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Contrasting *in vitro* lymphocyte chemotactic activity of the hydroxyl enantiomers of 12-hydroxy-5,8,10,14-eicosatetraenoic acid

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1 The chemotactic activity of 12(**R**)-hydroxy-5,8,10,14-eicosatetraenoic acid (12(**R**)-HETE), 12(S)-HETE and leukotriene B_4 (LTB_4) for human mixed peripheral blood lymphocytes has been assessed in a 48-well microchemotaxis assay. Responses to the standard lymphocyte chemoattractants, zymosan-activated plasma, casein and N-formyl-methionyl-leucyl-phenylalanine (fMLP) were also measured.

2 12(**R**)-HETE was shown to be chemotactic for lymphocytes over the range 5×10^{-7} to 5×10^{-5} M. In contrast, negligible chemotactic responses to 12(S)-HETE were obtained.

3 LTB_4 was 200 times more potent than 12(**R**)-HETE as a lymphocyte chemoattractant, although maximal responses to the two agonists were similar.

4 12(**R**)-HETE and LTB_4, which are present in extracts of samples from the skin lesions of psoriasis, may be, at least in part, responsible for the lymphocyte infiltrate which is a characteristic feature of this disease.

Introduction

The accumulation of neutrophils and lymphocytes in lesional skin is characteristic of the inflammatory and scaling skin disease psoriasis (Helwig, 1958; Ragaz & Ackerman, 1979; Chowaniec *et al.*, 1981) and may be induced by the local generation of one or more chemoattractant compounds. The monohydroxy metabolite of arachidonic acid, 12-hydroxy-5,8,10,14-eicosatetraenoic acid (12-HETE), has been found in elevated levels in samples from lesional psoriatic skin, when compared to those from normal skin (Hammarstrom *et al.*, 1975; Camp *et al.*, 1983) and stereochemical analysis of psoriatic scale-derived 12-HETE has shown that the major stereoisomer present is 12(**R**)-HETE (Woollard, 1986). Analysis of the biological activity of 12(**R**)-HETE has shown it to be a more potent chemokinetic agent for human neutrophils *in vitro* than the platelet product, 12(S)-HETE (Cunningham *et al.*, 1986, Cunningham & Woollard, 1987; Evans *et al.*, 1987).

In the present study, in which a 48-well microchemotaxis chamber was used, the chemotactic activity of 12(**R**)-HETE for mixed human peripheral blood lymphocytes has been compared with that of its hydroxyl enantiomer 12(S)-HETE, as well as 5(S),12(**R**)-dihydroxy-6,8,10,14-eicosatetraenoic acid (leukotriene B_4, LTB_4), also found in extracts of samples from psoriatic lesions (Brain *et al.*, 1984a,b). Responses to the standard lymphocyte chemoattractants, zymosan-activated plasma (ZAP), casein and N-formyl-methionyl-leucyl-phenylalanine (fMLP), have also been measured, initial experiments being carried out to determine the optimal incubation time and cell concentration required. This work has been presented in a preliminary form to the British Pharmacological Society (Bacon *et al.*, 1987a,b).

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Methods

Cell preparation

Mixed human peripheral blood lymphocytes were separated from heparinized venous blood (usually 20 ml samples) by Ficoll-Hypaque density gradient centrifugation (500 *g*, 20 min). The mononuclear cell layer was removed and washed twice, first with excess phosphate buffered saline (PBS, pH 7.4) and subsequently with Eagle's Minimal Essential Medium (MEM), buffered to pH 7.4 with 30 mM N-2-hydroxyethylpiperizine-N'-2-ethanesulphonic

acid (HEPES) buffer, and containing 2% heat inactivated foetal calf serum (FCS). The cells were then resuspended in HEPES-buffered MEM containing 10% FCS and monocytes were removed by adherence to 90 mm plastic petri dishes for one hour at 37°C in 5% CO_2. The non-adherent cells were harvested and resuspended at $2 \times 10^6\,ml^{-1}$ in HEPES-buffered MEM containing 10% FCS, $100\,u\,ml^{-1}$ penicillin and $100\,\mu g\,ml^{-1}$ streptomycin, for overnight culture in $25\,cm^2$ plastic tissue culture flasks. After 18 h the non-adherent cells were collected and resuspended at $2 \times 10^6\,ml^{-1}$ in serum-free, HEPES-buffered MEM before assay.

