxic effects of some chemotherapeutic agents. 14 In the future, dietary intake of n-3 fatty acids may be recognized as an inhibitor of tumor growth and may be used to increase the efficiency of chemotherapy.

Several explanations exist for how n-3 fatty acids may affect cell proliferation; docosahexaenoic acid (DHA) may increase membrane permeability in tumor cells, and render the cells more susceptible to anticancer drugs or to destruction by the immune system. 17 Conversion of fatty acids to eicosanoids or lipid peroxidation products may influence cellular processes such as proliferation, chemotaxis, and motility. 18,20 Influence by fatty acids on signal transduction through interactions with protein kinases, lipases, or G proteins have become a subject of investigation. 21,22 Furthermore, influence on gene transcription by fatty acids and/or their derivatives through interaction with nuclear receptors in the peroxisomal proliferator activated receptor (PPAR) family may be of importance. 23

The human promyelocytic cell line HL-60 24-25 has been used to study the effect of different agents on differentiation and proliferation of transformed myeloid cells. 26 When the cells are exposed to agents such as retinoic acid 27 (RA) or dimethyl sulfoxide, 28 they differentiate into granulocyticlike cells. Exposed to phorbol esters, the cells differentiate to macrophagelike cells. 29 In the present study, we used HL-60 cells to investigate possible mechanisms responsible for the inhibition of cell proliferation by very long chain polyunsaturated fatty acids. We describe the effects of arachidonic acid (AA; n-6) and eicosapentaenoic acid (EPA; n-3) on proliferation, differentiation, necrosis, and apoptosis and compare these to the effects of RA. Results show that polyunsaturated fatty acids inhibit the proliferation rate and induce differentiation, necrosis, and apoptosis in HL-60 cells, but to different extents and possibly through different mechanisms.

MATERIALS AND METHODS

Materials. Stearic acid (SA), oleic acid, (OA), linoleic acid (LA), AA, arachidonic acid (AdA), α-linolenic acid (LnA), EPA, DHA, all-trans RA, bovine serum albumin (BSA; essentially fatty acid free), nitroblue tetrazolium (NBT), trypan blue, phorbol 12-myristate 13-acetate (PMA), guanidinium isothiocyanate, 3(N-morpholino) propane-sulfonic acid, formamide, formaldehyde, Hoechst 33258, cytochrome c, superoxide dismutase, indomethacin, nordihydroguaiaretic acid (NDGA), vitamin E, and butylated hydroxytoluene (BHT) were all purchased from Sigma Chemical Co, St Louis, MO. Agarose was obtained from FMC Bioproducts, Rockland, MN, and (13H)thymidine and (α32P)dCTP from DuPont-NEN Research Products, Du Pont Scandinavia AB, Stockholm, Sweden. The probe for glyceraldehyde 3-phosphate dehydrogenase (G3PDH) was supplied from Clontech Laboratories, Palo Alto, CA. The third exon of the c-myc gene was obtained from Dr Kari Blomhoff. The open reading frame of serglycin was obtained via polymerase chain reaction from a U-
937 library. A leukotriene B₄ kit was obtained from Amersham, UK.

**Cells.** All human cell lines used were purchased from ATCC, Rockville, MD, except for the monocytic cell line U937-cloned 1, which was obtained from Dr K. Nilsson, University of Uppsala, Sweden. U937-1 is the most mature of the U937 sublines available. HL-60 cells were cultured in Iscove’s medium from Whittaker Bioproducts, Walkersville, MD. The medium was supplemented with 10% heat-inactivated fetal calf serum (FCS; Gibco, Paisley, Scotland), 1-glutamine (2 mmol/L), and gentamycin (0.1 mg/mL). U937-1 cells and the monocytic cell line THP-1 were cultured in RPMI 1640 with 10% FCS, 100 IU/mL penicillin, 100 μg/mL streptomycin, and 2 mmol/L 1-glutamine (all from Whittaker). The human colon carcinoma cell line CaCo-2 was cultured in Dulbecco’s modified Eagle’s medium with 20% FCS, 10 μg/mL of insulin, 1% nonessential amino acids and antibiotics, and 1-glutamine as for U937-1 and THP-1 cells. Fatty acids were added to the cells and treated with concentrations from 10 μM to 100 μM.

