Activated lymphocytes increase expression of 5-lipoxygenase and its activating protein in THP-1 cells

WILLIAM L. RING, CARL A. RIDDICK, JOSEPH R. BAKER, CHRISTOPHER K. GLASS, AND TIMOTHY D. BIGBY
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Ring, William L., Carl A. Riddick, Joseph R. Baker, Christopher K. Glass, and Timothy D. Bigby. Activated lymphocytes increase expression of 5-lipoxygenase and its activating protein in THP-1 cells. Am. J. Physiol. 273 (Cell Physiol. 42): C2057–C2064, 1997.—The aim of this study was to investigate the regulation of the 5-lipoxygenase pathway of arachidonic acid metabolism by lymphocytes using the monocyte-like cell line, THP-1. When THP-1 cells were incubated over 4–7 days in 10% supernatant from lectin-activated human lymphocytes, their capacity to synthesize 5-lipoxygenase products was significantly increased. In contrast, the supernatant from nonactivated lymphocytes had no effect. The increase in capacity to synthesize 5-lipoxygenase products was mimicked by the addition of either granulocyte/macrophage colony-stimulating factor (GM-CSF) or interleukin-3. These increases in synthetic capacity reflected increased enzymatic activity. Increased immunoreactive protein and mRNA for the enzymes 5-lipoxygenase and 5-lipoxygenase-activating protein were also found in cells conditioned with activated lymphocyte supernatants. Furthermore, the increase in mRNA for both enzymes was not blocked by cycloheximide, suggesting that the effect on steady-state mRNA levels does not require the synthesis of new protein. The increase in mRNA could be reproduced by GM-CSF. We conclude that lymphocytes can regulate the expression of 5-lipoxygenase in THP-1 cells over a period of days via the release of soluble factors.

THE 5-LIPOXYGENASE PATHWAY produces leukotrienes, which are oxygenated metabolites of arachidonic acid. Leukotrienes have been implicated in a wide range of inflammatory diseases (7). The enzyme 5-lipoxygenase, which acts on free arachidonic acid, is responsible for catalyzing the first two steps in the synthesis of leukotrienes, namely the conversion of arachidonic acid to 5(S)-hydroperoxy-6,8,11,14-eicosatetraenoic acid (5-HPETE) and then 5-HPETE to leukotriene A₄ (LTA₄). The synthesis of 5-lipoxygenase products in intact cells is also dependent on a nuclear membrane-associated protein, 5-lipoxygenase-activating protein (FLAP) (6). The mechanism by which 5-lipoxygenase expression and activity is controlled is still poorly understood.

THP-1 cells are a well-established line of human tumor cells derived from a Japanese pediatric patient with acute monocytic leukemia (23). These cells possess characteristics of mononuclear phagocytes: they are nonspecific esterase positive, they produce lysozyme, and they are phagocytic (1). However, THP-1 cells have not been viewed previously as an adequate in vitro model for examination of 5-lipoxygenase pathway regulation. Specifically, this cell line has been reported to either lack 5-lipoxygenase activity or to have 5-lipoxygenase activity that cannot be modulated (13, 14, 19).

We recently reported that human peripheral blood monocytes, when grown in coculture with human lymphocytes for 2 days, developed a threefold increase in their capacity to synthesize 5-lipoxygenase products (16). Moreover, when the supernatant from lectin-activated lymphocytes, but not from nonactivated lymphocytes, was added to monocytes at a 10% concentration (vol/vol) for 2 days, there was a large increase in the capacity of the monocytes for 5-lipoxygenation. This increase in capacity was associated with significant increases in 5-lipoxygenase and FLAP proteins in conditioned cells compared with control cells, as assessed by immunoblot analysis. Likewise, Northern blot analysis of total RNA showed significant increases in 5-lipoxygenase and FLAP mRNA in conditioned monocytes compared with control monocytes. Additional work revealed that granulocyte/macrophage colony-stimulating factor (GM-CSF) and interleukin-3 (IL-3), present in supernatants from activated lymphocytes, were responsible for most of the observed increases in 5-lipoxygenase and FLAP (16). However, further investigations into the regulation of the 5-lipoxygenase pathway expression have been limited by the difficulties inherent in working with primary cell isolates and by practical limits to the number of human monocytes that can be harvested. These difficulties preclude further in-depth studies of mRNA levels or of the mechanism(s) whereby transcription is regulated in primary isolates of human mononuclear phagocytes.

