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Lyso-PAF acetyltransferase activity in neutrophils of patients during acute asthma and after recovery

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Lyso-PAF acetyltransferase activity in neutrophils of patients during acute asthma and after recovery. N.L.A. Misso, R.L. Gillon, G.A. Stewart, P.J. Thompson. ©ERS Journals Ltd 1996.

ABSTRACT: The production of platelet-activating factor (PAF) by inflammatory cells is regulated by lyso-PAF acetyltransferase, and the activity of this enzyme is increased in neutrophils of stable asthmatic patients. The aim of this investigation was to determine whether acetyltransferase activity is further upregulated in asthmatic patients experiencing acute symptoms.

A radioenzymatic assay was used to measure the enzymatic affinity constant (K_m) and maximal enzymatic activity (V_{max}) for acetyltransferase from unstimulated and Ca^{2+} ionophore (A23187)-stimulated neutrophils from 16 patients with acute asthma, and the measurement was repeated at the time of discharge (n=9) and after recovery from the acute episode (n=13).

During acute asthma, K_m (median 93.8 (interquartile range 64.1–109.7) µM) was lower than that measured in nonasthmatic subjects in a previous study using identical methods (155.1 (122.2–179.9) µM; p=0.0001), and in 10 out of 13 acute patients K_m for unstimulated neutrophils increased following recovery. In A23187-stimulated neutrophils, K_m during acute asthma (84.3 (73.6–100.2) µM) and at discharge (83.9 (83.1–94.8) µM) were similar, but K_m after recovery was increased (115.0 (95.6–119.5) µM; p=0.02). The change in K_m following stimulation with A23187 was also significantly less during acute asthma than previously measured in nonasthmatic subjects (p=0.003). Although V_{max} during acute asthma (12.9 (interquartile range 10.5–22.5) nmol·min^{-1}·mg^{-1} protein) did not differ significantly from that at discharge (14.4 (12.3–20.4) nmol·min^{-1}·mg^{-1}) or after recovery (17.3 (12.3–18.4) nmol·min^{-1}·mg^{-1}), both median K_m and V_{max} tended to be lowest during acute asthma and increase at discharge and after recovery.

An increase in lyso-PAF acetyltransferase activity alone may not account for increased systemic PAF concentrations during acute asthma. However, the reduction in the enzymatic affinity constant and its smaller change following *in vitro* stimulation suggest that alterations in the affinity of acetyltransferase for acetyl-coenzyme A (CoA) and in the regulation of enzyme activity may be occurring during acute asthma.

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Platelet-activating factor (PAF) is an ether-linked phospholipid with numerous biological activities which suggest that it plays a significant role in the pathogenesis of asthma [1]. PAF causes bronchoconstriction and, in some studies, has been shown to induce a persistent increase in bronchial hyperresponsiveness in humans [2, 3]. This may relate to the potency of PAF as a chemoattractant for eosinophils [4], since these cells appear to play a significant role in the development of airway hyperresponsiveness [5, 6]. However, other studies, both in normal and asthmatic subjects [7, 8], have failed to demonstrate any increase in airway responsiveness after PAF inhalation. PAF also causes airway microvascular leakage and oedema [9], stimulates tracheal mucus secretion [10], and inhibits mucociliary clearance in normal subjects [11].

There is much indirect evidence implicating PAF in the pathogenesis of asthma, but it has been more difficult to obtain direct evidence based on measurements of this mediator and its precursor, lyso-PAF, in biological fluids [12]. However, PAF has been detected in bronchoalveolar lavage fluid (BAL) from asthmatics [13], and allergen challenge has resulted in high levels of lyso-PAF in nasal fluids [14], and increased plasma PAF concentrations [15]. A number of recent studies have also suggested that systemic PAF concentrations may be increased in symptomatic asthmatic subjects [16–18]. Despite these observations, the interpretation of such measurements is complicated by factors such as the short half-life of PAF, the fact that lyso-PAF is both a precursor and metabolite of PAF, the likelihood that PAF acts mainly at localized sites of inflammation and that it may be rapidly metabolized and reincorporated by surrounding inflammatory cells [19].

