

Effects of Leukotrienes on Neutrophil Migration, and on Production and Action of Lymphokines

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The effect of the leukotrienes LTB₄, LTC₄ and LTD₄ on the production of the lymphokine leukocyte migration inhibitory factor (LIF) from human peripheral blood mononuclear cells was tested. LTC₄ and LTD₄ failed to influence LIF production by cells stimulated by antigen or the polyclonal T cell activator, phytohemagglutinin (PHA). LTB₄, at concentrations 10⁻⁷ to 10⁻⁶ M, showed significant inhibition of antigen-induced LIF production but was without effect when cells were stimulated by PHA. The cell levels of cyclic adenosine monophosphate (cAMP) were unaffected by 2.5 × 10⁻⁷ M LTB₄ in the absence or presence of theophylline. These results indicate that LTB₄ acts on the early effector stage of the immune response without affecting the cellular cAMP content.

Key words: cAMP; leukocyte migration inhibitory factor (LIF); leukotrienes; lymphocyte activation; polymorphonuclear leukocytes.

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The leukotrienes are derived from arachidonic acid via the lipoxygenase pathway (14), and are released by human lung tissue (9) and peripheral leukocytes (8) stimulated by Ca²⁺ ionophore. The leukotrienes LTC₄, LTD₄ and LTE₄ have been shown to be the bioactive components of slow-reacting substance of anaphylaxis (10), and the leukotriene LTB₄ is a potent chemo-attractant and proaggregant for neutrophils (6, 11). A general regulatory role of the leukotrienes on the inflammatory reactions is indicated by recent studies showing an inhibitory effect of LTB₄, LTD₄ and LTE₄ on mitogen-induced lymphocyte transformation (12, 13, 17) and antigen-induced plaque-forming cell formation *in vivo* (17).

In the present study, the influence of LTB₄, LTC₄ and LTD₄ on the G₀ to G₁ effector stage of the immune response was measured by testing the elaboration of the lymphokine, leukocyte migration inhibitory factor (LIF) (1).

MATERIAL AND METHODS

Effects of leukotrienes on LIF production

PHA-pulse and antigen-induced production of LIF by human blood mononuclear cells (MNC) was carried out as previously detailed (1, 3). The antigen used was purified protein derivative of tuberculin (PPD; Statens Serum Institute, Copenhagen, Denmark) at a concentration of 50 µg/ml. Parallel cultures in medium alone were used as controls. The leukotrienes were added at the beginning of the culture period, and, in the case of PHA-pulse treatment, the cell suspensions were reconstituted with the leukotrienes after the 1 h pulse period. Appropriate controls without leukotrienes were always cultured in parallel. After 22 h at 37°C, the supernatants were collected by centrifugation. Before assay, the leukotrienes were removed by dialysis for 48 h with at least two changes of dialysates.

Bioassay of LIF activity

An indirect leukocyte migration agarose technique, using purified, human polymorphonuclear cells (PMNL) as indicators, was used as previously described (1). The migration index (MI) was determined by the formula: $MI = \text{mean PMNL migration area in antigen or lectin-stimulated cell supernatant} / \text{mean PMNL migration area in control supernatant}$. Generally, values of $MI < 0.85$ indicate the presence of LIF. All assays were done in triplicate, and the per cent error was less than 5%.

Effect of leukotrienes on PMNL migration and the expression of LIF activity

These experiments were performed as earlier described (3). The direct effect of leukotrienes on PMNL migration under agarose was determined as a migration index (MI) calculated as follows: $MI = \text{mean migration area of leukotriene-treated PMNL} / \text{mean migration area of PMNL in medium alone}$. The effect of leukotriene on LIF expression was measured by adding Sephadex G-100-purified LIF, and its control counterpart, to PMNL in the presence or absence of leukotriene. After 60 min at 37°C, the cells were allowed to migrate under agarose (1, 3). The MI's were calculated using the formula: $MI = \text{mean PMNL migration area in LIF-rich supernatants} / \text{mean PMNL migration area in the control counterpart}$.

Quantitation of cAMP

The same medium and cell density as for the elaboration of LIF were used. The MNC cyclic adenosine monophosphate (cAMP) content was assayed in duplicate on duplicate samples as previously described (15, 16). The data shown represent mean values of these four determinations (the SD values were below 10%).

RESULTS AND DISCUSSION

Effect of leukotrienes on PMNL migration and the expression of LIF activity

Table 1 shows the effects of LTB_4 , LTC_4 and LTD_4 on the migration of PMNL in the presence or absence of preformed LIF. LTB_4 , at concentration 10^{-6} M, caused a significant enhancement of migration. Furthermore, although the effect is slight, LTB_4 seems to reduce, directly, the function of LIF.