**RESULTS**

**Flow cytometry.** Blue fluorescence from Hoechst 33342 stain was measured at 550 nm in an Argus 100 flow cytometer (Skatron AS, Lier, Norway). After transferring filters to a Zeta-Probe membrane over night under the same conditions as described above, the membranes were washed for 15 minutes at room temperature with 2× SSC/0.1% SDS, 0.5× SSC/0.1% SDS, and 0.1× SSC/0.1% SDS successively. The size of the messenger RNA (mRNA) was determined with reference to 18S and 28S recombinant RNA (rRNA), which was visualized by ethidium bromide staining. After autoradiography (Fuji medical X-ray film, Japan) the densities of the signals were determined by scanning the film with a Bio-Image scanner and analyzing it with a program from Bio-Image (Millipore, Bedford, MA). To calibrate the mRNA signal levels with an internal standard in addition to total RNA, filters were stripped and rehybridized with a probe for G3PDH, which is regarded as a “housekeeping gene” because expression of this gene often remains refractory to many common gene transcription-inducing agents.

**Polyunsaturated fatty acids from the n-6 series—LA (18:2), AA (20:4), and AdA (22:4)—were also tested for their ability to inhibit DNA synthesis (Fig 1B). LA did not inhibit incorporation of (³H)thymidine after 1 and 3 days of exposure. In contrast, AdA reduced (³H)thymidine incorporation by 40% after day 1 and by 60% at days 2 and 3. AA was the strongest inhibitor among the n-6 fatty acids tested: after 1 and 3 days of incubation, (³H)thymidine incorporation was reduced by 85% and 90%, respectively.**

**Northern blots.** Total RNA was extracted via the guanidinium isothiocyanate method with centrifugation through a CsCl gradient and subsequent phenol/chloroform extractions. Concentration of RNA was determined by absorption at 260 nm. Twenty micrograms total RNA was denatured for 15 minutes at 50°C in 50% formamide and 6% formaldehyde, followed by 15 minutes on ice, and then transferred to a 1% agarose gel with 2.2 mol/L formaldehyde dissolved in 1X MOPS (10X MOPS = 0.5 mol/L 3(N-morpholino) propane-sulfonic acid, pH 7.0, and 0.01 mol/L EDTA, pH 7.5) for size fractionation. The presence of equal amounts of RNA in each lane was ensured by inspection after ethidium bromide staining.

**Polynsaturated fatty acids from the n-3 series—EPA (20:5) and DHA (22:6) caused a marked reduction in the incorporation of (³H)thymidine: 60% and 70% after 1 day and 85% and 95%, respectively, after 3 days. The amount of BSA used to present the fatty acids to the cells, reduced (³H)thymidine incorporation by 10% to 30%.
During cell-counting studies, BSA alone, in concentrations from 6 to 96 μmol/L, reduced cell numbers by up to 20% (not shown). However, the effects observed with BSA were at all times far less than those observed with the very long chain polyunsaturated fatty acids. Reduction in (\textsuperscript{3}H)thymidine incorporation was thus observed in cultures incubated with 60 μmol/L AdA, AA, EPA, and DHA. For further studies, we chose to use AA and EPA because they were the strongest inhibitors of (\textsuperscript{3}H)thymidine incorporation.

**Effects of EPA and AA on (\textsuperscript{3}H)thymidine incorporation and cell number.** HL-60 cells were incubated with AA and EPA in concentrations ranging from 15 to 240 μmol/L for 24 hours (Fig 2). At 15 μmol/L, no inhibitory effect on (\textsuperscript{3}H)thymidine incorporation was observed with either of these two fatty acids. However, a 10% to 20% reduction of the incorporation was observed with 30 μmol/L of both EPA and AA, and this effect increased further with higher concentrations. At 240 μmol/L, AA inhibited (\textsuperscript{3}H)thymidine incorporation by 67%, whereas the corresponding decrease in EPA-treated cells was 83%.

