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Platelet-activating factor-induced human eosinophil activation. Generation and release of cyclo-oxygenase metabolites in human blood eosinophils from asthmatics

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SUMMARY

The spontaneous and stimulated generation of fatty acid cyclo-oxygenase pathway-derived products of arachidonic acid from highly purified (91·6±1·3%, $n=23$) human blood eosinophils obtained from asthmatics were examined using combined gas chromatography/mass spectrometry. Under resting conditions, eosinophils spontaneously generated 0·24±0·10 pg prostaglandin E_2 (PGE_2), 0·51±0·20 prostaglandin D_2 (PGD_2), 0·35±0·10 pg prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and 8·5±2·2 pg thromboxane B_2 (TXB_2), the stable metabolite of TXA_2 per 10^6 cells. In contrast, 6-keto-prostaglandin $F_{1\alpha}$ and 9α, 11β-prostaglandin F_2 were not detectable. Stimulation of eosinophils with platelet-activating factor (PAF) for 5 min induced a two- to sixfold increase in the biosynthesis of prostanoids. More than 95% of the generated prostanoids were released into the surrounding medium. The response to PAF was inhibited by the PAF receptor antagonist WEB 2086 (1 μM). The fatty acid cyclo-oxygenase inhibitor, ibuprofen, abolished both the spontaneous and PAF-stimulated generation of prostanoids by eosinophils. LTB_4, PMA and calcimycin also produced an increase in prostanoid production, whereas lyso-PAF, the PAF precursor and metabolite, failed to induce prostanoid generation over basal production. In conclusion, the results demonstrate that PAF potently activates human eosinophils to generate and release several fatty acid cyclo-oxygenase metabolites of the arachidonic acid pathway, with TXB_2 being the most abundant. These data are in agreement with previous observations suggesting that PAF may be an important stimulus for prostanoid release by the eosinophil in allergic diseases such as asthma.

INTRODUCTION

There is substantial evidence to suggest that eosinophils may play an important role in the pathogenesis of the late phase response in bronchial asthma.[1] Recent investigations have focused upon eosinophil secretion and the effects of granule-associated proteins such as eosinophil peroxidase, major basic protein and eosinophil cationic protein. These cationic proteins have been shown to damage respiratory epithelium in humans[2] as well as in animal models of asthma,[3-5] and may contribute, in part, to the development of bronchial hyper-responsiveness.[6] In contrast, the putative role of eosinophils as producers of bioactive lipid mediators is less well defined.

Abbreviations: FCS, foetal calf serum; GC/MS, gas chromatography/mass spectrometry; PAGCM, Pipes-albumin-glucose-calcium-magnesium buffer; PAF, platelet-activating factor; PG, prostaglandin; PGE_2, prostaglandin E_2; PGD_2, prostaglandin D_2; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; TXB_2, thromboxane B_2; TXA_2, thromboxane A_2; 6-keto-$PGF_{1\alpha}$, 6-keto-prostaglandin $F_{1\alpha}$; 9α, 11β-PGF_2, 9α, 11β-prostaglandin F_2.

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Hubscher in 1975 demonstrated that human eosinophils secrete E-series prostaglandins.[7] Other studies have shown that human peritoneal fluid eosinophils produce TXB_2, PGE_2 and 6-keto-$PGF_{1\alpha}$.[8] In addition, eosinophils synthesize leukotriene C_4[9] and platelet-activating factor.[10] However, for most of these studies the non-physiological stimulus calcium ionophore A23187, a bacterial ether antibiotic which may be cytotoxic for eosinophils,[11,12] was employed.

Recent evidence suggests that platelet-activating factor (PAF) may play an important role in asthma by inducing broncho-constriction,[13,14] mucosal oedema,[15] sustained increase in bronchial reactivity[13,16] and recruitment of inflammatory cells.[17,18] In addition, PAF appears to be a potent modulator of eosinophil functions, inducing adherence to endothelial cells,[19] eosinophil chemotaxis *in vitro*[17] and *in vivo*,[20,21] generation of superoxide anions,[12] release of granular enzymes and proteins[12,22-24] and a shift in density from normodense to hypodense eosinophils.[25] In contrast, however, very little is known about the effect of PAF in inducing generation of fatty acid cyclo-oxygenase-derived arachidonic acid metabolites from endogenous precursors in human blood eosinophils.

