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LEUKOTRIENE B_4 AUGMENTS HUMAN NATURAL CYTOTOXIC CELL ACTIVITY

Marek Rola-Pleszczynski, Lyne Gagnon & Pierre Sirois

Laboratoires d'Immunologie et de Pharmacologie

Unité de Recherche Pulmonaire, Faculté de Médecine

Université de Sherbrooke

Québec, Canada J1H 5N4

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We have recently shown that leukotriene B_4 (LTB_4) activates T lymphocytes to become suppressor cells. We now report that LTB_4 also augments human natural cytotoxic cell activity against target cells infected with herpes simplex virus. This activity is partially inhibited by the lipoxygenase inhibitor nordihydroguaiaretic acid and the thromboxane synthetase inhibitor OKY-1581, but is augmented by indomethacin. We suggest that LTB_4 may play a role in early host defense responses during inflammatory and infectious disease processes.

Leukotrienes (LTs) are a novel family of compounds derived from the metabolism of arachidonic acid via the C-5 lipoxygenase pathway[1,2]. We have recently reported that LTB_4 activates T lymphocytes to become suppressor cells. These suppressor cells can inhibit the proliferative response of human lymphocytes to mitogens and require an adherent cell population for this activity [3]. Because suppressor cells may be generated concomitantly with cytotoxic effectors[4], and because they share some membrane markers[5], we studied the effect of LTB_4 on the cytotoxic activity of human lymphocytes. We now report that LTB_4 does also augment human natural cytotoxic cell activity.

MATERIALS AND METHODS

Leukotriene B_4: LTB_4 was kindly supplied by Dr J. Rokach, Merck-Frosst Laboratories. Its structure was verified by mass spectrometry. Bioactivity was assayed on lung parenchymal strips[6]. LTB_4 was stored in ethanol at -20°C and appropriately diluted in Earle's MEM medium (Flow Laboratories) immediately prior to the experiments.

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Leukocytes: Human peripheral blood mononuclear leukocytes (PBML) were obtained by density gradient centrifugation of heparinized venous blood on a Hypaque-Ficoll gradient [8]. They represented 75-90% lymphocytes and 10-25% monocytes. After three washes in phosphate buffered saline (PBS, pH 7.4), they were resuspended in appropriate concentrations in MEM medium supplemented with 10% fetal bovine serum.

Target cells: Target cells for the cytotoxicity assay consisted of the human prostatic adenoma MA-160 cell line, either uninfected or persistently infected with herpes simplex virus type 1 (HSV). These cells and their use have been previously described [4,7].

Cytotoxicity assay. Cytotoxic activity of PBML was measured using a ^{51}Cr-release microassay as described (4,7). Target cells were labeled with ^{51}Cr ($Na^{51}CrO_4$, New England Nuclear), washed three times and distributed into microtiter wells at a concentration of 5 X 10^3 cells per well. In most experiments, PBML were then added to half of the wells at a concentration of 2.5 X 10^5 cells per well, giving an effector to target cell ratio of 50:1. Alternate wells contained medium alone. Cytotoxicity was assayed by measuring the ^{51}Cr content of culture supernatants. Cytotoxic activity was expressed as % specific ^{51}Cr release using the following formula:

$$\% \text{ specific } {}^{51}Cr \text{ release} = \frac{R_E - R_S}{R_T - R_S} \times 100$$

where R_E represents experimental release, R_S, spontaneous release and R_T, total releasable ^{51}Cr.

Drug treatment: To study the effect of LTB_4 or metabolic inhibitors on the cytotoxic activity of PBML, appropriate concentrations of the drugs were prepared in medium MEM and added to additional sets of microtiter wells containing PBML-targets or medium-targets cultures. Cytotoxicity was expressed as above and cytotoxicity ratios were calculated by dividing the latter by the former.

Drug used: The following drugs were used: indomethacin (Merck-Frosst Lab., Montréal, Québec, 2 μ M) Nordihydroguaiaretic acid (NDGA, Sigma Chem., St-Louis, MD, 50 μg/ml) and OKY-1581 (ONO. Pharm., Japan, 50 μg/ml). The optimal concentrations of the drugs were previously determined by dose-response experiments.

RESULTS

When normal human PBML were incubated with increasing concentrations of LTB_4 during the 5-hr cytotoxicity assay, a very significant ($p < 0.01$) augmentation of their natural cytotoxic (NC) activity against HSV-infected targets was observed (Figure 1). Low concentrations of LTB_4, in the range of 10^{-8} to 10^{-12}M caused the strongest augmentation of NC cell activity, while concentrations above or below showed little effect. A similar, albeit weaker enhancement was observed when uninfected MA-160 target cells were used (data not shown).

