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HUMAN B LYMPHOCYTES POSSESS 5-LIPOXYGENASE ACTIVITY AND CONVERT ARACHIDONIC ACID TO LEUKOTRIENE B4

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Incubation of cell sonicates from monoclonal B cells with arachidonic acid led to the formation of leukotriene (LT) B4 and 5-hydroxy-eicosatetraenoic acid (5-HETE). In contrast, stimulation of intact B cells with the calcium ionophore A23187 +/- arachidonic acid did not, under similar conditions, lead to formation of LTB4. The identification of these products was based on reverse phase- and straight phase-HPLC analysis, UV-spectroscopy and gas chromatography-mass spectrometry. Cell sonicates of highly enriched human tonsillar B lymphocytes also converted arachidonic acid to LTB4 and 5-HETE. Activation of these cells with B cell mitogen and cytokines for three days led to an up-regulation of 5-lipoxygenase activity. This study provides evidence for the biosynthesis of LTB4 from arachidonic acid in B cell lines and in normal human tonsillar B lymphocytes.

Biosynthesis of leukotriene (LT) B4 proceeds via the intermediate LTA4. This epoxide is formed from arachidonic acid in two consecutive reactions, both catalyzed by the 5-lipoxygenase (1). Leukotriene A4 can be hydrolyzed either enzymatically to LTB4, a reaction catalyzed by the LTA4 hydrolase, or non-enzymatically to the epimers at C-12 of Δ^6-trans-LTB4 and the two isomers of 5,6-DHETE (2).

During the last decade, one of the most controversial matters in the leukotriene research field has been whether or not human lymphocytes can synthesize LTB4 from arachidonic acid. The first study suggesting synthesis of LTB4 by human peripheral T cells was published in 1981 (3). However, the utilized cell population contained about 20% non-T cells and thereby the question was raised whether contaminating monocytes were the true source of LTB4. In 1986, Goodwin and coworkers claimed that activated T cells produce LTB4 (4, 5). These reports initiated studies in many laboratories, including our own. However, the synthesis of LTB4 from arachidonic acid by highly purified B and T cells or monoclonal lymphoblastoid cell lines could not be observed by us or other investigators (6-10). Later, Goodwin and his colleagues retracted their original reports since they were unable to reproduce their initial observations (11,12). The biosynthesis of

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5-hydroxy-eicosatetraenoic acid (5-HETE) by lymphocytes was suggested by Parker et al in 1979 (13) and recently in Epstein-Barr virus (EBV) positive B cell lines (14). The biosynthetic pathway for the formation of 5-HETE, i.e. catalysis by cytochrome P-450 or 5-lipoxygenase, was not examined in these studies. As a result of these studies, the general consensus at present is that human lymphocytes can not metabolize arachidonic acid to LTB4. However, it has been demonstrated that lymphocytes can convert LTA4 into LTB4 (9, 10), and recently, evidence was presented that lymphocytes might play a role in the formation of LTB4 via conversion of monocyte-released LTA4 (15).

In highly purified B lymphocytes leukotriene B4 has been shown to stimulate the expression of the activation-associated surface antigen CD23, thymidine incorporation, proliferation and immunoglobulin secretion (16), an observation recently confirmed by other investigators (17). Several studies have also shown that LTB4 induces natural killer (NK) and suppressor/cytotoxic T cell activity (18-21) and influences the release of certain cytokines (22, 23).

The present report describes the biosynthesis of LTB4 from arachidonic acid in sonicates of human tonsillar B cells and lymphoblastoid B cell lines.

MATERIALS AND METHODS

Materials Synthetic standards of 5-HETE and LTB4 were obtained from Biomole Inc (Plymouth Meeting, PA). The calcium ionophore A23187 was from Calbiochem-Behring (La Jolla, CA).

Isolation of lymphocytes and PMNL Isolation of human B lymphocytes from tonsils was performed as described (24). This cell preparation contained more than 98 % B cells and less than 0.2 % monocytes, based on immunofluorescence staining as described (9). Polymorphonuclear leukocytes (PMNL) from leukocyte concentrates were isolated as described (15, 25).

Activation of B cells Isolated tonsillar B cells were incubated for 72 h at 37°C with a cocktail of heat inactivated *Staphylococcus aureus* Cowan I (SAC) diluted 1:80,000 plus 10 U/ml of recombinant interleukin-2 (rIL-2) and rIL-6, respectively and 25% (v/v) of B cell stimulatory factor from T helper cell MP6 supernatants (BSF-MP6) (24), containing thioredoxin as an active cytokine (Rosén et al, to be published).