We have recently reported[26] that guinea-pig eosinophils release TXB_2 and E-series prostaglandins, as measured by

radioimmunoassay, when stimulated with PAF. Using combined capillary gas chromatography/mass spectrometry (GC/MS), the present report extends these studies and examines the effect of PAF and other stimuli on the generation and release of a spectrum of prostanoids by human blood eosinophils obtained from allergic subjects.

MATERIALS AND METHODS

Materials

Hanks' balanced salt solution (HBSS) was purchased from Flow Laboratories Ltd (Rickmansworth, U.K.); Percoll (polyvinyl-pyrrolidene-coated silica gel) and dextran T70 were purchased from Pharmacia Fine Chemicals AB (Uppsala, Sweden). Triton X-100 was purchased from BDH Ltd (Poole, U.K.). PAF, leukotriene B_4 and ibuprofen were obtained from Biomol Research Laboratories (Plymouth Melting, PA). Calcium ionophore A23187 (calcimycin), 4-phorbol-12-myristate 13-acetate (PMA), 4α-phorbol, piperazine-N,N′-bis-(2-ethane sulphonic acid) (Pipes), foetal calf serum (FCS), Tris-HCl, gelatin, activated charcoal (100–400 mesh), thiomerosal, dimethylsulphoxide (DMSO), human serum albumin (HSA), EGTA and digitonin were purchased from Sigma Chemicals Co. (St Louis, MO). WEB 2086 was kindly donated by Boehringer Ingelheim (Ingelheim, Germany). Platelet glycoprotein IIb was purchased from Dianova (Hamburg, Germany).

PAF, LTB_4 and WEB 2086 were all prepared by dissolution in ethanol in which they remained stable for several weeks at −20°. The agents were made up daily in PAGCM buffer. Calcimycin was dissolved in DMSO at 10 mM, stored at −20° and diluted to required concentrations in prewarmed PAGCM immediately before use. PMA and 4α-phorbol were prepared as stock solutions of 1 mM in DMSO and stored at −20°. Required dilutions were made daily in PAGCM, DMSO at the concentrations used in the experiments (<0·01%, v/v) neither affected the viability nor the functional responses of the cells.

The PAGCM buffer used in the experiments contained: 7·6 g/l Pipes, 6·4 g/l NaCl, 0·37 g/l KCl, 1·0 g/l glucose, 0·03 g/l HSA, 1·0 mM $MgCl_2$, 1·0 mM $CaCl_2$. Pipes medium used for Percoll solutions consisted of 25 mM piperazine-N, N′-bis-(2-ethane sulphonic acid), 110 mM NaCl, 5 mM KCl, 40 mM NaOH and 5·4 mM glucose. All buffers were adjusted to pH 7·4 at room temperature.

Preparation of eosinophils

Eosinophils were obtained from the peripheral blood of patients suffering from asthma and allergic rhinitis who were not currently undergoing steroid therapy. Heparinized venous blood was sedimented with 6% Dextran for 90 min and the buffy coat was collected and washed twice in Ca^{2+}- and Mg^{2+}-free HBSS. The cells were suspended in 2 ml Percoll (Pharmacia), density 1·065 g/ml, supplemented with 5% heat-inactivated FCS and overlayered on a 7-step discontinuous Percoll density gradient consisting of 1·5 ml, 1·000 g/ml, 2 ml 1·195 g/ml, 2 ml 1·090 g/ml, 2 ml 1·085 g/ml, 2 ml 1·080 g/ml, 2 ml 1·075 g/ml and 2 ml 1·070 g/ml in a 15-ml polyethylene tube. The gradients were centrifuged at 1600 g_{max} at 15° for 20 min and eight density fractions were collected. Contaminating platelets and erythrocytes were hypotonically lysed and the cells washed three times in ice-cold HBSS. The distribution of the cells in each density interface was determined by counting in a Neubauer haemocytometer using Kimura's stain.[27] Eosinophils were pooled from the 1·085 g/ml, 1·090 g/ml and 1·095 g/ml interfaces and had a mean purity of 91·6 ± 1·3% (n = 23). The viability of eosinophils was >98·3% using trypan blue exclusion. Density interfaces containing eosinophils were not contaminated by platelets, as assessed by electron microscopy (manuscript in preparation) and staining with the CD41b (platelet GP IIb).

