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LEUKOTRIENES C_4 AND D_4 INDUCE PROSTAGLANDIN AND THROMBOXANE RELEASE FROM RAT PERITONEAL MACROPHAGES

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The present study examined the effect of leukotrienes C_4 and D_4, the products of the 5′ lipoxygenase pathway on prostaglandins and thromboxane release from rat peritoneal macrophages. Incubation of rat peritoneal macrophages with leukotrienes C_4 and D_4 enhanced the release of prostaglandin E_2 (PGE_2), 6-keto $PGF_{1\alpha}$ and thromboxane B_2 (TxB_2) in a dose-dependent manner. The increase of PGE_2 was more pronounced than that of 6-keto-$PGF_{1\alpha}$ and TxB_2. Lipopolysaccharide, a known stimulator of these cells elicited a similar pattern of increase of the arachidonate metabolites assayed. These results suggest that leukotrienes C_4 and D_4 are potential activators of macrophages. Since leukotrienes C_4 and D_4 are produced by these cells, it is suggested that endogenous leukotrienes may be involved in activation of macrophages.

Introduction Leukotriene C_4 (LTC) and leukotriene D_4 (LTD), which are products of the 5′ lipoxygenase pathway, have been identified recently as the major compounds of slow-reacting substances of anaphylaxis, SRS-A (Samuelsson, Hammarstrom, Murphy & Borgeat, 1980). Both LTC and LTD have been shown to be produced by rat peritoneal macrophages when treated with a Ca^{2+} ionophore (Bach, Brashler, Hammarstrom & Samuelsson, 1980; Lewis, Austen, Drazen, Clark, Marfat & Corey, 1980). Furthermore, LTC has also been shown to be produced by mouse peritoneal macrophages in response to a phagocytotic stimulus (Rouzer, Scott, Cohn, Blackburn & Manning, 1980). These findings prompt the question as to the effect of these compounds themselves on macrophages. In order to examine this question we have studied the effect of LTC and LTD on prostaglandin and thromboxane release from rat peritoneal macrophages.

Methods *Cell culture* Fischer 344 rats (8–12 weeks) were inoculated (i.p.) with 5 ml of Fruend's incomplete adjuvant (Difco Lab). Four days later the cells were harvested by lavage with RPMI-1640 (50 ml), washed three times and purified on 50% Percol density gradient. Cells obtained in this manner are more than 90% macrophages, based on nonspecific esterase staining.

Cells in suspension: The cells were suspended in RPMI-1640 (supplemented with penicillin, streptomycin and glutamine) to 1×10^6 cells/ml; tubes containing 1 ml of this cell suspension were incubated for 3 h in a humidified atmosphere containing 5% CO_2 in air. Thereafter, the tubes were centrifuged, the supernatant was decanted and frozen (−20°C) until assay for prostaglandins.

Cells in monolayer: 3×10^6 cells/ml were suspended in RPMI-1640 (supplemented as above) plus 1% of foetal calf serum. One ml of the cell suspension was added to 16 mm wells and incubated for 4 h at 37°C as above. Thereafter the non-adherent cells were removed and the wells were washed three times with saline (0.9% w/v NaCl solution). One ml of serum-free RPMI-1640 (supplemented as above) was added to each well. The adherent cells were incubated for 20 h (as above) and then the supernatant was frozen.

The effect of LTD was evaluated on cells in suspension. The effect of LTC and lipopolysaccharide was examined on both cells in suspension and cells in monolayer.

Prostaglandin assay Prostaglandin content in the supernatant was determined by a direct radioimmunoassay (Grandstrom & Kindhal, 1976). The ^3H-labelled prostaglandins were purchased from New England Nuclear, Mass. Prostaglandin E_2 (PGE_2) antibody was purchased from Accurate Chemical & Scientific Corporation, N.Y. The antibody for 6-keto-$PGF_{1\alpha}$ was prepared in this lab and its cross reactivity with PGE_2 was 10%, $PGF_{2\alpha}$ 7.6% and thromboxane B_2 (TxB_2) less than 1.0%. Antibody for TxB_2 was kindly given by Dr L. Levine, Brandeis Univ., Boston, Mass. (Patrono, Ciabattoni, Pugliese, Pinca, Castrucci, De Salvo, Sutta & Parachini, 1980).

Lipopolysaccharide *E. coli* 055:BS was obtained from Difco. Leukotrienes C and D were kindly given by Dr J. Rokach, Merck Frosst Laboratories, Canada (Rokach, Girard, Guindon, Atkinson, Larue, Young, Masson & Holme, 1980).

The viability of the cells as tested by exclusion of trypan blue was higher than 90%.

Data in the text and figures represent means ± s.e. mean. Statistical evaluation was done by Student's *t* test.

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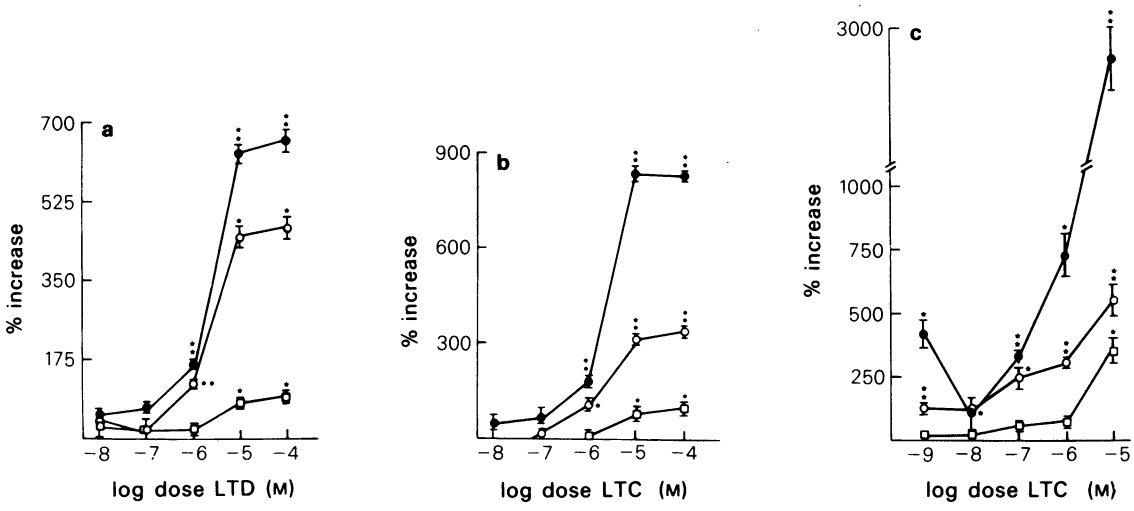

