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HUMAN B AND T LYMPHOCYTES CONVERT LEUKOTRIENE A_4 INTO LEUKOTRIENE B_4

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Incubation of human tonsillar B lymphocytes and peripheral blood T lymphocytes with leukotriene A_4 led to the formation of leukotriene B_4. The purity of these cell suspensions was more than 99%, containing less than 0.5% monocytes. Incubation of purified B or T lymphocytes with the calcium ionophore A23187 did not lead to the formation of any detectable amounts of leukotrienes. Several established cell lines of B and T lymphocytic origin were also found to convert leukotriene A_4 into leukotriene B_4, showing that monoclonal lymphocytic cells possess leukotriene A_4 hydrolase activity.

Biosynthesis of leukotrienes proceeds via the unstable intermediate leukotriene A_4 (1). This compound can be hydrolysed enzymatically into leukotriene B_4, by leukotriene A_4 hydrolase, or non-enzymatically into Δ^6-trans-leukotriene B_4 and 12-epi-Δ^6-trans-leukotriene B_4 (1). Leukotriene B_4 is a putative modulator of various lymphocyte functions. It can augment natural killer cell activity and induce human supressor T lymphocytes (2,3). However, it has been a matter of controversy whether this cell type is able to synthesize leukotrienes from arachidonic acid (4-6). Highly purified peripheral lymphocytes and T-cell lines were recently reported to lack the ability to generate leukotrienes from arachidonic acid (7,8). Although certain cells can not transform arachidonic acid into leukotrienes, they might play an important role in the metabolism of leukotrienes produced by other cells. Since leukotriene A_4 is not only an intracellular precursor but also an extracellular (9), it was of interest

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to examine if lymphocytes can metabolise this compound. Human endothelial cells (10,11), erythrocytes (12) and platelets (13), all devoid of 5-lipoxygenase activity, have been shown to convert leukotriene A_4 into leukotriene B_4 and/or leukotriene C_4. In this report we describe the ability of highly purified lymphocytes to metabolise leukotriene A_4 into leukotriene B_4. Furthermore, leukotriene A_4 hydrolase activity in established B and T lymphocytic cell lines is also described.

MATERIALS AND METHODS

Synthetic leukotriene A_4 and B_4 were kindly given by Dr. Frank Fitzpatrick, The Upjohn Company, Kalamazoo, MI, U.S.A. Alkaline hydrolysis of the methyl ester of leukotriene A_4 was carried out as described (14).

Isolation of lymphocytes: Blood from healthy human donors was collected in tubes containing EDTA (final conc. 6 mM). T lymphocytes were isolated in the following way: 1. Peripheral blood was fractionated on a Ficoll-Isopaque (Lymphoprep, Nyegaard and Co., Oslo, Norway) density gradient to separate mononuclear cells from erythrocytes and granulocytes (15). 2. The fraction containing mononuclear cells was added to plastic petri dishes (1 hr, 37^{0}C) to remove macrophages and monocytes. 3. Non-adherent cells were incubated with sheep red blood cells (1 hr;0^{0}C) and thereafter fractionated on a Ficoll-Isopaque density gradient to obtain T cells. 4. The rosetted T-lymphocytes in the pellet were subjected to hypotonic lysis in order to remove erythrocytes (0.5 ml destilled sterile water, 23^{0}C for 30 sec). Immediately after the lysis, the cells were diluted to 15 ml with phosphate-buffered saline (PBS, pH 7.4), centrifuged at 800 g for 5 min and resuspended in PBS. Human B-lymphocytes from tonsills were isolated as described (16).

Immunofluorescence: The purity of T and B lymphocytes separated from peripheral blood and tonsills was determined by indirect and direct immunofluorescence staining using mouse monoclonal antibodies against lineage specific markers (CD 14 for monocytes/macrophages and OKT 3 for T cells) plus rabbit anti mouse IgG-flourescein isothiocyanate (FITC) conjugates (Dako Immunoglobulins, Copenhagen, Denmark). B cells were stained by a direct immunofluorescence method using rabbit anti-human IgA-, IgM-, IgG-FITC (Dako Immunoglobulins, Copenhagen, Denmark). CD 14 antibodies were kindly provided by Dr. Manuel Patarroyo (17). OKT 3 antibodies were obtained from Ortho Diagnostic, New Jersey, U.S.A. (18). The purity of the periheral blood T lymphocytes and the B lymphocytes from tonsills was found to be better than 99%, containing less than 0.5% monocytes.

