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Identification on receptors for leukotriene B_4 expressed on guinea-pig peritoneal eosinophils

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SUMMARY

We have recently reported that guinea-pig eosinophil chemotactic factor of anaphylaxis (ECF-A), an activity present in diffusates from antigen-challenged sensitized lung, is largely accounted for by leukotriene B_4 (LTB_4) and to a lesser extent 8(S)15(S)-dihyroxy 5,9,11,13 (Z,E,Z,E) eicosatetraenoic acid. We characterized cell surface receptors for LTB_4 on guinea-pig eosinophils in order to demonstrate an association between receptor occupancy and eosinophiliotactic activity of guinea-pig ECF-A. Equilibrium binding studies showed that peritoneal eosinophils bound [^{3}H]LTB_4 in a cell concentration and time-dependent fashion. The binding was saturable and specific for LTB_4 as other eosinophil chemoattractants, i.e. platelet-activating factor (PAF) and 8(S)15(S)-diHETE, were unable to displace significant amounts of [^{3}H]LTB_4. In addition the binding was readily reversed by the LTB_4 receptor antagonist LY 255283 (K_i 4·30 nM). Scatchard plot analysis revealed two discrete populations of binding sites, high affinity ($K_{d1} = 0{\cdot}30$ nM; $B_{max} = 900$ sites/cell) and low-affinity sites ($K_{d2} = 140$ nM; $B_{max} = 60{,}000$ sites/cell). The major migratory component of LTB_4-stimulated eosinophil locomotion was chemotaxis, optimal at 1×10^{-7} M ($P < 0{\cdot}01$) with EC_{50} value of 3×10^{-9} M. A comparison of the profile of arachidonic acid metabolism by RP-HPLC analysis showed that following stimulation with calcium ionophore (A23187) guinea-pig eosinophils preferentially synthesized LTB_4 (10 ng/10^6 cells) while in contrast human eosinophils synthesized LTC_4 (10 ng/10^6 cells). Therefore our data show that guinea-pig eosinophils express both high- and low-affinity receptors for LTB_4 and that the chemotactic response to this mediator may be mediated by ligation of the high-affinity binding site. Furthermore guinea-pig peritoneal eosinophils can synthesize LTB_4, a mediator which constitutes >60% of guinea-pig ECF-A.

INTRODUCTION

A preponderance of eosinophils at the site of inflammation is a hallmark of IgE-associated allergic disease.[1] Through their ability to release granule-associated basic proteins and membrane-derived mediators, eosinophils have been implicated as the prime effector cells of epithelial damage and airway hyperreactivity, which are characteristic features of the pathology of asthma.[2,3] However despite the putative importance of eosinophils in the pathogenesis of bronchial asthma, mediators responsible for the preferential accumulation of eosinophils at the site of inflammation have not been characterized with certainty.

Using an *in vitro* model of immediate type hypersensitivity, Kay *et al.*[4–6] described a chemotactic activity present in diffusates from pre-sensitized guinea-pig or human lung fragments challenged with specific antigen, which appeared to be selective for eosinophils in mixed leucocyte populations. This activity was designated eosinophil chemotactic factor of anaphylaxis (ECF-A). A part of the activity associated with human ECF-A was attributed to a family of tetrapeptides namely Valine- and Alanine-Glycine-Serine-Glutamic acid.[7] However, by comparison with more recently described lipid mediators such as platelet-activating factor (PAF) and leukotriene B_4 (LTB_4) neither of these tetrapeptides has significant chemotactic activity for either human or guinea-pig eosinophils.[8,9] We have recently characterized guinea-pig ECF-A and identified its principal components as LTB_4 and 8(S)15(S)-dihyroxy 5,9,11,13 (Z,E,Z,E) eicosatetraenoic acid [8(S)15(S)-diHETE],[9] which account for >60% and >20% of the activity, respectively. LTB_4 was a strong chemoattractant for guinea-pig eosinophils, whereas PAF, a documented chemoattractant for human eosinophils,[8] histamine and human ECF-A tetrapeptides had negligible effects.

The aim of the present study was to characterize and quantify LTB_4 receptors on guinea-pig eosinophils and establish their association with chemotactic responsiveness. In addition, we investigated the pathway of arachidonic acid metabolism by

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guinea-pig eosinophils to determine whether they have the potential to function in an autocrine capacity, promoting their own migrational response through the generation of LTB_4.

