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Enhanced leukotriene synthesis in leukocytes of atopic and asthmatic subjects

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1 We have investigated the capacities of peripheral leukocytes from atopic asthmatic (AA) ($n = 7$), atopic non-asthmatic (AN) ($n = 7$), and normal (N) ($n = 7$) subjects to generate the bronchoconstrictor and proinflammatory mediators leukotrienes (LTs) B_4 and C_4.

2 Mixed leukocyte preparations containing 61–84% neutrophils, 2.4–15% eosinophils, and 13-29% mononuclear cells were incubated *in vitro* at 37°C in the presence of calcium ionophore A23187. Synthesis of LTB_4 and LTC_4 was quantitated by radioimmunoassay.

3 Both in dose-response experiments (0–10 μM A23187 for 5 min), and in time-course investigations (2 μM A23187 for 0–30 min), the mixed leukocytes of the AA and AN subjects generated on average 4- to 5-fold more LTB_4 and 3- to 5-fold more LTC_4 than the normal leukocytes ($P < 0.01$ in all cases; ANOVA).

4 This enhanced LT synthesis by the AN and AA leukocytes was not due to differences in the counts of leukocyte sub-types, or to altered rates of LT catabolism between the subject groups.

5 LTB_4 synthesis correlated significantly with LTC_4 synthesis in the leukocytes of the AN and AA subjects ($r = 0.81$, $n = 14$, $P < 0.01$), but not in those of the normal subjects ($r = 0.19$, $n = 7$, $P > 0.05$).

6 Our results demonstrate an up-regulation of the leukotriene synthetic pathway in the circulating leukocytes of atopic non-asthmatic and atopic asthmatic subjects, which may have important implications in the pathophysiology of asthma and allergy.

Keywords leukotrienes atopic asthma leukocytes GM-CSF

Introduction

Leukotrienes (LTs) are a family of lipid mediators derived by 5-lipoxygenation of arachidonic acid, and are generated principally by inflammatory leukocytes. Their potent actions, including polymorphonuclear (PMN) leukocyte chemotaxis and degranulation (Czarnetzki & Rosenbach, 1986; Martin *et al.*, 1989), mucus hypersecretion (Coles *et al.*, 1983), bronchial smooth muscle contraction (Barnes *et al.*, 1984), and increased microvascular permeability (Woodward *et al.*, 1983), have stimulated interest in their possible role as mediators in human bronchial asthma.

Leukotrienes B_4 and C_4 have been detected in the bronchoalveolar lavage fluid of asthmatic subjects (Lam *et al.*, 1988), and urinary excretion of LTE_4 increases markedly following allergen challenge and during acute exacerbations of asthma (Taylor *et al.*, 1989). Inhalation of LTE_4 by asthmatic subjects results in increased bronchial responsiveness to histamine for up to 1 week (Arm *et al.*, 1988). Recently, LT synthesis inhibitors and receptor antagonists have been shown to reduce bronchoconstriction induced by allergen, cold dry air, and exercise, and to inhibit allergen-induced bronchial hyperresponsiveness (Israel *et al.*, 1990; Manning *et al.*, 1990; Taylor *et al.*, 1991).

Few studies have compared the LT synthetic capacity of PMNs from asthmatic patients with that of normal PMNs. Mita *et al.* (1985) described increased 5-lipoxygenase activity in PMN homogenates of extrinsic and

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intrinsic asthmatics, but measured conversion of radiolabelled arachidonate into both LTB_4 and its biologically-inactive isomer (5S,12S)-diHETE. Tomioka *et al.* (1985) used radioimmunoassay to document a 16-fold increased release of LTC_4 from isolated asthmatic PMNs stimulated with calcium ionophore A23187, but the cellular composition of the mixed PMN preparations was not reported. The use of a crude bioassay to quantitate cysteinyl-LTs in the study of Wang *et al.* (1986) reduces confidence in their finding that A23187-stimulated LT synthesis by the PMNs and monocytes of asthmatic subjects correlates with the degree of atopy of the donor. Dramatic (22-fold) increases in the LTB_4 synthetic capacity of circulating PMNs in response to zymosan and A23187 have been reported after exercise challenge in susceptible subjects (Arm *et al.*, 1987), and much more modest enhancements (1.6-fold) in those of mild untreated stable asthmatics (Radeau *et al.*, 1990).

We have previously described a 2- to 3-fold enhancement of LTB_4 and LTC_4 release in a group of moderately-severe, symptomatic atopic asthmatics, using h.p.l.c. and radioimmunoassay analysis in an A23187-stimulated whole blood model (Sampson *et al.*, 1990); this enhancement was not due to differences in the counts of leukocyte sub-types, nor to differences in LT catabolic rates. We now report studies of the synthesis of LTs in A23187-stimulated mixed leukocytes isolated from the peripheral blood of atopic asthmatic, atopic non-asthmatic, and normal healthy controls.