The broad goal of our investigations was to further study the mechanism(s) whereby the expression and, in turn, the activity of the 5-lipoxygenase pathway are regulated over the long term. Therefore, the specific aim was to examine, using a stable cell line, the interaction we have previously demonstrated between lymphocytes and monocytes and to utilize the cell line to begin to define the molecular aspects of regulation of expression of the 5-lipoxygenase pathway in mononuclear phagocytes.

METHODS

Cell culture. THP-1 cells were cultured in RPMI 1640 medium with 10% fetal calf serum, to which 100 U/ml penicillin, 100 µg/ml streptomycin, 100 µg/ml gentamicin, and 50 µM 2-mercaptoethanol were added. Media were replaced every 2–3 days. Studies were performed at cell densities of 2.0 × 10⁶ to 2.5 × 10⁶ cells/ml. All cell prepara-
tions were performed at room temperature using sterile techniques in a laminar flow hood and endotoxin-free (<10 pg/ml) solutions.

Preparation of nonactivated and lectin-activated lymphocyte supernatant. Lymphocyte supernatants were prepared as previously described (16). Briefly, peripheral blood was obtained from normal healthy subjects without known allergies, who had not taken any medication for 2 wk and any aspirin products for 6 wk before blood donation. All subjects were volunteers and had given informed consent to a protocol approved by the Committee on Investigations Involving Human Subjects of the University of California, San Diego.

Lymphocytes were isolated by density gradient centrifugation over isocitric Percoll with a specific gravity of 1.077 followed by removal of monocytes by their adherence to serum-coated plastic. Lymphocytes were incubated for 4 days without (nonactivated) or with (lectin-activated) lectins. For all lectin stimulation studies, cells were incubated with 10 µg/ml concanavalin A and 10 µg/ml phytohemagglutinin. The media were then collected and centrifuged to remove cells and debris, and the supernatant was stored at 4°C. For THP-1 conditioning studies, lymphocyte supernatant was added to a final concentration of 10% (vol/vol).

Studies of disrupted cells. THP-1 cells were collected from tissue culture plates, and any nonadherent cells were gently released from tissue culture plastic with a sterile rubber policeman and added to the collected cells. The resulting cell suspension was centrifuged, and the cell pellet was disrupted by sonication at 4°C as previously described (15).

Radio labeling and assessment of arachidonic acid release. Labeling studies were performed by overnight incubation of THP-1 cells with 0.05 µCi of [14C]arachidonic acid, as described (9).

Assay of 5-lipoxygenase and LTA4 hydrolase activity in whole and disrupted cells. 5-Lipoxygenase activity and LTA4 hydrolase activity were measured in intact THP-1 cells as previously described (15). Cells were collected in warmed Hanks’ balanced salt solution (HBSS) and stimulated with 1 µM A-23187 at 37°C for 15 min. The supernatant was collected, and lipids were extracted from this supernatant using methanol and chloroform by previously described techniques (2, 12). Phosphatidylethanolamine, LTA4 hydrolase, and LTA4 were determined in disrupted cells by specific enzymatic assay, as previously described (12). For 5-lipoxygenase activity, samples were incubated in a 37°C shaking water bath with 2 mM CaCl2, 2 mM ATP, and 100 µM arachidonic acid for 15 min. For LTA4 hydrolase activity, samples were incubated in a 37°C shaking water bath in assay buffer at a final concentration of 100 mM tris(hydroxymethyl)aminomethane, 0.35% bovine serum albumin, and 15 µM LTA4 for 15 min. In both specific enzymatic assays, the reaction was then quenched by adding cold methanol, and precipitated protein was removed by centrifugation. Supernatants were removed and evaporated to dryness, and residues were dissolved in methanol for storage at −70°C until further analysis. For lipoxygenase and hydrolase assays in both intact and disrupted cells, lipoygenase metabolites were resolved and quantitated by reverse-phase high-performance liquid chromatography using previously described techniques (12).