MATERIALS AND METHODS

Materials

The following reagents were purchased as indicated: ovalbumin (OVA) (grade VI) and bovine serum albumin (BSA) [radioimmunoassay, (RIA) grade] from Sigma Chemical Co. (Poole, Dorset, U.K.); horse serum, Hanks' balanced salt solution (HBSS) and medium 199 from Gibco (Paisley, Renfrewshire, U.K.); Percoll and Optiphase 'High safe 3' scintillant cocktail from Pharmacia (Milton Keynes, Bucks, U.K.); silicone oil from General Electric Co. (Waterford, NY); trypan blue, light green, saponin, haematoxylin and chromotrope 2R from BDH Ltd (Dagenham, Essex, U.K.); HPLC standards: LTB_4, 6-trans-LTB_4, 12-epi-6-trans LTB_4, 20-OH-LTB_4, 8(S)15(S)-diHETE from Cascade Biochem Ltd (Reading, Berks, U.K.); methanol [high-performance liquid chromatography (HLPC) grade] and water (HPLC grade) from Rathburn Chemicals Ltd (Walkerburn, U.K.); tritiated leukotriene B_4 [14,15,$-^3$H(N)] (in ethanol, specific activity 32·8 Ci/mmol) stored at $-20°$ from NEN/Dupont (Stevenage, U.K.); LY 255283 (1-(5-ethyl-2-hydroxy-4-(6-methyl-6-(1H-tetrazol-5-yl)-heptyloxy)-phenyl)ethanone) was a gift from Lilly Research Laboratories (Indianapolis, IN).

Recruitment and purification of eosinophils

Eosinophils were obtained from the peritoneal lavage of Dunkin Hartley guinea-pigs (> 600 g) repeatedly sensitized with horse serum and purified on 6-step Percoll discontinuous gradients, as described previously.[9] Normal density eosinophils were recovered from the 1·090/1·095/1·100 g/ml interfaces, washed once with HBSS (without calcium or magnesium) to remove all traces of Percoll and exposed to hypotonic medium for 20 seconds (ice-cold water) to lyse residual erythrocytes. The cells were then washed twice in the buffer appropriate for the subsequent assay to be performed. The purity of the eosinophil population was $92 \pm 3\%$ ($n = 12$) with typical yields of $3–4 \times 10^7$ eosinophils/animal, as determined by toluidine blue/light green staining.[10] Macrophages were the main contaminating cell type.

Human eosinophils from normal donors were purified on Percoll discontinuous gradients, by a modification of the method of Gärtner,[11] as previously described by us.[12] In brief, leucocyte-rich plasma was recovered from heparinized (20 U/ml) venous blood (100 ml) using dextran sedimentation (4 parts blood: 1 part dextran) for 30 min at room temperature and washed twice with RPMI-1640 supplemented with HEPES (30 mM). The cells were then resuspended in Percoll (1·080 g/ml density) containing 2·5 mg/ml BSA at $5–10 \times 10^7$ cells/ml and overlayered (2 ml/gradient) onto 5-step discontinuous Percoll gradients composed of 1 ml of 1·120 g/ml, 3 ml of 1·100, 1·095 and 1·090 g/ml and 2 ml of 1·085 g/ml, and centrifuged at 1500 *g* for 20 min at 20°. The purity of normal density eosinophils recovered from the 1·095/1·100/1·120 g/ml interfaces was greatly enhanced by adjusting the osmolality of the 90% Percoll (9 parts 100% Percoll: 1 part × 10 HBSS) used to make the various density fractions to 313–315 mOsm/kg H_2O. The cells were then treated in the same way as described above for guinea-pig eosinophils.

Binding assay for [^{3}H]LTB_4

All binding assays were performed on ice since previous studies have indicated that granulocytes quantitatively metabolize LTB_4 at room temperature (> 23°), but not at 4°[13] and > 97% of [^{3}H]LTB_4 is recoverable from cells incubated with label at 4° (our findings, data not shown). In addition this minimized the reduction in cell-associated binding of [^{3}H]LTB_4 as a result of oxidation of the tritiated label. Cells at 1×10^7 cells/ml (unless otherwise stated) were suspended in Hanks' buffer solution (containing 1·0 mM $CaCl_2$; 0·7 mM $MgCl_2$) at 4° for 20 min prior to the assay. The cell suspension (950 μl) was then overlayered onto 400 μl silicone oil previously added to 1·5 ml polypropylene microfuge tubes and placed on ice. The cells were then treated simultaneously with 50 μl of [^{3}H]LTB_4 (31·6 pM) in the presence or absence of unlabelled LTB_4 or other competing ligands. Stock solutions of labelled and unlabelled ligands were stored in methanol at −20° and were evaporated to dryness over nitrogen and redissolved in HBSS (with $CaCl_2$ and $MgCl_2$) plus 2·5 mg/ml BSA immediately before use. Following an appropriate incubation period the cells were pelleted by centrifugation for 2 min in a Beckman model 152 microfuge (Beckman Instruments, Fullerton, CA). This method achieved rapid separation of cells and bound [^{3}H]LTB_4 with minimal contamination of the cell pellet by free [^{3}H]LTB_4 (< 0·5% of the total counts was recovered from the bottom of the microfuge tubes in control experiments without cells). An aliquot (200 μl) of the supernatants was collected and placed in scintillation vials and the remaining supernatant fluid and oil layers removed by aspiration and discarded. Eosinophil-bound radioactivity was measured in the cell pellets, obtained by clipping the tip of the microfuge tubes. The cell pellets and supernatant samples were individually mixed with 500 μl methanol for 10 min and 4 ml of scintillation cocktail and the radioactivity (d.p.m.) counted in a β_2-scintillation counter.