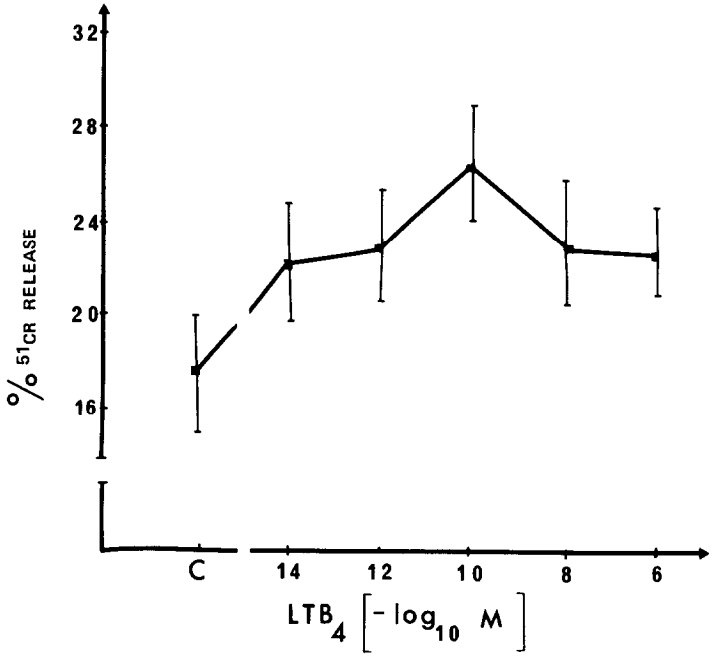

Figure 1. Effects of varying concentrations of LTB_4 on natural cytotoxic (NC) cell activity against HSV-infected target cells.

Continued presence of LTB_4 during the cytotoxic process was required as preincubation of PBML with LTB_4 for periods of 15-60 minutes, followed by washing before the onset of the cytotoxicity assay produced insignificant increases in NC activity. Similarly, addition of LTB_4 during the last 60-120 minutes of an ongoing cytotoxicity culture failed to modify NC activity suggesting that the initial hours of the cytotoxic process were sensitive to the enhancing effect of LTB_4.

In view of our recent findings that LTB_4-induced suppressor cells exerted their effect through synthesis of cyclooxygenase products (prostaglandins, thromboxanes)[8], we initiated an additional set of experiments to test whether a similar phenomenon occurred during LTB_4-enhanced NC activity. As presented in Figure 2, incubation of PBML with indomethacin (2μM), in order to block the synthesis of cyclooxygenase products during the 5 hr cytotoxicity assay, significantly enhanced by itself the NC activity of PBML ($p < 0.01$). Addition of LTB_4 did not

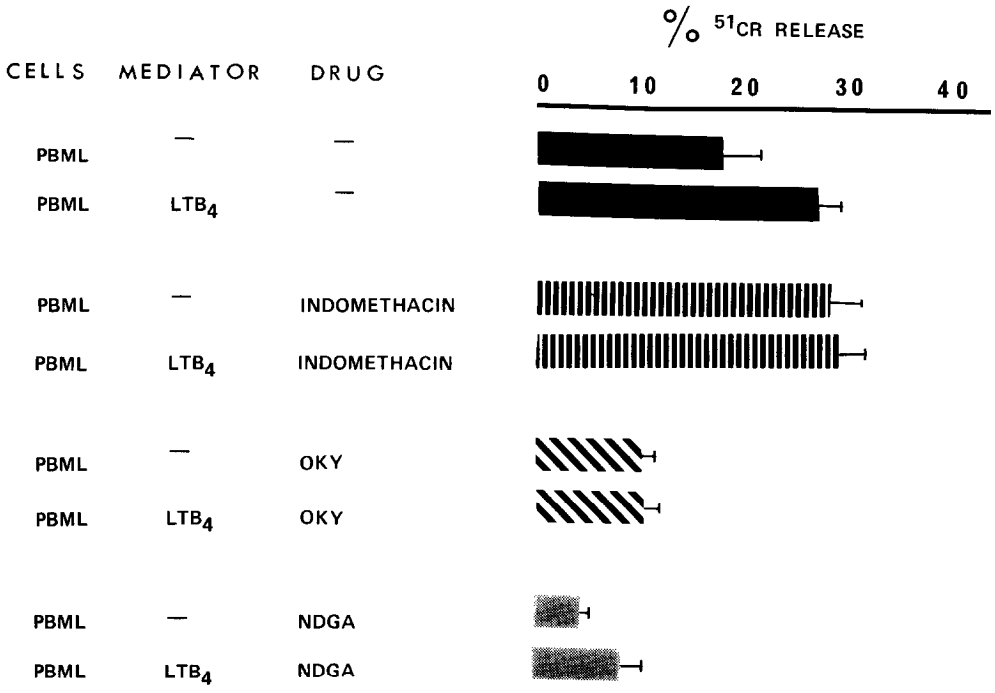

Figure 2. Effects of various inhibitors of arachidonic acid metabolism on spontaneous and LTB_4-augmented natural cytotoxic activity of PBML against HSV-infected target cells.