Preparation of neutrophils and macrophages

Neutrophils and macrophages were obtained as by-products of the eosinophil separation procedure from the gradient interfaces 1·080 g/ml (neutrophils) and 1·075 g/ml (macrophages). The cells were washed three times in HBSS and counted. Only fractions with a purity of at least 93% were used.

Incubation procedure

Purified eosinophils, monocytes and neutrophils were each resuspended in PAGCM at a concentration of $3·3 \times 10^7$ cells/ml, prewarmed for 5 min and dispensed as 90-μl aliquots. Stimulation was initiated by addition of buffer or stimulus (10 μl) as indicated and incubated for 5 min at 37°. Reactions were terminated by the addition of 1 ml ice-cold acetone. Samples were processed for measurement of prostanoid release by combined GC/MS spectrometry and the amount of mediator synthesis per 10^6 cells was calculated.

Gas chromatography/mass spectrometry

Sample preparation and derivatization for analysis by combined capillary GC/MS were performed by modification of a previously published procedure.[28] Mass spectrometric analysis of derivatized prostanoids was performed with a Finnigan MAT TSQ-700 GC/MS/MS/DS operated as a single quadrapole mass spectrometer. Prostanoid identification and quantification were based upon retention times of endogenous species relative to tetradeuterated analogues of PGE_2, $PGF_{2\alpha}$, PGD_2, TXB_2 and 6-keto-$PGF_{1\alpha}$ employed as internal standards. Concentration of the prostanoids was calculated as the integral area under the peak relative to signal of the internal standard (Fig. 1). Limit of detection for each prostanoid was 0·1 pg.

Lactate dehydrogenase assay

The activity of lactate dehydrogenase (LDH) in the supernatant was determined by measuring the reduction of pyruvate to lactate as described elsewhere.[29] Briefly, 0·1 ml of the supernatant was added to 2·85 ml phosphate buffer (pH 7·5) containing 200 μg NADH. The resultant solution was thoroughly mixed, incubated at 25° for 15 min, and dispensed into disposable cuvettes (1-cm path length). The reaction was initiated by the addition of 100 μl sodium pyruvate (22·7 mM) and the reduction of absorbance monitored spectrophotometrically over 25 min at 340 nm at 25° versus distilled/deionized water as reference. LDH activity was calculated from the linear part of the absorbance drop as described by Wroblewski & La Due.[29]

Statistics

Negative log EC_{50} values from each concentration–response curve were derived by linear regression analysis of percentage of the maximum response for the agonist versus log concentration at concentrations immediately above and below the 50% response level. The data shown in the text and figures refer to

mean ± SEM of n independent observations. The results were analysed non-parametrically by using the Mann–Whitney U-test for paired and unpaired variates. Where more than two groups of related data were compared, statistical evaluation was assessed by Kruskal–Wallace ANOVA. Significance was accepted when $P < 0.05$.

RESULTS

Profiles of prostanoid release in unstimulated and PAF-stimulated eosinophils

Eosinophils (3.3×10^7 cells/ml) were incubated for 5 min at 37° and assayed for the presence of PGE_2, PGD_2, TXB_2, 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and 9α, 11β-PGF_2 in the cells and buffer medium by GC/MS (Fig. 1). As summarized in Fig. 2a, TXB_2 was found to be the predominant cyclo-oxygenase product biosynthesized by resting human blood eosinophils, with 8·5 ± 2·2 pg being generated by 10^6 eosinophils ($n = 7$). In contrast, 17–34 times smaller amounts of PGE_2 (0·24 ± 0·10 pg/10^6 cells), PGD_2 (0·51 ± 0·20 pg/10^6 cells) and $PGF_{2\alpha}$ (0·34 ± 0·10 pg/10^6 cells) were produced under unstimulated conditions by blood eosinophils. 6-keto-$PGF_{1\alpha}$ and 9α, 11β-PGF_2 were not present in detectable quantities (i.e. < 0·01 pg/10^6 cells).

