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INDUCTION OF PLASMA EXUDATION AND INFLAMMATORY CELL INFILTRATION BY LEUKOTRIENE C_4 AND LEUKOTRIENE B_4 IN MOUSE PERITONITIS

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Abstract—Leukotriene induction of the fluid and cellular phases of the inflammatory response in the mouse was evaluated. Intraperitoneal injection of leukotriene C_4 (LTC_4 250 ng) led to dye extravasation but not polymorphonuclear leukocyte (PMN) infiltration, whereas injection of leukotriene B_4 (LTB_4 250 ng), led to PMN infiltration but not dye extravasation. The injection of both leukotrienes did not result in synergy. LTC_4 did not appear to induce significant release or formation of chemotactic mediators, but the dye extravasation induced by LTC_4 was inhibited by the vasoactive amine antagonist cyproheptadine and not by the eicosanoid inhibitors phenidone or naproxen. The response was markedly inhibited by the cytokine and eicosanoid inhibitors SK&F 86002 and SK&F 104493. PMN infiltration induced by LTB_4 was not inhibited by SK&F 86002 or phenidone but was abrogated by colchicine treatment. LTB_4 in this model did not appear to cause release or formation of vasoactive mediators. These leukotrienes appeared to be independent, complementary, and sufficient to mount a complete inflammatory response in the mouse.

INTRODUCTION

Many autocoids have been suggested to be candidates as mediators of the fluid and/or cellular phases of the inflammatory response. These have included lipid mediators such as platelet-activating factor (1), prostaglandins (2), and leukotrienes (3); vasoactive amines such as serotonin and histamine; small peptides such as bradykinin (4) and substance P (5); and proteins such as the interleukins and tumor necrosis factor (6). While the candidacy of each of these substances is supported by their ability to induce certain elements of the inflammatory response, it appears likely that they are not mutually exclusive but represent a

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cascade in which each or several have the capacity to induce the production and/or release of the others (7).

In addition, significant species differences appear to exist. For example, in the rat, a predilection to utilize prostanoids seems to be the basis of their well-described sensitivity to cyclooxygenase (CO) inhibitors (8, 9) and poor responsiveness to leukotrienes (10), while in the mouse, one can clearly demonstrate the effect of leukotrienes and the antiinflammatory activity of 5-lipoxygenase (5-LO) inhibitors and inhibitors of 5-LO/CO (11–14).

There is, however, some controversy concerning the chemotactic activity of leukotriene B_4 (LTB_4) in the mouse. Polymorphonuclear leukocyte (PMN) infiltration following intradermal injection of LTB_4 was demonstrated in man (15), mouse, rabbit, guinea pig, but not rat (16). In contrast, no significant infiltration of PMN was seen following intraperitoneal injection of LTB_4 in the mouse (17). Our recent experience has shown that intraperitoneal injection of LTB_4 does include PMN infiltration in mice and that it is of sufficient magnitude to conduct pharmacological studies (18). The current study described here provides data in support of the conclusion that leukotriene C_4 (LTC_4) and LTB_4 in consort can mediate both the fluid and cellular phases of the inflammatory response in the mouse.

MATERIALS AND METHODS

Animals. Male Balb/c mice were obtained from Charles River Breeding Laboratories (Kingston, New York). Within a single experiment the mice (20–25 g) were age-matched.

Dye Extravasation. Evan's blue dye (J.T. Baker), 0.2 ml of a 0.2% solution in saline was administered intravenously in the tail vein of Balb/c male mice. Shortly thereafter LTB_4 and/or LTC_4 in saline (0.25 ml) were injected intraperitoneally at doses of 25–1000 ng/mouse. Fifteen and/or 30 min later the mice were asphyxiated by carbon dioxide and the peritoneal contents were washed out with 3 ml cold Dulbecco's phosphate-buffered saline devoid of calcium and magnesium (DPBS/wo; Gibco, New York). The washouts were centrifuged at 2000 rpm for 10 min, and the optical density was read at 610 nm in a Perkin Elmer 552A UV/VIS spectrophotometer. Compounds were administered 30 min prior to the injection of Evan's blue dye.

PMN Infiltration. LTB_4 and/or LTC_4 (in 0.25 ml saline) were injected intraperitoneally into Balb/c male mice. Two hours later the peritoneal contents were washed out with 3 ml cold DPBS/wo. Leukocytes in the washout fluid were quantified using a Coulter counter (model ZBI, Coulter Electronics, Hileah, Florida). Cytospin slides were then prepared and PMN differential counts were performed. Compounds were administered 15–30 min prior to the injection of the leukotrienes.

