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On the expression and regulation of 5-lipoxygenase in human lymphocytes

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ABSTRACT **The expression of arachidonate 5-lipoxygenase (arachidonate:oxygen 5-oxidoreductase, EC 1.13.11.34) and the 5-lipoxygenase-activating protein (FLAP) genes in human tonsillar B cells and lymphoblastoid B-cell lines was demonstrated at the transcriptional level by reverse transcription–PCR analysis. Also, five lymphoblastoid T-cell lines were investigated and found to express the FLAP gene but not the 5-lipoxygenase gene, suggesting that the transcriptional regulation of these two genes is different. Western blot analysis of the cytosolic proteins from a lymphoblastoid B-cell line with an antiserum raised against purified human leukocyte 5-lipoxygenase revealed an immunoreactive band that comigrated with recombinant human 5-lipoxygenase. Intact B cells produced very low amounts of leukotriene B_4 and 5-hydroxyeicosatetraenoic acid upon stimulation with the calcium ionophore A23187 and arachidonic acid, in comparison to the amounts formed by sonicates of these cells. However, preincubation of intact lymphoblastoid B cells with the glutathione-depleting agents azodicarboxylic acid bis(dimethylamide) or 1-chloro-2,4-dinitrobenzene prior to the addition of the calcium ionophore A23187 and arachidonic acid led to similar amounts of leukotriene B_4 as were formed by sonicated cells. In contrast, the glutathione synthesis inhibitor buthionine sulfoximine diminished the cellular level of glutathione by >90% but did not influence the production of leukotriene B_4 or 5-hydroxyeicosatetraenoic acid in intact cells. These results demonstrate that certain drugs affecting the redox status can stimulate the cryptic 5-lipoxygenase activity in intact lymphoblastoid B cells but that the mechanism of this activation is unclear and appears not to be directly related to intracellular glutathione levels.**

Leukotriene (LT) B_4 mediates certain inflammatory and immunological reactions (1, 2). The key enzyme in LTB_4 biosynthesis, arachidonate 5-lipoxygenase (EC 1.13.11.34), possesses two catalytic activities, (*i*) conversion of arachidonic acid to 5-hydroperoxyeicosatetraenoic acid, which can be degraded to 5-hydroxyeicosatetraenoic acid (5-HETE), and (*ii*) the subsequent formation of LTA_4 (3). This enzyme requires Ca^{2+} and ATP for maximal activity (4). Also, 5-lipoxygenase-activating protein (FLAP) appears to play an important role in the cellular synthesis of LTs (5–7). A threshold level of hydroperoxides is required for the activation of 5-lipoxygenase (8). Therefore, the redox status of the cell is of importance for 5-lipoxygenase activity, and glutathione-depleting agents have been shown to activate the 5-lipoxygenase in human polymorphonuclear leukocytes (PMNLs), possibly through increased levels of hydroperoxide products (9, 10).

The second enzyme involved in LTB_4 synthesis from arachidonic acid, LTA_4 hydrolase, is expressed in a variety of cells, including human lymphocytes (11, 12). Activated human monocytes can release LTA_4, and studies on monocyte–lymphocyte interactions have revealed that this compound can be utilized by lymphocytes for the formation of LTB_4 (13).

Biosynthesis of LTs from arachidonic acid has been thought to be restricted to cells of myeloid lineage. Recently, however, we reported that tonsillar human B lymphocytes and lymphoblastoid B cells possess 5-lipoxygenase activity and can convert arachidonic acid to LTB_4 (14). Stimulation of intact B cells with the calcium ionophore A23187 and arachidonic acid led to very low levels of LTB_4 in comparison to the amount formed by sonicated B cells, indicating a cryptic 5-lipoxygenase activity in intact B cells. It was also suggested that cytokine activation of tonsillar B lymphocytes leads to an upregulation of 5-lipoxygenase activity. These results led us to initiate studies on the expression of 5-lipoxygenase and FLAP in human B and T lymphocytes and on the mechanism of activation of the cryptic 5-lipoxygenase activity in B cells.