Methods

Subjects

Subjects were recruited into atopic asthmatic (AA), atopic non-asthmatic (AN), and normal (N) groups. All were volunteers who gave informed consent to donate blood. Ethical permission was obtained from the Ethics Committees of King's College Hospital and the Hunterian Institute, Royal College of Surgeons.

AA subjects (4M/3F; mean age 30.7 years, range 17–49) had a clinical history of asthma and allergy, with airflow obstruction reversible by at least 15% with inhaled β_2-adrenoceptor agonists, but were stable at the time of blood-taking. All were atopic as judged by positive skin-prick tests (wheal diameter >3 mm after 10 min) to at least two common aeroallergens. None was receiving oral corticosteroids, methylxanthines, sodium cromoglycate, or non-steroidal anti-inflammatory drugs, but all were using inhaled salbutamol as required, and four out of seven were controlled with low-dose inhaled beclomethasone. AN subjects (4M/3F; mean age 34.9 years, range 22–57) had no history of chronic respiratory disease and had normal lung function, but were judged atopic on the basis of positive skin-prick tests to at least two common allergens and a family history of allergic disease. None was receiving medication at the time of venesection. Normal controls (2M/5F; mean age 31.6 years, range 19–51) were healthy subjects with no history of respiratory disease, normal lung function, and no medication at the time of blood-taking. All had negative skin-prick tests to a battery of ten common allergens.

Leukocyte isolation

Blood was taken from a forearm vein into a syringe containing preservative-free sodium heparin (10 iu ml^{-1}), and added to one-fifth volume of 5% dextran (grade B, MW 150–200 kD) in calcium- and magnesium-free phosphate-buffered saline (PBS). Erythrocytes were allowed to sediment for 45–60 min at room temperature before removal of the leukocyte-rich supernatant by Pasteur pipette. Supernatants were centrifuged (500 *g*) in an equal volume of PBS for 10 min, and cell pellets were resuspended in ammonium chloride solution (0.87% w/v) for 10 min to lyse remaining erythrocytes. Leukocytes were washed a further three times in PBS containing D-glucose (5 mM) and bovine serum albumin (BSA; 0.1% w/v), including two low-speed centrifugations (150 *g*) to deplete remaining platelets. Leukocyte viability was estimated by counting the proportion of cells excluding Trypan Blue (0.1%) in a total count of at least 200 cells. Total cell counts were performed with an improved Neubauer haemocytometer. Differential cell counts were performed by morphology on smears after fixation in 100% methanol and Giemsa staining.

Leukocyte incubations

Mixed leukocytes were resuspended at 5×10^6 cells ml^{-1} in tissue culture medium RPMI 1640 containing HEPES buffer (25 mM) and BSA (0.1% w/v). L-serine (20 mM) was included to eliminate oxidative metabolism of cysteinyl-LTs (Owen *et al.*, 1987). Aliquots (1 ml) were incubated in sterile polypropylene tubes in a shaking water-bath at 37°C for 15 min, before the addition of A23187 (0–10 μM) in dimethylsulphoxide (DMSO; final concentration <0.2% v/v). Control incubations were performed with the DMSO vehicle alone. Incubations were terminated after 0–30 min by immersion of the tubes in ice and the addition of 3 vol of ice-cold methanol. Cells were pelleted by centrifugation at 1500 *g* for 20 min; the methanolic supernatants (containing LTs released by cells) and extracts of cell pellets treated with 100% methanol for 18 h at 4°C (containing LTs retained intracellularly) were evaporated to dryness under reduced pressure (UniVap), and stored at −70°C before radioimmunoassay.

In parallel experiments to investigate the catabolism of LTs, $[^3H]$-LTB_4 (10 nCi; 32 Ci $mmol^{-1}$) or $[^3H]$-LTC_4 (10 nCi; 39 Ci $mmol^{-1}$) were added to cell suspensions immediately prior to stimulation with A23187.

Leukotriene quantitation

Leukotrienes B_4 and C_4 were quantitated by radioimmunoassay. Deproteinised samples were reconstituted in Tris-HCl buffer (pH 7.4) containing gelatine (0.1% w/v), and aliquots (100 μl) were incubated in duplicate for 18 h at 4°C with the radiolabelled LT (2.5–4 nCi) and the appropriately diluted anti-LT serum in a total volume of 400 μl. Unbound radiolabel was removed by centrifugation after the addition of dextran-coated

charcoal, and supernatants were counted for at least 2 min each in 5 ml Optiphase HiSafe 3 scintillant. Unknown LT was quantitated by interpolation on the standard curve (8-4096 pg/tube).

