Eicosanoid Production by the Human Gastric Cancer Cell Line AGS and Its Relation to Cell Growth

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ABSTRACT

Eicosanoids have the ability to stimulate or inhibit the proliferation of epithelial cells, and they have been shown to modulate the growth characteristics of certain tumor cell lines. In addition, many epithelial cells have the ability to produce eicosanoids, which may then serve as autocrine growth factors. We have measured the eicosanoids produced by the human stomach cell line AGS using reverse-phase high-performance liquid chromatography. AGS cells were incubated with [3H]arachidonic acid and stimulated to release eicosanoids by the calcium ionophore A23187. Unlike its counterpart from the normal stomach, the AGS tumor cell line produced prominent amounts of the leukotrienes D4, C4, and B4; 6-keto-prostaglandin F1α; thromboxane B2; hydroxyeicosatetraenoic acids; and smaller amounts of other prostaglandins in response to A23187. Under basal condition (in the absence of calcium ionophore), hydroxyeicosatetraenoic acid was produced in greatest relative amount compared with the other eicosanoids.

To elucidate the potential autacoid role of these agents, exogenous eicosanoids were added to AGS cells, and proliferation was measured. Prostaglandins D2 and E2 suppressed the growth of AGS cells in a dose-dependent manner. On the other hand, leukotrienes D4 and C4 had a dose-dependent proliferative effect on cell growth. The lipooxygenase inhibitor nordihydroguaiaretic acid (10⁻⁴, 10⁻³ M) and hydrocortisone (10⁻³ M) had dose-dependent suppressive effects on growth, whereas indomethacin (10⁻⁴ M and 10⁻³ M) had no effect. These results suggest that AGS cells preferentially metabolize arachidonic acid through the 5-lipoxygenase pathway, which results in the production of growth-stimulatory autacoids. Agents that selectively block this arm of eicosanoid metabolism might be useful therapeutic agents in the treatment of certain gastrointestinal cancers.

INTRODUCTION

Eicosanoids are important autacoids that are known to regulate a wide range of physiological processes in gastrointestinal epithelia including the secretion of fluid and electrolytes, mucosal blood flow, and cell proliferation (1, 2). Cells vary in their ability to metabolize AA, and it is becoming apparent that each cell type may have a unique ability to produce PGs, LTs, and other related eicosanoids. It has long been known, for example, that normal gastric epithelium produces certain PGs, but has a relatively limited ability to produce LTs, which may be prominently produced by certain inflammatory cells (3).

Many eicosanoids have been shown to affect the growth of tumor cell lines. For example, certain members of the PGA, PGE, and PGD series (including 12-PGD2, the ultimate metabolite of PGD) are potent inhibitors of growth for certain cultured tumor cells (4–7). PGE2, which is abundantly produced by gastric tissue, has a significant suppressive effect on the growth of the human stomach cancer cell line Kato III (8). Although not studied previously in gastrointestinal epithelium, it has been suggested that some lipoxygenase products may participate in the stimulation of the human leukemia cell line HL-60 (9). Work by several laboratories has suggested that tumors of the gastrointestinal tract may synthesize eicosanoid products that differ from those found in the corresponding normal tissue (10, 11). However, these studies have not definitively ascertained the profile of eicosanoids derived from tumor tissue itself, because of the presence of inflammatory cells in fresh tumor tissue (12, 13). A cultured cell line permits a determination of eicosanoid production by the epithelial cells unambiguously; however, it is possible that cultured tumor cells do not perfectly reflect the behavior of tumors in vivo.

We have hypothesized that some tumor cells may gain a growth advantage by losing their ability to synthesize growth-suppressive PGs and by producing greater amounts of growth-stimulatory eicosanoids. To test this hypothesis, we measured the eicosanoid-metabolizing capacities of the human gastric cancer cell line AGS using [3H]AA as a metabolic precursor and identified the eicosanoid metabolites using reverse-phase HPLC. We found that AGS cells produced a different profile of eicosanoid metabolites than what has been reported previously from normal gastric mucosal cells, that members of the PG family suppressed the growth of AGS cells, and that LTs had a proliferative effect on these cells.