In preliminary experiments, we established that PAF-induced prostanoid generation reached a plateau 2–3 min post-challenge (data not shown). Thus, in all subsequent experiments eosinophils were incubated for 5 min. The cumulative data of seven independent experiments are shown in Fig. 2b. PAF (1 μM) increased the generation of the cyclo-oxygenase products PGE_2 (5·8-fold), PGD_2 (twofold), $PGF_{2\alpha}$ (twofold) and TXB_2 (3·3-fold) over basal prostanoid levels (Fig. 1). Portions of the increased levels of PGE_2, PGD_2 and $PGF_{2\alpha}$ may result, in part, from hydrolysis of PGH_2 before enzymatic transformation to TXA_2. Again, 6-keto-$PGF_{1\alpha}$ and 9α, 11β-PGF_2 did not reach detectable levels. Preincubation with the competitive PAF receptor antagonist, WEB 2086, at 1 μM for 5 min resulted in a 62·3 ± 11·3% ($n = 3$) inhibition of PAF-induced TXB_2 generation and abolished the generation of other prostanoids. WEB 2086 alone had no effect on spontaneous prostanoid levels.

Concentration–response curve

Since the major cyclo-oxygenase product generated by human eosinophils in response to PAF was TXB_2, this prostanoid was chosen as a marker to characterize the concentration–response curve. Figure 3 shows the non-cumulative effect of PAF on the generation of TXB_2. PAF (0·1 nM to 10 μM) stimulated the synthesis of TXB_2 in a concentration-dependent manner with an EC_{50} value of 11·9 ± 2·2 nM, $n = 5$). Maximal generation of TXB_2 was observed with 1 μM PAF. PAF at 10 μM was significantly ($P < 0.001$) less effective at stimulating TXB_2 biosynthesis. Lyso-PAF, the inactive precursor and metabolite of PAF, at concentrations up to 10 μM, was ineffective at stimulating prostanoid generation in human eosinophils.

To rule out whether prostanoid generation was a consequence of some cytotoxic property of PAF, LDH activity was measured in the supernatant of control and PAF (1 μM)-stimulated eosinophil samples. LDH activity released from eosinophils did not exceed 5% of total enzyme activity and was not significantly different ($P > 0.05$, Mann–Whitney U-test) in the supernatants of control and PAF-stimulated cells.

Prostanoid generation versus release

In order to evaluate the amount of synthesized prostanoids being released from the cells, stimulated eosinophils were