Compounds and Reagents. LTB_4, LTC_4, SK&F 86002 [5-(4-pyridyl)-6(4-fluorophenyl)-2,3-dihydroimidazo (2,1-b)thiazole] and SK&F 104493 [2-(4-methoxyphenyl)-3-(4-pyridyl)-6,7-dihydro-[5H]-pyrrolo[1,2-a]imidazole] were synthesized at SmithKline Beecham (King of Prussia, Pennsylvania). Naproxen, phenidone, cyproheptadine, and colchicine were obtained from Sigma (St. Louis, Missouri). Compounds were administered orally in 0.5% tragacanth or parenterally in saline at the dosages indicated.

Statistical Analysis. Experimental alterations of extravasated dye and PMN infiltration were analyzed using Students t test, with $P < 0.05$ considered significant.

RESULTS

The ability of LTB_4 and LTC_4 to induce increased vascular permeability was examined following intraperitoneal injection. As seen in Table 1, LTB_4 (250 ng/mouse) did not induce protein-bound dye extravasation. In contrast, LTC_4 induced a significant extravasation of dye (sixfold). The dye extravasation seen with LTC_4 was corroborated and found to be dose-related (Fig. 1). The dye extravasation induced by LTC_4 was inhibited by the vasoactive amine antagonist, cyproheptadine, at doses of 2.5–10 mg/kg, per os (57–70% inhibition, Table 2). In contrast, the dye extravasation induced by LTC_4 injection was not inhibited by near-maximum tolerated doses (100 mg/kg, per os) of the 5-lipoxygenase (5-LO) inhibitor, phenidone, or the cyclooxygenase (CO) inhibitor, naproxen. The cytokine and 5-LO/CO inhibitors, SK&F 86002 and SK&F 104493, at 50 mg/kg, per os, however, did significantly inhibit the LTC_4-induced dye extravasation (78% and 65%, respectively, Table 2).

The ability of these leukotrienes to induce PMN infiltration also was studied. As seen in Table 3, LTB_4 but not LTC_4 caused significant PMN infiltration into the peritoneal cavity at doses of 250 ng/mouse. Coadministration of LTC_4 and LTB_4 did not cause enhanced PMN infiltration.

Examination of the dose-response using doses of 125–1000 ng/mouse of LTB_4 revealed a dose-related influx of PMN (Figure 2). Maximal influx (3.5-fold over background) was seen with a dose of 250 ng/mouse. As anticipated, the infiltration of PMNs induced by LTB_4 (250 ng) was not inhibited by the administration of SK&F 86002 (80 mg/kg, per os) or phenidone (100

Table 1. Ability of LTB_4 and LTC_4 to Induce Peritoneal Extravasation of Evans Blue Dye

Treatment[a]	N	$Absorbance_{610nm}$
Saline	3	0.024 + 0.004
LTB_4	4	0.026 + 0.004 NS[b]
LTC_4	5	0.144 + 0.025[c]

[a] Peritoneal washouts (1.5 ml saline) were collected 15 min after intraperitoneal inoculation with saline or leukotriene (250 ng). Data are the mean ± *SEM.*
[b] NS, not significantly different from saline treated.
[c] Significantly different from saline treated at $P < 0.05$.

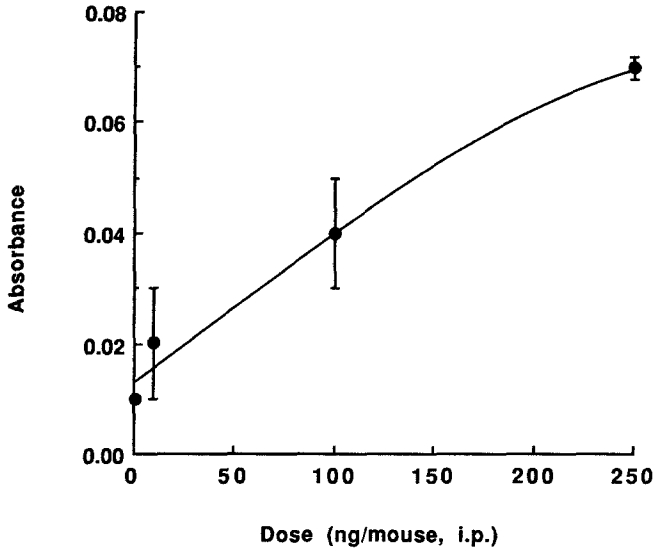

Fig. 1. Extravasated Evans blue dye in response to intraperitoneal injection of graded doses of LTC_4 was quantified spectrophotometrically (absorbance at 610 nm) in the mouse. Saline washout aliquots (1.5 ml) were collected 15 min after the administration of LTC_4. The data are the mean ± SEM from two to three animals per dose level.

