Control of Polyclonal Immunoglobulin Production from Human Lymphocytes by Leukotrienes; Leukotriene B_4 Induces an OKT8(+), Radiosensitive Suppressor Cell from Resting, Human OKT8(−) T Cells

Durgaprasadarao Atluru and James S. Goodwin
Department of Medicine, University of New Mexico School of Medicine, Albuquerque, New Mexico 87131

Abstract. We report that leukotriene B_4 (LTB_4), a 5-lipoxygenase metabolite of arachidonic acid, is a potent suppressor of polyclonal Ig production in pokeweed mitogen (PWM)-stimulated cultures of human peripheral blood lymphocytes, while LTC_4 and LTD_4 have little activity in this system. Preincubation of T cells with LTB_4 in nanomolar to picomolar concentrations rendered these cells suppressive of Ig production in subsequent PWM-stimulated cultures of fresh, autologous B + T cells. This LTB_4-induced suppressor cell was radiosensitive, and its generation could be blocked by cyclohexamide but not by mitomycin C. The LTB_4-induced suppressor cell was OKT8(+), while the precursor for the cell could be OKT8(−). The incubation of OKT8(−) T cells with LTB_4 for 18 h resulted in the appearance of the OKT8(+) on 10–20% of the cells, and this could be blocked by cyclohexamide but not by mitomycin C.

Thus, LTB_4 in very low concentrations induces a radiosensitive OKT8(+) suppressor cell from OKT8(−) cells. In this regard, LTB_4 is three to six orders of magnitude more potent than any endogenous hormonal inducer of suppressor cells previously described. Glucocorticosteroids, which block suppressor cell induction in many systems, may act by inhibiting endogenous production of LTB_4.

Address reprint requests to Dr. Goodwin.

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Introduction

The leukotrienes are a recently discovered class of arachidonic acid metabolites that result from oxidation at the C-5 position (1). This results in the production of a labile epoxide, leukotriene A_4 (LTA_4),[1] which in turn is converted to either LTB_4 by enzymatic hydrolysis or to LTC_4 by conjugation with glutathione. LTC_4 is in turn converted to LTD_4 and LTE_4 (1–3). The biologic potency of these compounds is impressive and diverse (1, 2). LTC_4, LTD_4, and LTE_4 are together the active components of the slow reacting substance of anaphylaxis (1), while LTB_4 has powerful chemoattractant and aggregating properties for neutrophils (4, 5).

Because phospholipase A_2 activation, the first step in arachidonic acid metabolism, is as ubiquitous a response to stimuli as is adenylate cyclase activation, it is safe to assume that the eventual list of biologic actions of the leukotrienes and other lipoxygenase metabolites of arachidonic acid will be considerably longer than it is today. One area that has been relatively uninvestigated is the action of leukotrienes on humoral and cellular immune responses. Webb et al. (6) reported that LTD_4 and LTE_4 in concentrations as low as 10^{-12} M caused >50% inhibition of phytohemagglutinin (PHA)-induced ^{3}H-thymidine incorporation in mouse splenic T cells, while much higher concentrations (10^{-7} M) caused inhibition of the formation of antibody-forming cells against sheep erythrocytes in Mishell-Dutton cultures. In contrast, Payan and Goetzl (7) found no inhibition of human T cell proliferation in PHA-stimulated cultures by LTC_4 or LTD_4, but did find a modest amount of inhibition with high concentrations (10^{-7}–10^{-6} M) of LTB_4. Rola-Pleszczynski et al. also reported a modest

1. *Abbreviations used in this paper:* ELISA, enzyme-linked immunosorbent assay; FCS, fetal calf serum; LT, leukotriene; LTA_4, LTB_4, LTC_4, LTD_4, LTE_4, leukotrienes A_4, B_4, C_4, D_4, and E_4; PBMC, peripheral blood mononuclear cells; PHA, phytohemagglutinin; PWM, pokeweed mitogen; PGE, prostaglandin E.

inhibition of PHA-stimulated mitogenesis in human T lymphocytes, by LTB_4 (8). More importantly, these authors observed that T cells that were preincubated with low concentrations ($\sim 10^{-12}$ M) of LTB_4 suppressed the mitogen response of fresh T cells.

These preliminary reports prompted us to examine in detail the role of leukotrienes in the control of polyclonal Ig production by human peripheral blood lymphocytes. We found that LTB_4, but not LTC_4 or LTD_4, inhibits IgG and IgM production in concentrations as low as 10^{-12} M. LTB_4 induces a resting OKT8(−) T cell to become an OKT8(+), radiosensitive suppressor cell.