MATERIALS AND METHODS

Materials

The following reagents were purchased from Sigma Chemical Co. (St. Louis, MO): calcium ionophore A23187; DMSO; PGE2, PGD2, BSA, HBSS, PCA; trifluoroacetic acid; 6-keto-PGF1α, PGE2, PGD2, PGF2α, TXB2; trypan blue; indomethacin; NGDA; BrdU; and hydrox- yurea. LTB4, LTC4, LTD4, 12-HHT and 12- and 15-HETE were purchased from Cayman Chemical (Ann Arbor, MI). IMDM and trypsin (0.25%) EDTA (1 mm) were purchased from Gibco Laboratories (Grand Island, NY). Heat-inactivated FBS was from HyClone, Logan, UT. [3H]AA (243 mCi/mmol) and [3H]thymidine were purchased from New England Nuclear (Wilmington, DE). HPLC-grade acetonitrile and methanol were purchased from Mallinckrodt, Inc. (Paris, KY). The tissue culture plates were purchased from Costar (Cambridge, MA). Anti-BrdU monoclonal antibody was purchased from Vector Laboratories (Burlingame, CA). Protein was measured using the Coomassie blue reagent of Pierce (Rockford, IL). All reagents were of highest grade available, and all water was deionized and passed through a MilliQ purification system (Waters Associates, Milford, MA) prior to use.
Methods

Eicosanoids Synthesis by AGS Cells

Cell Culture and Loading with [3H]AA. Two ml of an AGS cell suspension (2 x 10^5 cells/ml) were plated in tissue culture wells, loaded with 0.5 mCi of [3H]AA per well, and incubated at 37°C under 5% CO2 and 95% air. After 18 h of incubation, the labeling medium and floating cells were removed, and the cells were washed twice with HBSS containing 2% BSA and once with HBSS containing 0.1% BSA to remove free unincorporated [3H]AA.

Extraction of [3H]-labeled Eicosanoids. The AGS cell (14) monolayers were incubated in 2 ml of IMDM supplemented with 10% FBS in the presence or absence (using vehicle as control) of 2.0 or 5.0 mM A23187 in DMSO (at a final DMSO concentration of 0.02%) for 1 h. The extraction from culture medium of radiolabeled eicosanoids produced by the [3H]AA-labeled AGS cells was performed by the method of Wescott et al. (15). After 1 h of incubation, culture media from three wells were pooled and added to two volumes of icold methanol. The mixture was centrifuged at 1500 rpm for 5 min, and the methanolic supernatant was diluted in 0.1 M sodium phosphate buffer, pH 7.4, to yield a final methanol concentration of 20%. This 80% aqueous mixture was applied to Sep-Pac C18 cartridges (Waters Associates, Milford, MA) that had been prewashed sequentially with 20 ml of methanol and 20 ml of deionized water. The loaded cartridges were washed with 5 ml of 20% methanol in 0.1 M sodium phosphate buffer followed by 5 ml of deionized water, both of which were discarded. Eicosanoids were eluted with 3 ml of 80% methanol in water. Extracts were then evaporated to dryness under nitrogen, and residues were stored at −70°C pending eicosanoid separation by HPLC.

Separation of [3H]AA Metabolites by HPLC. Eicosanoid separation was accomplished according the method of Peters-Golden and Thebert (13). For identification of radiolabeled eicosanoids, the extracted samples were redissolved in 300 μl of acetonitrile:water:trifluoroacetic acid (33:67:0.1) and subjected to reverse-phase HPLC using a 30- x 0.4-cm Waters reverse-phase column (Waters Associates, Milford, MA). The mobile phase consisted of acetonitrile:water in trifluoroacetic acid at a flow rate of 1 ml/min. Cyclooxygenase metabolites were eluted during an initial isocratic phase (acetonitrile:water:trifluoroacetic acid, 33:67:0.1); the lipoxigenase metabolites and free AA were eluted using a continuous gradient of increasing acetonitrile to 100:0:0:1.