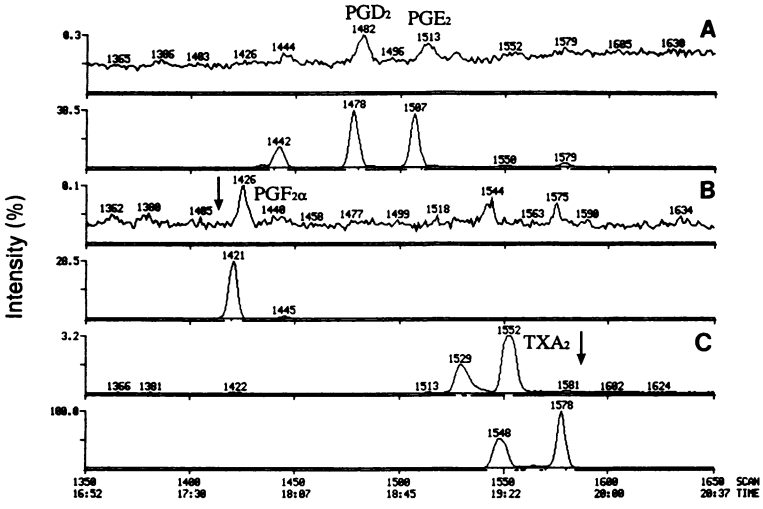

Figure 1. Capillary gas chromatography-negative ion mass spectrometry of prostanoids generated by human eosinophils. Fragment ions were monitored simultaneously at six different masses: m/z 524 (characteristic of PGD_2 and PGE_2); m/z 528 (derived from $^2H_4$ PGE_2; panel A), m/z 569 (common ion generated from 9α, 11β-PGF_2 and $PGF_{2\alpha}$; panel B), m/z 573 (fragment of $^2H_4$ $PGF_{2\alpha}$; panel B), m/z 614 (fragment ion common to TXB_2 and 6-keto-$PGF_{1\alpha}$; panel C), and m/z 618 (fragment ion from $^2H_4$-6-keto-$PGF_{1\alpha}$; panel C) were monitored simultaneously. Signal intensity (ordinate) was normalized in each panel to that derived from the deuterated analogues employed as internal standards. Retention times in minutes and seconds are indicated along the abscissa. Arrows indicate the position at which 9α, 11β-PGF_2 (1411) and 6-keto-$PGF_{1\alpha}$ (1586) would elute if they were present. The mass chromatograph is representative of the profiles obtained in nine experiments. The sensitivity of the assay was 1 pg.

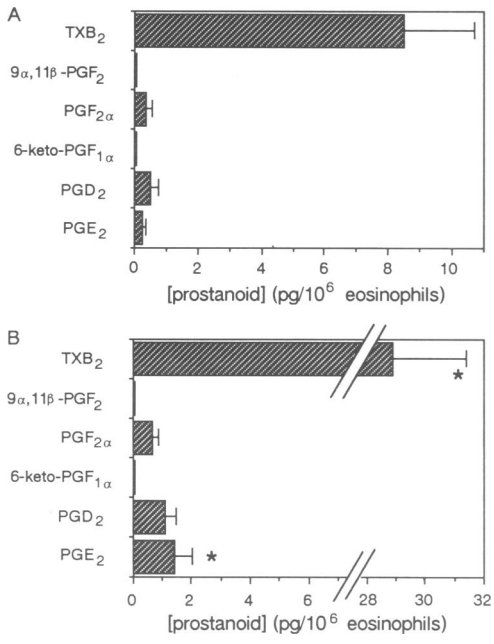

Figure 2. (A) Profile of spontaneous prostanoid generation by human blood eosinophils. Eosinophils (3×10^6 cells) suspended in PAGCM buffer were incubated at 37° in 100 μl and lysed after 5 min by the addition of 1 ml ice-cold acetone. Samples were dried under nitrogen, derivatized and analysed as described in the Materials and Methods. Histograms shown represent mean ± SEM of seven independent determinations. (B) Effect of PAF on prostanoid generation by human blood eosinophils. Eosinophils (3×10^6) were stimulated with PAF (1 μM) for 5 min at 37°. Reactions were stopped and samples processed for measurement of prostanoid biosynthesis as described in the Materials and Methods section. Data shown represent mean ± SEM obtained from seven independent experiments. * A significant increase over respective control values at $P < 0.05$.

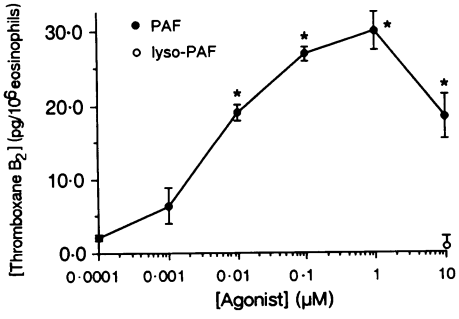

Figure 3. Concentration–response curve of PAF-induced TXB_2 generation by human blood eosinophils. Purified cells suspended in PAGCM buffer were allowed to equilibrate for 5 min at 37° and then stimulated with increasing concentrations of PAF (0·1 nM to 10 μM) for 5 min in buffer. Samples were processed as described in Fig. 1. Each data point represents the mean ± SEM of five experiments. * A significant increase over respective control values at $P < 0.05$.