The LTB_4 antiserum showed negligible cross-reactivity for (5S,12S)-LTB_4, 6-trans-LTB_4, 20-hydroxy-LTB_4, 20-carboxy-LTB_4, 5-HETE, cysteinyl-LTs, and arachidonate (Rokach *et al.*, 1984). Fifty percent binding of radiolabel occurred at an LTB_4 concentration of 140 pg/tube. The LTC_4 antiserum raised in this laboratory (Zakrzewski, Thomas & Piper, unpublished data) recognised (5S,6R)-LTC_4 and (5R,6R)-LTC_4 (100%), LTC_4-sulphone (69%), and LTD_4 (29.4%), but did not cross-react with LTE_4, *N*-acetyl-LTE_4, LTB_4, 6-trans-LTB_4, (5S,12S)-LTB_4, 20-hydroxy-LTB_4, 20-carboxy-LTB_4, 5-HETE, various prostanoids, arachidonate, or glutathione (<0.05%). Fifty percent binding of radiolabel occurred with 150 pg/tube LTC_4.

Leukotriene metabolism experiments

In parallel experiments, reverse-phase high performance liquid chromatography (h.p.l.c.) was used to characterise the catabolism of LTB_4 and LTC_4 in stimulated cell preparations spiked with the tritium-labelled LT; these samples were reconstituted in the h.p.l.c. solvent system (methanol/water/acetic acid, 75/25/0.01 v/v/v, pH 5.6), and pumped isocratically at 1 ml min^{-1} through a C18 Techsphere column (250 × 4.6 mm) using a Waters 6000A pump. Ultraviolet absorbance was monitored at 280 nm, and radioactivity emerging in thirty 1 ml fractions was detected by scintillation counting. Typical retention times were: LTC_4 (5.5 min), LTD_4 (21.0 min), LTE_4 (16.5 min), LTB_4 (13.0 min), and 20-carboxy-/20-hydroxy-LTB_4 (3.5–4.0 min).

H.p.l.c. validation of radioimmunoassays

Twenty-five representative leukocyte supernatants (at least one from each subject) were selected arbitrarily for analysis by h.p.l.c. to confirm the identity of LTs generated by A23187 stimulation. Samples were chromatographed as described above, with the addition of trace amounts of [^{3}H]-LTB_4 and [^{3}H]-LTC_4 (3 nCi). Aliquots of fractions corresponding to peaks of u.v. absorbance and radiolabel with the same retention times as synthetic standards were subjected to radioimmunoassay for LTB_4 and LTC_4. After correcting for losses during chromatography (10–15%), LTB_4 and LTC_4 immunoreactivity eluting in these peaks was not significantly different to that detected by direct r.i.a. of the same cell supernatants without a prior h.p.l.c. purification step.

Statistical analysis

In preliminary experiments, up to 40% and 10% of synthesised LTs were retained intracellularly at the 5 min and 30 min time-points respectively, in agreement with other reports where A23187 was used as the stimulus (Owen *et al.*, 1987; Williams *et al.*, 1985). Intracellular and extracellular LT concentrations were therefore added to give total LT levels. As neutrophils are the predominant source of leukotriene B_4 in mixed leukocyte preparations (Shaw *et al.*, 1984), total LTB_4 levels were expressed as nanograms of LTB_4 per million neutrophils. Similarly, LTC_4 concentrations were corrected for the number of eosinophils within individual leukocyte preparations, as the numbers and/or LTC_4 synthetic capacity of neutrophils, monocytes (Shaw *et al.*, 1984) and basophils are limited. All results are given as mean ± s.e. mean for $n = 7$ unless otherwise indicated. Comparisons between groups were made by Mann-Whitney U-test (M-W) on the areas under the curves (AUC), and by multiple analysis of variance (ANOVA), using the Minitab statistical software package (Minitab Inc., State College, PA, USA).

Materials

The following were used: LTB_4 antiserum (gift of Dr A. W. Ford-Hutchinson, Merck Frosst), synthetic LTB_4 and LTC_4 (Cascade Biochem. Ltd.), [^{3}H]-LTB_4, [^{3}H]-LTC_4 (New England Nuclear), PBS, A23187, DMSO, activated charcoal, heparin, Trypan Blue (Sigma Chem. Co.), Dextran grade B (BDH), RPMI 1640 (Flow Labs.), methanol (FSA Labs.), Optiphase HiSafe 3 (Pharmacia), Giemsa stain (R. A. Lamb) and Techsphere ODS columns (HPLC Technology Ltd).

Results

Leukocyte preparations

The mean compositions of the mixed leukocyte preparations obtained by dextran sedimentation, ammonium chloride lysis, and three-fold washes in Ca/Mg-free PBS are shown in Table 1 for the asthmatic, atopic, and normal subjects. The proportions of neutrophils and mononuclear cells were similar for the three groups; mean eosinophil count was normal (<5%) in the N subjects, but was elevated in the AN and AA groups, ranging up to 9.1% and 14.9% respectively. However, this did not reach statistical significance due to considerable overlap between the groups. The variability of leukocyte preparations (by Trypan Blue exclusion method) was always greater than 95% immediately prior to incubations, and remained above 90% for up to 1 h after stimulation with A23187 (2 μM).