Data analysis

Non-specific binding was determined by measuring the amount of [^{3}H]LTB_4 bound to the intact eosinophils in the presence of excess (100 nM) unlabelled LTB_4. Specific binding was determined by subtracting non-specific binding from total binding. Scatchard analysis plotted fmol bound/fmol free ratios versus fmol bound to the cells using 31·6 pM [^{3}H]LTB_4 and increasing concentrations of unlabelled LTB_4. Data from Scatchard plot analysis were compatible with a two-receptor model.[13,14] The low-affinity binding site was a straight line (coefficient of linearity > 0·85) as determined by the method of least squares using data obtained at high concentrations of LTB_4 (10–100 nM). The amount of LTB_4 bound to the high-affinity receptors was estimated by subtracting low-affinity binding from total binding observed at each (low) concentration of LTB_4 (i.e. 0·068–3·16 nM). The resulting values were used to construct a straight line for high-affinity sites by the method of least squares and the correlation coefficient for linearity was > 0·92. The quantitation of the total number of binding sites (B_{max}) and estimation of equilibrium dissociation constant (K_d) were calculated from the Scatchard plot. Confirmation of the binding parameters was obtained by the LIGAND computerized program analysis.[15] In addition, the significance of fitting the data into a two-binding site versus a single-binding site model was tested by this program.

Eosinophil chemotaxis assay

Cell migrational responses were assessed using 48-well microchemotaxis chambers (Neuro Probe, Inc., Cabin John, MD) as previously described.[9] In brief, 50 μl of the cell suspension resuspended at 4×10^6/ml in medium 199 plus 4 mg/ml OVA was added to the upper well and separated from 29 μl of chemoattractant in the lower well by an 8-μm pore size nitrocellulose filter (Sartorius, Göttingen, Germany). Synthetic LTB_4 was stored at $-80°$ in solution with methanol and was evaporated to dryness over nitrogen and redissolved in medium 199 plus 2·5 mg/ml BSA (pH 7·4) immediately before use. The microwell chambers were incubated for 90 min at 37° after which the filters were washed, fixed and stained with haematoxylin/chromotrope 2R.[16] Chromotrope 2R stain was used to distinguish eosinophils from other contaminating cell types. The migrational response of the cells was assessed as the number of cells in random high-power fields ($\times 400$) that had migrated through to the underside of the filter.

Arachidonic acid metabolism by eosinophils

Eosinophils (4×10^6 cells/ml) were equilibrated in HBSS containing 1 mM $CaCl_2$ and 0·7 mM $MgCl_2$ for 5 min at 37° prior to stimulation with calcium ionophore A23187 (5 μM), for 15 min at 37°. The reaction was stopped by placing the cells on ice for 15 min, centrifuging at 1500 ***g*** for 5 min. The cell supernatant was recovered and added to an equal volume of methanol and stored overnight at $-80°$ in 1·5 ml polypropylene tubes. The mixture was then centrifuged at 1500 ***g*** for 5 min in order to pellet the precipitated protein and the resultant supernatant collected, dried in a vacuum concentrator (Speedvac; Savant Inc., Hicksville, NY), and reconstituted in HPLC running solvent.