Cell purity and viability

The viability of the lymphocyte population obtained was $99.3 \pm 0.1\%$ ($n = 8$), as assessed by Trypan blue exclusion. The purity of the lymphocyte population obtained was assessed by staining for monocytes using a modification of a non-specific esterase method (NSE) (Knowles *et al.*, 1978) and by immunocytochemical detection, using specific monoclonal antibodies and immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes) (a modification of the methods of Cordell *et al.*, 1984). Briefly, cytocentrifuged smears of cell suspensions from either the mixed mononuclear cell layer obtained after centrifugation on Ficoll-Hypaque, or the purified lymphocyte population, were air dried for a minimum of 18 h, fixed in acetone (2 min, −20°C), and transferred to Tris buffered saline, pH 7.6 (0.9% w/v aqueous sodium chloride, buffered with 0.05 M Tris HCl). The fixed cytocentrifuged smears were incubated with either anti-Leu-M1, anti-Leu-M5, or Dako-Macrophage antibodies in PBS, or PBS alone as a negative control, for a minimum of 18 h in a moist chamber, then with unlabelled rabbit anti-mouse immunoglobulin and finally with the APAAP complex, the latter two steps each requiring an incubation time of 1 h at room temperature. The cells were then incubated for 30 min at room temperature with 10 ml of a freshly prepared solution of naphthol AS-MX phosphate (10 mg) dissolved in 10 ml dimethylformamide and diluted to 50 ml in 0.1 M Tris HCl (pH 8.2, containing 12 mg levamisole), and containing 10 mg Fast-Red salt. The slides were counterstained for 30 s with haematoxylin and mounted in Apathy's Medium.

Chemotaxis assay

Lymphocyte chemotaxis was assessed by use of a 48-well microchemotaxis chamber (Neuro Probe, Cabin John, Maryland, U.S.A.). The bottom wells, containing 25 μl of chemoattractant, were separated from the top wells, containing 50 μl cell suspension, by a polyvinylpyrrolidone-free polycarbonate filter (8 μm pore size) (Harvath *et al.*, 1980). Each concentration of chemoattractant was tested in quadruplicate wells in the microchemotaxis chamber. After incubation for one hour at 37°C the filter was removed from the chemotaxis apparatus, the top wiped to remove residual cells, and the filter then fixed in methanol for two minutes. Cells were stained using a modified Field's method (Field, 1940). The area of the lower surface of the filter occupied by cells was measured with an AMS 40-10 image analyser (Analytical Measuring Systems, Saffron Walden, Cambridgeshire), the filter being viewed through a 10× objective, with a VG-9 green filter and ND 35% neutral density filter (Microscope Service and Sales, Egham, Surrey), placed in the incident light path (Harvath *et al.*, 1980). Lymphocyte migration has been expressed either as the area of the lower surface of the filter occupied by cells (mm^2) or as a migration index (area of lower surface of filter occupied by cells following stimulation with chemoattractant (mm^2)/area of filter occupied by randomly migrating cells (mm^2)). The range of area covered by cells in response to medium alone was between 0.02–0.05 mm^2, with a mean ± s.e.mean area of $0.03 \pm 0.001\,mm^2$, corresponding to an apparent mean ± s.e.mean of 1747 ± 80 cells ($n = 26$). This number of cells represents approximately 1.8% of the number added to the top wells of the microchemotaxis chamber. As the maximal migration index obtained in response to agonist was approximately 3.4 (see Results section), this indicates that less than 10% of the total cell number introduced into upper wells was present on the lower surface of the filter after optimal stimulation. In quantitating lymphocyte migration by image analysis, the area covered by cells has been used in preference to the apparent number of cells on the undersurface of the filter. The area method was adopted because groups of clumped cells are counted as single cells by the image analysis system, which therefore underestimates the extent of cell migration, when analysing cell numbers. Student's *t* test, or, where the data were found not to be normally distributed, the Mann-Whitney U-test, was used to compare differences between mean migration indices for statistical significance.