Methods

Preparation of lymphocytes. Peripheral venous blood was drawn in syringes that contained preservative-free heparin. Peripheral blood mononuclear cells (PBMC) were separated from heparinized blood of healthy normal donors by differential centrifugation over Ficoll-Hypaque and were washed three times with phosphate-buffered saline (PBS). Glass adherent cells were removed by incubation at 37°C for 45 min on glass petri dishes in RPMI 1640 with 20% fetal calf serum (FCS). T cells were isolated from these nonadherent cells by rosetting with 2-aminoethylisothiouronium bromide-treated sheep erythrocytes followed by centrifugation over Ficoll-Hypaque for 30 min at 300 *g*. The sheep erythrocytes were lysed with Tris-ammonium chloride. The nonrosetting cells left at the interface were termed B cells. The percentage of Ig-bearing cells in this population was between 40 and 60%.

Monoclonal antibodies. The monoclonal antibodies OKT8 and OKT4 were used. OKT8 detects suppressor and cytotoxic T cells (9, 10); OKT4 is a monoclonal antibody that reacts with the helper/inducer subset of human T cells (10, 11). There is no overlap between OKT8 and OKT4 in peripheral blood of normal donors (12). Subpopulations of human T cells were separated with a monoclonal antibody rosetting technique (11, 13). Ox erythrocytes were coupled to affinity-purified goat anti-mouse IgG (Tago Inc., Burlingame, CA) with the chromium chloride method (11) and resuspended as a 0.5% suspension in RPMI 1640 with 10% FCS. After incubation for 30 min with monoclonal antibody, the T cells were washed two times with cold PBS in a refrigerated centrifuge, resuspended to a concentration of 4×10^6 cells/ml in RPMI 1640 that was supplemented with 10% FCS, and mixed with an equal volume of the sensitized ox erythrocytes. The mixture was centrifuged for 10 min at 200 *g*, incubated for 30 min on ice, and gently resuspended. Rosetting cells were separated from nonrosetting cells by centrifugation over Ficoll-Hypaque for 30 min at 300 *g*. The T cell subsets were isolated by either positive or negative selection, e.g., the OKT4-positive helper T cells were isolated as either OKT4(+) cells or OKT8(−) cells. OKT4(+) cell populations that were isolated positively were >92% OKT4(+) and <3% OKT8(+), while OKT4(+) populations that were isolated by negative selection were >90% OKT4(+) and <1% OKT8(+). OKT8(+) cell populations that were isolated positively were >90% OKT8(+) and <5% OKT4(+), while those that were isolated negatively were >86% OKT8(+) and <5% OKT4(+).

Indirect immunofluorescence analysis of T cells on FACS 111. A cell pellet that contained 10^6 cells was incubated with 50 μl of an appropriate dilution of the monoclonal antibody for 30 min on ice. The cells were washed twice in a refrigerated centrifuge with PBS that contained 0.01% azide. The pellet was further incubated with 50 μl of fluorescein-conjugated goat anti-mouse IgG (Becton-Dickinson Monoclonal Center, Mountain View, CA) for 30 min on ice. The cell pellet was washed twice, resuspended in 1 ml PBS-paraformaldehyde, and analyzed with the FACS 111 (Becton-Dickinson & Co.).

Leukotrienes. LTB_4, LTC_4, and LTD_4 were kindly supplied by Dr. J. Rokach (Merck-Frosst Laboratories, Dorval, Canada), stored in ethanol at −20°C, and appropriately diluted in RPMI 1640 medium immediately before use. This resulted in ethanol concentrations in the cultures of from 100 μl to 10 nl/liter. Control cultures always contained appropriate amounts of ethanol, which had no measurable effect on Ig production.

Cell cultures for Ig production. Cultures that contained 10^6 PBMC or combinations of 10^5 T cells or T cell subsets with 2.5×10^5 B cells were incubated at 37°C in 5% CO_2 in 1 ml RPMI 1640 that was supplemented with L-glutamine, 10% FCS, and penicillin-streptomycin. Cultures were set up in 10 × 75-mm culture tubes (Fisher Scientific Co., Pittsburgh, PA). Pokeweed mitogen (PWM) (Gibco Laboratories, Grand Island, NY) was added in final concentrations of 1/400; cultures were stopped after 7 d; the supernatants were collected; and IgG and IgM were determined by an enzyme-linked immunosorbent assay (ELISA) method (14). All cultures were performed in duplicate, and each culture was further split for duplicate analysis of Ig concentration by ELISA.