PAF is synthesized by a variety of cell types, including neutrophils, eosinophils, alveolar macrophages, monocytes and endothelial cells, in response to stimuli, such as the calcium ionophore A23187, opsonized zymosan

[19], granulocyte/macrophage colony-stimulating factor (GM-CSF) [20], bradykinin, tumour-necrosis factor-α (TNF-α) and interleukin-1α (IL-1α) [21, 22]. Stimulated inflammatory cells synthesize PAF in a two-step process catalysed by the enzymes phospholipase A_2 and acetyl-coenzyme-A (CoA):lyso-PAF acetyltransferase [23]. Acetyltransferase activity can be increased *in vitro* by A23187, TNF-α and IL-1α, and *in vivo* activation of this enzyme could result in the production of excessive amounts of PAF [19]. Thus, upregulation of acetyltransferase activity may possibly explain the increased PAF concentrations reported in some studies of symptomatic asthmatics.

In a previous study from our laboratory, increased acetyltransferase activity was observed in neutrophils from clinically stable atopic asthmatic subjects compared with nonasthmatic control subjects, suggesting that in these nonsymptomatic asthmatics, neutrophils may be subject to chronic priming [24]. It is not clear, however, whether disease exacerbation further modulates acetyltransferase activity. Thus, the aim of the present investigation was to measure neutrophil acetyltransferase activity in a group of asthmatic subjects experiencing acute disease exacerbations.

Methods

Subjects

Sixteen subjects presenting to the Emergency Department with a clinically diagnosed acute exacerbation of asthma were recruited for the study, which was approved by the Committee for Human Rights of the University of Western Australia. Informed consent was obtained from all patients in the study group which comprised 13 females and 3 males (mean age 39±15 yrs, range 19–69 yrs). The clinical details and drug treatments were noted for all patients, and spirometry and oximetry data were obtained for most patients (table 1). Patients were bled prior to receiving treatment and a second blood sample was obtained at the time of discharge from those patients admitted to hospital (n=9). A further blood sample was obtained from patients (n=13) approximately 8 weeks after the acute exacerbation, when their asthma was considered to be stable and they had not been taking oral corticosteroids for at least 2 weeks. Three subjects could not be contacted after discharge and it was, therefore, not possible to obtain a follow-up blood sample from these subjects.

Isolation of neutrophils

Whole blood, anticoagulated with ethylenediamine tetra-acetic acid (EDTA), was layered on a discontinuous Percoll gradient (densities 1.082, 1.094) and centrifuged (500×g for 30 min). The neutrophil band was recovered and contaminating erythrocytes were lysed in 0.2% (w/v) saline for 30 s. An equal volume of 1.6% (w/v) saline was added and the cells were again centrifuged (500×g for 10 min). Neutrophils were washed in hydroxyethylpiperazine ethanesulphonic acid (HEPES)-buffered Hank's balanced salt solution (HBSS) (HEPES 4.2 mM, NaCl 137 mM, KCl 2.6 mM, glucose 5.6 mM, pH 7.4) and resuspended in HEPES-HBSS supplemented with $CaCl_2$ (1.3 mM) and $MgCl_2$ (1 mM). Neutrophils were counted and diluted to 11×10^6 cells·mL^{-1}. Purity and viability were consistently >95%.

Assay of acetyltransferase activity

Acetyltransferase activity of neutrophils was assayed as described previously [24]. Briefly, unstimulated neutrophils, and neutrophils stimulated with 5 µM Ca^{2+} ionophore (A23187) to induce maximum acetyltransferase activity, were incubated at 37°C for 10 min. Cells were centrifuged and resuspended in 0.25 M sucrose containing 1 mM dithiothreitol. They were disrupted

Table 1. – Lung function, oximetry and drug treatment for patients with acute asthma

