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The involvement of platelet activating factor in endotoxin-induced pulmonary platelet recruitment in the guinea-pig

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1 Exposure of conscious guinea-pigs to an aerosol of endotoxin (25–100 $\mu g\ ml^{-1}$) resulted in a dose-related, progressive accumulation of platelets in the thoracic region. Accumulation of ^{111}indium oxine labelled erythrocytes was not observed following exposure to an aerosol of endotoxin (50 $\mu g\ ml^{-1}$).

2 Pretreatment of guinea-pigs with the selective platelet activating factor (Paf)-antagonists. CV-3988 or brotizolam resulted in a dose-related inhibition of endotoxin-induced pulmonary platelet recruitment. Pretreatment of guinea-pigs with the selective Paf-antagonist BN 52021 resulted in significant inhibition of endotoxin-induced pulmonary platelet recruitment, although the effects of BN 52021 were not dose-related.

3 Pretreatment of guinea-pigs with indomethacin at doses known to inhibit cyclo-oxygenase did not inhibit endotoxin-induced pulmonary platelet recruitment, whereas higher doses of indomethacin produced a reduction in platelet recruitment in the lung.

4 Pretreatment of guinea-pigs with the anticoagulant heparin and the prostacyclin analogue ZK 36374 inhibited endotoxin-induced platelet recruitment.

5 These observations suggest that endotoxin-induced pulmonary platelet recruitment in the guinea-pig is secondary to the release of platelet activating factor, but not to cyclo-oxygenase products of arachidonic acid and may also involve activation of the coagulation cascade.

Introduction

The exposure of experimental animals and man to aerosols of bacterial endotoxins (lipopolysaccharide, LPS) or dusts contamined with LPS (such as cotton dust) elicits bronchoconstriction. A prolonged exposure may cause subjective symptoms of chest 'tightness' (byssinosis). Furthermore, increased bronchial reactivity to spasmogens such as methacholine has been demonstrated in one study (Haglind *et al.*, 1983). Such respiratory changes are associated with inflammatory events including the appearance of neutrophils on the surface of the respiratory epithelium (Merchant *et al.*, 1973), a decrease in the number of circulating platelets (Bomski *et al.*, 1971) and a subsequent accumulation of platelets in the pulmonary capillaries (Lantz *et al.*, 1985).

In experimental animals, inhalation of LPS is followed by the activation of alveolar macrophages with consequent release of chemotactic factors and recruitment of platelets and neutrophils into the airways (Rylander & Snella, 1983). In particular, inhalation of LPS has been shown to initiate the production of the ether-linked phospholipid platelet activating factor (1-*o*-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine) (Paf) (Rylander & Beijer, 1987), a substance known to activate a variety of cells including platelets and neutrophils (Morley *et al.*, 1984). Additionally, Paf has potent effects on the respiratory system including the induction of acute bronchoconstriction (Vargaftig *et al.*, 1982) and a non-selective increase in airway reactivity in both experimental animals (Mazzoni *et al.*, 1985; Chung *et al.*, 1986; Barnes *et al.*, 1987) and man (Cuss *et al.*, 1986). Exposure of the lungs to Paf also results in a long lasting inflammatory response (Camussi *et al.*, 1983) and a pulmonary recruitment of platelets (Robertson & Page, 1987).

Since Paf has been shown to contribute to endotoxin-induced pathology in other circumstances (Terashita *et al.*, 1985; Handley *et al.*, 1986; Lagente *et al.*, 1987) we have investigated the contribution of Paf

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to endotoxin-induced pulmonary platelet recruitment. Pulmonary recruitment of ^{111}indium oxine labelled platelets into the thoracic area was monitored with external scintillation detectors after inhalation of LPS and the effect of various Paf antagonists and anti-platelet drugs investigated for their ability to influence this phenomenon. Some of these results have been presented to the British Pharmacological Society (Beijer *et al.*, 1987).

Methods

Dunkin-Hartley guinea-pigs of either sex (300–500 g body weight) were used throughout these experiments.