Suppressor cell assay. In some experiments, varying concentrations of LTB_4 were added at the initiation of cultures with PWM and left throughout the 7-d culture period. Most experiments were designed to study the induction of suppressor cells. In these experiments, T cells or T cell subsets were preincubated for 18 h with varying concentrations of LTB_4, washed three times with PBS, and added to fresh PWM-stimulated B + T cell cultures. When specified, preincubated cells were either treated with mitomycin C (Sigma Chemical Co., St. Louis, MO, 50 μg/ml) or with cyclohexamide (Gibco Laboratories, 50 μg/ml) for 30 min at 37°C, or irradiated (2,000 rad) before addition to the fresh B + T cell cultures. The viabilities of the preincubated T cells or T cell subsets was >95%, as determined by trypan blue dye exclusion test.

ELISA for IgG-IgM measurements. Flat-bottom polyvinyl flexible microtiter plates (Flow Laboratories, Inc., McLean, VA) were incubated overnight at 4°C with 120 μl affinity purified goat anti-human IgG or anti-human IgM (Tago Inc.) in a concentration of 1 μg/ml (diluted in PBS). After washing the plates five times in PBS, 100 μl supernatant from the lymphocyte incubations, diluted 1/10 in PBS with 5% gamma-globulin free bovine serum (Gibco Laboratories), was added to the wells. Standards were prepared from human serum. After 2 h incubation at room temperature (in a moisture chamber) the plates were washed five times in PBS. 100 ml of peroxidase-labeled goat anti-human IgG or anti-human IgM (Tago Inc.) were added to the wells. These antibodies were diluted to 1 μg/ml with PBS that contained 5% γ-globulin-free bovine serum. After a 1-h incubation at room temperature in a moisture chamber, the plates were washed five times with PBS, and 100 μl freshly prepared substrate was added. The substrate was prepared by mixing 10 ml citric acid (2.3 g%) with 100 μl 0.5% H_2O_2 and 100 μl of a 2 g% solution of 2,2'-azido-di-(3-ethyl-benzthiazolin-solfonate) (Boehringer Mannheim Biochemicals, Indianapolis, IN). The reaction was stopped after 30 min and the absorption of each well was measured in microelisa autoreader MR 580 (Dynatech Laboratories Inc., Alexandria, VA) at 415 nm, and optical densities were translated to nanograms by plotting from the standard curve. Duplicate samples were assayed and were always within 10% of each other.

Results

Effect of leukotrienes on PWM-stimulated immunoglobulin production. Fig. 1 demonstrates the effects of adding various concentrations of LTB_4, LTC_4, and LTD_4 on IgG production in PWM-stimulated cultures of PBMC. Concentrations of LTB_4 as low as 10^{-12} M caused small but significant inhibition of IgG production, while concentrations in the 10^{-8}–10^{-10} M range caused substantial inhibition. In contrast, LTC_4 and LTD_4 caused only a small amount of inhibition, even at 10^{-8} M concentrations. Analogous results were obtained with IgM production (data not shown).

Effect of preincubating T cells with leukotrienes on PWM-stimulated immunoglobulin production. We next asked whether LTB_4 was inhibiting PWM-stimulated Ig production by acting primarily on B cells or T cells. Accordingly, we measured the effect of preincubating T cells with leukotrienes, then of adding them back to fresh autologous B cells in PWM-stimulated cultures. As shown in Table I, T cells that were preincubated for 18 h with LTB_4 no longer supported IgG and IgM production by autologous B cells in subsequent PWM-stimulated cultures. As little as 10^{-11} M LTB_4 caused >30% inhibition of subsequent Ig production. Once again, LTC_4 and LTD_4 in high concentrations had little effect in this system.

The LTB_4-induced decrease of Ig production could be due either to a decrease in T helper function or to an increase in T suppressor function. Table II shows that T cells preincubated with LTB_4 suppressed Ig production in PWM-stimulated cultures of fresh, autologous B + T cells, which suggested that LTB_4 inhibits Ig production by stimulating suppressor cell function.