Table 1 Differential leukocyte counts of atopic asthmatic (AA), atopic non-asthmatic (AN), and normal (N) subjects

	AA (n = 7)	*AN* (n = 7)	*N* (n = 7)
Neutrophils	73.9 (60.9–83.8)	76.3 (70.0–81.7)	74.9 (63.9–81.2)
Eosinophils	6.01 (2.8–14.9)	5.28 (2.8–9.1)	4.56 (2.4–6.1)
Mononuclear cells	20.1 (13.2–28.0)	18.5 (14.4–24.5)	20.5 (14.9–29.4)

Figures represent counts of each cell-type expressed as mean percentage of total isolated leukocytes. Range in parentheses.

Dose-response to ionophore

Both LTB_4 and LTC_4 were generated dose-dependently in response to stimulation with A23187 (0–10 μM) for 5 min. Maximal levels of LTB_4 (ng/10^6 neutrophils) attained with 5 μM A23187 were 40.9 ± 20.9 (AN), 32.8 ± 10.0 (AA), and 9.8 ± 2.0 (N) (Figure 1). Production of LTB_4 was significantly higher (by ANOVA) in the AN group ($P < 0.01$) and in the AA group ($P < 0.01$) compared with the N subjects. There was no significant difference in LTB_4 synthesis between the AN and AA groups ($P > 0.05$).

Leukotriene C_4 production was maximal at 10 μM A23187, with levels (ng/10^6 eosinophils) of 227 ± 31.0 (AN), 151 ± 35.6 (AA), and 78.0 ± 23.2 (N) (Figure 2). Multiple ANOVA demonstrated significantly higher LTC_4 levels in the AN group ($P < 0.01$) and the AA group ($P < 0.01$) compared with the N subjects. There was a tendency for greater LTC_4 synthesis in the AN subjects compared with the AA group, but this did not reach statistical significance ($0.05 < P < 0.10$).

Time-course investigations

Mixed leukocytes from the three subject groups were incubated with a sub-maximal dose of A23187 (2 μM) for periods up to 30 min. In all groups, mean LTB_4 levels rose rapidly to a peak at 5 min, followed by a gradual fall in levels to 30 min (Figure 3). There was clear evidence of enhanced LTB_4 synthesis in the AN and AA groups, with maximal levels (ng/10^6 neutrophils) of 31.6 ± 12.1 (AN), 28.5 ± 9.8 (AA), and 6.38 ± 1.37 (N). Using the AUC as a measure of production, mean LTB_4 synthesis was 4.9-fold greater than normal in the AN subjects ($P < 0.05$, M-W), and 4.5-fold greater than

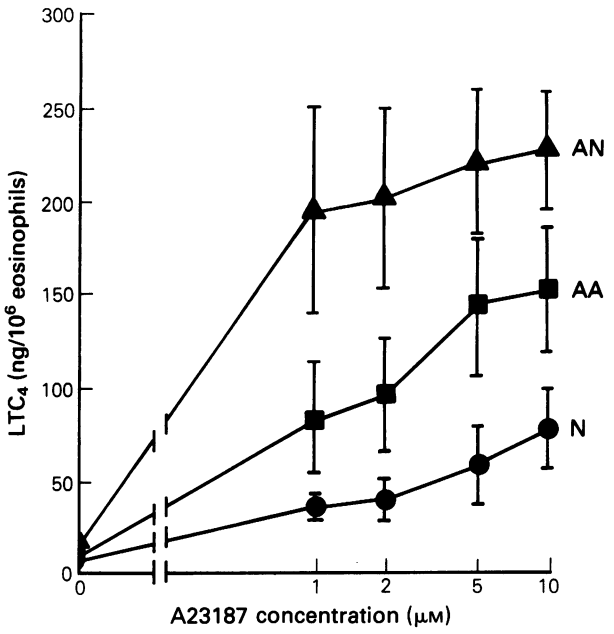

Figure 2 Synthesis of LTC_4 (ng/10^6 eosinophils) induced by A23187 (0–10 μM; 5 min) in mixed leukocytes (5 × 10^6/ml) of atopic asthmatic (■), atopic non-asthmatic (▲), and normal (●) subjects. Mean ± s.e. mean (n = 7).