Figure 1 (a) Effect of leukotriene D_4 (LTD) on release of prostaglandin E_2 (PGE_2), 6-keto-$PGF_{1\alpha}$ and thromboxane B_2 (TxB_2) from rat peritoneal macrophages in suspension. Peritoneal cells (1×10^6) were incubated for 3 h with various doses of LTD. PGE_2 (●); 6-keto-$PGF_{1\alpha}$ (○); TxB_2 (□). Points show means; vertical lines indicate s.e. mean. $n=4$. $^*P<0.01$; $^{**}P<0.001$. (b) Effect of leukotriene C_4 (LTC) on release of PGE_2, 6-keto-$PGF_{1\alpha}$ and TxB_2 from rat peritoneal macrophages in suspension. Peritoneal cells (1×10^6) were incubated for 3 h with various doses of LTC. Symbols as in (a). Points show means; vertical lines indicate s.e. mean. $n=4$. $^*P<0.01$; $^{**}P<0.001$. (c) Effect of LTC on release of PGE_2, 6-keto-$PGF_{1\alpha}$ and TxB_2 from rat peritoneal macrophages in monolayer. Peritoneal cells (3×10^6) were adhered for 4 h. Thereafter the non-adherent cells were removed and the adherent cells were incubated for 20 h with various doses of LTC. Symbols as in (a). Points show means; vertical lines indicate s.e. mean. $n=4$. $^*P<0.05$; $^{**}P<0.01$.

Results Rat peritoneal macrophages in suspension released: PGE_2 0.89±0.05; 6-keto-$PGF_{1\alpha}$ 2.08±0.17 and TxB_2 3.82±0.17 ng/10^6 cells during 3 h. Thus, TxB_2 and 6-keto-$PGF_{1\alpha}$ were found to be the major metabolites produced by these cells. Incubation of macrophages in suspension with various doses of LTD (Figure 1a) or LTC (Figure 1b) elicited a dose-dependent release of all the metabolites measured. The increase in PGE_2 was more pronounced than the increase in 6-keto-$PGF_{1\alpha}$ and TxB_2 (Figure 1a and b).

Macrophages in monolayer released: PGE_2 0.036±0.01; 6-keto-$PGF_{1\alpha}$ 0.343±0.02 and TxB_2 0.387±0.036 ng/3×10^6 cells during 20 h. Incubation of these cells with LTC elicited a dose-dependent increase of all metabolites assayed (Figure 1c). The responsivenes of the cells in monolayer to LTC was higher than that of the cells in suspension. The cells in monolayer responded significantly to 10^{-9}M of LTC whereas cells in suspension responded significantly only to 10^{-6}M of LTC.

Lipopolysaccharide, a common stimulator of these cells increased the release of prostaglandins from cells in suspension as follows: PGE_2 3.4 fold; 6-keto-$PGF_{1\alpha}$ and TxB_2 2.54 and 2.32 fold respectively ($n=4$, $P<0.001$ for all the ratios). The responsiveness of the cells in monolayer to lipopolysaccharide was markedly higher: PGE_2 was increased 180 fold, 6-keto-$PGF_{1\alpha}$ and TxB_2 were increased 12 and 15 fold respectively ($n=4$, $P<0.001$ for all the ratios). Thus, the pattern of release induced by either LTC and LTD was similar to that elicited by lipopolysaccharide.

Discussion The present study shows for the first time that LTD and LTC stimulate prostaglandin and thromboxane release from rat peritoneal macrophages. Prostaglandin release from macrophages has been shown to be correlated with activation of these cells (Hsueh, Kuhn & Needleman, 1979; Scott, Zrike, Hamill, Kempe & Cohn, 1980). Thus, our data indicate that LTC and LTD are potential activators of macrophages.

Both LTC and LTD had been shown to be produced by rat macrophages upon stimulation with a Ca^{2+} ionophore (Bach *et al.*, 1980 Lewis *et al.*, 1980) and a phagocytotic stimulus (Rouzer *et al.*, 1980). Furthermore, the importance of endogenous leukotrienes in mediating biological functioning of immunoreactive cells is suggested by a recent study which showed that lysosomal enzyme release from neutrophils induced by arachidonic acid was blocked by an inhibitor of the lipoxygenase pathway (Walenga, Showell, Feinsten & Becker, 1980). Moreover, 5-HETE, the precursor of LTC and LTD has

been shown to induce degranulation (lysosyme release) in human neutrophils (Stenson & Parker, 1980).

This evidence is in accord with our results and it leads to the hypothesis that endogenous leukotrienes might be involved in the activation process of macrophages. Furthermore, the fact that the pattern of the increase of arachidonic acid metabolites by the leukotrienes was similar to that elicited by lipopolysaccharide, may further suggest the possible role of endogenous leukotrienes in mediating the effect of lipopolysaccharide on macrophages.

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