MATERIALS AND METHODS

Molecular biology reagents were obtained from Pharmacia unless otherwise mentioned in the text. PCR primers were obtained from Scandinavian Gene Synthesis AB (Köping, Sweden). The polyclonal anti-human 5-lipoxygenase rabbit antiserum was a gift from Jilly F. Evans, Merck Frosst Centre for Therapeutic Research (Quebec). HPLC solvents were from Rathburn Chemicals (Walkerburn, U.K.) and synthetic standards of 5(*S*)-HETE and LTB_4 were from Biomol (Plymouth Meeting, PA). The calcium ionophore A23187 was obtained from Calbiochem–Behring. Azodicarboxylic acid bis(dimethylamide) (diamide), 1-chloro-2,4-dinitrobenzene (Dnp-Cl), buthionine sulfoximine, 5-bromo-4-chloro-3-indolyl phosphate, and nitroblue tetrazolium were purchased from Sigma.

Isolation of Cells. Human tonsillar B lymphocytes were isolated as described (15). The resting B cells were activated with a mixture of cytokines (14). The B-cell preparation contained >98% B cells and <0.2% monocytes, as judged from immunofluorescence staining (11). PMNLs (from leukocyte concentrates obtained from Karolinska Hospital) and human endothelial cells were isolated as described (13, 16, 17).

Lymphoblastoid B and T Cells. The B-cell lines BL41-E95-A (18) and U266 (19) have been characterized. The T-cell lines MOLT-3, MOLT-4, CCRF, 1301, and Jurkat (kindly provided by G. Klein, Department of Tumor Biology, Karolinska Institutet) have been described (20–23). Cells were cultivated at 37°C in an atmosphere of 5% CO_2. Culture medium was RPMI 1640 supplemented with penicillin (100

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Abbreviations: FLAP, 5-lipoxygenase-activating protein; LT, leukotriene; HETE, hydroxyeicosatetraenoic acid; Dnp-Cl, 1-chloro-2,4-dinitrobenzene; diamide, azodicarboxylic acid bis(dimethylamide); PMNL, polymorphonuclear leukocyte.

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units/ml), streptomycin sulfate (100 μg/ml), and 6% fetal bovine serum. All cultures were seeded at a density of 2×10^5 cells per ml and grown for at least 4 days prior to the experiments. After harvesting, cells were washed in phosphate-buffered saline (PBS, Dulbecco's formula, pH 7.4), and resuspended in Ca^{2+}-free PBS.

Incubation of Sonicated Lymphocytes. Cells (10^7) suspended in Ca^{2+}-free PBS (1 ml) containing EDTA (1 mM) were sonicated twice for 5 sec on ice. Thereafter, ATP was added (1 mM) and samples were preincubated for 30 sec at 37°C. Incubations were started by the addition of $CaCl_2$ (2 mM) and arachidonic acid (40 μM). The enzymatic reaction was allowed to proceed for 10 min at 37°C before termination by the addition of 1 volume of methanol.

Incubation of Intact Lymphocytes. Cells (10^7) were preincubated for 30 sec at 37°C in Ca^{2+}-free PBS (1 ml) prior to the addition of $CaCl_2$ (1 mM), arachidonic acid (40 μM), and ionophore A23187 (5 μM). Subsequently, the cells were incubated for 10 min at 37°C and the incubations were terminated by the addition of 1 volume of methanol. In experiments with diamide and Dnp-Cl, the cells were preincubated for 10 min at 20°C in the presence of these drugs.

Quantitative Analysis of Glutathione. Total intracellular content of glutathione and glutathione disulfide (GSSG) was determined in disrupted cells by the 5,5'-dithiobis(2-nitrobenzoic acid (DTNB)–GSSG reductase recycling assay (24) modified by the addition of 2 mM EDTA and 100 μg of bovine serum albumin per ml. In order to examine the effects of glutathione depletion, cells were cultivated for 24 hr in the presence of the inhibitor of γ-glutamylcysteine synthase, buthionine sulfoximine (2 mM) dissolved in dimethyl sulfoxide (25). Control cells were cultivated in parallel with the addition of dimethyl sulfoxide only.

Analysis of LTs and Monohydroxy Acids. Precipitated proteins and cell fragments were removed by centrifugation and the supernatant was extracted by using a disposable octadecyl reverse-phase column (Chromabond, C_{18}, 200 mg; Macherey & Nagel) (26). The samples were thereafter analyzed in a reverse-phase HPLC system equipped with a Radial-Pak cartridge (5 × 100 mm) packed with 4-μm Novapak C_{18} material, guarded by a Novapak C_{18} guard column, both of which were obtained from Waters. The mobile phase was methanol/water/trifluoroacetic acid (70:30:0.007, by volume) and the flow rate was 1.2 ml/min. Qualitative analysis was performed by comparison with retention times of synthetic standards and by on-line analysis of ultraviolet spectra of eluted compounds (Waters 991 diode-array spectrophotometer). Quantitative determinations were performed by computerized integration of the area of eluted peaks.