Established cell lines: Raji (19) and Namalwa (20) are Burkitt lymphoma derived B-cell lines. U 698 (21) and B6 (22) are B-lymphoblastoid cell lines. MP-6 is a T_4-T hybridoma (16) and Molt 4 is a T-lymphoma (23).

Experimental conditions: Cells were suspended in PBS (pH 7.4) containing 0.5% albumin (human fraction V; Sigma Chemical Co., St. Louis, MO) to stabilize leukotriene A_4 (24). Incubations were carried

out in the presence of leukotriene A_4 or ionophore A23187 (final conc. 10 μM) at 37°C for 10 min.

Assay for leukotrienes: Incubations were terminated by the addition of one volume of methanol. Samples were then centrifuged and subjected to RP-HPLC (reverse phase high performance liquid chromatography) without further purification according to the method previously described (25). Briefly, HPLC was performed on a Constametric III equipment with a CI-10 integrator from LDC/Milton Roy with a 3μ Nucleosil C_{18} column (Skandinaviska Genetec AB, Sweden) guarded by a 10μ Bondapak C_{18} guardcolumn (Waters Inc.). The eluent was acetonitrile/methanol/water (29.5/19.5/51) supplemented with acetic acid 1% and adjusted to pH 5.6 with 30% NaOH. UV-monitoring was carried out at 280 nm and quantitative analysis was performed by integration of the elution profile. UV-spectral properties of leukotrienes were assayed with a Hewlett-Packard 8451 computerized scanner, on-line with the HPLC eluent.

RESULTS

Purified tonsillar B lymphocytes (6 x 10^6 cells; < 0.5 % monocytes) were incubated with leukotriene A_4 (0.5 nmol) for 10 min at 37°C in the presence of 0.5% albumin. The RP-HPLC chromatogram of the products formed showed three peaks which had elution times corresponding to standards of the two non-enzymatic products , Δ^6-trans-leukotriene B_4 (peak I) and 12-epi-Δ^6-trans-leukotriene B_4 (peak II), and synthetic leukotriene B_4 (Fig.1 A). The UV-spectrum of the material in this peak was also in agreement with the spectrum reported for leukotriene B_4 (26). The amount of leukotriene B_4 formed was 21 pmol, giving 35 pmol/10^7 cells. No formation of leukotrienes was observed after stimulation with the calcium ionophore A23187 for 10 min (data not shown).

Highly purified peripheral blood T lymphocytes (15 x 10^6 cells; <0.5 % monocytes) also transformed leukotriene A_4 (0.5 nmol) into leukotriene B_4 (Fig. 1 B). The pattern of products formed was similar to that obtained by tonsillar B-lymphocytes (Fig.1 A) and the amount of leukotriene B_4 formed was 25 pmol/10^7 cells.

Addition of the same amount of leukotriene A_4 to phosphate-buffer alone (with 0.5% albumin) yielded almost exclusively the two non-enzymatic products (peaks I and II; Fig. 1 C) with only a small peak corresponding to the elution time of synthetic leukotriene B_4 (Fig. 1 C). Another unidentified peak, lacking a conjugated triene spectrum and with a retention time of about 16 min, was observed in all samples (Fig. 1). This peak was probably derived from material present in the albumin batch.

Different lymphocytic cell lines were also incubated with leukotriene A_4. Raji, an established B-lymphocytic Burkitt lymphoma cell line, efficiently converted leukotriene A_4 into leukotriene B_4

(Fig.2 A). Incubation of these cells with leukotriene A_4 (0.7 nmol) for 10 min led to the formation of 307 pmol leukotriene $B_4/10^7$ cells. According to these preliminary data, Raji possesses the highest capacity to produce leukotriene B_4 of all tested cell lines. Other B-lymphocytic cell lines that produced significant amounts of leukotriene B_4 were U-698, Namalwa and B6 (data not shown).

Also MP-6, an established T_4-T hybridoma cell line, formed leukotriene B_4 after incubation with leukotriene A_4 (0.7 nmol) for 10 min (Fig. 2B). The level of leukotriene B_4 was 44 pmol/10^7 cells. Similar results were obtained by another T-cell line, Molt-4 (data not shown). None of these B- and T-cell lines produced leukotriene B_4 after stimulation with the calcium ionophore A23187 for 10 min (data not shown).