The eluate was continuously monitored for UV absorbance (210 nm for cyclooxygenase products and free AA, 280 nm for leukotrienes, and 235 nm for mono-HETEs). The retention times (min) for authentic eicosanoids standards were as follows: 6-keto-PGF_1α, 7 to 8; TXB_2, 13 to 15; PGF_2α, 16; PGE_2, 18 to 19; PGD_2, 21 to 22; LTC_4, 44; LTD_4, 46; LTB_4, 48 to 49; 12-HHT, 58 to 59; 15-HETE, 72 to 75; 12-HETE, 77 to 78; and 15-HETE, 99 to 100. Cochromatography with authentic eicosanoid standards was used with each sample to control for any variation in retention and elution.

One-ml eluted fractions were collected, and [3H]-labeled products were quantified by liquid scintillation counting in 5 ml of scintillant (Ecolume; ICN Biomedicals, Inc., Irvine, CA).

AGS Cell Growth in Response to Exogenous Eicosanoids

Count of Cell Number and Protein Assay. Suspensions of 2 x 10^5 AGS cells in 3 ml of IMDM supplemented with 10% FBS were plated in tissue culture plates and incubated at 37°C under 5% CO2:95% air overnight ("the preincubation period"). The next day ("time 0"), the medium was replaced with 3 ml of fresh medium containing the following eicosanoids: PGD_2 or PGE_2 (0.5, 1.0, 2.5, 5.0 μg/ml); LTC_4 or LTD_4 (0.01, 0.05, 0.1, 0.25, 0.5 mg/ml); indomethacin (10^−6, 10^−5 M); NDGA (10^−5, 10^−4, 10^−3 M); hydrocortisone (10^−3 M); or vehicle only as control. The medium was adjusted to a final methanol concentration of 0.5%. After 96 h of incubation, cells were harvested using trypsin/EDTA and counted using a hemocytometer; the protein content of the cells was measured. The initial cell numbers at time 0 (1.85 ± 0.27 x 10^5) (mean ± SD, n = 9) and protein contents were not significantly different in each series of experiments.

[3H]Thymidine Incorporation. AGS cells (2 x 10^5) prepared as described above were incubated for 24 h, to which [3H]thymidine was added at a specific activity of 0.5 mCi/ml. To estimate nonspecific incorporation or adherence of thymidine, 10 ml of hydroxyurea (10 mg/ml) were added to some wells. Two h later, 2 ml of ice-cold saline were added, aliquots were removed, and cells were washed twice with PBS. After counting cell numbers, 2 ml of 5% PCA were added, and the dpm value in the acid-insoluble fraction was measured.

BrdU LI. To measure DNA synthesis, 2 x 10^6 AGS cells were prepared as described above, after which BrdU was added at a final concentration of 100 μg/ml for 4 h. To terminate BrdU labeling, ice-cold PBS was added to the wells, and the supernatant was discarded. BrdU-labeled cells were washed twice with PBS and removed from the wells with trypsin/EDTA. Cells were attached to glass slides by centrifugation using a Cytospin-2 centrifuge (Shandon, Pittsburgh, PA) and fixed in 70% ethanol. Cells on the glass slides were treated with 4 N HCl for 30 min to denature the DNA, and immunohistochemical detection of BrdU incorporation was performed using the anti-BrdU monoclonal antibody and the avidin-biotin-peroxidase complex method (16). To calculate the BrdU LI, photographs were taken of at least four different highpower fields randomly selected under the light microscope. Total cells and BrdU-positive nuclei were counted in each field. The LI was defined as the ratio of BrdU-labeled cells to total cells counted.

Data Analysis. The data were expressed as the mean ± SEM of at least 3 to 5 different series of experiments. Individual measurements were done in duplicate or triplicate. Differences between control and treatment groups were compared by the Student t test, and P < 0.05 was regarded significant.

