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Leukotriene B: a potential mediator of inflammation

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Mediators of acute inflammation have been classified into those agents affecting vascular permeability and those with leucotactic properties (Ward 1974). Substances that either mediate or modulate changes in vascular permeability include vasoactive amines, kinins and prostaglandins. Leucotactic agents include the complement derived peptide C5a, bacterial chemotactic factors, as exemplified by the synthetic peptide F-met-leu-phe and monohydroxyeicosatetraenoic acids derived from arachidonic acid (Gallin & Quie 1978; Goetzl & Sun 1979). The results of the present work suggest that to this category must be added leukotriene B (5,12-dihydroxy 6,8,10,14-eicosatetraenoic acid).

Rat and human polymorphonuclear leucocytes (PMNs) when exposed to the calcium ionophore A23187 release a product of the lipoxygenase pathway which causes the aggregation and chemokinesis of fresh PMN suspensions (Bray et al 1980). This product has been identified as leukotriene B and is active over the concentration range 10 pg to 5 ng ml^{-1} (Ford-Hutchinson et al 1980). It shows comparable activity on a molar basis in vitro to that observed for C5a and F-met-leu-phe and is between 100 and 1000 times

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Table 1. The effects of leukotriene B on the chemotaxis of human PMN in vitro. Chemotaxis of human PMNs prepared from dextran sedimented venous blood was assessed using the agarose plate technique described by Nelson et al (1975). Results are calculated as a chemotactic ratio A/B, where A is the distance migrated towards the stimulant and B the distance migrated towards the control well. The concentrations of leukotriene B required to produce positive chemotaxis appear to be higher than those required for maximal chemokinetic response (Ford-Hutchinson et al 1980) but this reflects dilution of the leukotriene B along the chemotactic gradient rather than a difference in sensitivity. Results are expressed as means ± s.e.m. ** $P < 0.005$. n = no of determinations.

Concn of leukotriene B in outer well, ng ml^{-1}	A/B	n =
0	0·92 ± 0·07	10
0·3	0·92 ± 0·09	5
1	1·08 ± 0·03	4
3	1·07 ± 0·02	5
10	1·66 ± 0·13**	5
30	1·6 ± 0·14**	5
100	1·71 ± 0·08**	5
300	1·84 ± 0·02**	5
1000	1·52 ± 0·05**	5

Table 2. Effects of leukotriene B and F-met-leu-phe on the accumulation of leucocytes into the guinea-pig peritoneum in vivo. Leucocyte movement in vivo was assessed following intraperitoneal injection into 200–250 g Dunkin Hartley guinea-pigs of leukotriene B or F-met-leu-phe (Sigma Chemical Co., Poole, Dorset, U.K.) dissolved in 0·4 ml of Hank's balanced salt solution. Cells were harvested 1 and 5 h after injection as previously described (Goetzl et al 1979) and total and differential counts performed. Results obtained after 5 h have been expressed above as mean total cell counts per animal $\times 10^6$ ± s.e.m. In a parallel experiment no increase in leucocyte migration was observed after 1 h incubation. * $P < 0.05$. ** $P < 0.01$.

Treatment	Dose per animal	n	Total white cell count	Macrophages	Polymorphonuclear leucocytes	Eosinophils
Vehicle alone	—	8	9·5 ± 0·6	6·1 ± 0·5	1·1 ± 0·2	2·2 ± 0·3
Leukotriene B	1 μg	7	30·8 ± 4·4**	14·0 ± 3·2*	13·1 ± 3·9**	3·6 ± 0·7
Leukotriene B	100 ng	9	16·9 ± 3·0**	7·7 ± 1·0	7·8 ± 1·9**	2·2 ± 0·5
F-met-leu-phe	1 μg	4	15·5 ± 2·5*	11·1 ± 1·8**	0·8 ± 0·4	3·5 ± 1·0
F-met-leu-phe	100 ng	4	11·4 ± 2·0	6·6 ± 1·5	1·0 ± 0·1	3·7 ± 1·0*

more active as a chemokinetic agent than any of the monohydroxyeicosatetraenoic acids (Ford-Hutchinson et al 1980).

We have now shown that in vitro, leukotriene B, prepared from rat PMNs stimulated with the calcium ionophore A23187 as previously described (Ford-Hutchinson et al 1980), stimulates the chemotaxis as well as the chemokinesis of human PMNs (Table 1). It also causes dose-related increases in the chemokinesis of human monocytes and a second mononuclear cell population (rat macrophages elicited by sodium caseinate) over the range 30 pg to 10 ng ml^{-1}. Leukotriene B also elicits leucocyte migration in vivo and a significant increase in total white cell counts was observed 5 h after the intraperitoneal injection of either 100 ng or 1 μg per guinea pig (Table 2). This effect was mainly due to PMN migration although at the higher dose a significant increase in the number of macrophages was also observed. In contrast, the injection of 1 μg of F-met-leu-phe did not produce an accumulation of PMNs but an increase in macrophage numbers was observed. Finally, we have not found any evidence that leukotriene B alters vascular permeability. Intradermal injection of 100 ng of leukotriene B in Tyrode solution into the rat did not produce any visible reaction and no differences in paw volume over 5 h were observed between groups of rats injected with carrageenan and leukotriene B (100 ng or 1 μg per rat) compared with rats injected with carrageenan alone.

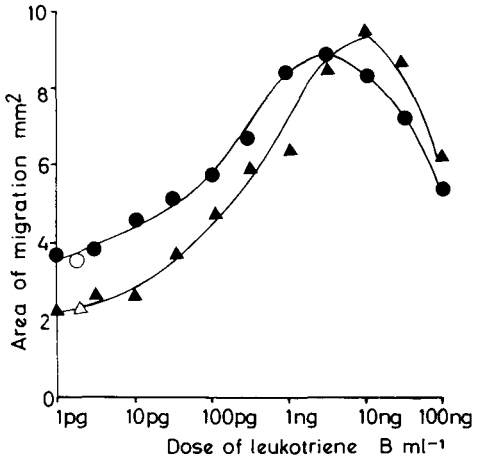

FIG. 1. Effects of leukotriene B on the chemokinesis of human monocytes and rat macrophages in vitro. Rat macrophages were prepared from peritoneal exudates 3 days after the injection of 6 ml of 12% sodium caseinate. Human monocytes were prepared from dextran sedimented blood and both cell types were further purified by Ficoll-Hypaque density gradient centrifugation to produce populations containing > 85% of the relevant cell type. The effect of leukotriene B on the chemokinesis of human monocytes (▲) and rat macrophages (●) was assessed using an agarose microdroplet technique (Bray et al 1980; and Smith & Walker 1980) using incubation times of 6 and 24 h respectively. Open symbols represent migration in the presence of medium alone. Results are shown as mean area of migration (n = 6) in mm^2 (ordinate) and the abscissa shows the concentration of leukotriene B. Standard errors were always < 10% of the mean.

Leukotriene B is therefore a leucotactic agent, in vitro and in vivo, towards both PMNs and mononuclear cells. It is comparable in potency in vitro to C5a and F-met-leu-phe and in vivo preferentially stimulates the movement of PMNs when compared with F-met-leu-phe. It differs from prostaglandins and other vasoactive mediators in not affecting the early vascular effects of inflammation.

We are grateful to the Arthritis and Rheumatism Council for financial support (M.A.B.).

May 20, 1980

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