Immunoblot analyses. Immunoblotting was performed on total protein using previously described techniques (15). Immunoblots were probed with antibodies (generously provided by Dr. Jilly Evans, Merck-Frosst Centre for Therapeutic Research, Point Claire-Dorval, Quebec, Canada) that were raised against either recombinant human leukocyte 5-lipoxygenase or amino acid residues 41–52 of the human FLAP sequence (1:5,000 dilution). The specificity of these antibodies has been extensively characterized (8, 17, 24). Bands were quantitated by scanning laser densitometry (LKB Instruments, Uppsala, Sweden).

Northern blot analyses. Poly(A)+ RNA was isolated from total cell extracts on oligo(dT)25 cellulose as previously described (4). Northern blotting was performed as previously described (18, 22). Northern blots were probed with cDNA probes for 5-lipoxygenase (a generous gift from Dr. Richard A. Dixon; Merck Sharp & Dohme Research Laboratories, West Point, PA), FLAP (a generous gift from Dr. Jilly Evans), LTA4 hydrolase (a generous gift from Dr. Takao Shimizu, University of Tokyo, Japan), and β-actin. In some cases, 10 µg/ml cycloheximide was added to the cells before conditioning with lymphocyte supernatant, and mRNA was isolated. Bands were quantitated by scanning laser densitometry (LKB Instruments) and were normalized to bands observed with the β-actin probe.

Materials. HBSS and RPMI 1640 were obtained from BioWhittaker (Watersville, MD); penicillin and streptomycin were from the Cell Culture Facility, University of California, San Diego; gentamicin was from Gemini Bioproducts (Calabasas, CA); Percoll was from Pharmacia Fine Chemicals (Piscataway, NJ); A-23187 was from Calbiochem-Behring (La Jolla, CA); redissolved-in-grade chromatography solvents were obtained from Burdick and Jackson Division, Baxter (Muskegon, MI); 5(S)-hydroxy-6-trans-8,11,14-eicosatetraenoic acid (5-HETE) was from Biomol (Plymouth Meeting, PA); LTA4, LTB4, and LTC4 were generous gifts from Merck-Frosst Centre for Therapeutic Research, Point Claire-Dorval, Quebec, Canada; arachidonic acid was from NuChek Prep (Elysian, MN); and recombinant human GM-CSF and IL-3 were from R & D Systems (Minneapolis, MN). All other chemicals were from Sigma Chemical (St. Louis, MO) and were of the finest grade available. All cell lines were obtained from American Type Culture Collection (Rockville, MD).

Data analysis. Data are expressed as means ± SE in all circumstances where mean values are compared. The differences between two means were analyzed by an unpaired t-test. The time course study and the GM-CSF/IL-3 intact cell study were analyzed with a repeated measures analysis of variance using a Fisher’s post hoc test. Differences were considered significant when P < 0.05 (20).

RESULTS

Effect of lectin-activated lymphocyte supernatants on 5-lipoxygenase activity in intact THP-1 cells. A variety of inflammatory cell lines were screened for an effect on the regulation of 5-lipoxygenase activity by activated lymphocyte supernatants. These cells included HL-60 cells (a promyelocytic leukemic cell line), KG-1 cells (an acute myelogenous leukemic cell line), U-937 cells (a histiocytic lymphoma cell line), and THP-1 cells. Cells were cultured for 7 days either in media alone or with media containing 10% supernatant from lectin-activated lymphocytes, and then A-23187-stimulated intact cell 5-lipoxygenase activity was assessed. In these screening studies, only THP-1 cells, when conditioned with lectin-activated lymphocyte supernatant, demonstrated a significant increase in 5-lipoxygenase activity compared with control cells (Fig. 1). Moreover, there was no significant difference in 5-lipoxygenase activity between control THP-1 cells, THP-1 cells conditioned directly with lectins, or THP-1 cells conditioned with the supernatant from nonactivated lymphocytes (Fig. 1). Specifically, over a period of 7 days, the supernatant...
from lectin-activated lymphocytes caused a 15-fold increase in the capacity of THP-1 cells to synthesize the 5-lipoxygenase product, 5-HETE (P < 0.05). Similar results were obtained for leukotriene B4 (LTB4; 0.0 vs. 0.0 vs. 0.0 vs. 2.6 ± 0.5 pmol LTB4/10⁶ cells; data are for THP-1 cells, THP-1 cells treated with lectins, supernatant from nonactivated lymphocytes, and supernatant from lectin-activated lymphocytes, respectively; n ≥ 4). There was no detectable leukotriene C4 (LTC4) release from THP-1 cells under the conditions studied. There was also no detectable 5-HETE or LTB4 in supernatants from either lectin-activated or control lymphocytes. Over a period of 7 days, the supernatant from lectin-activated lymphocytes did not significantly change the number of cells present in culture compared with control cells (101.2 ± 11.7% vs. 100.0 ± 16.2%, total cells in culture as a percentage of the mean number of control cells, for conditioned vs. control cells, respectively).