Pt No.	Sex	Age yrs	Blood taken	FEV1 acute % pred	S_{a,O_2} %	FEV1 recovery % pred	Drug treatment Preadmission	Drug treatment Discharge
1	F	19	a, r	62	98	102	ba	ba, pn, Bf, th
2	F	32	a, d, r	38	93	122	ba, Bc	ba, pn, Bc
3	F	19	a	58	95	ND	ba, ip, Bf	ba, Bf
4	F	22	a, r	41	98	93	ba	ba, pn, Bf
5	F	59	a, d, r	53	91	90	ba, th	ba, pn, Bf
6	M	31	a, d, r	22	90	78	ba, Bc	ba, pn, Bf
7	F	25	a	16	94	ND	ba	ba, ip, pn, Bf, th
8	F	69	a, d, r	53	99	ND	ba, pn, bu	ba, Bf
9	M	58	a, d, r	22	ND	95	ba, pn, th	ba, pn, Bf, th
10	F	28	a	ND	94	ND	ba	ba, pn, Bf
11	M	29	a, d, r	38	92	92	ba	ba, pn, Bf
12	F	47	a, r	63	97	72	ba, pn, th, Bf	ba, pn, th, Bf
13	F	52	a, d, r	82	ND	78	ba, pn, Bf	ba, pn, Bf
14	F	47	a, d, r	31	98	49	ba, Bf	ba, pn, Bf
15	F	48	a, r	54	ND	91	ba, ip, Bf, cr, th	ba, fp, th, cr
16	F	41	a, d, r	72	ND	87	ba, sm, fp	ba, pn, sm, fp

Pt: patient; F: female; M: male; a: acute; d: discharge; r: recovery; ND: not determined; ba: β_2-adrenoceptor agonists; Bc: Becotide; Bf: Becloforte; bu: budesonide; pn: prednisolone; fp: fluticasone propionate; th: theophylline; cr: cromoglycate; sm: salmeterol; ip: ipratropium bromide; FEV1: forced expiratory volume in one second; % pred: percentage of predicted value; S_{a,O_2}: arterial oxygen saturation. Subjects from whom a blood sample was taken after recovery had ceased prednisolone for at least 2 weeks.

by sonication for 20 s on ice, and the neutrophil lysates were assayed in duplicate for acetyltransferase activity in a reaction mixture containing lyso-PAF (40 µM) and [^{3}H]acetyl-CoA (50–500 µM) in a final volume of 0. 5 mL HEPES-HBSS containing 0.25% (w/v) bovine serum albumin (BSA). After incubation at 37°C for 10 min, the reaction was stopped by addition of methanol-chloroform-acetic acid (2:1:0.04, by volume). Lipids were extracted into chloroform [25], the extract was washed twice with 2 mL of 0.9% (w/v) saline-methanol-chloroform-0.1 M sodium acetate (1.0:2.5:3.75:1.0, by volume) and [acetyl-^{3}H]PAF was measured by liquid scintillation counting. Losses during lipid extraction were corrected for by extracting a control sample, to which [alkyl-^{3}H]PAF was added. The mean recovery was 84±4%. Acetyltransferase activities were expressed as [acetyl-^{3}H]PAF produced (nmol·min^{-1}·mg^{-1} protein) after subtraction of the radioactivity in blank incubations which contained no lyso-PAF. Protein concentrations of neutrophil lysates were determined using the Coomassie blue protein assay (Bio-rad, Hercules, CA, USA).

Acetyltransferase activity, as measured in this assay, has previously been shown to increase linearly over the range of protein concentrations used and also over the incubation period of 10 min [24]. In addition [acetyl-^{3}H]PAF produced in this assay has been characterized on the basis of co-chromatography with authentic PAF standards on thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) and by stimulation of human platelet aggregation, which was inhibited by the specific PAF receptor antagonists WEB 2086 and STY 2108 [24].