HPLC analysis

Lipoxygenase products were analysed by RP-HPLC performed isocratically on a 5-μm Nucleosil C-18 (Microsorb) column ($125 \times 4{\cdot}6$ mm) (Ranin, Woburn, MA) eluting at 0·5 ml/min with methanol/water/acetic acid (70:30:0·07) running buffer, adjusted to pH 5·4 with ammonia. Lipoxygenase products were identified on the basis of their co-migration with synthetic standards and UV absorbance at either 270 or 280 nm. The limit of detection was 0·5 ng for LTB_4, 1 ng for $LTC_4/D_4/E_4$ and 10 ng for the HETE. Quantitation was performed by comparison of peak height of product with that of known amounts of the corresponding synthetic standard after correction for the recovery of internal standard. The recovery of $[^3H]LTB_4$ was consistently $>85\%$ and $>75\%$ for $[^3H]LTC_4$.

Statistical analysis

Experimental results are summarized as mean $\pm$ sem. Data for the chemotaxis assay were analysed for statistical significance by a two-way analysis of variance test.

RESULTS

The binding of $[^3H]LTB_4$ to intact eosinophils recovered from guinea-pig peritoneal cavity showed that both total and specific binding of the label increased with cell concentration, in a linear fashion (Fig. 1). This demonstrated that specific binding was directly proportional to the number of cells present. Thus, cell concentrations of $5–10 \times 10^6$/ml, which bound between 4 and 7% of the total ligand (31·6 pM), were routinely used in all subsequent experiments. Label that remained cell associated in the presence of 3000-fold excess unlabelled LTB_4 (100 nM) represented non-specific binding and accounted for $<14\%$ of the total $[^3H]LTB_4$ bound (Fig. 1).

Incubation of 5×10^6/ml eosinophils with $[^3H]LTB_4$ (31·6 pM) at 4° produced a time-dependent increase in the total

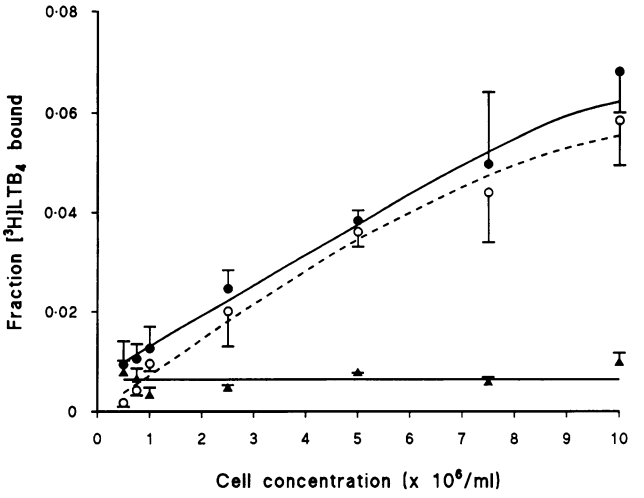

Figure 1. Cell concentration dependent binding of $[^3H]LTB_4$ to guinea-pig peritoneal eosinophils. A range of concentrations of eosinophils was incubated with 31·6 pM $[^3H]LTB_4$ for 40 min at 4°, in the presence (▲) or absence (●) of 100 nM LTB_4. Specific binding (○) was determined by subtracting non-specific binding from total binding. Values represent the mean $\pm$ SEM of three separate experiments, and the average purity of eosinophils was $91 \pm 2\%$.

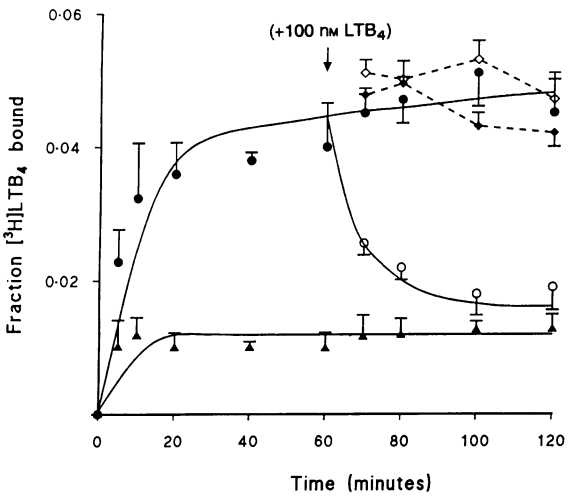

Figure 2. Time-course and reversibility of $[^3H]LTB_4$ binding to guinea-pig eosinophils. Cells (5×10^6/ml) were incubated with 31·6 pM $[^3H]LTB_4$ at 4° in the presence (▲) or absence (●) of excess unlabelled LTB_4 (100 nM). To some reaction tubes either unlabelled LTB_4 (100 nM) (○) or 8(S)15(S) diHETE (100 nM) (◇) or PAF (100 nM) (◆), were added after 60 min of incubation. Each data point represents mean $\pm$ SEM of five separate experiments for LTB_4, and two separate experiments for 8(S)15(S) diHETE and PAF, respectively. The purity of eosinophil preparations use was $93 \pm 2\%$.