Time course of the effect of lectin-activated lymphocyte supernatant on 5-lipoxygenase activity in THP-1 cells. THP-1 cells were cultured with or without 10% supernatant from lectin-activated lymphocytes for various lengths of time, and then A-23187-stimulated intact cell 5-lipoxygenase activity was assessed. There was no difference between conditioned cells and control cells at 15 min and 1 day (Fig. 2). However, at 2 days there was a 3.2-fold increase in the capacity of conditioned cells to release 5-HETE, compared with controls (n ≥ 7; P < 0.05). This effect of lectin-activated lymphocyte supernatant was maximal at 7 days and persisted for at least 14 days (the longest time period examined). There was no detectable A-23187-stimulated release of LTB4 from either control or conditioned THP-1 cells at 15 min through 4 days. However, after 7 days of conditioning, the LTB4 released was enhanced in conditioned vs. control cells as described above, and this effect again persisted for at least 14 days. As noted above, there was no detectable LTC4 release from either conditioned or control cells at any time.

Effect of GM-CSF and IL-3 on 5-lipoxygenase activity in THP-1 cells. We have shown that GM-CSF and IL-3 are the principal substances in the supernatant from lectin-activated lymphocytes responsible for increasing 5-lipoxygenase activity in monocytes (16). Therefore, we asked whether these cytokines also regulate 5-lipoxygenase activity in THP-1 cells. THP-1 cells were cultured with or without either 10 ng/ml GM-CSF or 10 ng/ml IL-3 for 7 days, and then A-23187-stimulated intact cell 5-lipoxygenase activity was assessed. Both GM-CSF and IL-3 caused an ~12-fold increase in the capacity of THP-1 cells to synthesize the 5-lipoxygenase product 5-HETE (P < 0.05; Fig. 3). Parallel results were obtained for LTB4 (0.0 vs. 1.5 ± 0.2 vs. 1.1 ± 0.8 pmol LTB4/10⁶ cells at 0, 1, and 7 days, respectively).

![Graph showing effects of lymphocyte supernatants on 5-lipoxygenase activity in THP-1 cells.](http://ajpcell.physiology.org/)

![Graph showing time course of effect of lectin-activated lymphocyte supernatant on 5-lipoxygenase activity in THP-1 cells.](http://ajpcell.physiology.org/)
pmol LTB₄/10⁶ cells, control vs. GM-CSF vs. IL-3, respectively; n ≥ 3). The magnitude of this increase in
capacity to synthesize 5-lipoxygenase products by each
cytokine was comparable with that exerted by 10%
supernatant from lectin-activated lymphocytes (Fig. 3).
Neither cytokine was able to stimulate detectable LTC₄
release.

Effect of lectin-activated lymphocyte supernatant on
5-lipoxygenase activity and LTA₄ hydrolase activity in
disrupted THP-1 cells. To determine whether an in-
crease in 5-lipoxygenase activity and/or LTA₄ hydrolase
activity was responsible for the increased release of
5-HETE and LTB₄ observed in intact conditioned THP-1
cells, cells were cultured with or without 10% superna-
tant from lectin-activated lymphocytes for 7 days and
then disrupted by sonication. Over this period of 7 days,
the supernatant from lectin-activated lymphocytes did
not significantly change the total protein per cell com-
pared with control cells (3.1 ± 0.4 vs. 3.3 ± 0.4 mg/10⁶
cells of total protein, for conditioned vs. control cells,
respectively). However, disrupted fractions from condi-
tioned cells had 2.5 more 5-lipoxygenase activity than
that found in disrupted fractions from control cells, as
assessed by 5-HETE production (P < 0.05; Fig. 4).
There was no difference in LTA₄ hydrolase activity
between samples prepared from conditioned vs. control
cells (117.4 ± 34.3%, LTB₄ production as percent of
control; n = 5; P = not significant).