The effect of AA and EPA was also evaluated by cell counting for 5 days. A 75% decrease was noted in the number of cells incubated with 1 μmol/L RA after 5 days as compared with day 1 (Fig 3A). Cells incubated with 15 μmol/L AA (Fig 3B) or EPA (Fig 3C) had a growth curve similar to that observed for untreated cells. However, when cells were exposed to 30 and 60 μmol/L of the respective fatty acids, a 40% to 50% reduction in the number of cells was observed as compared with untreated cells after 5 days. Exposure to 120 and 240 μmol/L AA for 5 days reduced the number of cells by 71% and 82%, respectively. The corresponding figures with EPA were 93% and 97%. Results obtained by cell counting were thus in agreement with (\textsuperscript{3}H)thymidine incorporation studies; AA as well as EPA inhibited (\textsuperscript{3}H)thymidine incorporation and the proliferation rate in a concentration-related manner. In both types of ex-
experiments, EPA was a more potent inhibitor than was AA at the highest concentrations tested.

Effects of EPA and AA on other cell types. To evaluate whether the inhibitory effects of polyunsaturated fatty acids on cell proliferation were a general phenomenon, the monocytic cell lines U937-1, THP-1, and the colon carcinoma cell line CaCo-2 were exposed to OA, AA, and EPA. From Table 1 it is evident that no decrease was seen in the incorporation of (3H)thymidine after 1 day of exposure to 60 μmol/L OA, EPA, or AA in the three human cell lines tested. In contrast, the proliferation of HL-60 cells was inhibited in the presence of EPA and AA but not in the presence of OA. Pilot studies with cell count experiments on the human acute myelogenous leukemia cell lines KG-1 and KG-1a showed no inhibition of proliferation using 60 μmol/L AA and EPA (results not shown).

Flow cytometry. To study the inhibition of proliferation by flow cytometry, cells were incubated with 120 μmol/L AA or EPA or 1 μmol/L RA for 5 days. Cells were analyzed daily for the percentage distribution in the various phases of the cell cycle. Among untreated cells, 48% were in the G1 phase, 39% in the S phase, and 13% in the G2/M phase after 24 hours of incubation (Fig 4A). Only minor changes in this distribution could be observed in control cultures during the incubation period. Similar results were observed with cells exposed to 48 μmol/L BSA for 5 days (not shown). Treatment of HL-60 cells with 1 μmol/L RA (Fig 4B), increased the percentage of cells in the G1 phase to 83% after 1 day. Concomitantly, the percentage of cells in the S and G2/M phases decreased to 9.6% and 7.5%, respectively. Through the rest of the incubation period, an elevated proportion of cells in the G1 phase was observed in RA-treated cells.

An increased proportion of cells in the G1 phase was also observed after treating cells with AA or EPA (Fig 4C and D). After exposure to 120 μmol/L AA for 1 day, the number of cells in the G1 phase was 58% and after 5 days 79%. During the same period, the percentage of cells in the S phase decreased from 30% to 10%. Similarly, after 1 day of incubation with 120 μmol/L EPA, the percentage of cells in the G1 and S phases was 57% and 34%, respectively. After 3 days, 76% of the cells were in the G1 phase compared with only 62% with AA. After 5 days of exposure to EPA, 80% of the cells were in the G1 phase. EPA was thus most effective in causing an increase of cells in the G1 phase with a concomitantly reduced number of cells in the S and G2/M phases.

Decreased proliferation and cell death. It is possible that a decrease in the proliferation of HL-60 cells incubated in the presence of AA or EPA could be attributable to initiation of apoptosis or necrosis. To investigate this possibility further, cells treated with RA, AA, or EPA for 3 days were analyzed by flow cytometry. By analyzing the light-scattering properties of the cells, it is possible to distinguish between apoptotic and necrotic cells. Little apoptosis or necrosis was observed in untreated cells (Fig 5A). When HL-60 cells were exposed to 1 μmol/L RA for 3 days, a portion of the cells went into apoptosis. The position of the apoptotic cells is indicated for the RA-treated cells in Fig 5B. In contrast, both apoptosis and necrosis could be observed in HL-