Table 2. Effect of Cyproheptadine, SK&F 86002, SK&F 104493, Naproxen, and Phenidone on LTC_4-Induced Dye Extravasation

Treatment[a]	Dose (mg/kg, per os)	*N*	Dye extravasation absorbance$_{610nm}$ (mean ± *SEM*)	Change (%)
Vehicle control		6	0.23 ± 0.09	—
Cyproheptadine	10.0	5	0.10 ± 0.01[b]	−57
	5.0	5	0.07 ± 0.01[b]	−70
	2.5	5	0.10 ± 0.01[b]	−57
	2.0	5	0.16 ± 0.04 NS[c]	−30
SK&F 86002	50.0	5	0.05 ± 0.01[b]	−78
SK&F 104493	50.0	5	0.08 ± 0.02[b]	−65
Phenidone	100.0	4	0.16 ± 0.02 NS	−30
Naproxen	100.0	3	0.21 ± 0.05 NS	−9

[a] Compounds were administered 15–30 min before LTC_4 was injected intraperitoneally as described in Materials and Methods.
[b] Statistically significant at $P < 0.001$.
[c] NS, not significant.

Table 3. Induction of Peritoneal PMN Infiltration by LTB_4 and LTC_4

Treatment[a]	N	60 min (PMN × 10^4/ml)	N	120 min (PMN × 10^4/ml)
Saline	3	4.0 ± 0.8	2	3.2 ± 5.0
LTB_4	5	10.2 ± 2.4[b]	3	24.0 ± 5.4[b]
LTC_4	5	3.3 ± 0.9 NS[c]	3	3.6 ± 1.1 NS
LTB_4 and LTC_4	4	7.6 ± 1.5 NS	5	10.3 ± 2.4[b]

[a] Peritoneal washouts (3.0 ml saline) were collected 60 and 120 min after intraperitoneal inoculation with saline or leukotriene (250 ng). Data are the mean ± *SEM*.
[b] Significantly different from saline treated at $P < 0.05$.
[c] NS, not significantly different from saline treated.

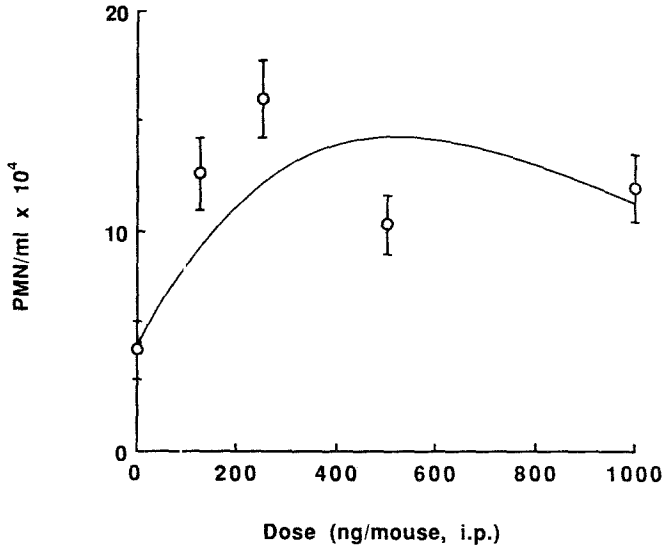

Fig. 2. The infiltration of PMN induced by graded doses of LTB_4 injected intraperitoneally was quantified. Three milliliters, 4°C DPBS/wo washouts were collected 2 h after the administration of LTB_4. The data are the mean ± SEM from groups of four to five animals at each dose level.

mg/kg, per os), whereas the administration of 1 mg/kg colchicine markedly inhibited the PMN infiltration (76%, Table 4).

