Reduction in leukotriene B_4 generation by bronchoalveloar lavage cells in asthma

Louise J Restrick, Anthony P Sampson, Priscilla J Piper, John F Costello

Abstract

Background **– Leukotrienes are inflammatory mediators implicated in the pathogenesis of asthma. The capacity of inflammatory cells within the airways to generate leukotrienes may be altered in asthma. This hypothesis was tested using bronchoalveolar lavage (BAL) to sample cells within the airways from atopic asthmatic and normal subjects, and by measuring their capacity to generate leukotriene B_4 (LTB_4) and leukotriene C_4 (LTC_4) in response to A23187, a potent stimulus of leukotriene generation.**

Methods **– Bronchoalveolar lavage was performed in 12 mild asymptomatic atopic asthmatic patients and 12 normal subjects. Mixed BAL cell aliquots (approximately 80% alveolar macrophages) were incubated with 0–20 μM A23187 for 10 minutes and with 4 μM A23187 for 0–30 minutes, and leukotrienes were measured by radioimmunoassay and high performance liquid chromatography.**

Results **– Mixed BAL cells from asthmatic subjects generated less LTB_4 than cells from normal subjects in dose response and time course experiments (area under the curve 81·5 (0·0–228·5) ng · min · 10^{-6} cells in asthmatic subjects and 197·9 (13·9–935·6) ng · min · 10^{-6} cells in normal subjects. There were no differences in LTC_4 generation between BAL cells from asthmatic and normal subjects.**

Conclusions **– Generation of LTB_4 by BAL cells from atopic asthmatic subjects in response to A23187 was reduced. As the alveolar macrophage is the major source of LTB_4 in BAL cells, these results probably reflect reduced generation of LTB_4 by alveolar macrophages from asthmatic patients. This may be a consequence of monocyte migration into the lung, or altered alveolar macrophage function in asthma, or both.**

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Keywords: asthma, alveolar macrophage, leukotriene B_4.

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Asthma is characterised by reversible airflow obstruction, bronchial reactivity, and airways inflammation in which a range of mediators, including leukotrienes, is implicated.[1] Leukotriene B_4 (LTB_4) is chemotactic for neutrophils, eosinophils, and mononuclear cells, and activates neutrophils and eosinophils, but also stimulates T suppressor cell function.[2] The cysteinyl leukotrienes – leukotriene C_4 (LTC_4), leukotriene D_4 (LTD_4), and leukotriene E_4 (LTE_4) – cause bronchoconstriction, increased vascular permeability, and increased mucus secretion.[3] The leukotrienes are derived from arachidonic acid by the 5-lipoxygenase pathway and their involvement in asthma is suggested by their increased levels in body fluids of asthmatic patients – for example, LTB_4 and LTC_4 in bronchoalveolar lavage (BAL) fluid[4] and LTE_4 in urine.[5] The most convincing evidence comes from studies of leukotriene antagonists and 5-lipoxygenase inhibitors in asthmatic subjects. These agents reduce bronchoconstriction induced by exercise[6] and cold air[7] and improve lung function in chronic asthma.[8]

Leukotrienes are produced by a range of cells present in the airways, including eosinophils, mast cells, macrophages/monocytes, and neutrophils.[9] Leukotriene generation independent of receptor-associated processes can be studied in vitro using calcium ionophore (A23187). Leukotriene generation by peripheral blood leucocytes from atopic asthmatic subjects is increased in response to ionophore compared with that in normal subjects.[10] We postulated that the capacity of inflammatory cells in the bronchi of asthmatic subjects to generate leukotrienes might also be increased. Inflammatory cells within the bronchi – most of which are alveolar macrophages – can be sampled by BAL and their function studied in vitro. The aim of this study was to investigate whether the capacity of BAL cells to generate leukotrienes when stimulated with ionophore is altered in asthmatic subjects.