Exposure to endotoxin (LPS)

LPS was dissolved in distilled water and the solution placed in a Collison atomizer. The resulting aerosol contained no particles detectable with a Royco optical particle size analyser (cut off size 0.5 μm), indicating that all particles were in the respirable size range. Conscious guinea-pigs were exposed for 40 min in a continuous flow exposure chamber to aerosols derived from solutions containing 25, 50 and 100 μg LPS. Control animals were exposed to an aerosol of distilled water for 40 min. For drug studies, guinea-pigs were exposed to 50 μg LPS following prior treatment with either drug or solvent. All drugs were administered i.v. 10 min before aerosol challenge except BN 52021 which was administered i.p. 1 h before to minimize the effects of the solvent, dimethyl sulphoxide (DMSO).

Measurement of pulmonary platelet and erythrocyte recruitment

Platelet and erythrocyte accumulation was measured in the thoracic region by a slight modification of the technique previously described (Page *et al.*, 1982). Guinea-pig platelets or erythrocytes were isolated from citrated (3.8%) arterial blood by cardiac puncture. Platelets or erythrocytes were washed with modified Tyrode solution containing prostaglandin E_1 (PGE_1; 300 ng ml^{-1}) and radiolabelled with 25–50 μCi ^{111}indium oxine. Radiolabelled platelets or erythrocytes were administered i.v. via a foot vein into non-heparinized recipients and the animals, when conscious exposed to the aerosol 1 h later.

Immediately after aerosol exposure, animals were anaesthetized with urethane (7 ml kg^{-1} of a 25% w/v solution i.p.). Radioactivity was monitored continuously in the thoracic region of the animal with an external collimated 1" crystal scintillation detector (GP6, Nuclear Enterprises Ltd., Edinburgh), linked to an automated isotope monitoring system comprising a multi-channel spectrometer (Nuclear Enterprises Ltd) interfaced to a microcomputer (IBM PC, AIMS 8000; Mumed Ltd). The detector was located as close to the animal as possible without restricting respiration.

The number of radioactive counts was registered in the thoracic area every 10 min for up to 2.5 h following cessation of the aerosol exposure. Platelet or erythrocyte accumulation into the thoracic region was expressed as a percentage difference between the initial measurement and all subsequent measurements. The effect of treatments was compared by calculating the mean area under the curve values for the entire time course using computer assisted planimetry.

Statistical analysis

Data are presented as mean ± s.e.mean of n observations. Significance was assessed by Student's t test and a P value of less than 0.05 was considered significant.

Drugs

LPS from *Escherichia coli* 026:B6 was obtained from Difco laboratories, urethane, indomethacin and prostaglandin E_1 (PGE_1) from Sigma, BN 52021 ([3-(1,1-dimethylethyl) hexahydro-1,4,4 7B-trihydroxy-8-methyl-9H-1,7(epoxymethanol)-14, 6α H_2- cyclopenta (c)furo[2,3b] furo [3',2'3,4] cyclopenta [1,2-d] furah-5,-9,12(4H)-trione) was a gift from Dr P. Braquet, Institute Henri Beaufort, Le Plessis Robinson, France, CV-3988 ((**RS**)-2-methoxy-3-(octadecylcarbamoyloxy) propyl 2-(3-thiazolio) ethyl phosphate) was a gift from Takeda Chemicals, Japan and brotizolam a gift from Boehringer Ingelheim KG. ZK 36374 (5-[(E)-(15,55,6**R**, 7**R**)-7-hydroxy-6-/(E)-(35,4**RS**)-3-hydroxy-4-methyl-oct-1-en-6-yn-yl-bicyclo -(3,3,d)-octane-3-yldenepentanoic acid) was a gift from Schering Laboratories. In addition heparin (Paines and Byrne) and ^{111}indium oxine from Amersham International plc were used. Drugs were prepared as follows: indomethacin (0.5% Na_2CO_3), BN 52021 (2% DMSO), brotizolam (20% 2-propylene glycol), CV-3988 (distilled water), ZK 36374 and heparin (Saline).