Effect of lymphocyte supernatant on arachidonic acid
release by THP-1 cells. Supernatants from lectin-
activated lymphocytes caused a greater increase in
5-lipoxygenase product release from intact cells than
the increase in enzymatic activity measured in dis-
rupted cells. Therefore, to determine whether a change
in arachidonic acid release contributed to the increased
release of 5-lipoxygenase products by intact condi-
tioned THP-1 cells, cells were cultured for 7 days with
media alone or with media and 10% supernatant from
lectin-activated lymphocytes. The cells were also radio-
labeled with [¹⁴C]arachidonic acid during the last day of
conditioning. The cells were then stimulated with 1 μM
A-23187 for 15 min, and release of label was assessed as
a percent of the total label incorporated. Similar quan-
tities of label were released from conditioned vs. control
cells (Fig. 5). Thus enhanced release of the substrate,
arachidonic acid, is unlikely to account for the in-
creased release of 5-lipoxygenase products from condi-
tioned cells.

Effect of lectin-activated lymphocyte supernatant on
immunoreactive 5-lipoxygenase and FLAP in THP-1
cells. To determine whether lectin-activated lympho-
cyte supernatant increases immunoreactive 5-lipoxy-
genase and FLAP, THP-1 cells were cultured for 7 days
with or without 10% supernatant from lectin-activated
lymphocytes and were then disrupted by sonication.
Immunoblotting of these sonicates revealed a reproduc-
ible increase in 5-lipoxygenase and FLAP protein when
immunoblots from conditioned cells were compared
with those from control cells (Fig. 6). Densitometric
analysis of autoradiographs was perfomed. There was
a 3.3 ± 1.2- and 2.3 ± 0.3-fold increase in 5-lipoxy-
genase and FLAP protein, respectively, when immunob-
blots from conditioned cells were compared with those

C2060 LYMPHOCYTES REGULATE 5-LIPOXYGENASE IN THP-1 CELLS
from control cells (means ± SE of at least 4 experiments).

Effect of lectin-activated lymphocyte supernatant on 5-lipoxygenase and FLAP mRNA in THP-1 cells. To determine the effect of lectin-activated lymphocyte supernatant on steady-state mRNA encoding for 5-lipoxygenase, FLAP, and LTA₄ hydrolase, THP-1 cells were cultured with or without 10% supernatant from lectin-activated lymphocytes for various lengths of time. Northern blot analysis of poly(A)⁺ RNA from these cells revealed no effect on mRNA for either 5-lipoxygenase or FLAP after 4 h of conditioning, compared with control cells (Fig. 7). However, there was an approximately threefold increase in mRNA for both 5-lipoxygenase and FLAP at 1 day of conditioning, and this increase persisted without change through 2, 4, and 7 days. In contrast, supernatants from lectin-activated lymphocytes had no effect on levels of LTA₄ hydrolase mRNA (110%, 80%, 120%, and 90% of control at 4 h, 1 day, 2 days, and 7 days, respectively).

Effect of GM-CSF on 5-lipoxygenase and FLAP mRNA levels in THP-1 cells. To determine the effect of GM-CSF on steady-state mRNA encoding for 5-lipoxygenase and FLAP, THP-1 cells were cultured with or without 10 ng/ml of GM-CSF for 2 days. Northern blot analysis of total RNA from these cells revealed an increase in mRNA for both 5-lipoxygenase and FLAP (Fig. 8). There was no effect on mRNA for LTA₄ hydrolase (90% of control).