Methods

SUBJECTS

Twelve mild asymptomatic atopic asthmatic subjects and 12 normal human volunteers were studied. Between two and seven days before fibreoptic bronchoscopy each subject was assessed for the presence and severity of asthma. Clinical history and examination, standard skin prick tests (Bencard, Idis, Kingston, Surrey, UK), full blood count and differential white blood count, serum IgE, biochemical screen, chest radiography (if not performed within the previous year), spirometry and methacholine challenge were performed. Forced expiratory volume in one second (FEV_1) was measured using a Vitallograph dry wedge bellows spirometer; baseline FEV_1 was recorded after three reproducible values were obtained. Methacholine challenge was performed by the two minute tidal breathing method.[11] FEV_1 was measured at one and three minutes after inhalation of physiological saline and doubling

Table 1 Subject characteristics

	Asthmatic subjects	*Control subjects*	*p value of difference*
No. of patients	12	12	
M:F	4:8	3:9	
Age (years)*	24 (20–29)	25 (20–32)	NS
PC_{20} (mg/ml)**	0·84 (0·43–1·63)	>8	
FEV_1 (litres)†	3·60 (0·71)	3·66 (0·77)	NS
FEV_1 (% predicted)†	102 (12)	104 (10)	NS
Skin tests: positive	12/12	1/12	
Serum IgE (kU/l)‡ (Normal <100 kU/l)	219 (17–867)	17 (0–42)	0·001
Blood eosinophils (10^6/ml)‡	0·2 (0·0–0·9)	0·0 (0·0–0·4)	NS
Blood eosinophils (% total)‡	4·0 (0·0–17·0)	0·0 (0·0–5·1)	0·05

PC_{20} = concentration of methacholine producing a 20% fall in FEV_1; FEV_1 = forced expiratory volume in one second.
*Mean (range); **geometric mean (CI); † mean (SD); ‡ median (range).

concentrations of methacholine to obtain the lowest post-inhalation value of FEV_1. The provocative concentration of methacholine producing a 20% fall in FEV_1 (PC_{20}), relative to the post-diluent FEV_1, was obtained from the log dose-response curve.

All subjects were non-smokers or ex-smokers who had not smoked for the preceding three months. The asthmatic subjects were using only inhaled salbutamol (as required) as treatment for their asthma and all had an FEV_1 >80% of predicted. None of the asthmatic subjects had used inhaled or oral steroids for the previous three months. Atopy was defined by two positive (>3 mm) skin prick tests from a standard range or a raised total serum IgE (>100 kU/l). Subject characteristics are shown in table 1. This study was performed with the permission of the ethics committee of King's College Hospital and all patients gave their informed written consent.

BRONCHOALVEOLAR LAVAGE (BAL)

Bronchoalveolar lavage was performed during fibreoptic bronchoscopy by the same clinician (LJR) using a standard procedure. Each subject fasted from 24.00 hours the previous day and bronchodilators were withheld for 24 hours (not required for symptom control in any patient during this period). FEV_1 was measured to check that it was greater than 80% of baseline immediately before fibreoptic bronchoscopy and inhaled salbutamol (200 μg) was administered to all subjects, both asthmatics and controls, 20 minutes before bronchoscopy. An intravenous cannula was sited in a peripheral vein and atropine (0·6 mg) and diazemuls (5–15 mg according to body weight) were administered intravenously. Fibreoptic bronchoscopy (Olympus BF P20D, Olympus Optical Co, Tokyo, Japan) was performed between 09.30 and 11.30 hours using lignocaine as a local anaesthetic to the nose and airways. The bronchoscope was wedged in the medial segment of the middle lobe and BAL was performed by instilling three 60 ml aliquots of sterile isotonic saline (0·85% w/v) at 37°C through the bronchoscope. Dwell time was minimised for each aliquot to less than 90 seconds and suction was also maintained at less than 200 cm H_2O.

During bronchoscopy continuous oxygen was administered at 2 l/min via nasal cannulae with ECG and oximetry monitoring. Following bronchoscopy, measurement of FEV_1 was repeated immediately and again after two hours of observation, prior to discharge. No subject was discharged before the FEV_1 had returned to 80% of baseline.

BAL CELL PREPARATION

All BAL fluid was collected into a siliconised glass vessel on ice and centrifuged at 400 *g* for 10 minutes at 4°C. The cell pellet was resuspended in phosphate buffered saline (PBS; Sigma Chemical Co, Poole, Dorset, UK) containing 5 mM D-glucose and 0·1% bovine serum albumin (BSA; Sigma). After centrifugation at 300 *g* for 10 minutes at 20°C, washing in calcium-free and magnesium-free Hank's balanced salt solution (HBSS; Gibco, Paisley, UK) containing 0·1% BSA, and further centrifugation at 300 *g* for 10 minutes at 20°C, the cells were resuspended in HBSS with BSA. A total cell count was performed using an improved Neubauer haemocytometer and viability measured by Trypan Blue exclusion. A differential cell count was performed using "Diff-Quik" stain (Baxter, Browns, Reading, Berks, UK) by one observer blinded to the subject's identity. Cells were classified as macrophage/monocyte lineage, lymphocytes, eosinophils, and neutrophils and expressed as a percentage of the total leucocytes. Epithelial cells were also enumerated and expressed as a percentage of the total cell count. Four counts of >400 cells were performed for each subject and the mean taken for each cell type.