DISCUSSION

Leukotriene formation has been described for several types of leukocytes, i.e. neutrophils, eosinophils, basophils and monocytes/-macrophages (1). The ability of lymphocytes to synthesize leukotrienes has been a controversial matter (4-8). In this report we describe the ability of highly purified B lymphocytes from tonsills and peripheral blood T lymphocytes to hydrolyse the epoxide intermediate leukotriene

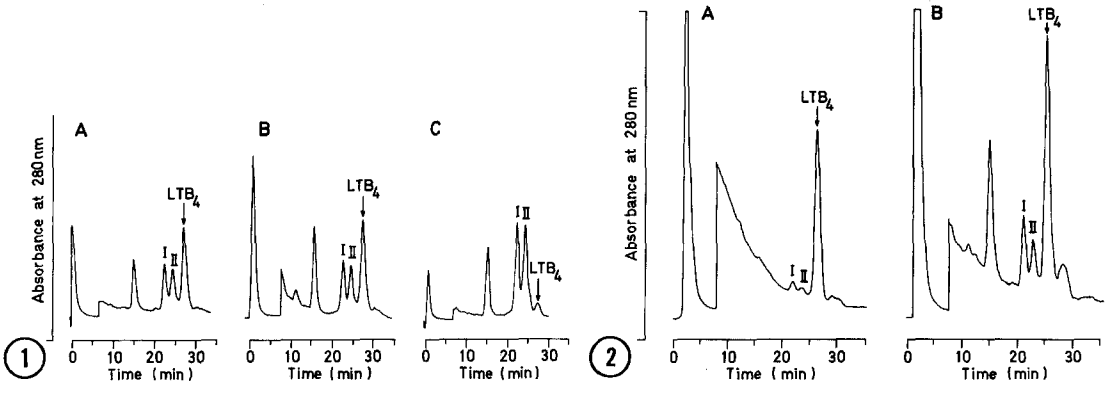

Fig. 1. RP-HPLC chromatograms of the products formed by (A), B lymphocytes from tonsills (6 x 10^6 cells); (B), peripheral T lymphocytes (15 x 10^6 cells); and (C), PBS without cells, after incubation with 0.5 nmol leukotriene A_4 for 10 min in the presence of 0.5% albumin. Leukotriene B_4 was identified by cochromatography with synthetic standard, as indicated, and UV-spectroscopy. Peaks designated I and II are Δ^6-trans-leukotriene B_4 and 12-epi-Δ^6-trans-leukotriene B_4, respectively. The chromatograms depict one typical experiment out of four.

Fig. 2. RP-HPLC profile of the products generated by (A), Raji 6 (8_6 x 10^6 cells), an establish B-cell line, and (B), MP-6 (12.5 x 10^6 cells), an T_4-T hybridoma cell line, after incubation with 0.7 nmol leukotriene A_4 for 10 min in the presence of 0.5% albumin. Roman numbers I and II are Δ^6-trans-leukotriene B_4 and 12-epi-Δ^6-trans-leukotriene B_4, respectively. The retention time for synthetic leukotriene B_4 is indicated. The chromatograms represent one typical experiment out of three.

A_4 into the biologically active leukotriene B_4 (Fig. 1). The identification of leukotriene B_4, throughout this study, was based on RP-HPLC and UV-spectroscopy. Stimulation of these cells with the calcium ionophore A 23187 did not lead to any significant formation of leukotriene B_4. The purity of the present lymphocyte preparations was determined by using monoclonal antibodies directed against monocytes, B lymphocytes and T lymphocytes, respectively, showing that the preparations of B lymphocytes from tonsills and peripheral blood T lymphocytes contained less than 0.5 % monocytes.

Several established B- and T-cell lines were found to produce leukotriene B_4 after incubation with leukotriene A_4 (Fig. 2), demonstrating that monoclonal lymphocytic cells had the capacity to hydrolyse leukotriene A_4 into leukotriene B_4. Thus it appears unlikely that contaminating cells are the source of the observed leukotriene A_4 hydrolase activity in tonsillar and peripheral blood lymphocyte preparations. Preliminary data indicated that certain established lymphocytic cell lines, especially Raji, possess higher capacity to produce leukotriene B_4 from leukotriene A_4 than resting lymphocytes (Figs. 1,2). A possible explanation could be that activation / proliferation of lymphocytes might, in certain cases, be associated with an increased leukotriene A_4 hydrolase activity.

In summary, the present study suggests putative cell-cell interactions where leukotriene A_4, released from cells containing 5-lipoxygenase activity, could be metabolised by B and T lymphocytes into leukotriene B_4. This might be of importance for the regulation of certain immunological and inflammatory reactions.

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