Experiments were performed to determine the optimal incubation time and cell concentration for lymphocyte migration in this assay, using ZAP as the chemoattractant. Cells which had detached from the filter after one or three hour incubations were recovered from the lower wells, counted by use of a haemocytometer and expressed as a percentage of the total number of cells introduced into the top well (10^5). Having established optimal conditions, chemo-

Table 1 Comparison of percentage monocytes in (a) the mixed mononuclear cell layer obtained on Ficoll-Hypaque centrifugation, and (b) non-adherent, purified, mixed peripheral blood lymphocytes

a Mixed mononuclear cells

Subject	Antibody Anti-Leu M1	Anti-Leu M5	Dako-macrophage	Non-specific esterase
1	6.4	14.7	15.6	17.4
2	8.4	5.0	7.6	12.3
3	19.6	21.5	25.6	32.0
4	12.2	17.6	23.0	16.6
5	19.5	14.7	19.0	21.2
Mean ± s.e.mean	13.2 ± 2.7	14.7 ± 2.7	18.1 ± 3.1	19.9 ± 3.3

b Purified lymphocytes

Subject	Antibody Anti-Leu M1	Anti-Leu M5	Dako-macrophage	Non-specific esterase
1	0.7	1.3	1.1	0.4
2	0.6	0	0	0.4
3	0.2	0.9	1.1	1.8
4	0.7	0	0.8	0.8
5	0.7	0	1.0	0.6
Mean ± s.e.mean	0.5 ± 0.07	0.4 ± 0.2	0.8 ± 0.2	0.8 ± 0.2

The data were determined by immunoenzymatic labelling using APAAP complexes and histochemically, using non-specific esterase staining ($n = 5$).

tactic responses to ZAP (0.1–25.0%, $n = 8$), casein (0.1–2.0 mg ml^{-1}, $n = 5$) and fMLP (10^{-10}–10^{-6} M, $n = 4$), were determined. The effects of 12(R)-HETE (5×10^{-7}–5×10^{-5} M) were compared to 12(S)-HETE (5×10^{-7}–5×10^{-5} M, $n = 5$) and in a further series of experiments, to LTB_4 (5×10^{-9}–5×10^{-7} M, $n = 4$).

In separate experiments ($n = 3$), mixed mononuclear cells obtained after Ficoll-Hypaque centrifugation, or purified lymphocytes obtained after the two adherence steps were prepared. Using 12(R)-HETE, 12(S)-HETE and LTB_4 as chemoattractants, the percentages of monocytes on the undersurface of the filter after 1 h were determined histochemically in order to assess the contamination of the migrating cell population with monocytes. Briefly, using a modification of the non-specific esterase method of Knowles *et al.* (1978), cells on the undersurface of each chemotaxis filter were stained for monocytes and counterstained with 1% methyl green in 0.1 M sodium acetate adjusted to pH 4.2 with HCl. The percentages of monocytes on the filter were determined by counting 100–200 cells under 400× magnification.

In order to demonstrate whether active lymphocyte locomotion was involved in the responses measured, the area of the filter covered by cells was related to the number of cells which had detached from the filter into the lower wells, in a series of experiments using a 1 h incubation time, purified lymphocytes and LTB_4, 12(R)- and 12(S)-HETE as agonists ($n = 3$).