Table 1. TXB_2 biosynthesis and release by human blood eosinophils in pg/10^6 eosinophils

Stimulus	Supernatant	Pellet
Control (none)	6·2 ± 1·4	ND
Calcimycin (1 μM)	43·9 ± 6·7	2·1 ± 0·6
PAF (1 μM)	22·9 ± 4·8	0·9 ± 0·2
FMLP (1 μM)	13·0 ± 1·6	ND

Prostanoid generation versus release. Eosinophils were stimulated for 5 min at 37° with calcimycin (1 μM), PAF (1 μM) and FMLP (1 μM). After 5 min, cells and supernatants were separated by rapid centrifugation and processed in parallel for prostanoid concentration. Data represent mean ± SEM of three independent experiments.
ND, not detectable.

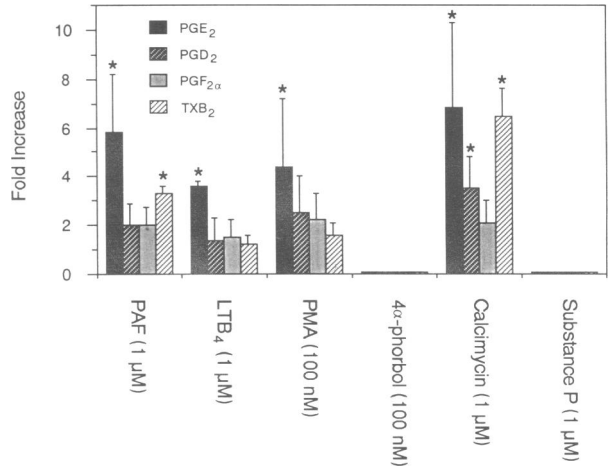

Figure 4. Comparison of PAF with LTB_4, PMA, 4α-phorbol, calcimycin and substance P on prostanoid biosynthesis by human eosinophils. Purified eosinophils were stimulated with different agonists at concentrations indicated, and the samples were treated as described in the Materials and Methods section. Data represent mean ± SEM obtained from seven independent experiments. * A significant increase over respective control values at $P < 0.05$.

centrifuged for 30 seconds at 4000 g_{max} for 30 seconds. Supernatant and cell pellet were analysed separately for prostanoids in three independent experiments. As shown in Table 1 for TXB_2, more than 93% of the total amount of prostanoids generated spontaneously or after challenge with calcimycin (1 μM), PAF (1 μM) and FMLP (1 μM) were released into the supernatant.

Effect of ibuprofen

Pretreatment of the eosinophils for 5 min with ibuprofen (8 μM), an irreversible cyclo-oxygenase inhibitor, abolished both the basal and PAF-stimulated prostanoid release (data not shown). This finding is consistent with the hypothesis that prostanoids detected in human eosinophils are derived *de novo* from cyclo-oxygenase metabolism of arachidonic acid in these cells.

Table 2. Prostanoid biosynthesis by blood neutrophils, monocytes, and eosinophils in resting and activated cells stimulated with 1 μM PAF (pg/10^6 cells)

	Neutrophils ($n=6$)		Monocytes ($n=5$)		Eosinophils ($n=5$)	
	Resting	Activated	Resting	Activated	Resting	Activated
TXB_2	0·8±0·1	1·0±0·1	1·1±0·2	1·6±0·5	8·4±2·3	21·1±5·8
9α, 11β-PGF_2	0·3±0·0	1·1±0·2	ND	0·4±0·6	ND	ND
$PGF_{2\alpha}$	0·1±0·0	0·8±0·2	ND	ND	0·3±0·1	0·8±0·2
6-keto-$PGF_{1\alpha}$	0·1±0·0	0·1±0·0	ND	0·2±0·0	ND	ND
PGD_2	ND	0·5±0·2	ND	0·3±0·0	0·5±0·3	1·3±0·4
PGE_2	ND	1·5±0·4	ND	0·8±0·0	0·3±0·1	1·4±0·6

PAF-induced formation of cyclo-oxygenase products by human peripheral blood monocytes, neutrophils and eosinophils. Data represent mean±SEM obtained from *n* independent experiments.