RESULTS

Profile of Eicosanoids Derived from AGS Cells. Fig. 1 shows the elution of [3H]-labeled eicosanoids after separation on the HPLC column. Relative molar amounts of each metabolite may be measured from the profiles. The lower curve (thin, dashed line) indicates eicosanoids produced in the unstimulated state, and upper curves (the dashed and solid bold lines) indicate eicosanoid production after stimulation with 2.0 mM and 5.0 mM calcium ionophore A23187, respectively. The calcium ionophore produced dose-dependent increases in eicosanoid metabolites. Although one cannot measure the total mass of eicosanoids, the relative molar proportions of all AA metabolites may be determined using this method. The major metabolites produced by AGS cells after A23187 stimulation were LTD_4, followed by progressively smaller amounts of 6-keto-PGF_1α, LTC_4, LTB_4, 12-HETE, PGE_2, and PGE_1. Under basal conditions, the profile showed 12-HETE > LTB_4 > LTC_4 > PGE_2 > PGE_1 ≥ 6-keto-PGF_1α > TXB_2 ≥ LTD_4 ≥ 15-HETE > PGD_2 (Fig. 1).

Influence of Exogenous Eicosanoids on Cell Growth. The time course of AGS cell growth is shown in Fig. 2. Both PGD_2 and PGE_2 (5.0 μg/ml) significantly suppressed cell growth at 24, 48, and 96 h (P < 0.01 versus control). However, LTC_4 and LTD_4 (0.5 mg/ml) significantly stimulated cell growth at 48 and 96 h (P < 0.05 versus control).

Dose-Response for Modulating AGS Cell Growth by Eicosanoids. The dose-response relationships for growth suppression by PGD_2 and PGE_2 are depicted in Fig. 3. Increasing the PG concentration from 0.5 to 5.0 μg/ml produced a progressive inhibition of cell growth by both PGD_2 and PGE_2. The 50% effective dose for growth inhibition was in the range of 2.5 to 5.0 μg/ml for each prostanoid.

The change in protein content of the AGS cells produced by PGD_2 and PGE_2 is shown in Table 1. Both PGS reduced the
EICOSANOIDS SYNTHESIS BY AGS CELLS

Fig. 1. Profile of eicosanoids derived from AGS cells. The AGS cells were incubated for 1 h in the presence or absence of the calcium ionophore A23187. The [3H]AA metabolites in the medium released by 6 x 10^6 AGS cells were separated by reverse-phase HPLC. The lowest line (thin, dashed line) represents the elution profile in the absence of A23187; the bold dashed line and bold solid line represent the elution profile in the presence of 2.0 μM and 5.0 μM A23187, respectively. The arrows point to the elution of authentic standards, as labeled.

Fig. 2. Growth curve of AGS cells incubated with PCs or LTs. Lines represent the numbers of AGS cells after incubation with LTD₄, LTC₄, control, PGE₂, and PGD₂ from upper to lower lines, respectively. *, P < 0.05; **, P < 0.01; ***, P < 0.005, compared with control.

Fig. 3. Dose-response of AGS cell growth in response to exogenous PCs. Columns express the mean cell counts (bars, SEM) (n = 3 to 5) expressed as the percentage of control cell number. ■ and ■, PGD₂ and PGE₂, respectively. *, P < 0.05; **, P < 0.01; ***, P < 0.005, compared with control. CONT., control.

Fig. 4. Dose-response of AGS cell growth in response to exogenous LTs. Columns express the mean (bars, SEM) (n = 3) of the percentage of control cell number. ■ and ■, LTC₄ and LTD₄, respectively. *, P < 0.05; **, P < 0.01; ***, P < 0.005, compared with control (CONT.).

The dose-response relationships for stimulation of cell growth by LTC₄ and LTD₄ are shown in Fig. 4. Increasing the LT concentration from 0.01 to 0.5 mg/ml produced progressive increases in cell number for LTD₄, which reached significance at a concentration of 0.05 mg/ml, and a 36.7% increase in cell number was observed at 0.5 mg/ml. Significant growth stimulation was seen for LTC₄ only at the 0.5 mg/ml concentration. In contrast, no concentration of LTC₄ or LTD₄ showed a significant increase in the total protein content of AGS cells (data not shown).