Analysis of data and statistics

Acetyltransferase activity was measured over a range of acetyl-CoA substrate concentrations both for unstimulated and A23187-stimulated neutrophil lysates. For each subject, the maximal enzymatic activity (V_{max}) and the enzymatic affinity constant (K_m) for unstimulated and A23187-stimulated neutrophils were determined from double reciprocal plots of acetyltransferase activity (1/V) against acetyl-CoA concentration (1/[S]). V_{max} and K_m values during the acute episode, at the time of discharge and following recovery are presented as medians and interquartile range (25th to 75th percentile). The degree of stimulation of acetyltransferase activity induced by A23187 was calculated as the ratio V_{max}-stimulated/V_{max}-unstimulated, and the change in K_m following stimulation with A23187 was calculated as the ratio K_m-unstimulated/K_m-stimulated. Differences in median V_{max} and K_m values and ratios for the 16 patients with acute asthma, the nine patients at discharge and the 13 patients at recovery were evaluated for statistical significance by nonparametric analysis of variance (Kruskal-Wallis test) using the InStat computer program (GraphPad Software, San Diego, CA, USA). The Mann-Whitney test was used to compare V_{max} and K_m values and ratios with data obtained previously using identical methodology, for 20 nonasthmatic subjects [24]. For statistically significant differences ($p<0.05$), the differences in median values and the associated 95% confidence intervals (95% CI) are presented.

Results

Acetyltransferase activity in unstimulated and A23187-stimulated neutrophils increased with increasing acetyl-CoA substrate concentration, reaching a plateau at 200 µM acetyl-CoA (fig. 1). For each subject, V_{max} and K_m values were determined from double reciprocal plots, which did not deviate significantly from linearity, indicating that the enzyme followed Michaelis-Menten kinetics both in unstimulated and A23187-stimulated neutrophils for acetyl-CoA concentrations of 50–500 µM and a fixed lyso-PAF concentration (40 µM). In the acute asthma group, the median K_m for acetyltransferase in unstimulated neutrophils (93.8 (64.1–109.7) µM; n=16) was not significantly different to the value measured at discharge (104.6 (95.4–115.1) µM; n=9) or after recovery (107.2 (96.7–126.9) µM; n=13) (fig. 2). However, an increase in K_m was observed in 10 of the

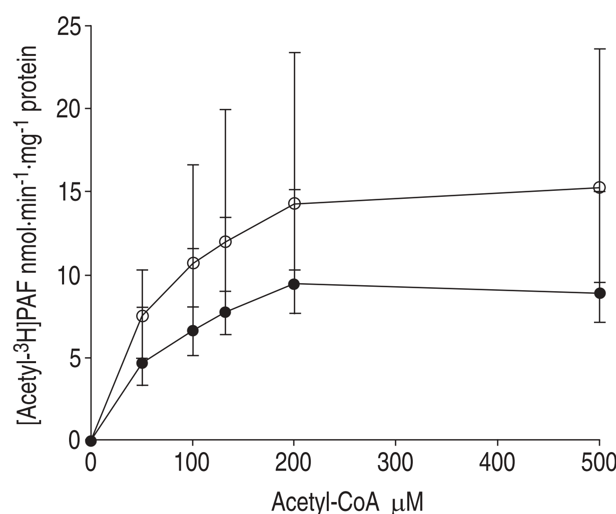

Fig. 1. – Lyso-PAF acetyltransferase activity as a function of acetyl-CoA concentration in unstimulated (—●—) and A23187-stimulated (—○—) neutrophils from patients with acute asthma. Values are medians and interquartile ranges of data from 16 patients. PAF: platelet-activating factor; CoA: coenzyme A.

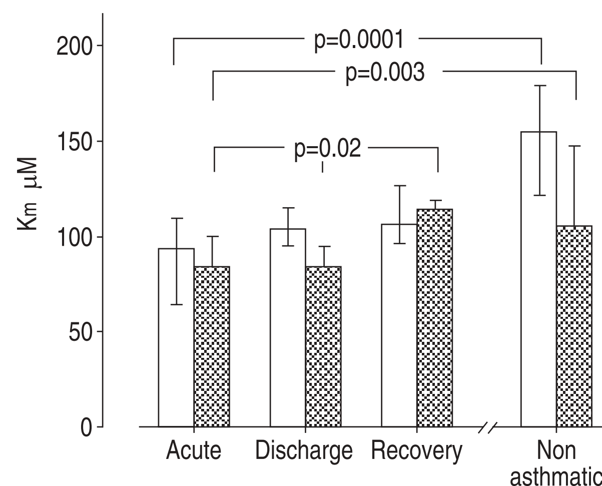

Fig. 2. – Median (interquartile range) K_m values for acetyltransferase in unstimulated (□) and A23187-stimulated (▩) neutrophils from patients with acute asthma (n=16), at discharge (n=9), and after recovery (n=13), compared with the values measured previously [24] in nonasthmatic subjects (n=20). K_m: enzymatic affinity constant.