Reverse Transcription and PCR. Total cellular RNA was isolated (27) and cDNA was produced by reverse transcriptase. A 40-μl reverse transcription mixture containing 2 μg of total RNA, 1× PCR buffer (50 mM KCl/10 mM Tris·HCl, pH 8.3/2 mM $MgCl_2$/0.01% gelatin), 5 mM dithiothreitol, 0.5 mM dNTPs, 37 units of RNasin (Promega), 200 ng of $(dT)_{12-18}$, and 200 units of Moloney murine leukemia virus reverse transcriptase (Bethesda Research Laboratories) was incubated at 37°C for 60 min, heated to 95°C for 5 min, and chilled on ice.

PCR was carried out in a total volume of 50 μl containing 2 μl of the reverse transcription reaction mixture, 1× PCR buffer, 0.2 mM dNTPs, and 0.5 μM 5' and 3' primers. The mixture was overlaid with a drop of mineral oil and the amplification was performed as follows: first cycle, denaturation at 94°C for 5 min, addition of 2 units of *Taq* polymerase, annealing at 60°C for 2 min 30 sec, and extension at 72°C for 3 min 30 sec; subsequent cycles, denaturation at 94°C for 1 min, annealing at 60°C for 2 min 30 sec, and extension at 72°C for 3 min 30 sec; last cycle, denaturation at 94°C for 1 min, annealing at 60°C for 2 min 30 sec, and extension at 72°C for 10 min.

Twenty-two cycles were carried out for β-actin, 28 for FLAP, and 32 for 5-lipoxygenase determinations. The following primers were used: β-actin, 800-base-pair (bp) primer set from the β-actin control amplimer panel (Clontech); FLAP, 5'-GGC-CAT-CGT-CAC-CCT-CAT-CAG-CG and 5'-GCC-AGC-AAC-GGA-CAT-GAG-GAA-CAG-G-3'; 5-lipoxygenase, 5'-ACC-ATT-GAG-CAG-ATC-GTG-GAC-ACG-C-3' and 5'-GCA-GTC-CTG-CTC-TGT-GTA-GAA-TGG-G-3'. Aliquots of the PCR mixtures (10 μl) were analyzed by electrophoresis in 2% agarose gels. The expected DNA fragments for 5-lipoxygenase and FLAP had sizes of 488 bp and 352 bp, respectively. All cDNAs were first amplified using the β-actin primers, as a quality control (data not shown).

Immunoblot Analysis of 5-Lipoxygenase. Cells were washed once in Ca^{2+}-free PBS and resuspended in PBS with 2 mM EDTA. After sonication and centrifugation at 15,000 × *g* for 10 min, an aliquot of the supernatant was mixed with 1 volume of 1 M Tris·HCl, pH 7.5/1% (wt/vol) sodium dodecyl sulfate (SDS)/0.1 M 2-mercaptoethanol/25% (vol/vol) glycerol/0.02% (wt/vol) bromophenol blue and boiled for 2 min. SDS/PAGE and Western blotting were performed as described (28). After blotting, the nitrocellulose membrane was incubated overnight at 4°C with a 1:200 dilution of the anti-5-lipoxygenase rabbit polyclonal antiserum. The second antibody was anti-rabbit IgG coupled to alkaline phosphatase, and immunoreactive bands were visualized with 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium. Standard recombinant human 5-lipoxygenase (purity better than 75%) was obtained from *Escherichia coli* cultures (29).