### Table 1. Effect of Fatty Acids on (3H)Thymidine Incorporation (% of control) in Different Cell Types

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>OA (60 μmol/L)</th>
<th>AA (60 μmol/L)</th>
<th>EPA (60 μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaCo-2</td>
<td>103</td>
<td>106</td>
<td>107</td>
</tr>
<tr>
<td>HL-60</td>
<td>100</td>
<td>98</td>
<td>99</td>
</tr>
<tr>
<td>THP-1</td>
<td>103</td>
<td>97</td>
<td>95</td>
</tr>
</tbody>
</table>

Abbreviations: OA, oleic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid.
Untreated

Time (days)

Untreated

RA

AA

EPA

Cell distribution (% of total)

Time (days)

Fig 4. Flow cytometry. Symbol explanation is shown in the panel for untreated cells (A). HL-60 cells were incubated in the presence of 1 μmol/L RA (B), 120 μmol/L AA (C), or 120 μmol EPA (D) for 1 to 5 days and analyzed for percentage distribution of cells in different phases of the cell cycle. Similar results were obtained in three separate experiments.

60 cells treated with 120 μmol/L AA or EPA. After AA treatment only a minor portion of the cells were necrotic or apoptotic (Fig 5C), whereas a larger percentage of HL-60 cells responded to EPA by going into either apoptosis or necrosis. Data from Fig 5 were used to calculate the percentage of necrotic and apoptotic cells. From Table 2 it is evident that after RA treatment 21% of the cells were apoptotic and 10% were necrotic, whereas the corresponding values for cells treated with AA were 8% and 7%, respectively. However, when cells were exposed to EPA, 19% were apoptotic and 32% were necrotic. There are, accordingly, large differences in the effects of RA, AA, and EPA on the induction of apoptosis and necrosis in HL-60 cells.

Cell differentiation. HL-60 cells are bipotent cells and can differentiate to either monocytes/macrophages or granulocytes. The differentiated monocytes and granulocytes have the ability to reduce NBT and to produce superoxide anion upon stimulation with PMA. Cells were incubated with 120 μmol/L AA or EPA or 1 μmol/L RA for 3 days. Among untreated cells, 5% were NBT positive, and in cells treated with 1 μmol/L of RA, the corresponding number was 14%. Exposure to 120 μmol/L AA or EPA yielded 24% or 19% NBT-positive cells, respectively (Fig 6A).

The same cultures were also tested for their ability to go through respiratory burst, assessed by reduction of cytochrome c, after stimulation with PMA (Fig 6B). Incubation with 120 μmol/L AA or EPA enhanced the reduction of cytochrome c 4.2 and 4.7 times, respectively, when compared with untreated cells, whereas RA treatment increased respiratory burst twofold. Addition of superoxide dismutase resulted in a complete abrogation of cytochrome c reduction both with fatty acids and RA (not shown).

Antioxidants and (3H)thymidine incorporation. To examine whether the reduced incorporation of (3H)thymidine induced by AA and EPA was due to the generation of peroxidation products inhibiting DNA replication, the effect of antioxidants in combination with fatty acid was investigated. Increasing concentrations of vitamin E or BHT were added
to the cultures before the addition of 60 μmol/L AA or EPA. Neither of the two antioxidants affected the reduced (H)thymidine incorporation in HL-60 cells exposed to AA and EPA for 24 hours (Fig 7A and B).

Eicosanoids and (H)thymidine incorporation. The ability of AA and EPA to inhibit proliferation of HL-60 cells may be due to the generation of their respective eicosanoid products, which may interfere with regulation of cell division. Two key enzymes in the generation of eicosanoids (lipoxygenase and cyclooxygenase) may be inhibited with NDCA and indomethacin, respectively. Neither indomethacin nor NDGA alleviated the inhibition of (H)thymidine incorporation by AA or EPA (Fig 8A and B). In contrast, an additive effect of both AA and EPA with NDGA could be observed. Furthermore, NDGA alone promoted a dose-dependent inhibition of (H)thymidine incorporation. Control experiments showed that in both AA-treated and untreated HL-60 cells the release of leukotriene B4 was inhibited when cultured in the presence of NDGA (result not shown). An increase in the level of eicosanoids after AA or EPA treatment does not seem to be a mechanism by which these fatty acids decrease the proliferation rate of HL-60 cells.