Materials

Eagle's MEM was obtained from Wellcome Diagnostics (Dartford, U.K.), heat inactivated FCS from Gibco (Uxbridge, U.K.) and Ficoll-Hypaque (Histopaque 1077), HEPES, casein, fMLP, naphthol AS-MX phosphate, dimethylformamide, levamisole (levamisole hydrochloride), Tris HCl and Fast-Red TR salt from Sigma (Poole, U.K.). Apathy's medium and haematoxylin were obtained from BDH (Dagenham, Essex, U.K.). Rabbit anti-mouse immunoglobulin, Dako-macrophage antibody, APAAP complexes (monoclonal calf intestinal alkaline phosphatase-mouse anti-calf alkaline phosphatase), were all obtained from Dakopatts (Glostrup, Denmark). Anti-Leu-M1 and anti-Leu-M5 were obtained from Becton-Dickinson (Cowley, Oxford, U.K.). Penicillin (sodium benzylpenicillin BP) was from Glaxo (Greenford, Middlesex, U.K.), streptomycin (streptomycin sulphate BP) from Evans Medical (Beaconsfield, Buckinghamshire, U.K.) and heparin (sodium heparin injection BP; 5000 u ml^{-1}) from Leo Laboratories (Princes Risborough, Buckinghamshire, U.K.). A 2 mg ml^{-1} solution of casein was freshly prepared by incubating the casein for

Table 2 Effect of cell concentration on lymphocyte locomotion in response to increasing concentrations of zymosan-activated plasma (ZAP)

	Cell concentration (ml^{-1})					
	10^6		2×10^6		4×10^6	
ZAP (%)	*Area*[a]	*Mmi*	*Area*	*Mmi*	*Area*	*Mmi*
0	0.022 ± 0.0009	1	0.035 ± 0.002	1	0.0851 ± 0.0025	1
0.5	0.022 ± 0.001	1.0	0.067 ± 0.007	1.8	0.1140 ± 0.02	1.1
1.0	0.030 ± 0.002	1.4	0.084 ± 0.01	2.6	0.1158 ± 0.02	1.1
5.0	0.034 ± 0.001	1.5	0.109 ± 0.009	3.1	0.1300 ± 0.02	1.3

[a] Data are expressed as mean ± s.e.mean area of the lower surface of the filter occupied by cells (mm^2, $n = 3$). Mean migration indices (mmi) are also shown.

two hours at 37°C in serum-free MEM, with frequent agitation. A stock solution of 10^{-2} M fMLP in dimethyl sulphoxide (DMSO) was used. Zymosan-activated plasma was prepared from heparinized human venous blood, as previously described (Camp *et al.*, 1986). LTB_4 was a gift from Dr J. Rokach (Merck Frosst, Point Claire-Dorval, Canada). The 12(R) and 12(S) hydroxyl enantiomers were resolved from 12(R,S)-HETE prepared by photooxidation of arachidonic acid (Camp *et al.*, 1983) by chiral high performance liquid chromatography (h.p.l.c.) using a Chiracel OB chiral column (HPLC Technology, Macclesfield, U.K.) eluted with hexane/propan-2-ol/acetic acid (97.5/2.5/0.1 v/v) at 1 ml min^{-1}. Column effluent was monitored for u.v. absorbance at 236 nm, and the u.v. absorbing peaks collected. The individual enantiometers were identified by co-elution on chiral h.p.l.c. with standard 12(R)- and 12(S)-HETE, resolved from 12(R,S)-HETE in a similar manner to that previously described (Cunningham & Woollard, 1987), as well as with 12(S)-HETE, prepared by incubation of human platelets with arachidonic acid (Woollard, 1986) and 12(R)-HETE, prepared by total synthesis, a generous gift from Dr J. Rokach. There was approximately 3% cross contamination between the purified 12(R)- and 12(S)-HETE enantiomers resolved by this method. All stock solutions were stored at −20°C and serial dilutions of chemoattractants were prepared in serum-free, HEPES-buffered MEM before assay.

Results

The percentages of monocytes in the unpurified and purified populations determined using both the APAAP and non-specific esterase staining techniques are shown in Table 1. It can be seen that this two-step adherence method allows the preparation of purified lymphocyte suspensions containing less than 1% monocytes. Preliminary experiments established that a cell concentration of 2×10^6 ml^{-1} and incubation of the microchemotaxis chamber for 60 min allowed optimal dose-related responses to the chemoattractants to be obtained (Tables 2 and 3, respectively). Higher cell concentrations resulted in a decrease in the migration index, which appeared to be due, at least in part, to an increase in the area of the filter occupied by randomly migrating cells

Table 3 Cell migration and percentage of cells detached from the chemotaxis filters over two time periods in response to zymosan-activated plasma (ZAP)

	1 *h incubation period*[a]		3 *h incubation period*[a]	
ZAP (%)	*Migration index*	*% cells in bottom wells*	*Migration index*	*% cells in bottom wells*
0	1.0	4	1.0	19
0.1	1.4	5	1.3	21
0.5	2.0	4	1.2	21
1.0	3.3	3	1.3	23
5.0	3.9	1	1.5	20
10.0	3.1	2	1.4	27

[a] Data are expressed as mean migration index and mean percentage of total number of cells introduced into each upper chamber well (10^5) ($n = 2$).