RESULTS

Detection of 5-Lipoxygenase mRNA. To investigate the expression of the 5-lipoxygenase gene in human lymphocytes, total RNA was isolated from resting and cytokine-activated tonsillar B cells and lymphoblastoid B and T cells and subsequently converted into cDNA for PCR analysis (30). To prevent amplification of the 5-lipoxygenase genomic DNA, the two 5-lipoxygenase primers spanned intron M (31). Positive 5-lipoxygenase signals were observed for resting and cytokine-activated human tonsillar B lymphocytes and for the lymphoblastoid B-cell lines BL41-E95-A and U266 (Fig. 1). The intensities of the detected bands indicate that the 5-lipoxygenase gene was expressed to a higher extent in activated tonsillar B cells than in resting B cells. These results are in agreement with our earlier report about 5-lipoxygenase activity in these cells (14). The corresponding PCR analysis of cDNA from PMNLs (also 32 cycles) showed a band of the same size as for the B cells. PCR analysis using 5-lipoxygenase primers and cDNA templates derived from five lymphoblastoid T-cell lines (CCRF, MOLT-3, MOLT-4, Jurkat FHRC, and 1301) gave negative results for 5-lipoxygenase, in agreement with the observation that sonicates of these cells did not metabolize arachidonic acid to 5-lipoxygenase products (14). Also reverse transcription–PCR analysis of RNA isolated from human endothelial cells did not give any detectable 5-lipoxygenase signal (data not shown), in accordance with the observation that these cells apparently are devoid of 5-lipoxygenase activity (17).

Detection of FLAP mRNA. FLAP has been reported to be required for the synthesis of LTs in intact cells after calcium ionophore challenge (6). Therefore we investigated whether lymphocytes express the mRNA coding for FLAP. Reverse transcription–PCR analysis of tonsillar B lymphocytes and the lymphoblastoid B- and T-cell lines showed, in contrast to the findings for 5-lipoxygenase mRNA, that all lymphocytic

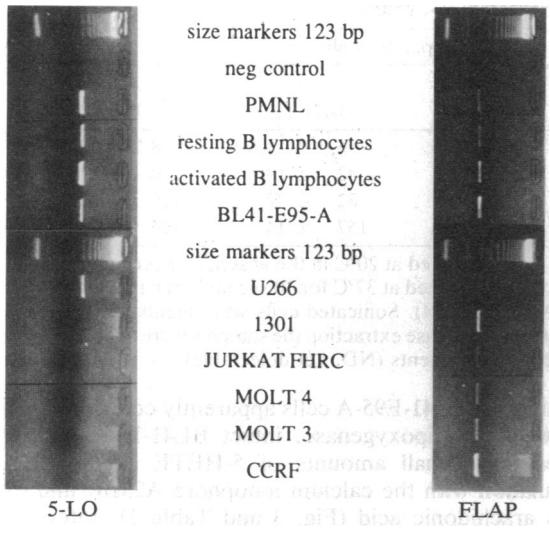

FIG. 1. Reverse transcription–PCR analysis of 5-lipoxygenase (5-LO) and FLAP mRNAs in lymphocytes. The samples were analyzed by 2% agarose gel electrophoresis. (*Left*) Products formed after 32 cycles of PCR using 5-lipoxygenase primers. (*Right*) Products formed after 28 cycles of PCR using FLAP primers.

cells expressed FLAP mRNA. However, no band was detected with human endothelial cells (data not shown).

Immunoblot Analysis of 5-Lipoxygenase in BL41-E95-A Cells. When the 15,000 × *g* supernatant from BL41-E95-A cells was analyzed by SDS/PAGE followed by Western blotting, an immunoreactive band appeared that comigrated with recombinant human 5-lipoxygenase (Fig. 2). The relative intensities of these bands give a rough estimate of the amount of 5-lipoxygenase in the sample, about 50 ng per 10^6 cells, or 0.5 μg/mg of soluble protein. Furthermore, only one band corresponding to 5-lipoxygenase was detected when the supernatant of BL41-E95-A cells was mixed with recombinant 5-lipoxygenase. Two different polyclonal antisera were used in these experiments, one developed against native 5-lipoxygenase purified from human leukocytes (see *Materials and Methods*) and another raised against recombinant 5-lipoxygenase (29). Similar results were obtained with these two antisera.