Phenotype of suppressor T cell generated by LTB4. The data presented above suggested that LTB_4 was inducing suppressor T cells. We next sought to determine the phenotype of those suppressor cells by incubating T cells with LTB_4; we then separated the T cells into helper T and suppressor T cell fractions using the OKT4 and OKT8 monoclonal antibodies,

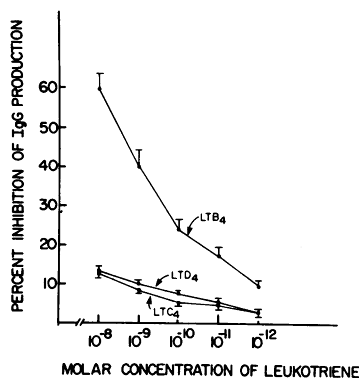

Figure 1. Effect of addition of leukotrienes on IgG production in PWM-stimulated cultures of human PBMC. LTB_4, LTC_4, or LTD_4 in the noted concentrations were added at the initiation of the cultures, and IgG production was measured by ELISA after 7 d. LTB_4 at all concentrations (10^{-8}–10^{-12} M) caused a significant inhibition of IgG production ($P < 0.01$ by paired t test). LTC_4 and LTD_4 caused a small but significant inhibition of IgG production at high concentrations. Data represent mean±SEM of the results of experiments on seven subjects.

Table I. Effect of Preincubating T Cells for 18 H with Leukotrienes on the Production of IgG and IgM in Subsequent PWM Cultures with Fresh Autologous B Cells

Cells	T cells preincubated with	IgG	% Inhibition	IgM	% Inhibition
		ng/ml		*ng/ml*	
B	—	93±10	—	23±6	—
B + T	0	1,101±124	—	315±39	—
B + T	10^{-12} M LTB_4	901±93*	18±4	285±31‡	9±3
B + T	10^{-11} M LTB_4	752±112*	32±4	208±27*	34±4
B + T	10^{-10} M LTB_4	531±102*	52±5	214±14*	32±3
B + T	10^{-9} M LTB_4	348±105*	68±5	191±19*	39±3
B + T	10^{-8} M LTB_4	258±98*	76±4	129±17*	59±4
B + T	10^{-9} M LTC_4	963±84*	12±3	287±46‡	9±3
B + T	10^{-8} M LTC_4	957±96*	13±6	288±33‡	8±4
B + T	10^{-9} M LTD_4	991±114*	10±4	291±34	8±4
B + T	10^{-8} M LTD_4	909±106*	17±4	278±41‡	12±3

T lymphocytes were incubated with leukotrienes LTB_4, LTC_4, or LTD_4 for 18 h, washed three times with PBS, and cultured with fresh, autologous B cells at 10^5 T cells plus 2.5×10^5 B cells. Viabilities of LTB_4 preincubated cells were >95.0% as determined by trypan blue dye exclusion test. Results are expressed as mean±SEM from experiments on cells from five different subjects.
* Significantly different from control by paired t test with $P < 0.001$.
‡ $P < 0.05$.

and added these fractions to PWM-stimulated cultures of fresh autologous B + T cells. As shown in Fig. 2, almost all of the suppressive activity of the T cells that were preincubated with LTB_4 is contained in the OKT8(+) fraction.

Phenotype of precursor of the LTB_4-induced suppressor T cell. In an analogous fashion, we next investigated the phenotype of the T cell that was induced to become an OKT8(+)

Table II. Effect of Preincubating T Cells with LTB_4 on IgG Production in PWM-stimulated Cultures of Fresh Autologous B Plus T Cells

Cells	Pre-incubated T cells	T cells preincubated with	IgG	% Inhibition
			ng/ml	
B + T	0	—	2,417±240	—
B + T	+	0	2,199±124	—
B + T	+	10^{-12} M LTB_4	1,938±97*	12±4
B + T	+	10^{-11} M LTB_4	1,728±181‡	21±6
B + T	+	10^{-10} M LTB_4	1,443±108‡	36±4
B + T	+	10^{-9} M LTB_4	1,088±110‡	50±5
B + T	+	10^{-8} M LTB_4	752±84‡	66±6

T lymphocytes were incubated with LTB_4 for 18 h, washed three times with PBS, and 10^5 of these T cells were added to cultures of fresh autologous B (2.5×10^5) plus T (10^5) cells. Cultures were incubated for 7 d with PWM at 1/400. Results are expressed as mean±SEM from 14 different experiments.
* Significantly different from control by paired t test with $P < 0.05$.
‡ $P < 0.001$.