Effect of NDGA, Hydrocortisone, and Indomethacin on Cell Growth. NDGA is an inhibitor of 5-lipoxygenase activity, and hydrocortisone inhibits PLA₂ and may have a variety of other effects on cell growth in culture. If LTs were important stimulators of AGS cell growth, NDGA or hydrocortisone might be expected to inhibit LT synthesis and inhibit cell proliferation. Increasing concentrations of NDGA (10⁻⁴ M, 10⁻³ M, 10⁻² M) and hydrocortisone (10⁻⁵ M) produced no significant effect on cell growth. Since LTs released into the medium may have influenced cell growth prior to the addition of inhibitors, experiments were designed to limit this effect, in which NDGA (10⁻³ M) or hydrocortisone (10⁻⁵ M) was added during the preincubation period. The results of those experiments are shown in Table 2. Increasing the concentration of NDGA (10⁻⁷ M, 10⁻⁶ M, or 10⁻⁵ M) in cells pretreated with 10⁻⁵ M NDGA produced a significant, dose-dependent inhibitory effect on cell growth (P < 0.005). Hydrocortisone (10⁻⁵ M), added to cells that were pretreated with hydrocortisone (10⁻⁵ M), revealed similar results, also shown in Table 2.

The addition of indomethacin (10⁻⁶ M and 10⁻⁵ M) to AGS cells reduced the cell numbers to 94 ± 5 and 97 ± 4% of control wells, respectively.

[3H]Thymidine Incorporation. To further document the effects of exogenous eicosanoids on cell growth, [3H]thymidine incorporation per 10⁶ AGS cells was measured. As demonstrated in Table 3, PGD₂ and PGE₂ (at 1.0 and 5.0 μg/ml) decreased [3H] thymidine incorporation significantly (P < 0.005), whereas LTD₄ (at 0.05 and 0.5 mg/ml) increased incorporation significantly.

<table>
<thead>
<tr>
<th>Concentration (μg/ml)</th>
<th>0.5</th>
<th>1.0</th>
<th>2.5</th>
<th>5.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGD₂</td>
<td>72.2 ± 7.9*</td>
<td>76.3 ± 4.6</td>
<td>61.4 ± 9.8*</td>
<td>45.6 ± 7.2*</td>
</tr>
<tr>
<td>PGE₂</td>
<td>73.3 ± 3.5b</td>
<td>63.1 ± 3.9</td>
<td>54.5 ± 9.4*</td>
<td>40.8 ± 5.7*</td>
</tr>
</tbody>
</table>

* Mean ± SEM of control wells (n = 3 to 4).
# P < 0.05.
¢ P < 0.01 compared with protein content in control wells.

Table 1 Growth response of AGS cells to PCs (total protein content as percentage)

Table 2 Growth response of AGS cells to PGs (total protein content as percentage)
EICOSANOIDS SYNTHESIS BY AGS CELLS

Table 2  Effect of inhibitors of eicosanoid metabolism on cell number and total protein contents of AGS cells

<table>
<thead>
<tr>
<th></th>
<th>NDGA</th>
<th>Hydrocortisone</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Control 10^-7 M 10^-4 M</td>
<td>Control 10^-7 M 10^-4 M</td>
</tr>
<tr>
<td></td>
<td>100 ± 3 e</td>
<td>118 ± 10</td>
</tr>
<tr>
<td></td>
<td>99 ± 8</td>
<td>90 ± 4</td>
</tr>
<tr>
<td></td>
<td>95 ± 4</td>
<td>91 ± 7</td>
</tr>
<tr>
<td>Protein content</td>
<td>100 ± 3</td>
<td>102 ± 7</td>
</tr>
<tr>
<td></td>
<td>95 ± 4</td>
<td>91 ± 7</td>
</tr>
<tr>
<td>Pretreated with inhibitors c</td>
<td>137 ± 5 e</td>
<td>89 ± 7</td>
</tr>
<tr>
<td></td>
<td>79 ± 3 e</td>
<td>57 ± 3 e</td>
</tr>
<tr>
<td></td>
<td>127 ± 3</td>
<td>75 ± 4 e</td>
</tr>
<tr>
<td>Protein content</td>
<td>99 ± 2</td>
<td>91 ± 6</td>
</tr>
<tr>
<td></td>
<td>82 ± 2 e</td>
<td>76 ± 8 e</td>
</tr>
<tr>
<td></td>
<td>113 ± 10</td>
<td>53 ± 13 e</td>
</tr>
</tbody>
</table>

* In the "nonpretreated" group, AGS cells were incubated with NDGA (10^-7 to 10^-4 M) or hydrocortisone (10^-7 M) for 96 h.
* Mean ± SEM.