Effects of Glutathione-Depleting Agents on the 5-Lipoxygenase Activity in Intact BL41-E95-A Cells. The observed discrepancy between intact and sonicated BL41-E95-A cells in the capacity to synthesize 5-lipoxygenase products (14) prompted us to initiate studies on the mechanism of activation of 5-lipoxygenase in intact B cells. Since it has been reported that the glutathione-depleting agents diamide and Dnp-Cl stimulate 5-lipoxygenase activity in intact human PMNLs (9, 10), the effects of these compounds on the synthesis of LTB_4 and 5-HETE in intact BL-E95-A cells were examined. Intact cells (10^7) were preincubated for 10 min in Ca^{2+}-free PBS in the absence or presence of diamide (500 μM) prior to a 10-min incubation with $CaCl_2$ (1 mM), arachidonic acid (40 μM), and ionophore A23187 (5 μM). Cell sonicates obtained from 10^7 cells (suspended in calcium-free PBS containing 1 mM EDTA) were incubated with ATP (1 mM), $CaCl_2$ (2 mM), and arachidonic acid (40 μM) for 10 min. Intact BL41-E95-A cells treated with diamide produced similar amounts of LTB_4 as were formed by sonicated cells (Fig. 3). However, the levels of the nonenzymatically formed Δ^6-trans isomers of LTB_4 were significantly lower in comparison to the amounts formed by the cell sonicates.

The results from another set of experiments are shown in Table 1. Under the conditions used in this study, stimulation of intact BL41-E95-A cells with the calcium ionophore

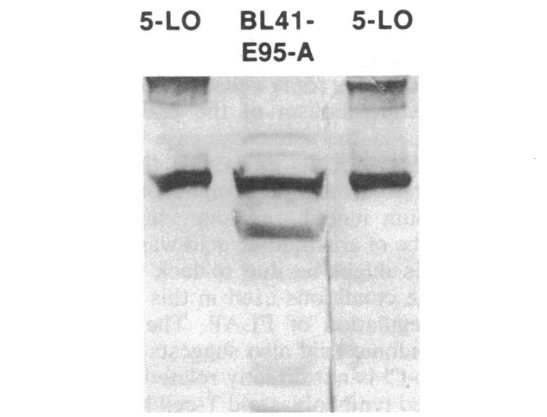

FIG. 2. Western blot analysis of 5-lipoxygenase in BL41-E95-A cells. An aliquot of the 15,000 × *g* supernatant from BL41-E95-A cells was electrophoresed in an SDS/polyacrylamide gel and electroblotted onto nitrocellulose. The membrane was incubated with a polyclonal antiserum against 5-lipoxygenase. The BL41-E95-A sample (180 μg of protein) corresponded to 2 × 10^6 cells. Recombinant human 5-lipoxygenase (5-LO, 100 ng) was used as standard.

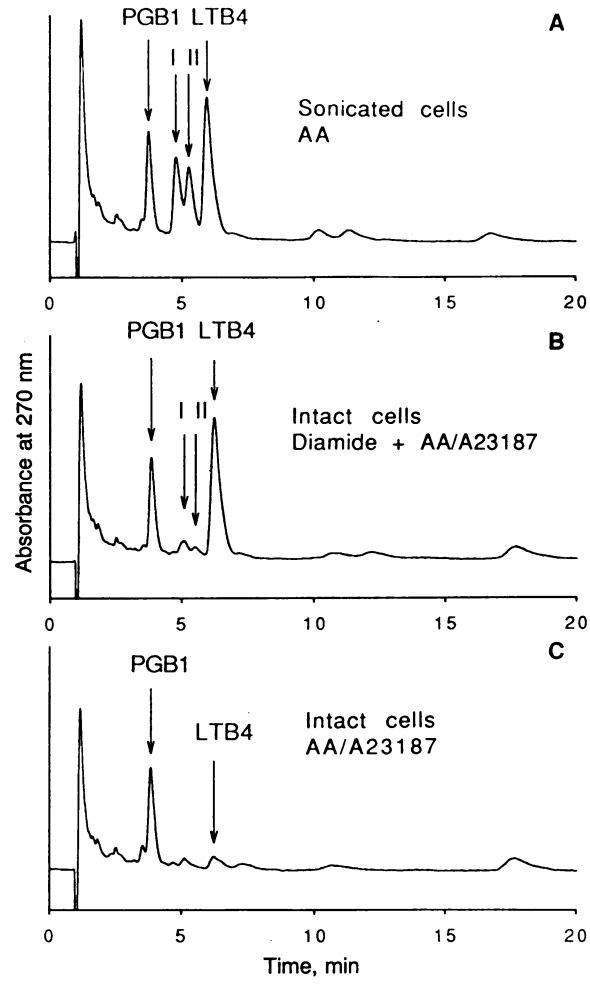

FIG. 3. Reversed-phase HPLC of the products formed by sonicated BL41-E95-A cells (*A*) and intact cells (*B* and *C*) after stimulation with indicated compounds for 10 min. AA, arachidonic acid (40 μM); calcium ionophore A23187 (5 μM); diamide (500 μM). Products were eluted from the HPLC column (Radial-Pak cartridge, 5 × 100 mm, packed with 4-μm Novapak C_{18}) with methanol/water/trifluoroacetic acid (70:30:0.007, by volume) at 1.2 ml/min. PGB1, prostaglandin B_1; I and II, Δ^6-*trans*-LTB_4 and 12-epi-Δ^6-*trans*-LTB_4.