Results

Effect of endotoxin

When animals receiving ^{111}indium oxine labelled platelets were exposed to an aerosol of distilled water the thoracic counts remained constant over the period of experimentation (Figure 1). Exposure of guinea-pigs to an aerosol of LPS (25–100 μg) induced a dose-related accumulation of radiolabelled platelets into the thoracic area evident as a progressive increase in the counts recorded per 10 min period over the experimental period (Figure 1 and Table 1). In con-

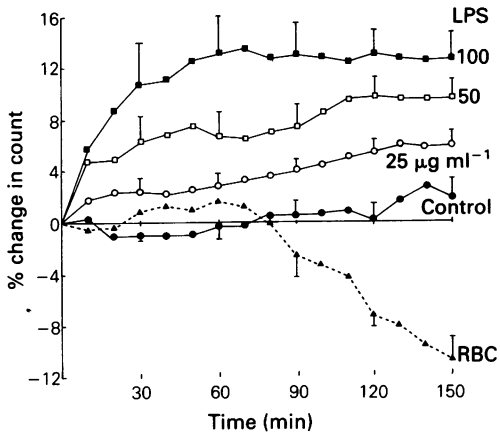

Figure 1 Time course of thoracic accumulation of ^{111}indium oxine labelled platelets following prior exposure to an aerosol of endotoxin (25–100 μg ml^{-1}; (○, □, ■) or distilled water (●). Time course of ^{111}indium oxine labelled erythrocyte accumulation following exposure to an aerosol of endotoxin (50 μg ml^{-1}, ▲) is shown for comparison. Results are expressed as mean percentage increase in thoracic counts for groups of $n = 6$–11 animals. Vertical lines represent s.e.mean.

Table 1 Effect of endotoxin on pulmonary platelet recruitment

Endotoxin (μg ml^{-1})	*Area under curve* (cm^2)	n	P	*Statistical level*
0(H_2O)	4.1 ± 1.0	6	—	—
25	10.8 ± 2.5	9	0.014	S
50	20.9 ± 4.5	11	0.002	S
100	34.6 ± 7.8	7	0.001	S

Results are presented as mean ± s.e.mean; S = Significant. against0 values.

trast, in animals receiving ^{111}indium oxine labelled erythrocytes, no significant accumulation of radioactivity was detected in the thoracic area following exposure to an aerosol of 50 μg ml^{-1} LPS (Figure 1).

Effect of Paf antagonists

In animals pretreated with the Paf antagonist BN 52021 (2 and 5 mg kg^{-1}, i.p.) a significant inhibition of LPS-induced pulmonary platelet recruitment was observed with both doses although the inhibition was not dose-related (Figure 2 and Table 2). In animals pretreated with the Paf antagonists CV-3988 (0.1 and 1.0 mg kg^{-1}, i.v.) (Figure 3 and Table 2) or brotizolam (0.5 and 1.0 mg kg^{-1}, i.v.) (Figure 4 and Table 2), there was a dose-related inhibition of endotoxin-induced pulmonary platelet recruitment. The respective solvents produced no significant effect,

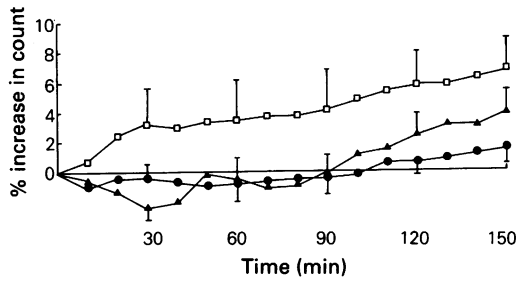

Figure 2 Time course of thoracic accumulation of ^{111}indium oxine labelled platelets following prior exposure to an aerosol of endotoxin (50 μg ml^{-1}) in the presence of dimethyl sulphoxide (□, $n = 5$) or BN 52021 (2 mg kg^{-1}, i.p. $n = 7$, ▲) or (5 mg kg^{-1} i.p. $n = 9$ ●). Results are expressed as mean percentage increase in thoracic counts. Vertical lines represent s.e.mean.