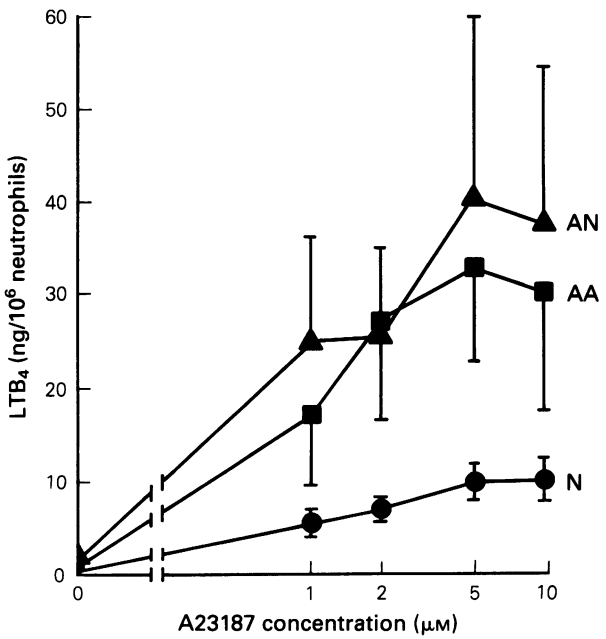

Figure 1 Concentrations of LTB_4 (ng/10^6 neutrophils) generated by A23187 stimulation (0–10 μM) for 5 min of mixed leukocytes (5 × 10^6/ml) from atopic asthmatic (■), atopic non-asthmatic (▲), and normal (●) subjects. Mean ± s.e. mean (n = 7).

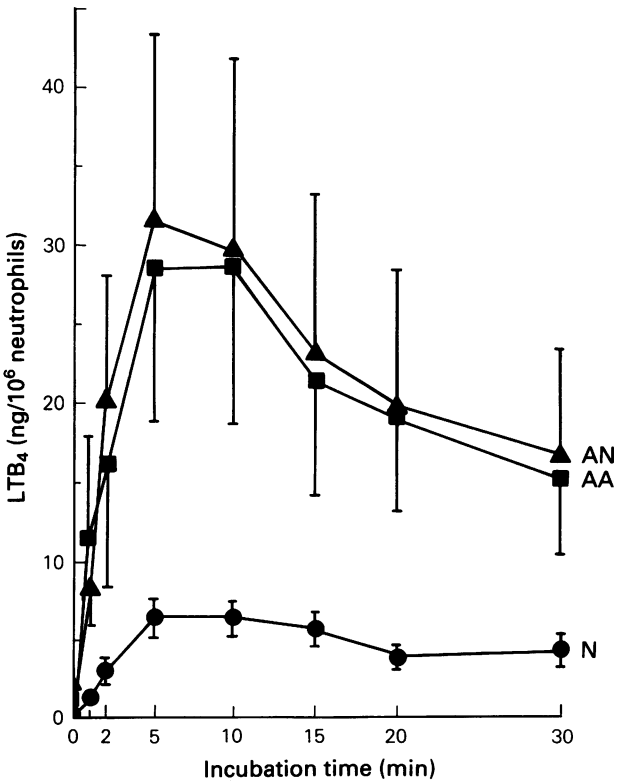

Figure 3 Time-course of synthesis of LTB_4 (ng/10^6 neutrophils) by A23187 (2 μM) stimulation for 0–30 min of mixed leukocytes (5 × 10^6/ml) from atopic asthmatic (■), atopic non-asthmatic (▲), and normal (●) subjects. Mean ± s.e. mean (n = 7).

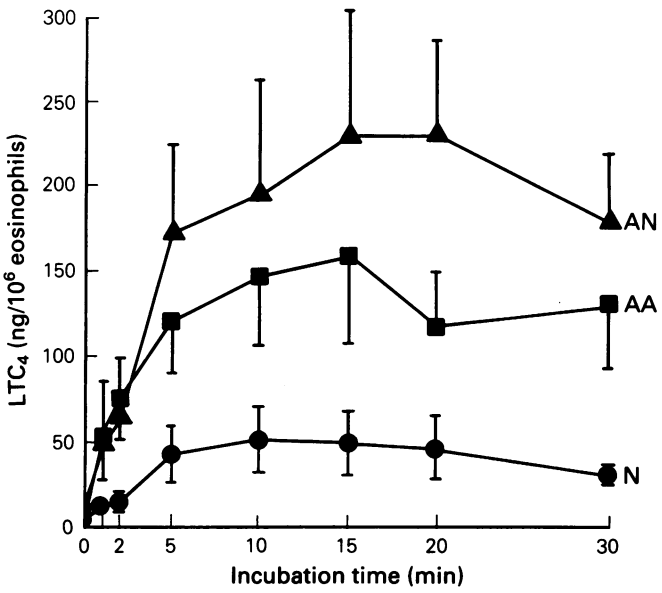

Figure 4 Time-course of synthesis of LTC_4 (ng/10^6 eosinophils) by A23187 (2 μM) stimulation for 0–30 min of mixed leukocytes (5 × 10^6/ml) from atopic asthmatic (■), atopic non-asthmatic (▲), and normal (●) subjects. Mean ± s.e. mean (n = 7).