ND, not detectable.

Comparison with other stimuli

In order to evaluate the relative potency of PAF on eosinophils, we compared its effects with that of optimal concentrations of LTB_4 (1 μM, as determined in preliminary experiments), calcimycin (1 μM) and the phorbol ester PMA (100 nM). The results shown in Fig. 4 demonstrate that LTB_4, PMA and calcimycin also induce the generation of prostanoids, whereas the tachykinin substance P, and 4α-phorbol, a non-tumour-promoting phorbol, were inactive at 1 μM and 100 nM, respectively. On a molar basis (1 μM), PAF was approximately twice as potent at generating TXB_2 as the protein kinase C activator, PMA (13·4±3·7 pg×10^6/cells, $n=4$), and three times more potent than LTB_4 (9·9±3·4 pg×10^6/cells, $n=5$). In contrast, calcimycin, a potent inducer of arachidonic acid metabolism, promoted the generation of approximately twice the amount of the TXB_2 per 10^6 eosinophils (50·8±9·3 pg×10^6/cells, $n=7$) as PAF.

Comparison with other leucocytes

In order to compare the spectrum of prostanoid synthesis by eosinophils with other leucocytes, density interfaces 1·075 g/ml and 1·080 g/ml containing highly pure (>93%) populations of macrophages and neutrophils, respectively, were analysed for the generation of cyclo-oxygenase products. As for eosinophils, stimulation with PAF (1 μM) results in the synthesis of prostanoids by both cell types (Table 2). However, the amount of mediators produced by neutrophils ($n=6$) and macrophages ($n=5$) differed significantly from those generated by eosinophils. In addition, both macrophages and neutrophils synthesized detectable amounts of 9α, 11β-PGF_2.

DISCUSSION

The present study demonstrates that PAF triggers the generation and release of PGE_2, PGD_2, TXB_2 and $PGF_{2\alpha}$ from human blood eosinophils obtained from allergic asthmatic subjects, with TXB_2 being the predominant cyclo-oxygenase product. This effect of PAF was rapid, selective, concentration-dependent and non-cytotoxic. On an equimolar basis, PAF was more potent than LTB_4 and PMA but less effective than calcimycin, while the neuropeptide substance P did not cause a detectable synthesis of prostanoids. In contrast to other inflammatory cells, significant concentrations of 6-keto-$PGF_{1\alpha}$ and 9α, 11β-PGF_2 were not detected in eosinophils. These results confirm and extend previous findings that human blood,[7,30] human peritoneal[8] and guinea-pig eosinophils[26] produce significant amounts of PGE and TXB_2.

Several products of the cyclo-oxygenase pathway have been implicated in broncho-constriction and bronchial hyper-responsiveness, characteristic of asthma. For instance, PGD_2, TXB_2 and $PGF_{2\alpha}$ have been shown to stimulate human airway smooth muscle contraction *in vitro*.[31] In addition, both PGD_2 and $PGF_{2\alpha}$ induce an increase in bronchial responsiveness to histamine and cholinergic agents in man.[32] Furthermore, TXA_2 has been implicated in bronchial hyper-responsiveness in various animal species, since the thromboxane synthetase inhibitor OKY-046 prevented the increased bronchial reactivity caused by both PAF[33] and allergen.[34] In asthma, bronchial hyperresponsiveness to histamine was suppressed by the cyclooxygenase inhibitor indomethacin[35,36] while oral administration of OKY-046 reduced bronchial hyper-responsiveness to acetylcholine.[37] Finally, prostaglandins have also been implicated in the modulation of cholinergic excitatory neuroeffector transmission in canine and human airway smooth muscle tissue.[38]