BAL CELL INCUBATION

Washed cell aliquots of $0·5 \times 10^6$ viable cells were resuspended in 0·5 ml RPMI-1640 containing calcium and magnesium (Flow Labs, Rickmansworth, Herts, UK) supplemented with HEPES buffer (20 mM) and 0·1% BSA. L-Serine (20 mM) was also added to prevent oxidative metabolism of LTC_4. Samples were incubated in Sarstedt tubes for 10 minutes at 37°C in a shaking waterbath. Preliminary experiments demonstrated that leukotriene generation by these samples without stimulation over 60 minutes was negligible. Samples were then incubated in the presence of A23187 dissolved in dimethylsulphoxide (DMSO; Sigma), with a final concentration of DMSO of 0·1% v/v. All incubations were performed between four and six hours after BAL, with no difference in the time between BAL and incubation between asthmatic and normal subjects. The response to A23187 was assessed by incubating the samples in the presence of various concentrations of A23187 (0, 2, 4, 10, 20 μM) for 10 minutes. Time course experiments were performed by incubating samples with a submaximal dose of A23187 (4 μM) for 0, 5, 10, 15, and 30 minutes, previous studies having demonstrated maximal generation of leukotriene within 30 minutes. The incubations were ended by rapid cooling of the tubes on ice and by the addition of 3 ml

methanol to extract intracellular leukotrienes. Samples were incubated at 4°C for 16–24 hours and then centrifuged at 1200 *g* for 20 minutes at 4°C to remove precipitated material. The supernatant was decanted and stored at −20°C. LTB_4 assays were performed within 15 weeks and LTC_4 assays within 23 weeks; there were no differences in the storage times between asthmatic and normal samples.

MEASUREMENT OF LEUKOTRIENES

The amounts of LTB_4 and LTC_4 in cell supernatants were measured by radioimmunoassay. Cell supernatant samples were evaporated to dryness in vacuo (Gyrovap, Howe, Banbury, Oxfordshire, UK) and resuspended in 1 ml Tris buffer at pH 8·6 for the LTB_4 assay and pH 7·4 for the LTC_4 assay. Aliquots were incubated at 4°C in duplicate in a total volume of 600 µl with ^{3}H-LTB_4 (NEN, Dupont, Stevenage, Herts, UK) or ^{3}H-LTC_4 (Amersham, Aylesbury, Bucks, UK) respectively (2·5–4·0 nCi, 30–39 Ci/mmol), and with the appropriate antiserum. The LTB_4 antiserum (kind gift of Dr A W Ford-Hutchinson, Merck Frosst Laboratories) was highly specific with negligible crossreactivity to 6-*trans*-LTB_4, (5S, 12S)-LTB_4, 20-OH-LTB_4 and 20-COOH-LTB_4, 5-HETE, LTC_4, LTD_4, LTE_4, and arachidonic acid. The LTC_4 antiserum (in house) recognised (5S, 6R)-LTC_4 and (5S, 6S)-LTC_4 (100%), LTC_4-sulphone (68·9%) and LTD_4 (29·4%), but did not crossreact with LTB_4, 6-*trans*-LTB_4, (5S-12S)-LTB_4, 20-OH-LTB_4, 20-COOH-LTB_4, LTE_4, *N*-acetyl-LTE_4, 5-HETE, various prostanoids, arachidonic acid, or glutathione. A 50% displacement of specific binding occurred at concentrations of 150–350 pg/tube (LTB_4) and 230–320 pg/tube (LTC_4). Unbound label was removed after 18 hours with dextran-coated charcoal. Bound radiolabel was counted in Optiphase Hi Safe 3 scintillant (Pharmacia, Milton Keynes, Bucks, UK) for at least two minutes.