DISCUSSION

The results of this study have shown that LTC_4 and LTB_4 induced increased vascular permeability and inflammatory cell infiltration, respectively. LTC_4 and LTB_4 do not share these activities and do not synergize, at least for PMN infil-

Table 4. Effect of SK&F 86002, Phenidone, and Colchicine on LTB_4-Induced Polymorphonuclear Infiltration into Peritoneum of Mice

Treatment[a]	Dose (mg/kg)	PMN $\times 10^4$/ml (mean $\pm$ *SEM*)	Change (%)
Vehicle		18.2 $\pm$ 2.7	
SK&F 86002	80 p.o.	13.9 $\pm$ 2.2 NS[b]	−24
Phenidone	100 p.o.	19.3 $\pm$ 4.0 NS	+6
Colchicine	1.0 i.v.	4.3 $\pm$ 1.1[c]	−76

[a] Compounds were administered 0–30 min before LTB_4 was given intraperitoneally as described in Materials and Methods.
[b] NS, not statistically significant from vehicle treated group.
[c] Statistically significant from vehicle-treated group at $P < 0.001$.

tration. Actually, the coadministration of both LTC_4 and LTB_4 led to a trend toward diminished PMN infiltration, although the changes seen were not statistically significant relative to LTB_4 alone. The fact that LTB_4 did not induce dye extravasation suggests that it does not release vasoactive amines or other substances capable of increasing vascular permeability. In contrast, the increase in vascular permeability seen with LTC_4 does appear to have a significant vasoactive amine component as shown by the inhibition of dye extravasation using cyproheptadine. Moreover, stimulation of fatty acid oxygenase activity following LTC_4 injection was not apparent since neither the 5-LO inhibitor, phenidone, nor the CO inhibitor, naproxen, inhibited dye extravasation. It was interest that the cytokine and eicosanoid inhibitors, SK&F 86002 (19) and its methoxyphenyl, pyrrole analog, SK&F 104493, both inhibited the response. Similar inhibition (data not shown) also was seen with dexamethasone, which also inhibits eicosanoid and cytokine production. It is tempting to speculate that cytokines may participate in this response; however, the time frame of 15–30 min would seem too short to allow for the de novo synthesis or molecules such as interleukin-1 (IL-1) and tumor necrosis factor (TNF). In addition, it is difficult to explain the absence of PMN infiltration with LTC_4 if IL-1 was generated. As an alternative, it is possible that inhibition of either pathway of arachidonic acid metabolism alone results in shunting of substrate to the other pathway, generation of products which cause dye extravasation, thus masking of the effect of phenidone and naproxen. Inhibition of both pathways might be necessary to be able to observe significant inhibition of dye extravasation. We have not tested this hypothesis by administration of both phenidone and naproxen. It is also possible and perhaps more likely that SK&F 104493, 86002, and dexamethasone have an effect upon mast cell vasoactive amine release.

The action of LTB_4 to induce PMN infiltration is more controversial. It should be noted that the magnitude of the response is considerably less than

that observed with less-defined stimuli, e.g., carrageenan or monosodium urate crystals (18). This response also should be contrasted with that of recombinant human IL-1 (17), in which the magnitude of response and the potency, even on a milligram per milligram basis, are greater. Given the facile nature of the oxidation of LTB_4, it would be expected that the concentration of LTB_4 would rapidly wane; making it difficult to judge the differences in chemotactic potency. However, the response to LTB_4 is clearly and reproducibly demonstrable.

The apparent plateau effect of PMN infiltration in the dose–response curve for LTB_4 (Figure 2) is interesting in light of the observation that myeloperoxidase (MPO) activity, used as neutrophil marker, did not exhibit a similar diminution with increasing doses of LTB_4 (data not shown). This discrepancy may be due to the activation and/or degranulation of PMNs by the higher doses of LTB_4. Thus, these cells may not be enumerated but could contribute to the MPO signal.

The results of this study demonstrate that LTB_4 induces PMN infiltration in the mouse and that the lack of activity seen in the previous study (17) is likely due to use of subthreshold doses of LTB_4. In support of these findings, LTB_4 was found to induce PMN infiltration in a mouse air pouch study (20). It should be pointed out, however, that the magnitude of the response and potency of LTB_4 is considerably less than recombinant human IL-1 (rhuIL-1), although the pharmacokinetics of LTB_4 make direct comparisons difficult.

It seems apparent that LTC_4 and LTB_4 can mediate, respectively, the fluid and cellular phases of the inflammatory response in the mouse in an independent manner and that pharmacological inhibition of their activity can be expected to result in antiinflammatory activity.

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