Expression of c-myc and serglycin mRNA. HL-60 cells contain amplified copies of the c-myc gene, which is down-regulated during in vitro differentiation. We wanted to investigate whether the decrease in proliferation rate and increase in capacity for oxidative burst was correlated to a decrease in the expression of c-myc in cells exposed to AA and EPA. However, the level of c-myc expression was not reduced after treatment with 60 μmol/L AA or EPA for 2 days (Fig 9A). In contrast, the level of c-myc was reduced to an undetectable level after exposure of the cells to 1 μmol/L RA for the same time period. Using 120 μmol/L concentrations of AA and EPA for 3 days did not lead to any down-regulation of the c-myc mRNA.

| Table 2. Percentage of Normal, Apoptotic, and Necrotic Cells After 3 Days of Incubation as Determined by Flow Cytometry |
|-----------------|-----------------|-----------------|
|                 | Normal | Apoptotic | Necrotic |
| Untreated       | 94     | 2        | 4       |
| RA (1 μmol/L)   | 60     | 21       | 10      |
| AA (120 μmol/L) | 85     | 8        | 7       |
| EPA (120 μmol/L)| 49     | 19       | 32      |

Abbreviations: RA, retinoic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid.
Fig 6. Cell differentiation. HL-60 cells were treated with 1 μmol/L RA, 120 μmol/L AA, or 120 μmol/L EPA for 3 days and subjected to the NBT test (A) or the respiratory burst test (B). Similar results were obtained with both tests in three separate experiments.

Serglycin is the major proteoglycan in hematopoietic cells, including HL-60 cells. Proteoglycan biosynthesis decreases in differentiated HL-60 cells and the expression of serglycin has recently been shown to decrease in the monocytic cell line U-937 after differentiation. We therefore investigated whether the expression of serglycin was affected after treatment of HL-60 cells with AA or EPA for 2 days. Both fatty acids and RA promoted reduced expression of the serglycin transcript. When the signal levels were calibrated with that of the G3PDH transcript, the serglycin mRNA observed after treatment with 60 μmol/L AA or EPA or 1 μmol/L RA was reduced by 65%, 80%, and 95%, respectively (Fig 9B).

DISCUSSION

Our findings demonstrate that very long chain polyunsaturated fatty acids from the n-6 and the n-3 series had the ability to reduce (3H)thymidine incorporation and proliferation.
tion rate of HL-60 promyelocytic leukemic cells. Reports on the effects of fatty acids on cancer cell proliferation are contradictory. In some studies, only n-3 fatty acids inhibited tumor cell proliferation, whereas n-6 fatty acids had no effect or even stimulated cell proliferation. Other reports showed inhibition of cancer cell proliferation with both n-3 and n-6 fatty acids similar to our findings. These discrepancies may be due to the use of different model systems, all expressing different sets of proteins. Some systems may be sensitive to n-3 fatty acids only, whereas others respond to both n-3 and n-6 fatty acids. It has been reported that the proliferation of T-lymphocytes is inhibited by n-3 as well as n-6 polyunsaturated fatty acids. Similar to the results presented here, inhibitors of enzymes involved in eicosanoid synthesis did not abrogate the reduced proliferation rate observed with long polyunsaturated fatty acids from both the n-3 and the n-6 family, nor did the addition of antioxidants. This finding is in contrast to several studies in which antioxidants or inhibitors of eicosanoid synthesis abrogated effects observed with n-6 and n-3 fatty acids. We were furthermore able to show that the inhibitory effects of AA and EPA were restricted to HL-60 cells and not seen in two different monocytic cell lines and one colon carcinoma cell line.

However, the data presented also showed that RA, AA, and EPA induced both apoptosis and necrosis in HL-60 cells. After 3 days of RA treatment, 21% of the cells became apoptotic and 10% necrotic, whereas AA treatment shifted 8% to apoptosis and 7% to necrosis. In contrast, EPA-treated HL-60 cells contained 19% apoptotic and 32% necrotic cells. The decrease in cell number observed both after EPA and RA treatment (Fig 3) can to a large extent be ascribed to the induction of cell death by these two different pathways. However, it is interesting to note that HL-60 cells treated with AA contained few necrotic cells and fewer apoptotic cells than seen in the RA-treated cultures. Although all three agents inhibited proliferation and induced differentiation to a certain extent, their effects on cellular death processes differed considerably. The differences in effects on apoptosis and necrosis suggest that they operate, at least partly, through different signal pathways.