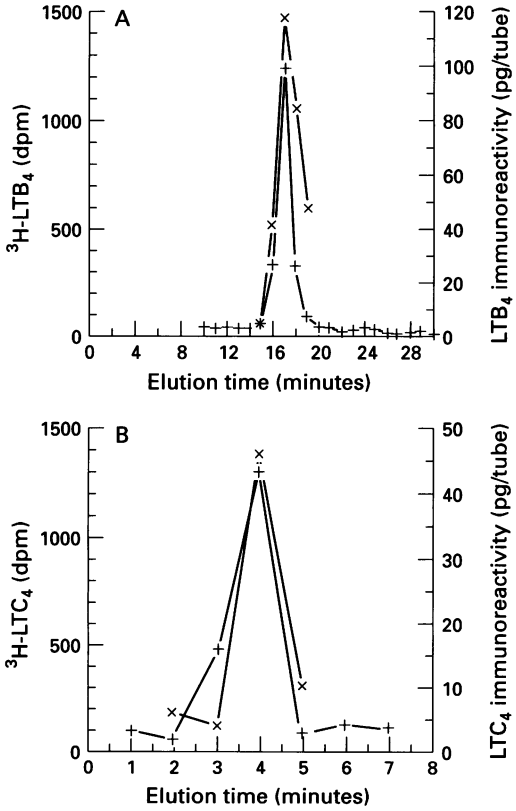

Figure 1 Co-elution of (A) endogenous LTB_4 immunoreactivity (—×—) with peaks of radioactivity of ^{3}H-LTB_4 internal standards (—+—), and (B) endogenous LTC_4 immunoreactivity (—×—) with peaks of radioactivity of ^{3}H-LTC_4 internal standards (—+—). Endogenous leukotrienes were generated by mixed bronchoalveolar lavage cells stimulated with A23187 (4 µM) for 30 minutes. The x axis indicates elution time in RP-HPLC analysis.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

The identities of both LTB_4 and LTC_4 measured by radioimmunoassay were confirmed qualitatively in representative samples (three asthmatic and three control subjects) by reversed phase high performance liquid chromatography (RP-HPLC). Dried samples were reconstituted in 0·5 ml HPLC solvent consisting of methanol/water/glacial acetic acid (70:30:0·01 or 75:25:0·01 v/v/v) adjusted to pH 5·7 with NH_4OH. Trace amounts of ^{3}H-LTB_4 and ^{3}H-LTC_4 were added as internal standards. Samples were chromatographed on a LiChrospher (Merck, Darmstadt, Germany) 5 µm ODS column with the degassed solvent at a flow rate of 1 ml/min. Fractions (1·0 ml) were collected for up to 30 minutes. Aliquots (0·9 ml) of each fraction were removed, added to Optiphase Hi Safe 3 scintillant (4 ml), and counted. The internal standard peaks were identified by comparing their retention times with a previously chromatographed ^{3}H-leukotriene standard and used to identify the fractions containing endogenous leukotrienes. The remaining aliquots (0·1 ml) of the fractions were evaporated to dryness and radioimmunoassay performed for measurement of leukotrienes. The peaks of immunoreactivity mirrored the shapes, and eluted with the same retention times, as the ^{3}H-labelled peaks, confirming the respective identities of the leukotrienes measured by radioimmunossay as LTB_4 and LTC_4. Figure 1A shows a representative result for LTB_4 and fig 1B the result for LTC_4 from one of the samples, with a retention time for LTB_4 of 17 minutes and for LTC_4 of 4 minutes.

STATISTICAL ANALYSIS

Mean (SD) FEV_1 was compared between groups by a two sample *t* test. PC_{20}, which has a log normal distribution, was described by the geometric mean and its confidence interval (CI). Median and range were used to describe all other variables including area under the curve (AUC) which was used to describe dose response and time course curves, and cell differentials.[12] Comparisons of non-parametric variables (including AUC) were performed using a Mann-Whitney U test. Spearman's correlation coefficient (r_S) was used to examine the relation between the generation of LTB_4