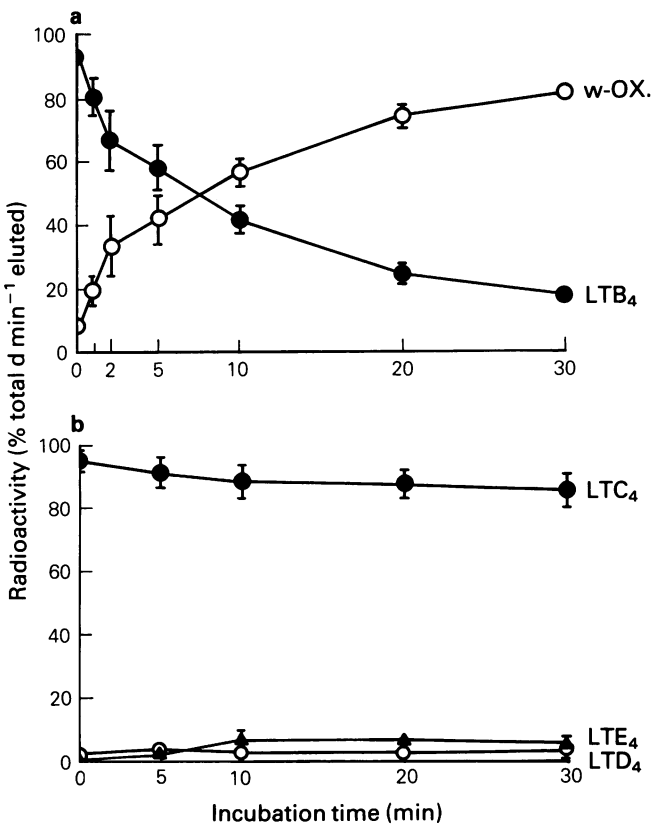

Figure 5 H.p.l.c. analysis of metabolism of a) [^{3}H]-LTB_4 and b) [^{3}H]-LTC_4 (10 nCi) incubated for 0–30 min with A23187-stimulated mixed leukocytes (5 × 10^6/ml) from normal donors. y-axis represents radioactivity associated with each peak expressed as a percentage of total d min^{-1} eluted. w-OX = omega-oxidation products (sum of 20-carboxy-LTB_4 and 20-hydroxy-LTB_4). Mean ± s.e. mean (n = 7). Similar results were obtained with leukocytes of atopic asthmatic and atopic non-asthmatic subjects (data not shown).

normal in the AA group ($P < 0.02$, M-W). These enhancements were confirmed by multiple ANOVA (both $P < 0.01$ compared with N group). There was no significant difference in LTB_4 synthesis between the AN and AA groups ($P > 0.10$, M-W).

The pattern of LTC_4 production in response to A23187 (2 μM) was different to that of LTB_4, with a more gradual rise to a peak at 15 min, followed by only small falls in levels over the remainder of the 30 min incubation period (Figure 4). Maximal levels (ng/10^6 eosinophils) were 230 ± 75.4 (AN), 161 ± 54.4 (AA), and 49.0 ± 19.2 (N). The AUCs were 4.5-fold greater than normal in the AN subjects ($P < 0.02$, M-W), and 3.0-fold greater than normal in the AA group ($P < 0.05$, M-W); these elevations were confirmed by ANOVA (both $P < 0.01$ compared with normals). There was again a tendency for greater LTC_4 synthesis in the AN group than in the AA subjects, but this did not reach statistical significance ($P > 0.10$, M-W).

Control incubations were performed in the absence of A23187 but in the presence of the DMSO vehicle (0.1% w/v) for periods up to 30 min. DMSO-induced generation of LTB_4 and LTC_4 was negligible, amounting to less than 10% of that induced by A23187 (2 μM) at all time-points.

Leukotriene catabolism experiments

The levels of LTs detected in leukocyte incubations represent the net balance of synthesis and degradation. To investigate the possibility of an apparent enhancement of LT synthesis arising due to differences in catabolic rates between the subject groups, parallel incubations were performed as above but with the addition of [^{3}H]-LTB_4 or [^{3}H]-LTC_4 (10 nCi) immediately prior to A23187 stimulation. Radiolabelled metabolites were separated by h.p.l.c. and identified by co-elution with synthetic standards.

Tritium-labelled LTB_4 was converted rapidly by leukocytes of all subject groups to two polar compounds which co-eluted with 20-carboxy-LTB_4 and 20-hydroxy-LTB_4, suggesting metabolism by the omega-oxidation pathway. The initial half-lives were approximately 4.5–7.0 min (Figure 5a). At the 5 min time-point, the proportion of total dpm associated with unmetabolised [^{3}H]-LTB_4 was 58.3 ± 8.4%, 68.4 ± 8.6%, and 63.3 ± 7.2% in the normal, atopic, and asthmatic groups respectively (n = 7).