13 subjects for whom K_m was measured both during the acute stage and after recovery, 8 weeks later (fig. 3a). The median K_m in acute asthma was significantly lower than the K_m values measured previously [24] in nonasthmatic subjects using identical methods (155.1 (122.2–179.9) µM; p=0.0001; median difference -64.9 (95% CI -98.4 to -38.4) µM) (fig. 2).

In acute asthma, at discharge and after recovery, median K_m values for A23187-stimulated neutrophils did not differ significantly from the values for unstimulated neutrophils (fig. 2), although A23187 stimulation was previously shown to cause a significant reduction in K_m in nonasthmatic subjects [24]. In A23187-stimulated neutrophils, the median K_m values in acute asthma (84.3 (73.6–100.2) µM) and at discharge (83.9 (83.1–94.8) µM) were similar, although the median K_m after recovery was increased (115.0 (95.6–119.5) µM; p=0.02, analysis of variance. The K_m for A23187-stimulated cells in acute asthma was also significantly lower than the value measured previously in nonasthmatic subjects (106.6 (98.1–148.7) µM; p=0.003; median difference -27.8 (95% CI -48.4 to -13.6) µM) (fig. 2).

The change in K_m following *in vitro* stimulation was measured as the ratio K_m unstimulated/K_m-stimulated.

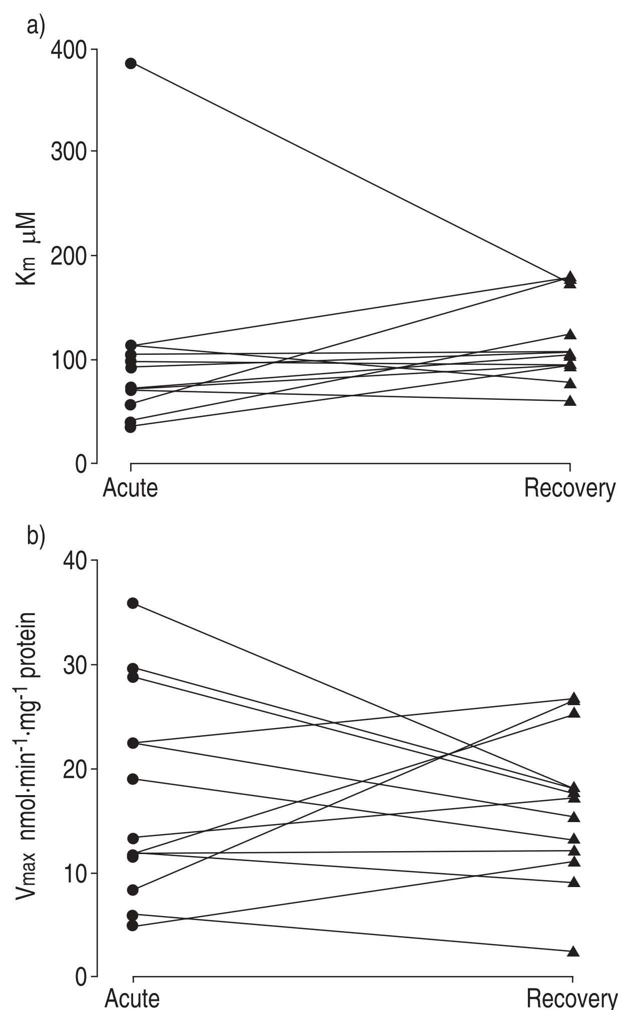

Fig. 3. – Changes in: a) K_m; and b) V_{max} values for neutrophil acetyltransferase in the 13 asthmatic patients for whom measurements were made both during the acute episode and following recovery. K_m: enzymatic affinity constant; V_{max}: maximal enzymatic activity.