Effect of cycloheximide on changes in mRNA encoding for 5-lipoxygenase and FLAP induced by conditioning with lectin-activated lymphocyte supernatant. To determine whether new protein synthesis was involved in the effect of lectin-activated lymphocyte supernatant on levels of mRNA for 5-lipoxygenase and FLAP, THP-1 cells were treated for 2 days with or without 10%
supernatant from lectin-activated lymphocytes, in the presence or absence of 10 µg/ml of a protein synthesis inhibitor, cycloheximide. Cycloheximide had no significant effect on the ability of lectin-activated lymphocyte supernatant to cause an increase in steady-state mRNA levels for either 5-lipoxygenase or FLAP (Fig. 9). There was no effect of cycloheximide- and/or lectin-activated lymphocyte supernatant on LTA4 hydrolase mRNA levels (117% and 96% of control cells, for cells conditioned with lectin-activated lymphocyte supernatant, with or without cycloheximide, respectively). Thus lectin-activated lymphocyte supernatant increased mRNA levels for 5-lipoxygenase by 6.0-fold in the presence of cycloheximide vs. 4.4-fold in the absence of cycloheximide. Parallel results were obtained for FLAP mRNA levels (4.6- vs. 3.4-fold increase in the presence and absence of cycloheximide, respectively).

**DISCUSSION**

We report that the human monocytic leukemia cell line, THP-1, has the capacity to synthesize 5-lipoxygenase products, has detectable levels of 5-lipoxygenase and FLAP, and expresses mRNA encoding for 5-lipoxygenase and FLAP. Furthermore, we report that the supernatant from lectin-activated human lymphocytes causes a large increase in the capacity of THP-1 cells to synthesize 5-lipoxygenase products, whereas supernatants from nonactivated lymphocytes have no effect. The increase in 5-lipoxygenase capacity is significant within 4–7 days, as measured by the release of 5-HETE and LTB4, and persists for at least 14 days of conditioning. The increase is also associated with significant increases in the expression of protein and mRNA for both 5-lipoxygenase and FLAP but is not associated with changes in levels of mRNA for LTA4 hydrolase. Similar increases in the capacity of THP-1 cells to synthesize 5-lipoxygenase products occur after 7 days of conditioning with either GM-CSF or IL-3, and GM-CSF also caused an increase in mRNA for both 5-lipoxygenase and FLAP. Thus THP-1 cells have a 5-lipoxygenase pathway that can be upregulated by activated lymphocytes and by GM-CSF or IL-3 via increases in expression of 5-lipoxygenase and FLAP.

The increased capacity for 5-lipoxygenation of arachidonic acid in THP-1 cells when conditioned with lectin-activated lymphocyte supernatants appears to be primarily associated with changes in 5-lipoxygenase and FLAP. Changes in availability of arachidonic acid do not appear to largely contribute to this increased capacity, as reflected by radiolabeling studies. Interestingly, in the conditioned THP-1 cells, there is a delay in the detection of increased LTB4 production compared with 5-HETE production. Although this might suggest a delayed effect of conditioning on expression of LTA4 hydrolase, this seems less likely, as there is neither a change with conditioning in LTA4 hydrolase activity by specific enzymatic assay nor a change in the level of mRNA for the enzyme. In fact, the delay in detecting LTB4 production may simply be due to differences in sensitivity of the intact cell assay. There is a greater increase in the capacity of conditioned THP-1 cells to generate 5-lipoxygenase products in the intact cell assay, which reflects changes anywhere in the pathway, compared with the specific enzymatic assays for 5-lipoxygenase and FLAP. Although this may suggest additional regulation at other levels of the pathway, it may also reflect differences in assay conditions.

Other investigators using THP-1 cells have suggested that they were a poor system for studying the 5-lipoxygenase pathway. Previous work has not demonstrated the presence of FLAP in unconditioned cells (14). Earlier studies have reported that, when THP-1 cells were conditioned with interferon-γ or with phorbol myristate acetate for 2 days, there was no detectable synthesis of 5-lipoxygenase products (13). Likewise, more recently, investigators have reported that THP-1 cells conditioned for 4 days with retinoic acid, interferon-γ, 1,25-dihydroxyvitamin D3, or combinations of these have no detectable synthesis of leukotrienes or HETEs (19). In contrast, we detected both 5-lipoxygenase and FLAP in immunoblots of THP-1 cells that had not been conditioned. In addition, we measured low levels of both 5-HETE and LTB4 synthesis in nonconditioned THP-1 cells stimulated with A-23187. This finding may be due to differences between sublines of this same cell line or differences in cell culture conditions. We also report a large increase in the capacity of THP-1 cells to synthesize 5-lipoxygenase products after 4 days of conditioning with supernatant from lectin-activated lymphocyte, GM-CSF, or IL-3. The differences between our findings and those published previously may be due to a number of factors, including an increased length of conditioning time and conditioning with presumably a wide range of soluble products produced by lectin-activated lymphocytes. However, previous work in the
area did not specifically examine the effect of GM-CSF or IL-3 on 5-lipoxygenase capacity in THP-1 cells.