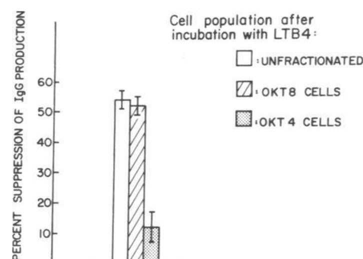

Figure 2. Effect of separating T cells that were preincubated with LTB_4 into OKT8(+) and OKT4(+) subsets on inhibition of IgG production in subsequent PWM-stimulated cultures of fresh, autologous B + T cells. T cells were incubated for 18 h with 10^{-9} M LTB_4, then washed, and part of the T cells were fractionated into OKT8(+)- and OKT4(+)-enriched subsets by the monoclonal antibody rosetting technique. These T cell subsets (10^5 cells) or unfractionated T cells (10^5 cells) were then added to fresh, autologous B + T cells (10^5 T plus 2.5×10^5 B) in PWM-stimulated cultures, and the percentage inhibition of IgG production was noted, compared with cultures in which T cells were preincubated in media alone, then divided into OKT8(+), OKT4(+), or unfractionated cells, and then added to fresh autologous B + T cells. Data represent results from four experiments. There was no difference in the degree of suppression that was caused by OKT8(+)-enriched T cells vs. unfractionated T cells ($P > 0.6$), but both caused significantly more inhibition than did OKT4(+)-enriched T cells ($P < 0.001$ by *t* test). In two experiments the OKT4(+) cells were isolated by positive selection and the OKT8(+) cells by negative selection, and in the other two experiments the OKT8(+) cells were isolated positively and the OKT4(+) cells negatively. The results did not vary with the method of cell isolation. Percentage of suppression is calculated as: (1 − [IgG production in cultures to which T cell or T cell subset preincubated with LTB4 is added]/[IgG production in cultures to which T cell or T cell subset preincubated with media is added]) × 100%.

suppressor T cell by LTB_4. T cells were separated into OKT8(+) and OKT4(+) fractions, then incubated for 18 h with LTB_4, and then added to fresh autologous B + T cells in PWM-stimulated cultures. As shown in Table III, after preincubation with LTB_4, either OKT4(+) T cells or OKT8(+) T cells inhibited Ig production in cultures of fresh autologous B + T cells. Unfractionated T cells that were preincubated with LTB_4 at any of three concentrations caused significantly more inhibition of Ig production than did OKT4(+) T cells.

Radiosensitivity of LTB4-induced suppressor T cells. Exposure of T cells to 2,000 rad γ-irradiation after preincubation with LTB_4 eliminated most but not all of the suppressive activity in subsequent PWM-stimulated cultures of fresh autologous B + T cells (Fig. 3), while mitomycin C pretreatment had little effect on suppressor cell generation. Similarly, exposure of T cells to 2,000 rad γ-irradiation before incubation with LTB_4 also resulted in an elimination of suppressor cell generation (data not shown).

Effect of LTB4 on T cell phenotype. The data presented thus far would suggest that the effector suppressor T cell generated by LTB_4 is OKT8(+), while the precursor can be OKT4(+) (because incubation of OKT4(+) T cells with LTB_4 results in generation of suppressor cells). Indeed when OKT4(+) cells were incubated with LTB_4, then separated into OKT4(+) or OKT8(+) fractions, the suppressive activity resided entirely in the OKT8(+) fraction (Fig. 4), which suggested that LTB_4 induced a phenotypic change from OKT4(+) to OKT8(+) T cells. We then tested this directly by measuring percentage of OKT8(+) T cells in OKT4(+) T cells after exposure to LTB_4. These results from two experiments are expressed in Fig. 5. After 4 h of incubation with LTB_4 at 10^{-8} or 10^{-9} M, T cells enriched for OKT4(+) cells by prior removal of OKT8(+) cells showed an increased percentage of cells staining with OKT8, compared with OKT4(+) T cells incubated in media. The percentage of OKT8(+) cells in the LTB_4 containing cultures continued to increase at 18 h of incubation, and then decreased at 40 h incubation. This increase in cells bearing the OKT8(+) phenotype after incubation with LTB_4 was blocked by prior irradiation of the cells with 2,000 rad or by addition of cyclohexamide (50 μg/ml) to the incubation media but not by prior treatment of the cells with mitomycin C, (Fig. 6).

Table III. Effect of Preincubating Either Whole T Cells, or T Cell Subsets with LTB_4 on IgG Production in PWM-stimulated Cultures of Fresh Autologous, B Plus T Cells

Preincubated cells	T cells/subsets preincubated with	IgG production	% Suppression
		ng/ml	
0	—	2,422±38	—
T cells	0	2,223±185	—
T cells	10^{-8} M LTB_4	785±106*	64±3‡
T cells	10^{-9} M LTB_4	1,114±92*	48±5‡
T cells	10^{-10} M LTB_4	1,551±114*	27±4‡
T_4(+) cells	0	2,255±132	—
T_4(+) cells	10^{-8} M LTB_4	1,116±120*	50±3
T_4(+) cells	10^{-9} M LTB_4	1,488±112*	35±4
T_4(+) cells	10^{-10} M LTB_4	1,818±99*	19±4
T_8(+) cells	0	1,994±174	—
T_8(+) cells	10^{-8} M LTB_4	1,141±117*	42±4
T_8(+) cells	10^{-9} M LTB_4	1,380±105*	30±5
T_8(+) cells	10^{-10} M LTB_4	1,580±103*	21±3