Table 2 Median (range) bronchoalveolar lavage (BAL) fluid return and cell counts

	Asthmatic subjects	*Control subjects*	*p value of difference*
BAL fluid return (ml) (maximum 180 ml)	124 (106–142)	133 (116–154)	NS
Total cells ($\times 10^6$)	21·5 (7·5–29·4)	17·4 (7·7–27·1)	NS
Viability (%)	91 (81–98)	96 (84–98)	NS
Macrophages (%)	73·1 (50·2–85·8)	83·6 (63·0–92·4)	NS
Lymphocytes (%)	25·0 (10·1–45·6)	15·3 (6·5–32·0)	NS
Neutrophils (%)	0·7 (0·2–1·6)	1.5 (0·4–3·9)	0·05
Eosinophils (%)	1·5 (0·3–5·2)	0·3 (0·1–1·4)	0·001
Epithelial cells (%)	2·1 (0·2–10·3)	1·1 (0·1–3·2)	NS

and LTC_4, the percentages of cell types, and FEV_1 and PC_{20}. For visual clarity in figs 2A, 2B, 4A, and 4B error bars represent mean (SE) values.

Results

BAL AND CELL COUNTS

Bronchoalveolar lavage was well tolerated by both asthmatic and normal subjects. There were no significant differences between the groups in the fall in Sao_2 (asthmatics 3 (0–8)%, controls 2 (0–7)%) or percentage fall in FEV_1 (asthmatics 13 (−7–29)%, controls 12 (−3–43)%). Table 2 shows that there were no significant differences between asthmatic and normal subjects in volume of BAL fluid returned, total cell counts, or viability. Cell viability was more than 80% in all subjects. Most of the cells in both subject groups were alveolar macrophages, with a trend ($p=0·1$) to fewer alveolar macrophages in asthmatic subjects (median 73%) than in controls (median 84%). The percentage of lymphocytes varied widely between subjects (7–46%), but there was no significant difference between asthmatic subjects and controls. There was no significant difference in the ratio of alveolar macrophages to lymphocytes in asthmatic subjects (3·0 (1·1–8·3)) and in normal subjects (5·5 (2·0–14·2)). The asthmatics had a significantly reduced percentage of neutrophils (0·7% *v* 1·5%; $p=0·05$), a highly significant increase in the percentage of eosinophils (1·5% *v* 0·3%; $p=0·001$), and a trend towards increased epithelial cells (2·1% *v* 1·1%) compared with control subjects. There were no significant correlations between the percentage of each cell type and either PC_{20} or FEV_1.

GENERATION OF LEUKOTRIENES

Figure 2A shows that LTB_4 was generated in a dose dependent manner in response to concentrations of A23187 from 0 μM to 20 μM by mixed BAL cells from asthmatic and control subjects. There was no difference in the basal level of LTB_4 generated by the asthmatic subjects (0·6 (0·0–4·3) ng/10^6 cells) and the control subjects (0·9 (0·0–2·5) ng/10^6 cells). However, mixed BAL cells from asthmatic subjects generated significantly less LTB_4 than mixed BAL cells from control subjects in response to increasing concentrations of A23187, with AUC for asthmatic subjects of 58·5 (0·0–153·2) ng · μM · 10^{-6} cells compared with 198·5 (2·9–955·1) ng · μM · 10^{-6} cells for control subjects ($p=0·01$). Figure 2B shows the amount of LTB_4 generated over 30 minutes in response