Table 1. Effects of diamide and Dnp-Cl on leukotriene synthesis in BL41-E95-A cells

Cells	Stimuli	pmol per 10^6 cells: Δ^6-trans isomers of LTB_4	LTB_4	5-HETE	Total
Intact	20:4 + A23187	ND	1.8 ± 0.3	6.9 ± 1.6	8.7 ± 1.7
	Diamide + 20:4 + A23187	2.8 ± 1.4	24 ± 3.3	42 ± 18	70 ± 22
	Dnp-Cl + 20:4 + A23187	6.5 ± 3.2	22 ± 2.3	82 ± 22	110 ± 28
Sonicated	20:4 + Ca^{2+} + ATP	20 ± 2.1	19 ± 4.3	157 ± 14	196 ± 18

Intact BL41-E95-A cells (10^7) were suspended in PBS without Ca^{2+} and incubated at 20°C in the absence or presence of diamide (500 μM) or Dnp-Cl (100 μM) for 10 min. Thereafter the cells were incubated at 37°C for 30 sec and then incubated for 10 min with Ca^{2+} (1 mM), arachidonic acid (20:4, 40 μM), and A23187 (5 μM). Sonicated cells were incubated with arachidonic acid (40 μM), $CaCl_2$ (2 mM), and ATP (1 mM) for 10 min. After solid-phase extraction the samples were analyzed by reversed-phase HPLC. Values are means ± SE of three independent experiments (ND, not detectable).

A23187 and arachidonic acid for 10 min led to the formation of relatively low but yet significant amounts of LTB_4 (1.8 ± 0.3 pmol per 10^6 cells) and 5-HETE (6.9 ± 1.6 pmol per 10^6 cells; mean ± SE, $n = 3$). Sonicated BL41-E95-A cells produced more than 10 and 30 times higher levels of LTB_4 and 5-HETE, respectively. In the presence of either diamide (500 μM) or Dnp-Cl (100 μM), intact BL41-E95-A cells synthesized similar amounts of LTB_4 but significantly lower levels of Δ^6-trans-LTB_4 plus 12-epi-Δ^6-trans-LTB_4, as compared with sonicated cells. Further, the formation of 5-HETE also increased when intact cells were treated with these drugs, although the amounts were still 2–4 times lower than those obtained from sonicated cells. In the absence of exogenously added arachidonic acid, neither diamide nor Dnp-Cl stimulated the synthesis of LTB_4 or 5-HETE by intact cells (data not shown). In the absence of Ca^{2+}, neither intact nor sonicated BL41-E95-A cells produced 5-lipoxygenase products. In contrast to the observation for intact cells, and in agreement with earlier studies with sonicated human PMNLs (10), diamide (500 μM) inhibited the 5-lipoxygenase activity in sonicated cells (data not shown).

To elucidate whether the cellular 5-lipoxygenase activity was directly related to the intracellular levels of glutathione, we investigated the effect of buthionine sulfoximine, an inhibitor of γ-glutamylcysteine synthase. BL41-E95-A cells were cultivated for 24 hr in the presence of buthionine sulfoximine (2 mM) without any effect on cell viability. The inhibitor reduced cellular glutathione levels from 6.4 ± 1.7 nmol per 10^6 cells to 0.52 ± 0.24 nmol/10^6 cells (mean ± SE, $n = 3$), but this was not accompanied by an upregulation of the 5-lipoxygenase activity in intact cells stimulated with arachidonic acid and calcium ionophore. Furthermore, relatively high concentrations of reduced glutathione were necessary to inhibit the formation of 5-HETE and LTB_4 in cell sonicates (IC_{50} varying from 2 to 12 mM; data not shown). In contrast, reduced glutathione (1 mM) slightly enhanced the stimulatory effect of diamide on the synthesis of 5-lipoxygenase products in intact cells (data not shown).