Table 2. – Ratios K_m-unstimulated/K_m-stimulated and V_{max}-stimulated/V_{max}-unstimulated in patients with acute asthma, at discharge and following recovery

Group	Pt n	Median K_m ratio	IQR	Median V_{max} ratio	IQR
Acute asthma	16	0.94*	0.80–1.08	1.59‡	1.16–2.11
Discharge	9	1.06	1.01–1.35	1.61	1.10–1.95
Recovery	13	0.95†	0.88–1.15	1.68	1.50–1.91
Nonasthmatic (previous study)	20	1.31	1.12–1.61	2.07	1.60–2.59

Values significantly different to corresponding value previously measured in nonasthmatic subjects [24]: *: median difference -0.39 (95% CI -0.62 to -0.19); p=0.003; †: median difference -0.33 (95% CI -0.53 to -0.1), p=0.006; ‡: median difference -0.46 (95% CI -0.88 to -0.06), p=0.029. K_m: enzymatic affinity constant; V_{max}: maximal enzymatic activity; IQR: interquartile range; 95% CI: 95% confidence interval.

Median K_m ratios did not differ significantly during acute asthma, at discharge or after recovery, but the ratios both during acute asthma and after recovery were significantly lower than the median K_m ratio measured previously in nonasthmatic subjects [24] (table 2).

At all acetyl-CoA concentrations, acetyltransferase activities of unstimulated and A23187-stimulated neutrophils from patients with acute asthma did not differ significantly from values measured at discharge or following recovery. In addition, although there was a trend for the median V_{max} for unstimulated neutrophils to increase from 12.9 (10.5–22.5) $nmol \cdot min^{-1} \cdot mg^{-1}$ protein in acute asthmatic patients to 14.4 (12.3–20.4) $nmol \cdot min^{-1} \cdot mg^{-1}$ protein at discharge, and 17.3 (12.3–18.4) $nmol \cdot min^{-1} \cdot mg^{-1}$ protein after recovery, with a corresponding reduction in the interquartile ranges, these median V_{max} values were not significantly different (fig. 4). V_{max} decreased in seven and increased in six of the 13 subjects in whom it was measured both during the acute stage and after recovery (fig. 3b). The median

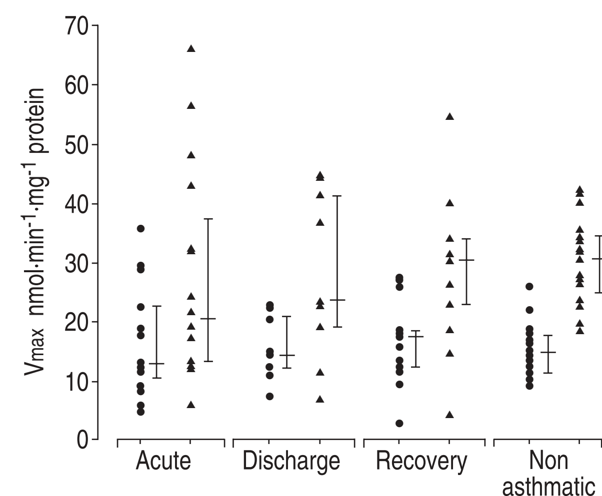

Fig. 4. – Scattergram showing V_{max} values for acetyltransferase activity in unstimulated (●) and A23187-stimulated (▲) neutrophils from patients with acute asthma (n=16), at discharge (n=9) and after recovery (n=13). For comparison, the values previously measured [24] in nonasthmatic subjects (n=20) are also indicated. Bars indicate median values and interquartile ranges. V_{max}: maximal enzymatic activity.