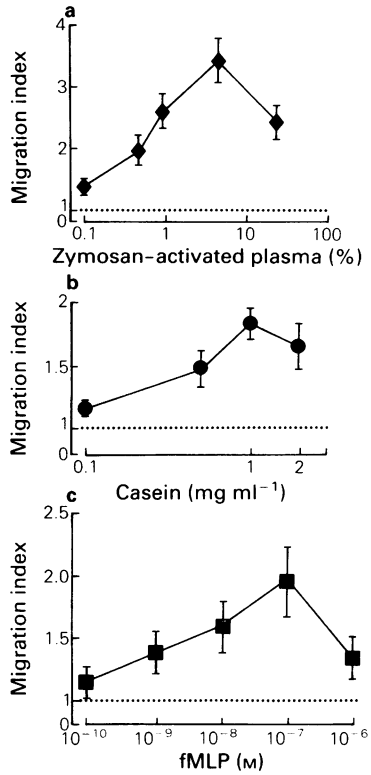

Figure 1 Concentration-response curves to (a) zymosan-activated plasma (0.1–25%; ◆; $n = 8$), (b) casein (0.1–2.0 mg ml^{-1}; ●; $n = 5$) and (c) N-formyl-methionyl-leucyl-phenylalanine (fMLP; 10^{-10}–10^{-6} M; ■; $n = 4$). Results are expressed as mean migration indices with vertical lines representing s.e.mean. Random migration is represented by the broken line.

(Table 2). Longer incubation periods resulted in detachment of cells from the lower surface of the filter and increased recovery of cells from the bottom wells as well as a loss of dose-related effects (Table 3).

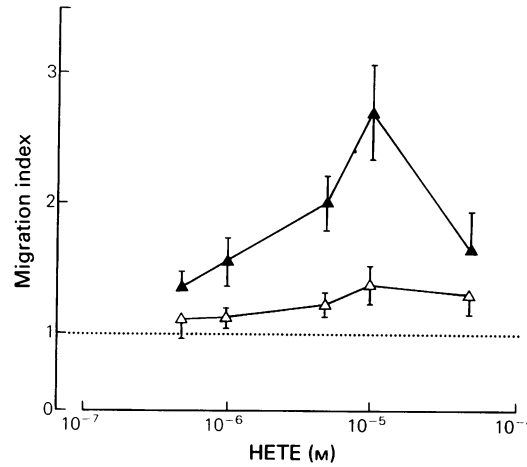

Figure 2 Comparison of lymphocyte chemotactic activity in response to 12(R)-HETE (▲) and 12(S)-HETE (△) (5×10^{-7}–5×10^{-5} M). Results are expressed as mean migration indices with vertical lines representing s.e.mean ($n = 5$). Random migration is represented by the broken line.

Concentration-related lymphocyte chemotaxis was obtained in response to ZAP (0.1–5%, $n = 8$), casein (0.1–1.0 mg ml^{-1}, $n = 5$) and fMLP (10^{-10}–10^{-7} M, $n = 4$) (Figure 1a, b and c, respectively). In all cases, higher concentrations of chemoattractant were inhibitory. Maximum migration indices in response to ZAP (3.4 ± 0.4) were significantly greater than those to either casein (1.8 ± 0.1) or fMLP (1.9 ± 0.3) ($P < 0.05$; Mann-Whitney U-test and $P = 0.02$; Student's t test, respectively).