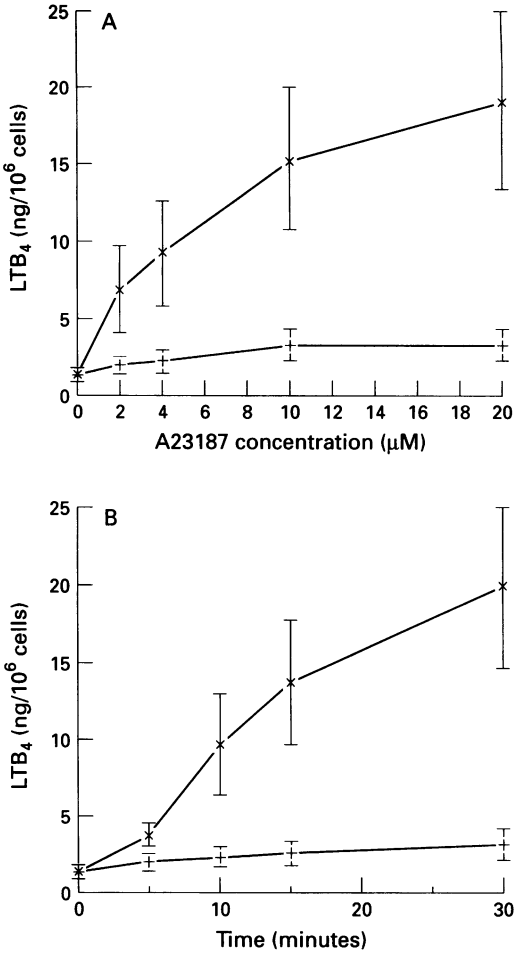

Figure 2 Mean (SE) LTB_4 generation by mixed bronchoalveolar lavage cells from asthmatic (—+—, n=12) and control subjects (—×—, n=11 in A, n=12 in B) (A) after incubation for 10 minutes with A23187 (0–20 μM) and (B) over 30 minutes stimulated by 4 μM A23187.

to 4 μM A23187 (a submaximal dose) by mixed BAL cells from asthmatic and control subjects. Again the mixed BAL cells from asthmatic subjects generated significantly less LTB_4 than mixed BAL cells from control subjects, with an AUC of 81·5 (0·0–228·5) ng · min · 10^{-6} cells for asthmatic subjects and of 197·9 (13·9–935·6) ng · min · 10^{-6} cells for controls ($p=0·01$). Peak levels of LTB_4, which were attained between 15 and 30 minutes, were 3·9 (0·0–9·5) ng/10^6 cells in the asthmatic group compared with 11·3 (0·8–48·8) ng/10^6 cells in the control group ($p=0·01$). When LTB_4 generation (AUC) was corrected for the proportion of alveolar macrophages in each BAL cell preparation and expressed per 10^6 macrophages, the differences between the groups remained significant ($p=0·02$) in both the dose response and time course experiments.

There was no consistent evidence of an association between the percentage of alveolar macrophages and amount of LTB_4 generated. While the Spearman's rank correlation coefficient (r_S) for the relation between the percentage of alveolar macrophages and amount of LTB_4 generated in the time course experiments (AUCs) by control subjects was significant ($r_S=-0·76$; $p<0·01$), it was not significant for asthmatic subjects ($r_S=-0·16$). Furthermore,

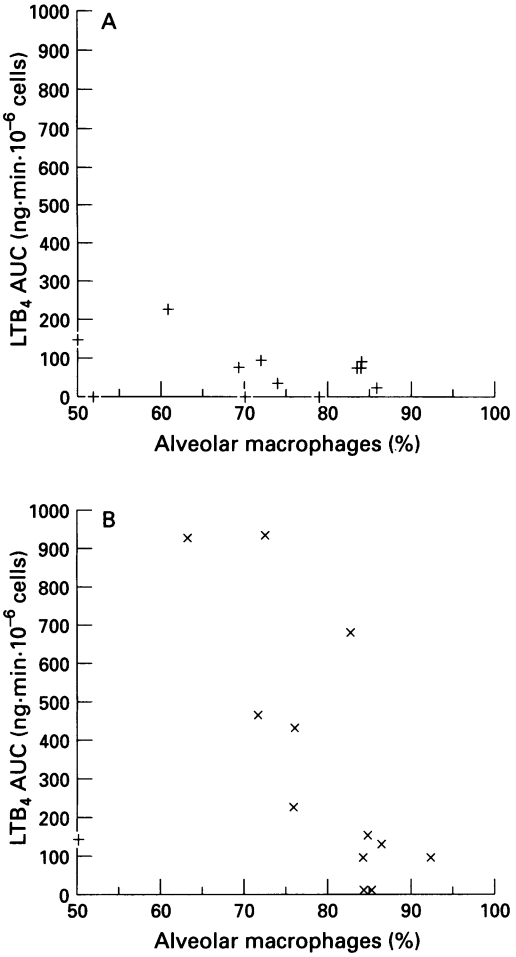

Figure 3 Relation between contribution of alveolar macrophages to the cell count (%) and LTB_4 generation by mixed BAL cells from (A) asthmatic (+, n=12) and (B) control subjects (×, n=12) over 30 minutes stimulated by 4 μM A23187. AUC=area under the curve; ng · min · 10^{-6} cells).