V_{max} value measured in acute asthmatic patients in this study was not significantly different to that measured previously [24] in nonasthmatic subjects (14.7 (10.9–17.4) $nmol \cdot min^{-1} \cdot mg^{-1}$ protein). There were no significant differences in the median V_{max} values for A23187-stimulated neutrophils during acute asthma, at discharge or after recovery, and the values were also not significantly different to those measured previously in nonasthmatic subjects.

The degree of stimulation of acetyltransferase activity induced by A23187 was calculated for each subject as the ratio V_{max}-stimulated/V_{max}-unstimulated. The median V_{max} ratios during acute asthma, at discharge and after recovery did not differ significantly but the V_{max} ratio during acute asthma was significantly lower than that measured previously in nonasthmatic subjects (table 2). The V_{max} ratio after recovery also differed from that of nonasthmatic subjects, although the difference did not quite reach statistical significance ($p=0.06$).

Discussion

Acetyl-CoA:lyso-PAF acetyltransferase activity was previously shown to be significantly increased in neutrophils from atopic, asthmatic subjects compared with a control group of nonasthmatic subjects [24]. The present study sought to extend these observations by measuring acetyltransferase activity of neutrophils obtained from patients experiencing symptoms of acute asthma. For most of these patients, it was possible to repeat the measurement of acetyltransferase activity after recovery from the acute episode, thus providing some indication of whether enzyme activity correlated with disease activity in asthmatic patients.

The median K_m in acute asthma was significantly lower than that measured previously in healthy nonasthmatic subjects, thus confirming the trend towards a reduction in K_m in stable asthmatic compared with nonasthmatic subjects [24]. Furthermore, in most acute subjects, the K_m increased on recovery, suggesting that decrease in K_m is a phenomenon associated with the acute asthmatic response. This was further supported by the observation that stimulation of neutrophils with A23187 resulted in very little change in median K_m either during acute asthma or after recovery, contrasting with the significant reductions in K_m following stimulation with A23187 of neutrophils from nonasthmatic and stable asthmatic subjects. These differences are highlighted by the ratios K_m-unstimulated/K_m-stimulated, which were close to unity during acute asthma and after recovery but significantly higher in stable asthmatic and nonasthmatic subjects. Therefore, it appears that during acute asthma, neutrophil acetyltransferase undergoes a structural or conformational modification that increases the affinity of the enzyme for acetyl-CoA and renders it less susceptible to *in vitro* modulation by the Ca^{2+} ionophore A23187.

Lyso-PAF acetyltransferase in human neutrophils is activated and inactivated by a phosphorylation-dephosphorylation mechanism involving Ca^{2+}-dependent protein kinase C [26, 27]. As neutrophils circulate through the lungs of patients experiencing acute asthma symptoms, they may be primed by proinflammatory cytokines, such as granulocyte macrophage colony stimulating factor (GM-CSF), that are known to be present in the lungs of asthmatic patients [28], and this priming or activation may result in the modification of acetyltransferase and its function. It is also possible that PAF released systemically during acute asthma may itself feedback to modify neutrophil acetyltransferase with a resultant lowering of the K_m. An analogous desensitisation of platelets to *in vitro* stimulation by PAF has been observed following allergen inhalation by asthmatic patients, which presumably causes systemic PAF release *in vivo* [29].

However, the reduction in K_m and increased substrate affinity during acute asthma does not appear to be associated with an increase in enzyme activity. Indeed, median V_{max} appeared to be lower during acute asthma and, in contrast to the previously measured [24] activity in stable asthmatics, was not significantly greater than the activity in nonasthmatic subjects. However, the median V_{max} appeared to increase at discharge and after recovery, although these changes were not statistically significant. The degree of stimulation obtained with A23187 as assessed by the ratio V_{max}-stimulated/ V_{max}-unstimulated was similar during acute asthma and after recovery but the ratios were smaller than those for non-asthmatic subjects. Thus, neutrophils from asthmatic patients appear to be inherently less sensitive to *in vitro* stimulation with A23187, supporting our previous findings [24].