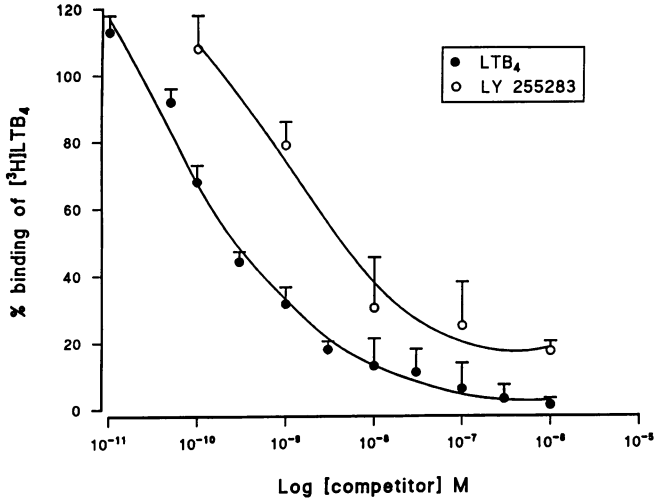

Figure 3. Competitive displacement of [^{3}H]LTB$_4$ by a specific LTB$_4$ receptor antagonist LY 255283. Intact eosinophils (1×10^7/ml) were incubated with [^{3}H]LTB$_4$ (31·6 pM) for 60 min at 4° in the presence of increasing concentrations of LY 255283 yielding a K_i value of 4·30 nM. Each data point represents the mean ± SEM of three separate experiments. The purity of eosinophils used was 94 ± 2%.

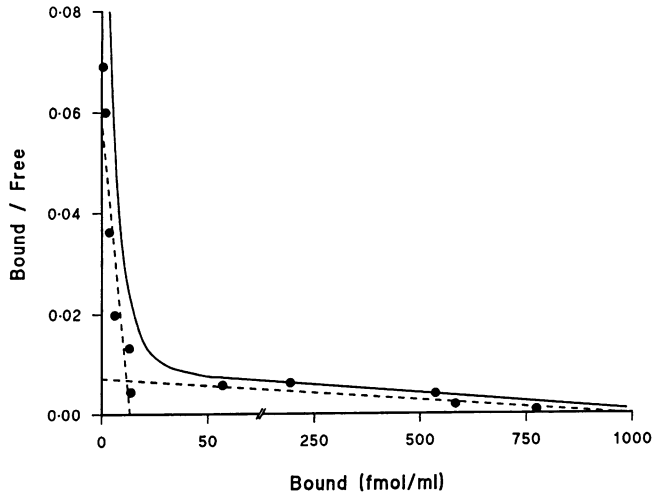

Figure 4. Scatchard plot analysis of [^{3}H]LTB$_4$ binding to guinea-pig eosinophils performed at equilibrium (60 min at 4°). Cells (1×10^7 cell/ml) were incubated with [^{3}H]LTB$_4$ (31·6 pM) and increasing concentrations of LTB$_4$ (68·4 pM–1000 nM). Bound [^{3}H]LTB$_4$ represents the concentration of specifically bound [^{3}H]LTB$_4$. Data represent the mean of six separate experiments, and the purity of eosinophils used was 93 ± 2%. The solid curve is a computerized best fit for a two-site model and two binding components are indicated by the straight lines.

binding. The amount of [^{3}H]LTB$_4$ bound to eosinophils increased rapidly within the first 5 min, and more gradually for the next 15 min. Apparent equilibrium occurred between 20 and 40 min and maximal binding was maintained for a further 80 min (Fig. 2). As depicted in Fig. 2, the addition of a large excess (100 nM) of unlabelled LTB$_4$ at 60 min displaced $\simeq$70% of specifically bound ligand within the first 10 min. Non-specific binding at equilibrium represented < 20% of the total [^{3}H]LTB$_4$ bound (Fig. 2).

[^{3}H]LTB$_4$ bound to eosinophils was not displaced by other chemically characterized eosinophil chemoattractants, including 8(S)15(S)-diHETE (100 nM) or PAF 100 nM) (Fig. 2). To demonstrate that these binding sites represented the specific LTB$_4$ receptors, competitive displacement assays were performed with a selective LTB$_4$ receptor antagonist, LY 255283.[17,18] As illustrated in Fig. 3, LY 255283 inhibited the binding of [^{3}H]LTB$_4$ in a dose-dependent manner, yielding a K_i of 4·30 nM.