Several experiments were performed to investigate whether AA and EPA induced differentiation in HL-60 cells similar to that of RA. Reduction of NBT and generation of oxidative burst were higher in cells incubated with 120 μmol/L AA or EPA for 3 days than in cells treated with 1 μmol/L RA, indicating a higher degree of differentiation in cells treated with fatty acids. Furthermore, the expression of serglycin, which is almost exclusively restricted to hemopoietic cells, is a useful marker for differentiation of these cells. Proteoglycan synthesis has been demonstrated to decrease when HL-60 cells differentiate, and the mRNA level for serglycin in HL-60 cells is similar to that found in cells from patients with acute myelogenous leukemia. If decrease in the serglycin mRNA level is used as a marker for differentiation, HL-60 cells differentiated in the presence of RA, EPA, and AA. DHA combined with RA, has been found to increase the rate of differentiation in HL-60 cells, supporting the notion that very long chain polyunsaturated fatty acids can influence the differentiation process in HL-60 cells.

Down-regulation of c-myc mRNA in differentiating HL-60 cells is well documented and seems to be important in the differentiation process. Use of c-myc antisense is sufficient to induce differentiation in this cell line. Our results show that only RA induced differentiation of HL-60 cells by that criteria. This is in contrast to what we observe when respiratory burst, NBT reduction, and decrease in serglycin mRNA expression are used as parameters; all three agents induce differentiation.
the regulation of differentiation and apoptosis is complex and dependent on cell type and specificity of stimuli. Expression of c-myc also seems to be necessary for several, but not all, forms of apoptosis. It has been demonstrated that activation-induced apoptosis in T-cell hybridomas was inhibited by c-myc antisense, whereas glucocorticoid-induced apoptosis was unaffected by such treatment. In order to define more clearly the differences between RA, AA, and EPA on the differentiation and apoptosis of HL-60 cells, studies are now being performed with flow cytometry together with measurements of mRNA levels of several genes shown to be involved in these processes.

Several reports have appeared on the importance of activation of protein kinase C (PKC) during HL-60 differentiation. Unsaturated fatty acids may also stimulate PKC and down-regulation of serglycin in HL-60 cells can be caused by activation of PKC, suggesting that this may be a mechanism by which fatty acid reduced the level of serglycin mRNA reported here. In pilot studies using a peptide inhibitor of PKC, we were unable to detect any effects on the inhibition of proliferation by AA and EPA. There is also another possible mechanism through which AA and EPA can regulate the serglycin mRNA level. In the promoter of the serglycin gene, a response element for two types of nuclear receptors was found: PPAR and the retinoid X receptor (RXR). These two types of receptors recognize a consensus sequence of six base pairs repeated twice with one intervening base pair. Fatty acids can activate PPAR and PPAR-like receptors found in humans, such as the NUCI or the human peroxisome proliferator activated receptor. The reduced level of serglycin mRNA observed with AA and EPA may thus be caused by an interaction between a fatty acid, a human analog to PPAR, and the serglycin gene promoter. Similarly, RA may, if converted to the 9-cis form, activate the RXR receptor and down-regulate serglycin transcription.

Fatty acids may exert their effects at several levels, both through signal transduction pathways and on gene transcription. At present it is not possible to outline the molecular basis for the differences between RA, AA, and EPA in con-
trolling apoptosis and necrosis in HL-60 cells while at the same time differently affecting the level of c-myc. It has been demonstrated that there is a close link between differentiation and apoptosis in HL-60 cells. Further use of AA and EPA in studies of cell proliferation and apoptosis should provide useful experimental tools to gain further insight into control mechanisms at the molecular level. Before polyunsaturated fatty acids can be considered for use in cancer therapy, or as a dietary support for conventional cancer treatment, these processes must be outlined in further detail.

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