augment NC activity beyond this enhanced level. In contrast, incubation of PBML with nordihydroguaiaretic acid (NDGA 50 μg/ml), in order to inhibit the endogenous lipoxygenase pathway, strongly impaired spontaneous NC cell activity as well as LTB_4-enhanced activity ($p < 0.01$). However, the enhancement of NC activity by LTB_4 in the presence of NDGA was significant ($p < 0.05$). OKY-1581 (50 μg/ml) an inhibitor of thromboxane synthesis, also depressed spontaneous NC activity, but similarly depressed LTB_4 enhanced cytotoxicity.

DISCUSSION

Leukotrienes are released by leukocytes and parenchymal cells during inflammatory and hypersensitivity reactions as well as upon stimulation by ionophore A23187[2]. Their release occurs quite rapidly and involves de novo synthesis from membrane phospholipids. Because they constitute very powerful mediators of immediate hypersensitivity responses, we were

interested in studying their potential role as immunomodulators. LTB_4 was indeed found to induce suppressor cell activity in human peripheral blood mononuclear leukocyte (PBML) cultures [3]. The induced suppressor cell was a T lymphocyte, but required the presence of adherent cells to exert its suppressive activity. Having previously shown that suppressor cells may be concomitantly generated with cytotoxic cells in an *in-vitro* sensitization system [4], we sought to investigate whether LTB_4 was also capable of augmenting natural cytotoxic (NC) cell activity in addition to its suppressor cell-inducing potential.

In this report, we present evidence that LTB_4 strongly augments natural cytotoxic activity of human PBML . This augmentation occurs at very low concentrations of LTB_4 and the pattern of NC cell sensitivity to LTB_4 closely parallels that of LTB_4-induced suppressor cells[3]. The reason for this bell-shaped dose-response curve is not apparent, but a similar pattern of response has also been reported in other systems of cytotoxicity [9] as well as in the LTB_4-generated suppressor cell activity recently described [5].

In other systems, natural killer cell activity against certain target cells can also be augmented by interferon [10,11] and interleukin-2[9,12]. These lymphokines were obviously not present in our cytotoxicity assays and the short duration (5h) of the ^{51}Cr-release assay precluded *in situ* synthesis and activity of these proteins.

It remained possible that LTB_4 did not act directly on NC cells, but triggered the rapid synthesis and release of other substances, such as products of the oxidative metabolism of arachidonic acid, which in turn would mediate the enhancing effect. To test this hypothesis, we used inhibitors of the cyclooxygenase or lipoxygenase pathways. Enhanced NC activity was observed when prostaglandin synthesis was inhibited by indomethacin. There are several reports in the literature indicating that prostaglandins of the E series are inhibitory for cytotoxic effector cells [13-15]. Furthermore, our observations that

NDGA, an inhibitor of lipoxygenase, strongly impaired NC activity lead us to hypothesize that PBML-mediated NC activity may be dependent upon endogenous lipoxygenase products and that, conversely, endogenous prostaglandin production causes a diminution of NC activity. This diminution is relieved by blocking prostaglandin synthesis. Furthermore, addition of exogenous LTB_4 may create an imbalance in the favor of lipoxygenase products and thus augments NC activity. As in other systems (16), LTB_4-induced activity may be mediated by thromboxanes, since inhibition of their synthesis by OKY-1581 abolished not only spontaneous but also LTB_4-induced cytotoxicity. Alternatively, the inhibition of thromboxane synthesis may lead to such an impairement of NC activity by allowing uninhibited prostaglandin synthesis. This implies that a delicate balance may normally exist between products of the cyclooxygenase and the lipoxygenase pathways in controlling NC cell activity.

We believe that the concomitant induction of cytotoxic and suppressor cell activities by LTB_4 may constitute part of the initial mechanisms for defense against foreignness (induction or augmentation of natural cytotoxicity) as well as for regulation of the immune response via a negative feedback (induction of suppressor cells). Studies are in progress to further define these mechanisms.

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