The equilibrium binding parameters of LTB$_4$ to peritoneal eosinophils were determined by incubating 10^7 cells/ml for 60 min at 4° with 31·6 pM [^{3}H]LTB$_4$ and increasing concentrations of LTB$_4$ ranging from 68·4 pM to 1000 nM. Scatchard plots derived from these displacement assays, performed at equilibrium, consistently yielded a curvilinear binding curve indicating the existence of two classes of binding sites for LTB$_4$ (Fig. 4). Results from LIGAND analysis confirmed this ($P < 0{\cdot}05$, for a two-binding site versus a one-binding site model) and indicated the presence of 900 high-affinity ($K_d = 0{\cdot}30$ nM) and 60,000 low-affinity ($K_d = 140$ nM) binding sites/cell (Fig. 4).

Using a modified Boyden microchemotaxis assay, we confirmed that LTB$_4$ was a highly effective chemoattractant for guinea-pig eosinophils, producing an optimal response at 10^{-7} M ($P < 0{\cdot}01$) (Table 1). We compared the relative contributions of chemotaxis and chemokinesis to LTB$_4$-induced eosinophil locomotion. Equal concentrations of LTB$_4$ above and below the filter (when any migration observed will be due to chemokinesis), were compared with locomotion observed with LTB$_4$ only in the lower well (where migration will be a combination of chemokinesis and chemotaxis) (Table 1). A significant difference between the migrational response due to chemotaxis plus chemokinesis and chemokinesis alone was observed at 1×10^{-8}–1×10^{-6} M, ($P < 0{\cdot}01$). These data suggest that the major component of the overall migratory response exerted by LTB$_4$ was chemotaxis, and the EC_{50} value for this response was 3×10^{-9} M (Table 1).

Representative profiles of lipoxygenase products obtained by RP-HPLC analysis of incubation media from guinea-pig and human eosinophils stimulated with A23187 (5 μM) are shown in Fig. 5. Guinea-pig eosinophils (98% pure) stimulated with A23187 (5 μM) produced LTB$_4$ (10 ng/10^6 cells) and the 6-trans and 6 trans-12-epi isomers of LTB$_4$. Sulphido-peptide leukotrienes, LTC$_4$/D$_4$/E$_4$ were not detected in any of the samples tested (Fig. 5a). Greater amounts of 5-lipoxygenase products were not produced by guinea-pig eosinophils following stimulation in the presence of both A23187 (5 μM) and arachidonic acid (20 μM) (data not shown). By comparison human eosinophils (98% pure) stimulated with ionophore (5 μM) produced predominantly LTC$_4$ (10 ng/10^6 cells) but no detectable amounts of LTB$_4$ (Fig. 5b).

DISCUSSION

In the present study we have used direct binding assays to establish the existence of specific binding sites for LTB$_4$ on guinea-pig peritoneal eosinophils. In addition, we have assessed the relationship between receptor occupancy and eosinophil migrational responses to LTB$_4$, *in vitro*. Under conditions where no metabolism of the ligand occurred, binding of [^{3}H]LTB$_4$ to

Table 1. Checkerboard analysis of LTB_4 induced eosinophil locomotion

LTB_4 below filter	Eosinophil migration with LTB_4 above the filter (cells/10 hpf)					
	Diluent	10^{-10} M	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M
Diluent	24±6	49±4	51±23	53±10	44±19	46±5
10^{-10} M	64±12	92±53	62±23	45±7	36±6	39±10
10^{-9} M	70±13	91±40	59±13	40±8	41±3	43±3
10^{-8} M	140±32*	201±57	162±42	86±13	55±27	60±29
10^{-7} M	238±39*	223±35	218±87	169±49	71±4	43±15
10^{-6} M	157±13*	179±17	180±30	145±69	156±45	68±2

Results represent eosinophil migration to varying concentrations of LTB_4 above and/or below the filter in modified chambers. A significant difference was observed between chemotaxis plus chemokinesis and chemokinesis at 10^{-8}–10^{-6} M; * $P<0.01$. Values are expressed as mean±SEM ($n=4$).