Effect of leukotrienes on the elaboration of LIF

As shown in Table 2, LTC_4 and LTD_4 failed to influence LIF production by MNC, whether stimulated by recall antigen or the polyclonal T lymphocyte activator, PHA (last data not shown). In contrast, LTB_4 , at concentrations at and above 10^{-7} M, induced a significant inhibition of LIF production by MNC stimulated by

Table 1
Effects of LTB_4 , LTC_4 and LTD_4 on PMNL migration and the function of preformed LIF

Conc. of leukotrienes	Migration index			
	0	10^{-8} M	10^{-7} M	10^{-6} M
LTB_4	1.00	0.96 ± 0.10	1.06 ± 0.15	$1.35 \pm 0.19^*$ (6)
LTB_4 + LIF	0.66 ± 0.05	—	0.68 ± 0.05	$0.83 \pm 0.03^*$ (6)
LTC_4	1.00	1.02 ± 0.03	1.00 ± 0.03	— (3)
LTC_4 + LIF	0.61 ± 0.07	0.60 ± 0.06	0.65 ± 0.06	— (3)
LTD_4	1.00	1.04 ± 0.06	1.03 ± 0.04	— (3)
LTD_4 + LIF	0.61 ± 0.07	0.60 ± 0.05	0.65 ± 0.04	— (3)

Migration indices were calculated as described in Methods. The leukotrienes and preformed LIF were added alone or in combination to the PMNL. The results are given as means $\pm$ 1 SD, based on the number of experiments shown in the brackets.

* $P < 0.05$ calculated by the Mann-Whitney rank sum test.

antigen (Fig. 1); however, it failed to influence the elaboration of LIF from PHA-stimulated cells (Table 2). These results are only in partial agreement with those recently reported by Payan & Goetzl (12), who found that of the leukotrienes B₄, C₄, D₄ and E₄, only LTB₄ prevented the elaboration of LIF from PHA-stimulated human MNC rather than the expression of LIF activity. The latter assumption was based on the finding that LIF activities in supernatants of LTB₄-treated T lymphocytes were similarly diminished before and after molecular sieve chromatography. However, this does not rule out an additional inhibitory function of LTB₄ on the expression of LIF activity, as we found.

Table 2

Effects of LTB₄, LTC₄ and LTD₄ on LIF production

Conc. of leukotrienes	Migration index	
	0	3×10^{-7} M
PHA + LTB ₄	0.65 ± 0.02	0.63 ± 0.01 (3)
PPD + LTB ₄	0.72 ± 0.03	$0.88 \pm 0.05^*$ (5)
PPD + LTC ₄	0.75 ± 0.05	0.74 ± 0.08 (4)
PPD + LTD ₄	0.72 ± 0.05	0.71 ± 0.06 (4)

MNC were stimulated by PHA-pulse (5 µg/ml of PHA for 1 h at 37°C) or PPD (50 µg/ml) with or without added leukotrienes at time zero. The results are given as means $\pm$ 1 SD. The numbers of experiments are shown in brackets.

* $P < 0.05$ (Mann-Whitney rank sum test).

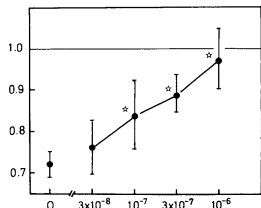


Fig. 1. Effect of LTB₄ on LIF production by PPD-stimulated human MNC. Results are means of five different experiments; vertical bars show the SD. * $P < 0.05$ (Mann-Whitney rank sum test). Ordinate: migration index. Abscissa: concentration of LTB₄ (M).

The reason for the discrepancy between our results and those of Payan & Goetzl (12) on the effect of LTB₄ on the LIF production by PHA-stimulated human MNC is at present unknown. However, in contrast to their study, we used a pulse-stimulation with PHA and a shorter incubation period for the elaboration of LIF. These differences may explain the results.

Effect of LTB₄ on MNC cAMP

Some arachidonic acid derivatives with immunosuppressive properties are known to in-

Table 3

Effects of LTB₄ and isoprotrenol on MNC cAMP levels

Time from stimulation	cAMP levels (pmol/10 ⁷ cells)											
	Basal				LTB ₄				Isoprotrenol			
	-theo		+theo		-theo		+theo		-theo		+theo	
2 min	9.0	5.4	14	8.4	8.8	4.7	15	9.0	12	6.6	84	30
10 min	8.5	4.6	15	9.0	8.4	4.6	14	9.0	11	8.4	54	17
30 min	9.0	4.4	15	9.8	8.6	4.4	15	9.8	12	6.2	34	14
60 min	8.4	5.0	14	9.8	8.8	4.8	15	9.8	10	5.0	28	12

MNC were incubated for 90 min at 37°C, or for 60 min followed by incubation with 4 mM theophylline (theo) for 30 min before stimulation. 2.5×10^{-7} LTB₄ and 4×10^{-6} M isoprotrenol were used. The table shows results from two such experiments.

crease the cellular levels of cAMP (7). Since cAMP effectively inhibits the elaboration of LIF (2), the inhibitory effect of LTB_4 might be mediated by leukotriene-induced elevated cAMP levels in MNC. We therefore tested the effect of LTB_4 on cAMP metabolism with and without the phosphodiesterase inhibitor, theophylline (18). Although strong potentiation of isoproterenol-induced cAMP generation was obtained by treatment with theophylline, no effect on the MNC levels of cAMP was seen following addition of LTB_4 (Table 3). Qualitatively, the same results were obtained with purified human T lymphocytes (results not shown).

In granulocytes, LTB_4 seems to affect the levels of cyclic nucleotides. Thus, under certain conditions, LTB_4 increases cAMP (5) as well as cGMP levels (4) within these cells. Therefore, one has to consider the possibility that LTB_4 increases the cGMP content also in MNC. Although this seems unlikely (cGMP and cGMP-elevating agents do not reduce the elaboration of LIF and, in fact, reverse the effect of cAMP (2)), one cannot at present exclude the possibility that LTB_4 influences the cAMP and/or cGMP metabolism in certain MNC subpopulations.

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