T cells or T cell subsets were incubated either in media alone or with LTB_4 at various concentrations for 18 h, then washed and added to fresh autologous B + T cells (10^5 preincubated T cells plus 10^5 fresh T cells plus 2.5×10^5 fresh B cells). Results are expressed as the mean±SEM for experiments on four subjects.

* Significantly different from control by paired *t* test with $P < 0.001$.

‡ Significantly greater (more inhibition) than for T4(+) or T8(+) cells, by paired *t* test, with $P < 0.05$.

Discussion

The results of the experiments presented above can be summarized as follows. LTB_4 in picomolar to nanamolar concentrations, but not LTC_4 or LTD_4, inhibits polyclonal IgG and IgM production in PWM-stimulated cultures of human peripheral blood lymphocytes. This inhibition is apparently due to the induction of an OKT8(+), radiosensitive suppressor cell

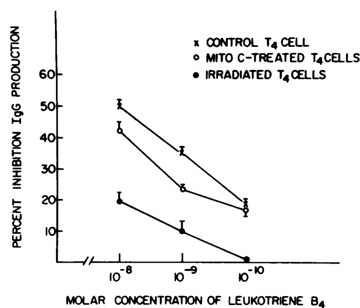

Figure 3. Effect of irradiation or mitomycin C treatment of OKT4(+) T cells after preincubation with LTB_4 on subsequent suppression of IgG production in PWM-stimulated cultures of fresh, autologous B + T cells. T cells were enriched for OKT4(+) cells by the monoclonal antibody rosetting technique. These cells were then incubated with media alone or with LTB_4 at various concentrations for 18 h. After this incubation, portions of these cells were exposed to 2,000 rad irradiation, or to mitomycin C, or to nothing, and then added to PWM-stimulated cultures of fresh autologous B + T cells. The exposure of the OKT4(+) T cells that were preincubated with media to irradiation or mitomycin C had no significant effect on subsequent IgG production in the PWM cultures of the fresh autologous B + T cells to which they were added. Preincubation of OKT4(+) T cells with LTB_4 resulted in a substantial suppression of IgG production in subsequent cultures. This suppression was greatly reduced, but not totally eliminated, by exposure of the cells to 2,000 rad. Treatment of the cells with mitomycin C resulted in a slight, nonsignificant decrease in degree of suppression of cells preincubated with 10^{-8} or 10^{-10} M LTB_4, and a slightly larger, significant ($P < 0.01$) decrease in degree of suppression of cells preincubated with 10^{-9} M LTB_4. Data represent mean±SEM of experiments on four subjects. Mitomycin C, mito C.