Cell lines Cells were cultivated at 37°C in an atmosphere of 5% CO_2. Culture medium was RPMI 1640, supplemented with 100 IU/ml penicillin, 100 µg/ml streptomycin and 5% fetal calf serum (final concentrations). After harvesting, cells were washed in phosphate-buffered saline (PBS, Dulbecco's formula, pH 7.4), and resuspended in calcium-free PBS.

Experimental conditions Lipoxygenase assay: Cells, suspended in calcium-free PBS containing 1 mM EDTA, were sonicated on ice. Thereafter, ATP was added to a concentration of 1 mM and samples were preincubated for 30s at 37°C. Incubations were started by the addition of calcium (2mM) and arachidonic acid (40 µM) and the enzymatic reaction was allowed to occur for 10 min at 37°C before termination by the addition of 1 vol of methanol.

Intact cells: Cells were preincubated for 30 s at 37°C in calcium-free PBS. Incubations were started by the subsequent addition of calcium (1mM), arachidonic acid (40µM) and ionophore A23187 (5µM) and stopped with 1 vol of methanol after 10 min of incubation.

Qualitative and quantitative analysis of leukotrienes and monohydroxy acids. Eicosanoids were extracted using C_{18} extraction cartridges as described (26), samples were subjected to reverse-phase (RP) HPLC. Mobile phase was methanol/water/ trifluoroacetic acid (70/30/0.007), flow-rate was 1.2 ml/min, columns were radial pak C_{18} from Waters Associates (Milford, MA) equiped with Novapak guard columns. On-line UV-spectra were recorded on a Waters 991 diode-array spectrophotometer.

Quantitative determinations were performed by integration of the area of eluted peaks. Straight-phase (SP) HPLC analysis was performed on methyl esters of the compounds (27).

Gas chromatography-mass spectrometry (GC-MS) Methyl esters of the compounds were converted into trimethylsilyl (Me_3Si) ethers by treatment with hexamethyldizilasane and trimethylchlorosilane in pyridine. GC-MS was carried out on a Hewlett-Packard 5970B mass selective detector connected to a Hewlett-Packard 5890 gas chromatograph, utilizing an ionizing energy of 70 eV.

RESULTS

Metabolism of arachidonic acid by monoclonal B cell lines

The metabolism of arachidonic acid in sonicates of various monoclonal B cells was investigated. Cells were homogenized by sonication on ice for 2 x 5 s and incubated at 37°C in the presence of ATP, calcium and arachidonic acid. After purification, the samples were subjected to RP-HPLC. Fig 1 shows a typical RP-HPLC chromatogram of the products formed after a 10 min incubation of sonicated cells from the EBV positive Burkitt's lymphoma-derived B cell line, BL41-E95A (19 $x10^6$ cells). Ultra-violet (UV)-monitoring was carried out at 270 nm (left panel) and 236 nm (right panel). Five peaks (I-V) containing a conjugated triene spectrum were identified. These had elution times corresponding to standards of Δ^6-trans-LTB4 (peak I), 12-epi-Δ^6-trans-LTB4 (peak II), LTB4 (peak III) and the two isomers of 5,6-DHETE (peaks IV and V), respectively. The material in peak III was collected and treated with diazomethane to yield methyl esters, and further analyzed on SP-HPLC, which gave a peak coeluting with a synthetic standard of LTB4 (data not shown). The mass spectrum of the Me_3Si derivative of this material had prominent ions at m/e 404, 383, 293, 267, 229, 217, 203, 191 and 129, which is in agreement with the reported mass spectrum of LTB4 (28). An additional peak was observed which coeluted with synthetic 5-HETE and had a diene spectrum with maximum at

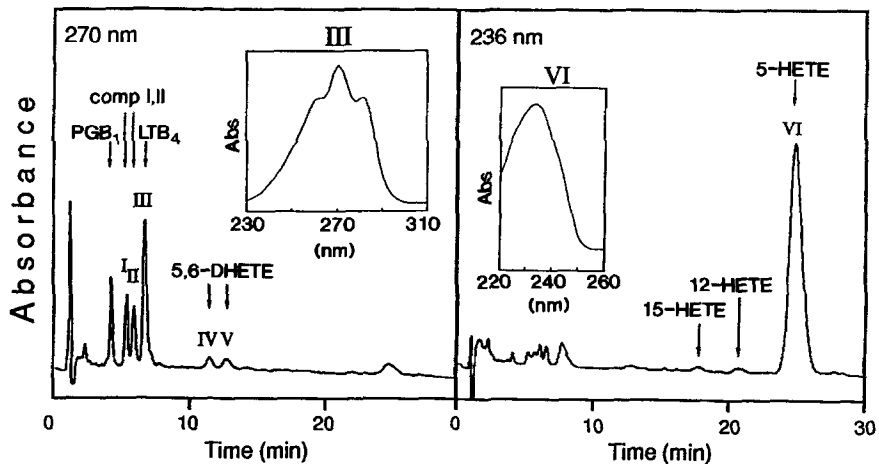

FIG. 1. RP-HPLC chromatogram of the products formed by sonicates of BL41-E95A cells after incubation with arachidonic acid (40μM) for 10 min. UV-monitoring was carried out at 270nm (left panel) and 236 nm (right panel). Insert: UV-spectrum of the materials in peak III (left panel) and peak VI (right panel). The arrows show the retention times for the indicated synthetic standards.