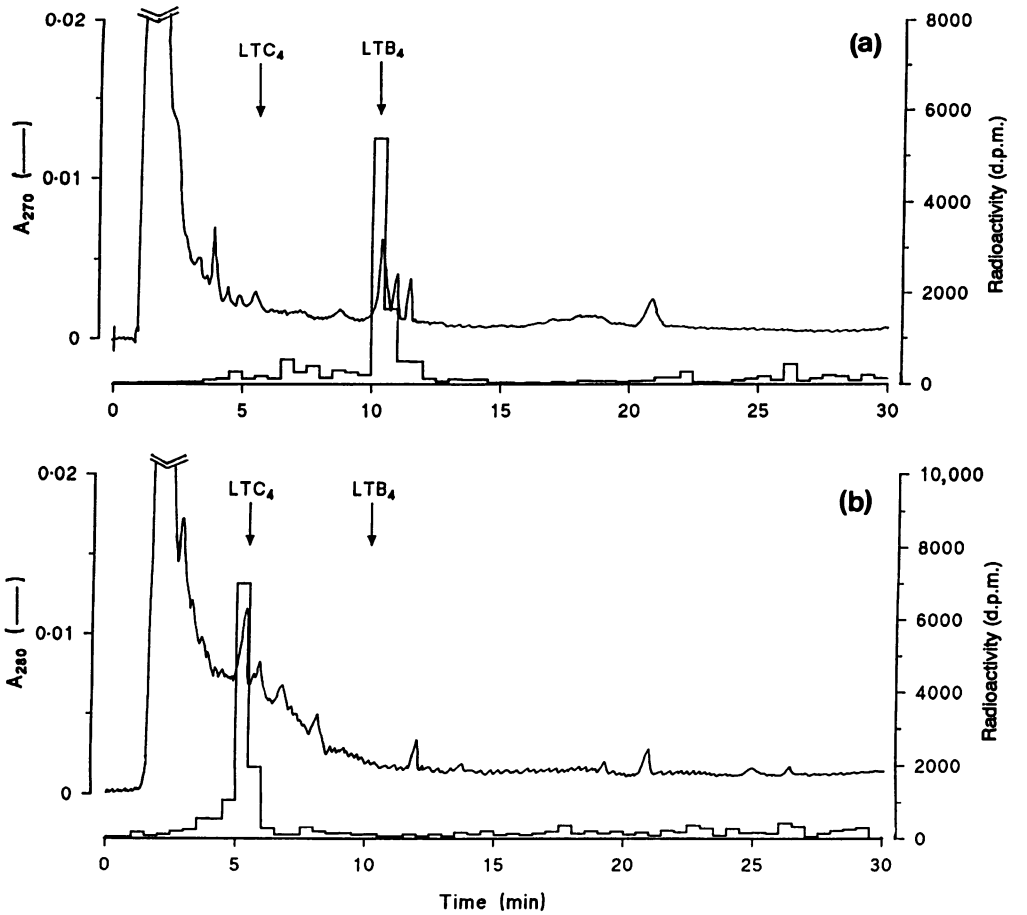

Figure 5. The profile on RP-HPLC of the methanol extracts of supernatants from the incubation of (a) guinea-pig eosinophils (>96%), (b) human eosinophils (>98% pure) (4×10^6 cells/ml) with calcium ionophore A23187 (5 μM) compared with the retention time of synthetic markers determined at 270 and 280 nm, respectively. Identity of the peaks was also determined by co-elution with radioactive standards (a) [^{3}H]LTB_4 (10,000 d.p.m.) or (b) [^{3}H]LTC_4 (10,000 d.p.m.).

eosinophils was saturable and readily reversed by the addition of excess unlabelled ligand (100 nM LTB_4) (Fig. 2). The rapid association and dissociation of specifically bound [^{3}H]LTB_4 (Fig. 2) indicated that the system was at equilibrium in the binding studies and that equilibrium parameters could therefore be derived accurately. Scatchard analysis of saturation binding studies showed that guinea-pig eosinophils expressed two discrete populations of binding sites for LTB_4, high-affinity (K_d 0·30 nM; B_{max} 900 sites/cell) and low-affinity binding sites (K_d 140 nM; B_{max} 60,000 sites/cell) (Fig. 4). Estimates of the binding parameters and statistical significance of the goodness of fit of a two-site model versus a single-site model were confirmed by the

LIGAND computerized program.[15] The specificity of binding was determined using LY 255283, a specific LTB_4 receptor antagonist.[17,18] LY 255283 competitively displaced [^{3}H]LTB_4 binding in a dose-dependent fashion yielding a K_i of 4·30 nM which was comparable with the K_d value calculated for the high-affinity binding site. As shown in Fig. 3, LY 255283 did not displace more than 85% of specifically bound [^{3}H]LTB_4 at concentrations up to 10^{-6} M. It is possible that if higher concentrations of the antagonist had been used (i.e. $> 10^{-5}$ M), a K_i value for the low-affinity binding site would have been obtained, however this was precluded by the limited solubility of LY 255283.