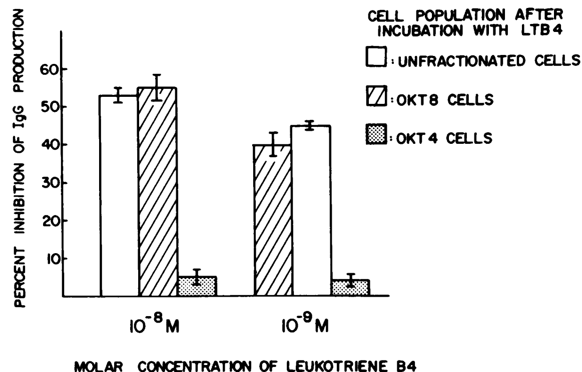

Figure 4. Effect of preincubating OKT4(+) T cells with LTB_4, then separating them into OKT4(+), OKT8(+), or unfractionated populations, on inhibition of IgG production in subsequent PWM-stimulated cultures of fresh, autologous B + T cells. The OKT4(+) cells were initially enriched with negative selection using the OKT8 antibody. These OKT4(+)-enriched cells were incubated with media alone or with LTB at 10^{-8} or 10^{-9} M for 18 h. After washing, the preincubated cells were used as unfractionated cells or were further divided into OKT8(+) and OKT4(+) fractions using the OKT8 antibody (so that the OKT8(+) cells were positively selected and the OKT4(+) cells were negatively selected). This procedure performed on OKT4(+) T cells that were preincubated in media alone produced almost no OKT8(+) cells; however, substantial amounts of OKT8(+) cells were recovered from the cultures where OKT4(+) T cells had been preincubated with LTB_4. The OKT4(+), OKT8(+), or unfractionated populations were then added to PWM-stimulated cultures of fresh, autologous B + T cells and degree of suppression of IgG production was calculated. There was no difference in the degree of suppression caused by unfractionated cells vs. OKT8(+)-enriched cells, but the OKT4(+)-enriched population caused almost no suppression. Thus, in cells that were first enriched for OKT4(+) cells, then incubated with LTB, the suppressive capability still remained in an OKT8(+) population, which suggests an OKT4 to OKT8 phenotypic switch that was stimulated by LTB_4. Data represent mean±SEM for experiments on three subjects.

by LTB_4. The precursor of this suppressor cell may be OKT4(+). Exposure of OKT4(+) T cells to LTB_4 results in an increase in the percentage of cells bearing OKT8(+) markers. This appearance of the OKT8 phenotype would appear to require new protein synthesis but not cell replication, because it is eliminated by cyclohexamide but not by mitomycin C.

Several low molecular weight hormones, most notably prostaglandin E (PGE) and histamine, have been shown to induce T suppressor cells in vitro. Webb et al. (15) reported that glass adherent mouse splenic T cells that were incubated with 10^{-5} M PGE became suppressive of subsequent in vitro assays of humoral and cellular immunity, and this PGE-induced suppressor cell acted by secreting a suppressor factor (16). Fischer et al. (17) has reported that 10^{-6} M PGE induces suppressor T cells from normal peripheral blood T cells. Other investigators have reported an analogous suppressor system activated by 10^{-4} M histamine (18–20).

What was striking to us about the findings in this present report is the very low concentrations of LTB_4 that were required for suppressor cell induction. As little as 10^{12} M caused significant inhibition of IgG production when added to PWM-stimulated cultures (Fig. 1) or when preincubated with T cells (Table I). No other endogenous substance is as potent an inducer of suppressor cells. Indeed it is three to six orders of magnitude more potent than either PGE or histamine. The potency of LTB_4 in inducing suppressor cells parallels its potency as a chemoattractant for polymorphonuclear leukocytes (4). It is interesting to contrast the extreme potency of LTB_4 in suppressor cell induction with its relative lack of effectiveness in in vitro assays of cellular immunity. Payan and Goetzl (7) reported a relatively modest inhibition of PHA-induced proliferation and lymphokine generation by 10^{-7} and 10^{-6} M LTB_4, with no effect of lower concentrations. Rola-Pleszczytnski et al. (8) also reported a small degree (~20%) of inhibition of PHA- or concanavalin A-induced proliferation of human lymphocytes by LTB_4, but these authors found essentially no dose-response relationship, with 10^{-12} M LTB_4 causing the same degree of inhibition (~20%) as did 10^{-6} M LTB_4. More recently, Gualde et al. (21) have found that another lipoxygenase product, 15-hydroperoxyeicosatetranoic acid, in 10^{-6}-M concentrations, causes induction of suppressor T cells from peripheral blood T cells. We feel that the relatively high concentration of 15-hydroperoxyeicosatetranoic acid that was required suggests that perhaps a metabolite such as a novel 14, 15

leukotriene is the active agent responsible for suppressor cell generation.

Incubation of OKT4(+) T cells for 18 h resulted in an increase in the percentage of cells staining with OKT8 (Fig. 5), and this OKT8(+) population contained all of the suppressor activity (Fig. 4). This increase in OKT8(+) cells could theoretically be secondary to a clonal expansion of the 2-3% residual OKT8(+) cells in the cell fraction enriched for OKT4(+) cells, or it could represent the expression of the OKT8 phenotype on cells previously not expressing OKT8. The fact that mitomycin C did not while cyclohexamide did prevent the LTB_4-stimulated increase in OKT4(+) cells makes us favor the latter explanation. We have not performed two-color immunofluorescence to determine if the OKT8(+) cells induced by LTB_4 also stain with OKT4. The fact that LTB_4 had no effect on cell viability or recovery indicates that the increase in the percentage of OKT8(+) cells is not due to an artifactually selective enrichment of these cells in the culture.