A comparison of the chemotactic activity of 12(R)- and 12(S)-HETE is shown in Figure 2. 12(R)-HETE produced concentration-related chemotactic responses (5×10^{-7}–10^{-5} M, higher concentrations being inhibitory), the approximate ED_{50} value being 5.2×10^{-6} M. In contrast, using cells from the same donors in the same assays, very little movement above random migration was seen in the presence of 12(S)-HETE (5×10^{-7}–5×10^{-5} M; $n = 5$). In order

Table 4 Assessment of the contribution of chemotaxis and chemokinesis to the 12(R)-HETE-induced locomotor response of purified human mixed peripheral lymphocytes

12(R)-HETE in lower compartment (M)	*12(R)-HETE in upper compartment* 0	10^{-6} M	5×10^{-6} M	10^{-5} M
0	1.0	0.9 ± 0.08	0.9 ± 0.1	0.9 ± 0.1
10^{-6}	1.4 ± 0.1	0.9 ± 0.7	1.0 ± 0.1	1.1 ± 0.3
5×10^{-6}	1.8 ± 0.3	1.4 ± 0.05	1.0 ± 0.06	1.1 ± 0.2
10^{-5}	2.4 ± 0.3	1.7 ± 0.2	1.5 ± 0.2	1.2 ± 0.3

Data are expressed as mean ± s.e.mean migration index ($n = 3$).

to determine the contribution of chemokinesis to the overall locomotor effect obtained in response to 12(R)-HETE in this assay, a checkerboard assay was performed ($n = 3$) (Table 4). The diagonal line defines the chemokinetic effect obtained when equal concentrations of 12(R)-HETE were present in the top and bottom wells. The responses seen suggest a chemotactic effect. In a further set of experiments ($n = 4$), responses to 12(R)-HETE and LTB_4 were compared using cells from the same donors in the same assays. Concentration-related lymphocyte migration was obtained in response to LTB_4 (5×10^{-9}–10^{-7} M), LTB_4 being at least 200 fold more potent than 12(R)-HETE (approximate ED_{50} values being 1.9×10^{-8} M for LTB_4 and 5.2×10^{-6} M for 12(R)-HETE). The mean maximal migration indices in response to the two agonists were similar (3.0 ± 0.4 and 3.0 ± 0.4 for LTB_4 and 12(R)-HETE, respectively) (Figure 3).

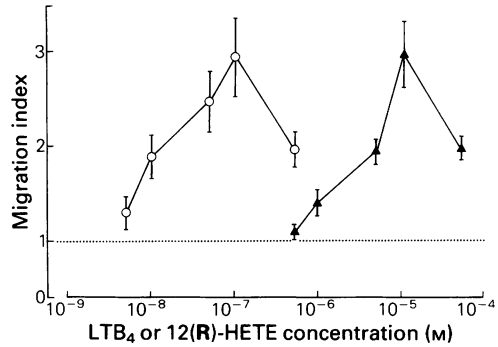

Figure 3 Comparison of lymphocyte chemotactic activity in response to 12(R)-HETE (▲) and leukotriene B_4 (LTB_4, ○) (5×10^{-9}–5×10^{-5} M). Results are expressed as mean migration indices with vertical lines representing s.e.mean ($n = 4$). Random migration is represented by the broken line.

The percentages of contaminating monocytes on the chemotaxis filters after incubation for 1 h, using mixed mononuclear cells or purified lymphocytes and 12(R)-HETE, 12(S)-HETE and LTB_4 as chemoattractants, are shown in Table 5. In contrast to experiments with the mixed mononuclear cell population, insignificant numbers of monocytes were seen on filters from experiments in which purified lymphocytes were used. Figure 4 shows the results of experiments in which the number of cells detaching into the lower well is related to the area of the filter covered by cells in response to a range of concentrations of 12(R)-HETE, 12(S)-HETE and LTB_4. It can be seen that a three fold increase in the area of the filter covered by cells in response to increasing concentrations of 12(R)-HETE and LTB_4, is associated with a less than two fold decrease in the number of cells detaching into the lower well (Figure 4a and c). This indicates that the concentration-related effects measured in response to 12(R)-HETE and LTB_4 involve an active locomotor process and not only increased adhesion of passively falling cells, although the slight reduction of cell numbers in the bottom wells, in response to increased concentra-