The identification of two distinct classes of plasma membrane-associated receptors for LTB_4 on guinea-pig peritoneal eosinophils is compatible with the findings reported for human neutrophils,[13,14,19] myeloid differentiated HL-60 cells[20] and guinea-pig alveolar eosinophils.[21] This suggests that a high degree of conservation is maintained in the expression of LTB_4 binding sites on different cell types isolated from various animal species. Our data however are not in agreement with a recent study which reported that both intact eosinophils and cell membrane preparations express a single high-affinity binding site.[22] The K_d values on intact cells and membrane preparations were $2{\cdot}8 \pm 0{\cdot}96$ nM and $1{\cdot}4 \pm 0{\cdot}2$ nM, respectively, and the corresponding B_{max} values were $40{,}000 \pm 6000$ receptors/cell and $1{\cdot}6 \pm 0{\cdot}4$ pmol/mg of membrane protein. The reason for this discrepancy is not clear, but may be attributable to the limited concentration range of [^{3}H]LTB_4 (up to 6 nM) used by these workers in the Scatchard binding assay.[22] Our data showed that the K_d value for the low-affinity binding site was 140 nM. Therefore it is unlikely that at concentrations as low as 6 nM of [^{3}H]LTB_4 significant binding to the low-affinity receptors would have been detected. The existence of a low-affinity binding site on guinea-pig peritoneal eosinophils is supported by the findings of Rabe *et al.*[23] who reported that the EC_{50} value for superoxide generation from guinea-pig peritoneal eosinophils was $60{\cdot}2 \pm 17{\cdot}3$ nM, which is comparable with a K_d value for the low-affinity site, estimated from the present study.

LTB_4 is a potent activator of leucocyte function and it has been preposed that the two classes of LTB_4 receptors on human neutrophils are associated with different biological responses.[19] The occupancy of high-affinity receptors transduces the chemokinetic and chemotactic stimuli, whereas binding of the low-affinity receptor triggers enzyme release and respiratory burst activity. In the present study we found that LTB_4 stimulated eosinophil locomotion (chemotaxis plus chemokinesis) in a dose-dependent fashion, optimal at 1×10^{-7} M ($P < 0{\cdot}01$). By checkerboard analysis, the major migratory component of LTB_4 was chemotaxis, with an EC_{50} value of 3×10^{-9} M. This value was comparable with the K_d for high-affinity binding sites ($0{\cdot}3 \times 10^{-9}$ M), indicating that occupancy of the high-affinity receptor may be involved in mediating eosinophil directional migrational responses towards LTB_4. Functional responses mediated by occupancy of the low-affinity binding sites for LTB_4 were not investigated in the present study. However, data from other studies using guinea-pig alveolar and peritoneal eosinophils have shown that occupancy of low-affinity binding sites may be associated with superoxide production and thromboxane A_2 generation.[21]

Although the preferential synthesis of LTB_4 by guinea-pig alveolar eosinophils has previously been reported,[24] it was not clear whether the observation was attributable to the local environment of the lung or a species-specific phenomenon. Therefore, we investigated the metabolism of arachidonic acid by purified guinea-pig peritoneal eosinophils. Our results, in agreement with observations of other workers,[25] clearly indicated that following stimulation with ionophore, LTB_4 (10 ng/10^6 cells) was the major leukotriene elaborated. In contrast, human eosinophils generated LTC_4 (10 ng/10^6 cells) almost exclusively. The inability of guinea-pig eosinophils to release peptido-leukotrienes is attributed to the lack of a glutathione transferase activity.[24] Since guinea-pig eosinophils have the capacity to synthesize LTB_4, it is plausible that the guinea-pig eosinophils function in an autocrine capacity to promote their own migrational responses during allergic inflammation. It is also noteworthy that while human eosinophils have a strong 15-lipoxygenase activity in the presence of exogenous arachidonic acid,[26] we found no evidence of this in the guinea-pig eosinophil preparations.

There is mounting evidence to suggest that LTB_4 plays an important role in the recruitment, activation and subsequent degranulation of eosinophils in the bronchopulmonary tissue in guinea-pig models of antigen-induced airway hyper-reactivity.[27-29] However the relevance of these findings to human airways disease must be interpreted with caution as considerable evidence exists showing marked species differences between guinea-pig and human eosinophils. In addition to the difference in the profile of arachidonic acid metabolism, guinea-pig eosinophils respond to chemotaxis to LTB_4, both *in vitro* and *in vivo*, but not to PAF, which is a potent chemoattractant for human eosinophils.[9,30]

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