Table 3  Effect of exogenous PGD2 and PGE2 on [3H]thymidine incorporation into AGS cells

<table>
<thead>
<tr>
<th></th>
<th>PGD2 (µg/ml)</th>
<th>PGE2 (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>0.5 1.0 5.0 50</td>
</tr>
<tr>
<td></td>
<td>10,815 ± 165 e</td>
<td>10,086 ± 207</td>
</tr>
<tr>
<td></td>
<td>9,520 ± 289</td>
<td>7,641 ± 715</td>
</tr>
<tr>
<td></td>
<td>9,400 ± 679</td>
<td>8,463 ± 225</td>
</tr>
<tr>
<td>P value vs. control</td>
<td>P = 0.05</td>
<td>P &lt; 0.005</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>0.5 0.1 5.0 1.0</td>
</tr>
<tr>
<td></td>
<td>75 ± 3</td>
<td>71 ± 7</td>
</tr>
<tr>
<td></td>
<td>87 ± 6</td>
<td>88 ± 3</td>
</tr>
<tr>
<td></td>
<td>89 ± 1</td>
<td>93 ± 2</td>
</tr>
<tr>
<td></td>
<td>100 ± 7</td>
<td>100 ± 7</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>8,034 ± 423</td>
<td>8,034 ± 423</td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.005</td>
<td>P &lt; 0.005</td>
</tr>
</tbody>
</table>

* Mean ± SEM.

Table 4  Effect of exogenous LTs on [3H]thymidine incorporation into AGS cells

<table>
<thead>
<tr>
<th></th>
<th>LTC4 (µg/ml)</th>
<th>LTD4 (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>0.05 0.5</td>
</tr>
<tr>
<td></td>
<td>10,761 ± 797</td>
<td>12,974 ± 289</td>
</tr>
<tr>
<td></td>
<td>121 ± 9.2</td>
<td>129 ± 3</td>
</tr>
<tr>
<td></td>
<td>140 ± 2</td>
<td>140 ± 2</td>
</tr>
<tr>
<td>P value vs. control</td>
<td>P = 0.065</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>13,798 ± 369</td>
<td>14,970 ± 178</td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.005</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

* Mean ± SEM.

DISCUSSION

This work reports the full range of eicosanoids produced by the human stomach tumor cell line AGS. Under basal conditions, AGS cells produce LTD4 as its principal eicosanoid metabolite, followed by progressively smaller molar quantities of 6-keto-PGF1alpha, LTC4, LTB4, 12-HETE, TxB2, PGF2alpha, PGE2, 12-HHT, 15-HETE, and PGD2. After stimulation with the calcium ionophore A23187, the principal eicosanoids products were (in order of relative molar abundance): 12-HETE; LTB4; LTD4; PGF2alpha; PGE2; 6-keto-PGF1alpha; TXB2; LTD4; 15-HETE; and PGD2. These results indicate that 5-lipoxygenase is more active than cyclooxygenase under both basal and ionophore-stimulated conditions in AGS cells. This is different from what has been reported in normal human gastric tissue in which PGE2 is the most prominent eicosanoid, followed by LTD4, and the sulfidopeptide-LTs (11). This also differs from reports of the prostanooids produced by gastric cancer tissue, in which 6-keto-PGF1alpha was the principal product, followed by smaller amounts of PGE2, PGF2alpha, and PGD2 (10). Pelus and Brockman (17) reported that macrophages from tumor-bearing animals have a markedly augmented capacity to metabolize arachidonic acid. Tumor tissue may be infiltrated with inflammatory cells, neutrophils, or macrophages, which would affect eicosanoid metabolism in a tumor cell line would be more likely to give an accurate measure of the endogenous capacity of the malignant epithelial