Table 5 Comparison of the percentage monocytes on the lower surface of chemotaxis filters, after 1 h incubations, in response to 12(R)- and 12(S)-HETE and leukotriene B_4 (LTB_4), using (a) mixed mononuclear cells obtained after Ficoll-Hypaque centrifugation and (b) purified mixed peripheral blood lymphocytes

a Mixed mononuclear cells[1]				
HETE (M)	*12(R)-HETE*[2]	*12(S)-HETE*[2]	*LTB_4* (M)	*LTB_4*[2]
0	27 ± 4	29 ± 4	0	24 ± 2
5×10^{-7}	31 ± 5	26 ± 1	5×10^{-9}	28 ± 1
10^{-6}	33 ± 8	28 ± 5	10^{-8}	25 ± 2
5×10^{-6}	30 ± 8	32 ± 6	5×10^{-8}	29 ± 2
10^{-5}	31 ± 3	31 ± 2	10^{-7}	24 ± 2
5×10^{-5}	29 ± 5	32 ± 5	5×10^{-7}	26 ± 3
b Purified lymphocytes[1]				
HETE (M)	*12(R)-HETE*[2]	*12(S)-HETE*[2]	*LTB_4* (M)	*LTB_4*[2]
0	0.9 ± 0.2	0.7 ± 0.2	0	1.0 ± 0.1
5×10^{-7}	0.7 ± 0.3	0.8 ± 0.2	5×10^{-9}	0.9 ± 0.1
10^{-6}	1.0 ± 0.04	0.9 ± 0.1	10^{-8}	0.5 ± 0.3
5×10^{-6}	0.6 ± 0.07	1.2 ± 0.2	5×10^{-8}	0.4 ± 0.2
10^{-5}	1.1 ± 0.2	1.0 ± 0.1	10^{-7}	0.5 ± 0.2
5×10^{-5}	1.0 ± 0.1	0.9 ± 0.1	5×10^{-7}	0.5 ± 0.3

[1] Monocytes were identified by non-specific esterase staining.
[2] % of monocytes on filter, in response to agonist indicated (each value represents mean ± s.e.mean, $n = 3$).

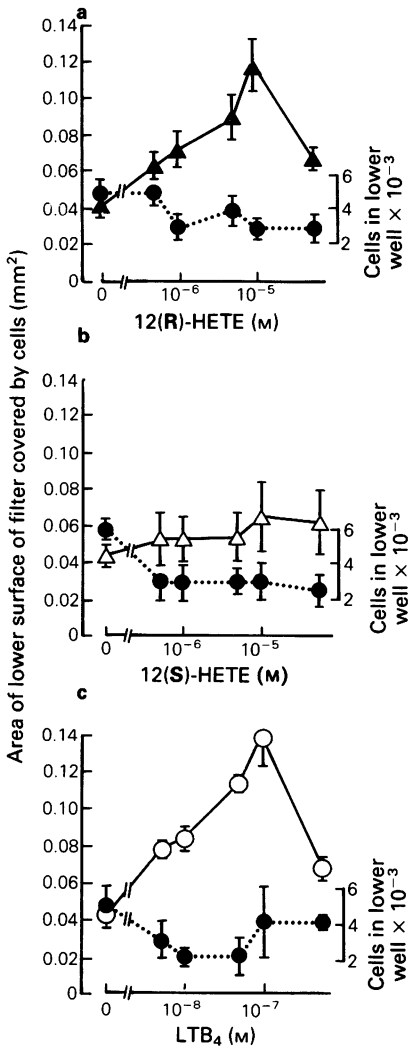

Figure 4 Comparison of area of lower surface of the chemotaxis filters occupied by cells (mm^2), using (a) 12(R)-HETE (▲), (b) 12(S)-HETE (△) and (c) leukotriene B_4 (LTB_4, ○) as chemoattractants, with percentages of detached cells in the lower wells (●) ($n = 3$).

tions of agonist, suggests that increased adherence may be contributing to the responses measured. Furthermore, the differences between the locomotor responses to 12(R) and 12(S)-HETE cannot be explained simply by greater adhesion of passively falling cells in response to 12(R)-HETE, since the reduction in the number of cells detaching into the lower wells with increasing concentrations of 12(S)-HETE, was greater than that seen with 12(R)-HETE (Figure 4a and b).