Tritium-labelled LTC_4 was converted slowly in the leukocyte preparations to two less polar compounds which co-eluted with [^{3}H]-LTD_4 and [^{3}H]-LTE_4, suggesting metabolism along the glutathione detoxification pathway (Figure 5b). After 5 min incubation, the proportion of total d min^{-1} associated with unmetabolised [^{3}H]-LTC_4 was 85.2 ± 6.4%, 87.0 ± 4.2%, and 91.3 ± 5.9% in the normal, atopic and asthmatic groups respectively. This stability of [^{3}H]-LTC_4 is a functional indication of the effectiveness of the leukocyte isolation procedures in removing platelets and plasma, both of which have significant capacity to metabolise LTC_4 (Edenius *et al.*, 1988; Zakrzewski *et al.*, 1989).

There were no significant differences between the subject groups in rates of metabolism of exogenous

[^{3}H]-LTB$_4$ or [^{3}H]-LTC$_4$, as assessed by Mann-Whitney U-test of the AUC and by multiple ANOVA.

Correlation of LTB$_4$ and LTC$_4$ synthesis

Using the areas under individual time-course curves, the relationship between the capacity for synthesis of LTB$_4$ and that of LTC$_4$ was examined in the stimulated leukocyte preparations of the three subject groups. Taking the 21 subjects as a whole, there was a highly significant correlation between LTB$_4$ and LTC$_4$ synthesis ($r = 0.837$, $P < 0.001$, $n = 21$) (Figure 6), which was largely due to strong relationships within the AN group ($r = 0.821$, $P < 0.05$, $n = 7$) and within the AA group ($r = 0.906$, $P < 0.01$, $n = 7$). Within the N group, the synthesis of LTB$_4$ and that of LTC$_4$ were not related ($r = 0.195$, $P > 0.5$, $n = 7$).

The absolute quantity of LTC$_4$ generated by 10^6 mixed leukocytes correlated with the percentage of eosinophils within the preparations ($r = 0.703$, $P < 0.001$, $n = 21$), suggesting that the eosinophil is a principal cellular source of LTC$_4$ (Shaw *et al.*, 1984). Moreover, when the LTC$_4$ levels were corrected for individual eosinophil counts, there was a weak correlation between LTC$_4$ synthesis (expressed as ng/10^6 eosinophils) and eosinophil counts, which was close to statistical significance ($r = 0.426$, $P = 0.054$, $n = 21$); this may indicate a possible relationship between the capacity of individual eosinophils to generate LTC$_4$ and the degree of eosinophilia of the donor.

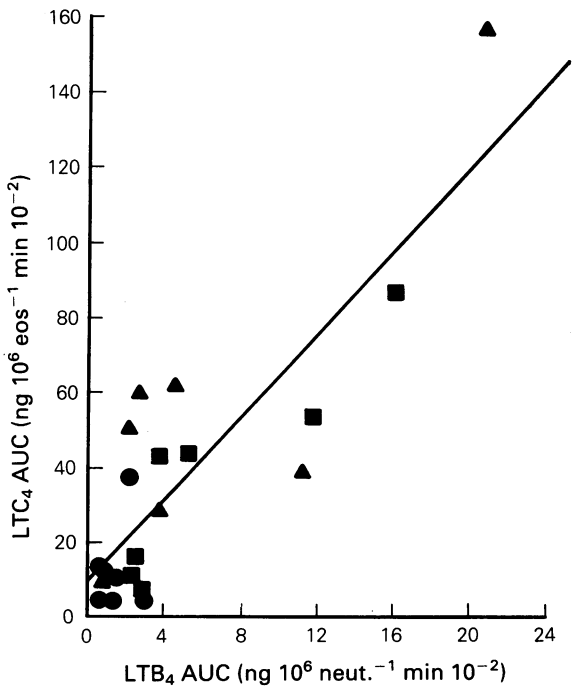

Figure 6 Correlation of synthesis of LTB$_4$ and LTC$_4$ by mixed leukocytes (5×10^6/ml) of atopic asthmatic (■), atopic non-asthmatic (▲), and normal (●) subjects, stimulated with 2 μM A23187 for 30 min. LT synthesis is expressed as the areas under the individual time-course curves. Equation of regression line: $y = 970 + 5.6x$. Correlation coefficient (r) = 0.837 ($P < 0.001$, $n = 21$).

Discussion

Our results demonstrate that peripheral leukocytes of atopic subjects with or without asthma have a significantly enhanced ability to synthesise LTB$_4$ and cysteinyl-LTs in response to calcium ionophore stimulation, when compared with those of healthy non-atopic subjects. If this enhancement were also apparent in PMNs resident in the asthmatic airway, increased bronchoconstrictor and inflammatory responses may occur following activation even in the absence of chronically-elevated PMN counts. Such a mechanism may underly the non-specific bronchial hyperresponsiveness and hypersensitivity to allergic stimuli characteristic of asthmatic and atopic subjects.