In recent years several reports have shown that PAF may be one of the most potent physiological stimuli for eosinophils. For instance, PAF induces eosinophil chemotaxis *in vitro* and has a greater effect on eosinophils than on neutrophils.[17,18] PAF is also a chemoattractant *in vivo* and causes eosinophil infiltration into the skin of atopic individuals,[20] and into lungs of primates following both local and systemic administration.[21,39-41] PAF also increased the adherence of eosinophils to cultured human umbilical vein endothelial cells.[19] PAF enhances eosinophil-mediated killing of schistosomula opsonized with immune serum[42] and increases the capacity of normodense eosinophils to bind IgE.[43] Furthermore, PAF induces a shift in density from normodense to hypodense eosinophils *in vitro*.[25] It also promotes the secretion of leukotriene C_4,[44] the release of eosinophil granule proteins,[12,22-24] and stimulates release of superoxide anions by human and guinea-pig eosinophils.[12] Finally, PAF has recently been shown to promote the synthesis and release of TXB_2 and E-series prostaglandins from guinea-pig eosino-

phils.[26] In addition to these findings, the results presented indicate that TXB_2 biosynthesis represents the major fatty acid cyclo-oxygenase product generated from endogenous arachidonic acid in PAF-stimulated human blood eosinophils.

PAF has many properties that are relevant to respiratory symptoms of asthma, such as broncho-constriction,[45,46] airway microvascular leakage[16,47,48] and a sustained increase in bronchial reactivity.[13,16,39,41] However, its mode of action is as yet not clear. The broncho-constrictor effect of intravenous PAF, for instance, is dependent on circulating platelets, suggesting that PAF acts indirectly through the release of spasmogens from platelets such as serotonin, histamine, or thromboxane A_2. However, the nature of these platelet-derived spasmogens remains uncertain, since neither anti-histamines, serotonin antagonists, nor indomethacin inhibit the broncho-constrictor response.[45] In contrast, the broncho-constrictor effect of aerosolized PAF is not inhibited by platelet depletion but is reduced by cyclo-oxygenase inhibitors in the guinea-pig[49] and by thromboxane synthetase inhibition in dogs.[34] Since PAF is a potent chemoattractant for eosinophils and promotes selective tissue accumulation of eosinophils, the effect of PAF may be mediated indirectly through the release of eicosanoids such as TXB_2 or leukotrienes[44] by eosinophils and other inflammatory cells.[50]

Probably the most interesting property of PAF is its unique ability to induce a sustained increase in bronchial responsiveness both in humans[13,34] and animals.[33,51,52] In normal and in asthmatic subjects, inhaled PAF causes a delayed increase of bronchial responsiveness within 3 days and persists for up to 4 weeks.[13] The observation that PAF is rapidly inactivated *in vitro*[52] raises the question as to whether PAF produces its effects on airway responsiveness via a series of secondary events.[50] Since PAF is a selective chemoattractant for eosinophils *in vitro*,[17] in primate lung[39] and human skin,[20] it may exert its effects on the airways through the recruitment and activation of eosinophils. This possibility is supported by the observation that eosinophil tissue accumulation, unlike neutrophils, occurs at the time of the late response and can persist for several days. Indeed it has been demonstrated that bronchial eosinophil infiltration is related at least in part to bronchial hyperresponsiveness.[1,2]

Eosinophils have been implicated as potent effector cells in the pathogenesis of chronic asthma.[1] Their basic granule constituents, such as EPO, MBP and ECP, damage respiratory epithelium,[1-6] which is a characteristic pathological feature in asthma.[1,3] In addition, eosinophil basic proteins stimulate mediator and enzyme release from basophils, mast cells, as well as from neutrophils, and induce complement activation.[1,54] Furthermore, a recent study by Gundel *et al.* suggests that MBP may directly induce an increase in airway responsiveness to inhaled methacholine.[55] Finally, eosinophils are one of the richest cellular sources of PAF.[10] The data presented here demonstrate that eosinophils may, in addition, contribute to the pathogenesis in asthma through the biosynthesis of significant quantities of TXB_2 and other prostanoids such as PGD_2.

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