TABLE 1. Formation of 5-HETE and LTB_4 in sonicates of various monoclonal B cells and PMNL

CELLS	5-HETE (pmol/10^6 cells)	LTB_4 (pmol/10^6 cells)
BL41	30±14	2.5±0.4
BL41-EHR-A	49±13	4.1±0.6
BL41-E95-A	124+29	14+1.0
BL41-E95-C	107±38	7.3±0.7
RAMOS Nut	4.5±0.5	0.4±0.2
AW-RAMOS	8.2±1	0.7±0.1
RAMOS-EHR-C	13±3	1.0±0.3
RAMOS-E95-C	72±19	6.0±1.6
RAJI	28±7	3.1±1.0
BL28	51±19	5.5±0.5
PMNL	239±19	27±3.0

Values depict mean ± range in one representative experiment out of three. Cell lines were characterized in ref. 34.

236 nm (right panel). The methylated material in this peak coeluted with 5-HETE on SP-HPLC (data not shown). The mass spectrum of the Me_3Si derivative of the methyl ester of this material showed ions of high intensity at m/e 406 (M), 391, 352, 305, 255, 203 and 129, thus confirming that the product was 5-HETE (29). The prominent ions in the total ion chromatograms of the materials in the HPLC-peaks III and VI coeluted with synthetic standards of LTB_4 and 5-HETE, respectively.

Table 1 shows the relative capacity of cell sonicates of some EBV negative B cell lines and their EBV-transformed counterparts to produce LTB_4 and 5-HETE. Cell sonicates of human polymorphonuclear leukocytes (PMNL) were about twice as efficient as BL41-E95A cells in their metabolizm of arachidonic acid to 5-HETE and LTB_4 (Table 1). Cell sonicates of other B lymphocytic cells which possessed the capacity to convert arachidonic acid to LTB_4 and 5-HETE were B type chronic lymphocytic leukemia (B CLL) and the B cell line Sultan (30) (data not shown). Intact B cells, in a number corresponding to those used in the lipoxygenase assay on sonicates, did not produce detectable amounts of 5-HETE or LTB_4. However, if a large number of intact BL41-E95-A cells (200 x 10^6 cells) were incubated with arachidonic acid (40 μM) and calcium ionophore A23187 (5 μM) for 10 min, barely detectable but yet significant amounts of LTB_4 and 5-HETE could be found.

Cell sonicates of seven T cell lines were also examined, i.e. MP-6, Molt3, Molt4, Jurkat DC, Jurkat-FHRC, CCRF-CEM and 1301 (kindly obtained from the lab of Dr. G. Klein, Dept. of tumor biology, Karolinska Inst.). In contrast to the tested B lymphocytes, none of these T cell line sonicates (5-9x10^6 cells per sample) produced any detectable amounts of LTB_4 or 5-HETE when incubated in a similar manner as the B lymphocytes.