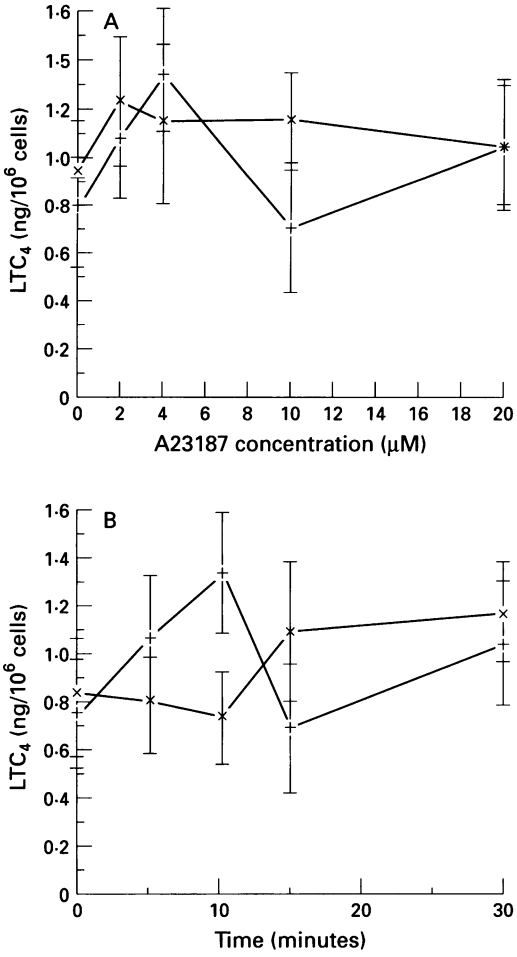

Figure 4 Mean (SE) LTC_4 generation by mixed bronchoalveolar lavage cells from asthmatic (—+—, n=12) and control subjects (—×—, n=11 in A, n=12 in B) (A) after incubation for 10 minutes with A23187 (0–20 μM) and (B) over 30 minutes stimulated by 4 μM A23187.

in the dose response experiments the correlation between the percentage of alveolar macrophages and amount of LTB_4 generated (AUCs) was not significant in either group ($r=-0{\cdot}56$ for control subjects; $r=-0{\cdot}10$ for asthmatic subjects). The relation between the percentage of alveolar macrophages and generation of LTB_4 over 30 minutes in response to 4 μM A23187 (AUCs) is shown in fig 3A and B for asthmatic and control subjects. There were also no consistent significant correlations between the percentage of lymphocytes, neutrophils, or eosinophils and LTB_4 generation.

Figure 4 shows the generation of LTC_4 in the same dose response and time course experiments. Basal generation of LTC_4 by mixed BAL cells from asthmatic subjects was 0·7 (0·0–2·4) ng/10^6 cells and by cells from control subjects was 0·9 (0·0–2·5) ng/10^6 cells, with peak amounts from asthmatic subjects of 1·3 (0·0–3·0) ng/10^6 cells and from control subjects of 1·3 (0·6–3·3) ng/10^6 cells. There were no significant differences in the generation of LTC_4, either basally or in response to A23187, between mixed BAL cells from asthmatic subjects and control subjects, in either dose response or time course experiments. There were no significant correlations between LTC_4 generation and the percentage of eosinophils in mixed BAL cells from asthmatic or control subjects, and there was no consistent relation between the capacity of mixed BAL cells to generate LTB_4 and their capacity to generate LTC_4.

Discussion

In this study mixed BAL cells from mild atopic asthmatic subjects showed a reduced capacity to generate LTB_4 in response to ionophore compared with those from normal subjects. LTC_4 was generated in much smaller quantities by BAL cells, with no dose response to ionophore and no difference between asthmatic and normal subjects. There was no consistent relation between the capacity of BAL cells to generate LTB_4 and LTC_4.

Alveolar macrophages are probably the major source of LTB_4 in this study. Not only are most of the BAL cells alveolar macrophages, but normal alveolar macrophages generate similar quantities of LTB_4 to those generated by mixed BAL cells from our normal subjects.[13] Lymphocytes, which have a negligible capacity for leukotriene synthesis,[2] accounted for most of the remaining cells. Neutrophils, like macrophages, preferentially generate LTB_4, but as they were present in small numbers and have

a lower capacity to generate LTB_4,[9] are unlikely to be a significant source. The likely sources of LTC_4 include eosinophils, which were few in number even in asthmatics (maximum 5%), and alveolar macrophages which generate much less LTC_4.[9] This, and metabolism of LTC_4 to LTD_4 and LTE_4, may explain the small quantities measured and the lack of dose response to ionophore.