Populations of mixed leukocytes were prepared which reflected the relative proportions of leukocyte subtypes found in whole blood, thus taking into account the possible effects of interactions between cell-types. Nevertheless, the levels of LTB$_4$ produced by normal leukocytes in response to A23187 were similar to those described in highly-purified neutrophils (>95%) by other workers (Gresele *et al.*, 1986; Radeau *et al.*, 1990; Shaw *et al.*, 1984), suggesting that the neutrophil is the overwhelming source of LTB$_4$ in our preparations. The lack of significant differences in the rate of omega-oxidation between the three groups demonstrates that the elevated levels of LTB$_4$ immunoreactivity observed in the AN and AA subjects were due to increased synthesis and not to a slower rate of catabolism than in the normal cells. This increased synthesis was highly statistically significant and apparent both in dose-response and time-course experiments.

Since the proportion of eosinophils to total leukocytes in peripheral blood is normally <5%, it is difficult to obtain highly purified eosinophils from normal control subjects. Indeed, studies characterising eosinophil leukotriene synthesis and release usually rely on cells isolated from patients with bronchial asthma, allergic rhinitis, eczema, and idiopathic hypereosinophilia; the levels of LTC$_4$ generated by A23187 from such cells (Mahauthaman *et al.*, 1988) are close to those observed by us in the mixed leukocytes of asthmatic and atopic subjects. The use of mixed leukocyte preparations, however, also allows quantitation of eosinophil LTC$_4$ synthesis in non-atopic normals, as the numbers and/or LTC$_4$ synthetic capacities of neutrophils, and basophils are limited, and in our preparations, LTC$_4$ generation significantly correlated with the percentage of eosinophils, suggesting that the eosinophil is the principal source of LTC$_4$. Monocyte synthesis of LTC$_4$, although small in comparison with that of eosinophils (Shaw *et al.*, 1984), may nevertheless have detracted from the correlation of LTC$_4$ synthesis with eosinophil percentage.

There was a significantly increased synthesis of LTC$_4$ in the AA and AN leukocytes compared with N cells, which was apparent in both the dose-response and time-course investigations; this was partly due to higher counts of eosinophils in the AA and AN groups, but the elevated levels remained significant after correction of LTC$_4$ levels for eosinophil counts. The eosinophil appears therefore to be inherently up-regulated with respect to LTC$_4$ synthesis in atopic and asthmatics. The

stability of [^{3}H]-LTC_4 incubated with mixed leukocytes eliminates the possibility that differences in the rate of conversion of LTC_4 to LTD_4 and LTE_4 might account for this up-regulation, and oxidative catabolism of LTC_4 by the peroxidase-dependent pathway was prevented by the presence of L-serine (Weller *et al.*, 1983).

The strong relationship between the capacity of each subject's leukocytes to generate LTC_4 and LTB_4, which was most apparent in the AA and AN groups, suggests concomitant priming of the 5-LO pathway in neutrophils and eosinophils in these subjects. This relationship was not seen in the N subjects, where LTB_4 and LTC_4 releasability appear to be independently regulated, although more N subjects need to be studied to assess this definitively. Our results should be viewed in the context of considerable evidence suggesting enhancements of other functional activities of peripheral blood eosinophils and neutrophils in asthma and atopy, including granule protein release (Durham *et al.*, 1989), and superoxide ion generation (Styrt *et al.*, 1988).

The mechanism of up-regulation of LT synthesis in asthma and atopy remains to be elucidated, but principal candidates for the putative factor involved are a variety of cytokines shown to have the ability to prime PMNs for elevated mediator release *in vitro*. In particular, granulocyte-monocyte colony-stimulating factor (GM-CSF) greatly augments survival of mature human eosinophils *in vitro*, resulting in conversion from the normodense to a hypodense phenotype (Vancheri *et al.*, 1989) and an increased capacity to synthesise LTC_4 (Silberstein *et al.*, 1986). Hypodense eosinophils are found in increased numbers in the circulation of asthmatic subjects (Fukuda *et al.*, 1985). Enhanced production of GM-CSF has been described in asthmatic alveolar macrophages and airway epithelial cells *in vitro* (Howell *et al.*, 1990; Mattoli *et al.*, 1991), and elevated levels of GM-CSF are present in BAL fluid of asthmatics (Mattoli *et al.*, 1990). Moreover, pre-incubation with GM-CSF also primes human neutrophils for increased LTB_4 synthsis triggered by A23187 and chemotactic factors (Dahinden *et al.*, 1988; DiPersio *et al.*, 1988). It is conceivable therefore, that our results might be explained by upregulation *in vivo* of phospholipase A_2 and/or 5-lipoxygenase activities by GM-CSF in the PMNs of atopic and asthmatic subjects. Further studies are underway to investigate this hypothesis.

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