It was possible that oral medications, such as corticosteroids and theophylline, administered either prior to or during the hospital admission may have influenced neutrophil acetyltransferase activity and the measurement of the enzyme activity at discharge was primarily performed in order to assess any such influence. The results indicating no significant differences in the K_m and V_{max} values at discharge compared with those measured during the acute stage and/or after recovery would suggest that the treatments received by these patients in hospital had little or no effect on acetyltransferase activity.

Studies with the oral PAF antagonists, WEB 2086 and modipafant, have shown little or no beneficial effect on lung function or asthma symptoms [30, 31], although a recent trial of another oral PAF antagonist, Y-24180 showed a significant reduction in bronchial hyperresponsiveness in asthmatic patients [32]. The importance of PAF as a mediator in asthma, therefore, remains controversial. While PAF may not be the primary effector in the asthmatic response, it may act as a priming agent in a network of lipid mediators and cytokines involved in the chronic inflammatory process [5, 19], and there is also evidence that systemic PAF concentrations are increased during acute asthma. A23187-stimulated granulocyte PAF production and plasma PAF levels are reported to be higher in children with asthma symptoms compared with asymptomatic or control children [16, 17], and in patients with mild asthma, blood PAF levels were increased following allergen-induced bronchoconstriction [15] and during spontaneous exacerbations [18].

Since *in vitro* studies indicate that acetyl-CoA:lyso-PAF acetyltransferase regulates PAF production in human neutrophils and endothelial cells [33, 34], it might be predicted that increased systemic PAF concentrations

in symptomatic asthma would result from upregulation of neutrophil acetyltransferase activity. However, the present study suggests that acetyltransferase activity is not increased, and may actually be reduced, during the acute episode compared with the activity in stable asthma. It is possible that increased inflammatory mediator or cytokine production consequent to the acute episode may initiate a negative feedback mechanism that reduces acetyltransferase activity as a means of controlling excessive PAF production. Following recovery, activity appeared to increase towards the chronically stimulated level previously observed in stable asthmatic subjects [24].

While upregulation of neutrophil acetyltransferase may, therefore, be involved in chronic PAF production in stable asthma, it may not be the major source of increased systemic PAF concentrations in acute asthma. It is possible that activated eosinophils in peripheral blood contribute significantly to systemic PAF production in acute asthma. Although the neutrophil preparations may have contained a few eosinophils, the present study does not permit any comment on whether acetyltransferase activity was increased in eosinophils alone, during acute asthma. Overall regulation of PAF biosynthesis in neutrophils may also depend on the activity of a CoA-independent transacylase in addition to lyso-PAF acetyltransferase [35, 36]. Increased PAF production during acute asthma may, therefore, result from increased CoA-independent transacylase activity, and an increased supply of lyso-PAF substrate for acetyltransferase, the activity of which is already increased in stable asthma. Alternatively, increased systemic PAF concentrations in asthma may reflect a reduced rate of PAF degradation due to decreased activity of PAF acetylhydrolase. The activity of this enzyme in plasma is reportedly decreased in asthma [37], systemic lupus erythematosus [38], and septic shock [39]. Plasma PAF acetylhydrolase was recently cloned and the recombinant enzyme was shown to block inflammation *in vivo*, suggesting the possibility of its therapeutic use as an anti-inflammatory agent [40].

Although the present study did not indicate any increase in lyso-PAF acetyltransferase activity during acute asthma, K_m was significantly reduced with a trend for K_m and V_{max} to increase on recovery. The change in the enzymatic affinity constant and maximal enzymatic activity following *in vitro* stimulation, was less in acute asthmatic patients compared with stable asthmatic and/or nonasthmatic subjects and, taken together, these observations suggest that alterations in the regulation of enzyme activity may be occurring. Although the precise mechanisms regulating lyso-PAF acetyltransferase are unclear, this may occur at the protein and possibly at the gene level. Systemic and tissue PAF concentrations are likely to be determined by the overall balance between the activities of the biosynthetic and catabolic enzymes. Therefore, further studies are required of lyso-PAF acetyltransferase activity and regulation in acute inflammation and in eosinophils, which are known to be associated with the development of airway hyperresponsiveness in asthma.

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