DISCUSSION

We previously reported that human tonsillar B lymphocytes and lymphoblastoid B cells possessed 5-lipoxygenase activity and metabolized arachidonic acid to LTB_4 (14). Here we report on the expression and regulation of 5-lipoxygenase and FLAP in human B and T lymphocytes. The expression of 5-lipoxygenase was demonstrated at the transcriptional and translational levels in B lymphocytes, by reverse transcription–PCR and Western blot analyses (Figs. 1 and 2). Activation of tonsillar B lymphocytes with cytokines seems to be associated with an increased expression of 5-lipoxygenase mRNA (Fig. 1), which is in accordance with the observation that activated B lymphocytes possess higher 5-lipoxygenase activity than resting B lymphocytes (14). Both resting and activated tonsillar B cells expressed FLAP mRNA (Fig. 1).

Although BL41-E95-A cells apparently contain substantial amounts of 5-lipoxygenase, intact BL41-E95-A cells produced very small amounts of 5-HETE and LTB_4 upon stimulation with the calcium ionophore A23187 and exogenous arachidonic acid (Fig. 3 and Table 1). Interestingly, intact osteosarcoma cells transfected with a 5-lipoxygenase expression vector synthesized considerable amounts of 5-lipoxygenase products after stimulation with calcium ionophore and exogenous arachidonic acid (32). It thus appears that B lymphocytes possess a system for downregulation of 5-lipoxygenase enzyme activity, which is absent in the osteosarcoma cells.

Treatment of intact BL41-E95-A cells with the two glutathione-depleting agents diamide and Dnp-Cl prior to stimulation with calcium ionophore and arachidonic acid remarkably increased the synthesis of 5-HETE and LTB_4, in comparison to the amounts formed by cells not preincubated with the drugs. This is apparently not due to a direct stimulatory effect on the enzyme, since diamide inhibits 5-lipoxygenase activity in sonicates of PMNLs (10) and in sonicates of BL41-E95-A cells. Furthermore, cultivation of the cells in the presence of buthionine sulfoximine led to reduction of the cellular glutathione levels by >90%, without any increase in 5-lipoxygenase activity in intact cells. The effect of diamide on the synthesis of LTB_4 by intact cells was not counteracted by addition of exogenous glutathione (1 mM). Instead, a stimulatory effect was observed. Thus, the actions of diamide and Dnp-Cl on 5-lipoxygenase activity appear not to be directly related to their effects on glutathione levels. A possible mechanism of action of these drugs would be to influence the redox status of a cellular factor, leading to the activation of 5-lipoxygenase.

The effects of diamide and Dnp-Cl on 5-lipoxygenase activity in intact cells were strictly dependent on the presence of exogenous arachidonic acid. Studies regarding the role of FLAP, utilizing osteosarcoma cells, indicate that FLAP is essential for the metabolism of the endogenous pool of arachidonic acid via the 5-lipoxygenase pathway (6, 32). Although BL41-E95-A cells expressed the FLAP mRNA (Fig. 1), cells pretreated with diamide or Dnp-Cl did not respond to calcium ionophore alone, suggesting that the endogenous source of arachidonic acid was not available for the enzyme. This might be due to lack of phospholipase activity under the conditions used in this study or to post-transcriptional regulation of FLAP. The requirement for exogenous arachidonic acid also suggests that the effect of diamide and Dnp-Cl is not directly related to FLAP.

The investigated lymphoblastoid T-cell lines (1301, Jurkat FHRC, MOLT-4, MOLT-3, and CCRF) expressed FLAP mRNA but not 5-lipoxygenase mRNA (Fig. 1). Based on studies on the expression of 5-lipoxygenase and FLAP during differentiation of the promyelocytic HL-60 cell line, it has been suggested that these two genes may respond to similar transcriptional control (7). However, our results indicate that the transcriptional regulation of these two genes is different

and that the inability of the investigated T cells to synthesize 5-lipoxygenase products is due to the absence of 5-lipoxygenase gene expression. Immunoblot analysis of FLAP in certain lymphoblastoid T cells failed to detect FLAP expression (7), suggesting either that those T cells were different from those used in the present study or that expression of FLAP is posttranscriptionally regulated.

In view of the complexity of 5-lipoxygenase, human B and T cells might provide a useful model system for studies on the regulation of this enzyme. An increased knowledge of the regulation of 5-lipoxygenase in human lymphocytes might also lead to a better understanding of the role of 5-lipoxygenase products in the immune system.

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