Several laboratories have found examples of OKT4(+) T cells differentiating into suppressor cells. Thomas et al. (22, 23) reported that PWM induces a subset of OKT4(+) T cells to become suppressor cells, but these suppressor cells retain their OKT4(+) OKT8(−) phenotype. Birch et al. (24) reported that a brief (30–60 min) exposure of human peripheral blood T cells to adenosine resulted in the generation of suppressor cells and also resulted in an increase in the percentage of OKT8(+) cells with a decrease in the percentage of OKT4(+) cells, which suggested an OKT4 to OKT8 conversion. Additional studies on whether the increase in the percentage of OKT8(+) required protein synthesis or cell replication were not performed. Burns et al. (25) described a probable change

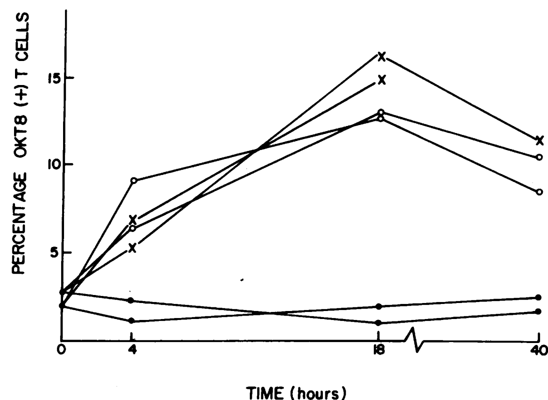

Figure 5. Effect of LTB_4 on percentage of cells expressing OKT8 marker among cell population enriched for OKT4(+) cells. OKT4(+) were enriched by negative isolation using OKT8 antibody. These OKT4(+) cells were then incubated for various times with media alone or with 10^{-8} or 10^{-9} M LTB_4. At 0, 4, 18, and 40 h the incubation was stopped and the percentage of OKT8(+) cells was calculated using an FACS. Individual data from experiments on two subjects is given. In both cases, either 10^{-9} or 10^{-8} M LTB_4 resulted in an increase in the percentage of cells that bore the OKT8 marker. •, Control; ○, 10^{-8} M LTB_4; ×, 10^{-9} M, LTB_4.

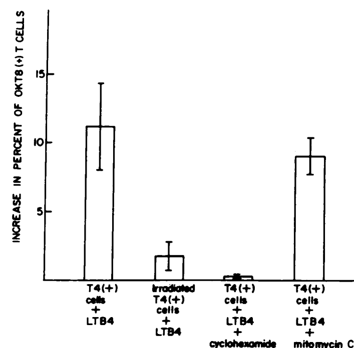

Figure 6. Effect of irradiation, mitomycin C, or cyclohexamide on the appearance of the OKT8(+) marker when OKT4(+) cells were cultured with LTB_4. OKT4(+) T cells were enriched from whole T cells by negative selection with OKT8. These cells were then exposed to mitomycin C, 2,000 rad irradiation, cyclohexamide, or nothing. These cells were then cultured in media alone or with 10^{-9} M LTB_4 for 18 h and the increase in percentage of OKT8(+) cells caused by LTB_4 was noted. Data represent the mean±SEM for experiments on four subjects. Both cyclohexamide or irradiation pretreatment blocked the LTB_4-induced increase in cells bearing the OKT8 phenotype, while mitomycin C had no appreciable effect.

in phenotype from OKT4 to OKT8 of OKT4(+)-enriched T cells in long-term culture.

While it is clear that physiologic concentrations of LTB_4 cause induction of suppressor cells in vitro, there is no evidence that endogenous LTB_4 plays a role in any in vitro or in vivo model of suppressor cell generation. It is interesting to note, however, that glucocorticosteroids inhibit suppressor cell generation in many in vitro and in vivo models (e.g., 26–28). It is currently thought that the action of steroids at the cellular level is mediated by the synthesis of a phospholipase A_2 inhibitory protein, termed lipomodulin (29) or macrocortin (30), and that this inhibitory protein prevents the release of arachidonic acid from membrane phospholipids. Thus, many or all of the actions of steroids may be due to inhibition of arachidonic acid metabolism. If steroids prevent suppressor cell generation by inhibiting arachidonic acid metabolism, then one would expect to find an arachidonic acid metabolite that at physiologic concentrations stimulated suppressor cell generation. Because cyclooxygenase inhibitors such as indomethacin do not inhibit suppressor cell generation (31–33), it is logical to assume that a lipoxygenase metabolite and not a cyclooxygenase metabolite of arachidonic acid is responsible for the suppressor cell generation. Our findings of suppressor cell generation by physiologic concentrations of LTB_4 are certainly consistent with that concept. Further work should be directed towards the question of whether addition of physiologic concentrations of LTB_4 can reverse the inhibition of suppressor cell generation or function caused by corticosteroids.

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