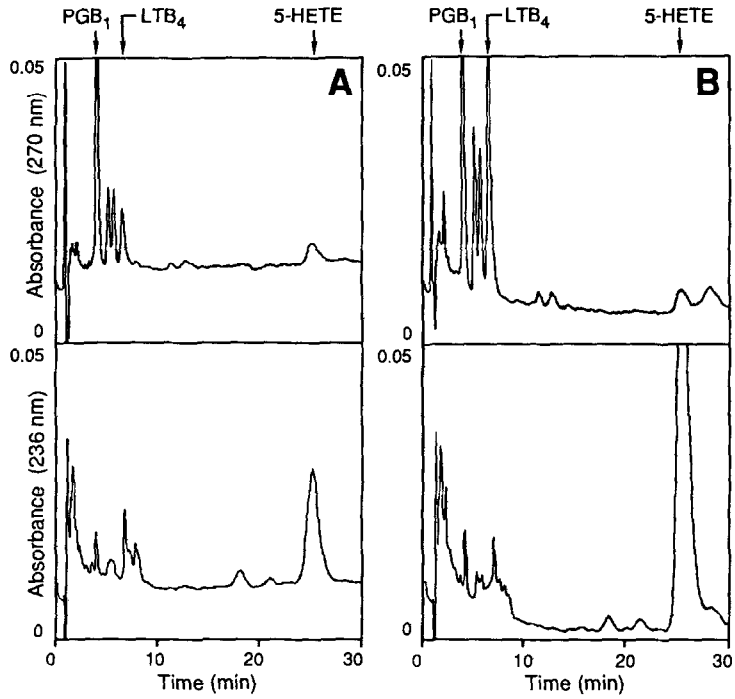

FIG. 2. RP-HPLC chromatograms of the products formed by sonicates of human tonsillar B lymphocytes after incubation with arachidonic acid for 10 min. (A) the products formed by freshly isolated B lymphocytes (7 x 10^6 cells) and (B) the products produced by tonsillar B cells (1 x 10^7 cells) after three days preincubation with SAC/rIL-2/rIL-6/BSF-MP6. UV-monitoring was carried out at 270 nm (upper panel) and 236 nm (lower panel). The arrows show the retention times for the indicated synthetic standards.

Cell sonicates of human tonsillar B lymphocytes

Human B lymphocytes of high density were isolated from tonsils (24). This preparation contains mainly resting non-activated B cells. Half of the cellpopulation was immediately sonicated and assayed for lipoxygenase activity. The second part was cultivated with a mixture of SAC/ rIL-2/ rIL-6/ BSF-MP6 for three days, as previously described (16). Thereafter, the cells were sonicated and incubated as above. As shown in Fig. 2, these activated B cells possessed a higher 5-lipoxygenase activity than those not preincubated with the cytokines. In the preparation containing resting B cells, the levels of LTB4 and 5-HETE were 5 pmol/10^6 cells and 38 pmol/10^6 cells, respectively (Fig. 2 left panel). Cell sonicates of cytokine-treated B cells produced 15 pmol LTB4/10^6 cells and 100 pmol 5-HETE/10^6 cells (Fig. 2 right panel).

DISCUSSION

The present report demonstrates that sonicates of human B lymphocytes from Burkitt's lymphoma-derived cell lines possess 5-lipoxygenase activity and have considerable capacity to convert arachidonic acid to 5-HETE and LTB4 (Fig. 1 and Table 1). The identification of 5-HETE and LTB4 was based on chromatographic properties, UV-absorbance and gas chromatography-mass spectrometry analysis (Fig.1).

Based on Northern and Western blot analysis, we suggested in a previous paper that Raji, a Burkitt's lymphoma-derived B cell line, did not express the 5-lipoxygenase gene (31). That report was apparently incorrect, probably due to the relatively low sensitivity of the utilized methods. In addition, recent studies with PCR (polymerase chain reaction) demonstrated the expression of the 5-lipoxygenase gene in Raji cells[1].

Studies on normal human tonsillar B lymphocytes indicated that these cells possess 5-lipoxygenase activity. Cell sonicates of resting tonsillar B lymphocytes had lower capacity to convert arachidonic acid to 5-HETE and LTB4 than B cells activated with the polyclonal B cell mitogen SAC and a mixture of rIL-2/rIL-6/BSF-MP6 (Fig.2). The cell preparations used in this study contained more than 98% B lymphocytes and less than 0.2% monocytes. In light of the high purity of B cells, the above described 5-lipoxygenase activity in various B cell lines and the observed upregulation of 5-lipoxygenase activity after cytokine treatment of tonsillar B cells, it is most unlikely that monocytes are the principal source of LTB4 and 5-HETE in the tonsillar B cell preparations.

In contrast to the previous studies on lymphocytes and LTB4 formation (3-14), we investigated the metabolism of arachidonic acid in both intact and sonicated B lymphocytes. Incubation of a very large number of intact BL41-E95A cells with arachidonic acid and calcium ionophore A23187 led to the formation of very low levels of 5-lipoxygenase products in comparison to those formed by sonicates of these cells. This was unexpected, since intact and sonicated PMNL's produce LTB4 in the same magnitude. We interpret these results to indicate that the ionophore is not a proper stimulus for LTB4 synthesis in B lymphocytes. Studies are in progress to elucidate the discrepancy between intact and sonicated lymphocytes in their capacity to produce LTB4 and 5-HETE.

In summary, the present report presents evidence showing that lymphoblastoid B cell lines as well as tonsillar B lymphocytes have the potential to convert arachidonic acid to LTB4. In the light of this study, the effects of LTB4 on certain lymphocyte functions (16-23) and the effects of 5-lipoxygenase inhibitors on allograft rejection (32, 33) are of considerable interest.

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