Macrophages are implicated in the pathogenesis of asthma. They have low affinity IgE receptors ($Fc_\varepsilon RII$),[14] are involved in antigen presentation, and release a wide range of relevant mediators.[9 14] The number of mononuclear cells is increased in bronchial biopsies from asthmatic subjects[15] compared with normal subjects, and in BAL fluid after allergen challenge.[16] Our results are not explained by differences in the number of alveolar macrophages; LTB_4 generation remained significantly less in asthmatics when expressed per million macrophages, and was not positively correlated with the percentage of alveolar macrophages.

Reduced generation of LTB_4 in the asthmatic subjects in this study might, however, be explained by altered composition, functioning, or both, of the macrophage population. A macrophage population containing a larger proportion of monocytes as a result of increased migration of monocytes into the asthmatic lung may produce less LTB_4, as monocytes have a lower capacity for ionophore-stimulated LTB_4 production than alveolar macrophages.[17 18] There is some evidence in support of this hypothesis. The proportion of mononuclear cells with a monocytic phenotype is increased in bronchial biopsies from asthmatic subjects[15] and in BAL fluid after allergen challenge.[16] There is also some evidence that alveolar macrophage function is altered in asthma, including an accessory cell role in cell-mediated immune responses,[19] impaired viability and zymosan phagocytosis,[20] and increased respiratory burst.[21] These differences may reflect macrophage activation, as may the increased number of hypodense alveolar macrophages[22] in asthma. Furthermore, there is some evidence that activated macrophages generate less LTB_4; alveolar macrophages from smokers generate less LTB_4,[23] as do rat alveolar macrophages activated in vivo[24] and in a model of silicosis.[25] It is therefore possible that reduced generation of LTB_4 by mixed BAL cells from asthmatic subjects is a result of altered alveolar macrophage function. This may be a consequence of exposure to other inflammatory mediators – for example, LTD_4 inhalation in normal subjects reduces ionophore-stimulated LTB_4 generation by alveolar macrophages.[26]

Our results differ from those of two studies of ionophore-stimulated LTB_4 generation by alveolar macrophages – one showing no difference[27] and one an increase[28] in asthmatic compared with normal subjects. In these studies alveolar macrophages were purified by adherence to plastic[27 28] which may account for the differences; adherence activates cells[29] and end stage macrophages adhere less readily than those closer to a monocytic phenotype. Hence, adherence may select subpopulations of macrophages which differ in number between asthmatic and normal subjects.

Mixed lavage cells were used in our study to reflect more closely the cellular milieu within the airways, especially as other cells – in particular lymphocytes – affect alveolar macrophage function.[30] In one study alveolar macrophage accessory function in asthma was associated with the percentage of lymphocytes in BAL fluid,[31] and alterations in viability and activation of alveolar macrophages have been correlated with the percentage of eosinophils.[20 21] We found no consistent relation between the percentage of lymphocytes or eosinophils in BAL fluid and the capacity of mixed BAL cells to generate LTB_4. Furthermore, while there was large variation in the number of lymphocytes between individuals in our study, there was no significant difference in the percentage of lymphocytes or the ratio of alveolar macrophages to lymphocytes between the groups. This suggests that differences in lymphocyte number do not explain the results. Differences in lymphocyte function were not investigated in our study. The relation between lymphocyte function and alveolar macrophage generation of LTB_4 is complex as LTB_4 itself stimulates the function and proliferation of T suppressor cells[2 32 33] which may be involved in suppression of the immune response to allergen in normal individuals. Thus, LTB_4 may even have a protective role in asthma, contrary to current thought but in keeping with our results.

In this study we have shown reduced generation of LTB_4 by mixed BAL cells from mild atopic asthmatics in response to ionophore stimulation which is not related to altered LTC_4 generation. The dramatic reduction in LTB_4 generation seen in such mild asthmatic subjects suggests a fundamental alteration in BAL cell function. This probably reflects reduced generation of LTB_4 by the alveolar macrophage which is the main cell type present in BAL fluid and has a considerable capacity to generate LTB_4. This difference may be a consequence of monocyte migration into the lung or alteration in the resident alveolar macrophage phenotype in asthma, or both. This study provides further evidence for an alteration of alveolar macrophage function in asthma and raises questions about the role of LTB_4 in its pathogenesis.

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