Discussion

In order to measure the migratory response of lymphocytes *in vitro*, preparation of suspensions containing less than 1% monocytes was necessary because the presence of larger numbers of monocytes allowed their preferential migration through the chemotaxis filters. The experimental conditions that have been established show that concentration-related lymphocyte locomotor activity may be measured in response to a range of agonists, without interference from the more highly motile monocytes. However, the monocyte-depleted lymphocyte population may contain a small proportion of low-density, transiently-adherent mononuclear and dendritic cells with veiled morphology (Knight *et al.*, 1986). The migratory properties of these cells have not been described and no assessment of their influence on lymphocyte chemotaxis, if any, has been made.

Concentration-related responses to the standard chemoattractants, ZAP, casein and fMLP, have been obtained using the 48-well microchemotaxis chamber in a novel assay incorporating polyvinylpyrrolidone-free polycarbonate membranes. These results confirm the observations of El-Naggar *et al.* (1980), who used blind-well Boyden chambers, and Miossec *et al.* (1984), who used a 24-well microchemotaxis chamber, both with nitrocellulose membranes. Use of the thin, optically superior polycarbonate membranes, in the present experiments, has allowed rapid and convenient quantification of migrated cells with an image analysis system. However, it is not possible to calculate the contribution of chemokinetic activity to the chemotactic response according to the method of Zigmond & Hirsch (1973), since distance measurements cannot be made using these filters.

The recent identification of 12(R)-HETE as the major stereoisomer of 12-HETE present in lesional psoriatic scale (Woollard, 1986) and the chemoattractant activity of this monohydroxy fatty acid for human neutrophils *in vitro* (Cunningham *et al.*, 1986; Cunningham & Woollard, 1987; Evans *et al.*, 1987), promoted the present investigation of the chemotactic activity of 12(R)-HETE for human lymphocytes, since neutrophilic and lymphocytic infiltrates are characteristic histological features of psoriasis.

In contrast to its epimer 12(S)-HETE, 12(R)-HETE showed dose-related chemotactic activity towards mixed human peripheral blood lymphocytes in the microchemotaxis assay, a finding which highlights the potential importance of 12(R)-HETE as a mediator of inflammation in human skin. Although adherence of lymphocytes to the substrata is likely to be an important physiological process involved in the locomotor responses measured in the present

experiments, the difference in the concentration-related activity obtained in response to 12(R)- and 12(S)-HETE and LTB_4 compared with the similarity in the percentages of cells detaching into the lower wells of the microchemotaxis apparatus, imply that the migratory responses observed are not simply due to differences in adhesion induced by the lipid mediators. Evans *et al.* (1987) have shown that 12(R)-HETE displaces [^{3}H]-LTB_4 from its receptor on human neutophils, but that 12(S)-HETE has a very low affinity for the same receptor. Since LTB_4 induced lymphocyte migration *in vitro* and was at least 200 times more potent than 12(R)-HETE, which in turn was more potent than 12(S)-HETE, 12(R)-HETE may act at an LTB_4 receptor on the human lymphocyte to elicit a migratory response.

The chemoattractant activity of LTB_4 for human lymphocytes has previously been described by Payan & Goetzl (1981) and by Ternowitz & Thestrup-Pedersen (1986), the latter using only a single concentration of LTB_4.

Since 12(R)-HETE and LTB_4 are found in $\mu g\,gm^{-1}$ and $ng\,gm^{-1}$ quantities, respectively, in psoriatic scale (Brain *et al.*, 1984a,b; Cunningham *et al.*, 1985; Woollard, 1986) and both are shown to have chemoattractant activity for human peripheral blood lymphocytes, it is possible that they may contribute to the chemoattractant activity responsible for the accumulation of lymphocytes in lesional psoriatic skin.

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