m) showed a nonsignificant effect on proliferation (94 ± 5 and 97 ± 4% of control BrdU, respectively).
cells themselves. We did not attempt to measure the total mass of eicosanoids made by the tumor cells; given the fact that the substances would act as autocrine factors, it is difficult to know how one would interpret these findings. One can indirectly estimate the dose-response relationships of the cells from the data obtained using exogenous LTs.

In this study, the growth and DNA synthesis of AGS cells were suppressed by exogenous PGE2 and PGD2, similar to the reported findings of other investigators using other tumor cell lines (4-8). It has been proposed that the antitumor activities of these prostaglandins are exclusively due to their ultimate metabolites, PGAs, and 12-PGJ2, which share a cyclopentenone ring structure (18). The growth-inhibiting effect of these PGs was independent of cyclic AMP, and its mechanism appeared to depend upon inhibition of entry into the G1 phase of the cell cycle (7, 19). Furthermore, Santro et al. (20) reported that a M, 74,000 protein was induced by PGA2 or PGJ2 in human K562 erythroleukemia cells; the protein has been identified as the heat-shock protein, and its appearance was associated with changes in cell proliferation. Ishioka et al. (21) reported that PG2 arrested HL-60 cells at the G0/G1 phase of the cell cycle, which was associated in a reduction in the mRNA for c-myc.

We have demonstrated that LTD4 (at concentrations from 0.05 to 0.50 mg/ml, or 10^-7 M to 10^-6 M) and LTC4 (at 0.50 mg/ml or 10^-6 M) significantly increased cell number and stimulated DNA synthesis in AGS cells. Dawson et al. (22) have reported that the 5-lipoxygenase inhibitor benoxaprofen suppressed the growth of various tumor cell lines in vitro and tumor growth in vivo after i.p. injection of mice with the T-cell lymphoma line EL4. Gati et al. (23) reported a similar inhibitory effect with the 5-lipoxygenase inhibitor NDGA on human glioma spheroids. Miller et al. (9) showed that NDGA, piriprost (a selective inhibitor of 5-lipoxygenase), and other inhibitors of peptide-LT (LTC4, LTD4, and LTE4) metabolism inhibited the growth of the human leukemia cell line HL-60, which was completely reversed by the daily addition of 10^-9 M LTD4. Ralph and Wojcik (24) have reported that various lipoxygenase and phospholipase A2 inhibitors (i.e., NDGA, hydrocortisone, and others) inhibited the growth of the P815 murine mastocytoma cell line, but that 0.16 nM LTC4, or 0.3 nM LTD4, failed to reverse this effect. In our study, we used relatively high dosages of LTs (up to 10^-6 M) and demonstrated that LTD4 was a more potent stimulator of AGS cell growth than LTC4, similar to what had been reported by Miller et al. (9). However, it is very difficult to know what represents a physiological concentration of a paracrine or autocrine substance.

Our data showed significant increases in AGS cell numbers after stimulation with LTs, but we did not demonstrate a significant increase in total protein content with any concentration of LT. The discrepancy between these two measures of cell growth may be a reflection of a reduction in cell size. AGS cells are somewhat pleomorphic, but after the addition of LTs, the cell size appeared subjectively smaller than in control wells. Therefore, LTs may stimulate cell division more rapidly than protein synthesis in these cells.

Nishizawa et al. (25) reported that the 5-lipoxygenase inhibitors NDGA and AA861 stimulated the growth of transformed murine Leydig cells, and that 5-HETE inhibited proliferation in these cells. In this study a single dose of NDGA (10^-7 M, 10^-6 M, or 10^-5 M) or hydrocortisone (10^-7 M or 10^-6 M) showed no significant effect on cell growth, but pretreatment with NDGA or hydrocortisone inhibited LT production during the preincubation period and produced a significant, dose-depend-
REFERENCES


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