Previous work in monocytes or the cell line MonoMac 6 have suggested a role for transforming growth factor-β in the regulation of the 5-lipoxygenase pathway (5, 21). Transforming growth factor-β is known to promote the maturation of THP-1 cells into macrophage-like cells (3) and may influence the 5-lipoxygenase pathway in these cells. However, this was not the focus of this investigation. We have reported that, in monocytes conditioned with lectin-activated lymphocyte supernatants, the increase in the capacity of monocytes to synthesize 5-lipoxygenase products is largely mediated by GM-CSF and IL-3 (16). In THP-1 cells, GM-CSF and IL-3 appear to play a similar role.

The protein synthesis inhibitor cycloheximide had no effect on the increased expression of both 5-lipoxygenase and FLAP mRNA seen in THP-1 cells after conditioning with lectin-activated lymphocyte supernatant. This suggests a direct effect on 5-lipoxygenase and FLAP expression by the conditioning agent(s) and argues against the stimulation of expression of an intermediary protein (transcription factor) modulating mRNA levels. This would be in keeping with recent work on GM-CSF and IL-3 signal transduction, which seems primarily to involve stimulated tyrosine phosphorylation of proteins already present within resting cells, including JAK2, STAT5a, and STAT5b (10, 11).

We have previously reported that the response to lectin-activated lymphocyte supernatant observed in THP-1 cells is also seen in human monocytes obtained from peripheral blood (16). However, in monocytes, there is an initial increase of 5-lipoxygenase synthesis capacity at 15 min that then returns to baseline values at 1 h. This rapid effect was not seen in THP-1 cells. In both monocytes and THP-1 cells, in contrast, there is a more prolonged effect on 5-lipoxygenase capacity. This begins at 1 day and persists at least through 4 days in monocytes. This failure to observe an initial increase in 5-lipoxygenase synthetic capacity in conditioned THP-1 cells compared with conditioned monocytes is as yet unexplained. It may be due to that fact that THP-1 cells are relatively immature macrophage precursors and that, therefore, this cell line responds somewhat differently than monocytes to the conditioning stimulus. In fact, the time course of increased capacity for 5-lipoxygenation of arachidonic acid in THP-1 cells is somewhat slower than for monocytes, requiring approximately three times as long before both the first detectable increase and the maximum effect. This again would suggest the possibility that THP-1 cells, before conditioning, are less mature than cultured monocytes. Moreover, the maximal effect of lectin-activated lymphocyte supernatant on THP-1 cells corresponds to a larger times increase in their capacity to synthesize 5-lipoxygenase products compared with the effect seen in monocytes. However, despite the minor quantitative and kinetic differences observed between THP-1 cells and monocytes, and, with the exception of the very early effect of lectin-activated lymphocyte supernatant seen in the latter cells, THP-1 cells appear to have broadly similar regulation of their 5-lipoxygenase pathway compared with monocytes.

In summary, THP-1 cells have the capacity to synthesize 5-lipoxygenase products, particularly after exposure of the cells to the supernatant from lectin-activated lymphocytes. The increased capacity of conditioned cells for 5-lipoxygenation is associated with increases in both protein and mRNA for both 5-lipoxygenase and FLAP and may be mediated by GM-CSF and/or IL-3. This effect of lectin-activated lymphocyte supernatant on the 5-lipoxygenase pathway of THP-1 cells is broadly comparable to effects observed with primary isolates of human peripheral blood monocytes. Thus lymphocytes can regulate the expression of 5-lipoxygenase and FLAP in THP-1 cells over a period of days via the release of soluble factors. The current observations advance the prior work in monocytes determining the time course of increases in mRNA and determining that increases in mRNA do not require intermediate synthesis of transcription factors that modulate gene expression. Moreover, THP-1 cells may be used for an in-depth analysis of the mechanisms whereby the expression of the 5-lipoxygenase pathway in inflammatory cells is regulated, providing additional insights into the mechanism by which 5-lipoxygenase expression and activity are controlled in mononuclear phagocytes.

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