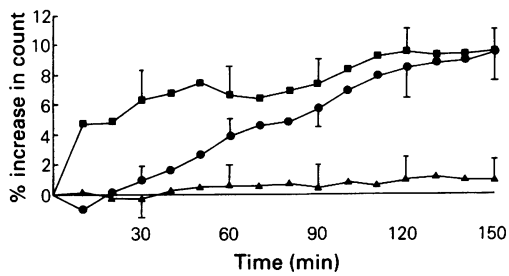

Figure 3 Time course of thoracic accumulation of ^{111}indium oxine labelled platelets following prior exposure to an aerosol of endotoxin (50 μg ml^{-1}) in the presence of saline ($n = 15$, ■) or CV-3988 (0.1 mg kg^{-1}, i.v. $n = 5$, ●) and (1.0 mg kg^{-1}, $n = 12$, ▲). Results are expressed as mean percentage increase in thoracic counts. Vertical lines represent s.e.mean.

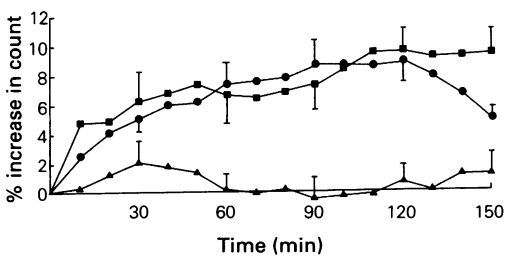

Figure 4 Time course of thoracic accumulation of ^{111}indium oxine labelled platelets following prior exposure to an aerosol of endotoxin (50 μg ml^{-1}) in the presence of 2-propylene glycol ($n = 15$, ■) or brotizolam (0.5 mg kg^{-1}, i.v. $n = 5$, ●) and (1.0 mg kg^{-1}, i.v. $n = 5$, ▲). Results are expressed as mean percentage increase in thoracic counts; Vertical lines represent s.e.mean.

Table 2 Effect of Paf antagonists and anti-platelet drugs on endotoxin-induced pulmonary platelet recruitment

Endotoxin (50 μg ml^{-1}) *plus treatment*		*Area under curve* (cm^2)	n	p	*Statistical level*
—		20.9 ± 4.5	11	—	—
CV-3988	0.1 mg kg^{-1}	12.4 ± 5.0	5	0.114	NS
CV-3988	1.0 mg kg^{-1}	2.5 ± 3.5	12	0.002	S
Brotizolam	0.5 mg kg^{-1}	19.7 ± 3.4	5	0.465	NS
Brotizolam	1.0 mg kg^{-1}	1.9 ± 3.2	5	0.002	S
DMSO	2%	11.1 ± 7.0	5	0.136	NS
BN 52021	2.0 mg kg^{-1}	0.8 ± 3.5	7	0.002	S
BN 52021	5.0 mg kg^{-1}	0.7 ± 2.5	9	0.001	S
Indomethacin	1.0 mg kg^{-1}	22.4 ± 4.7	5	0.444	NS
Indomethacin	5.0 mg kg^{-1}	3.4 ± 4.0	7	0.005	S
Heparin	100 u kg^{-1}	1.0 ± 6.8	5	0.020	S
ZK 36374	25 μg kg^{-1}	3.3 ± 4.0	7	0.005	S

Results are presented as mean ± s.e.mean; NS = not significant; S = Significant.

except wih DMSO where the inhibition obtained was not as marked as with BN 52021 (Table 2).

Effect of indomethacin

In animals pretreated with indomethacin (5 mg kg^{-1}, i.v.), there was significant inhibition of pulmonary platelet recruitment induced by LPS (50 μg) (Figure 5 and Table 2). In contrast, animals pretreated with indomethacin (1 mg kg^{-1}) or the diluent produced no significant inhibition of LPS-induced pulmonary platelet recruitment (Figure 5 and Table 2).

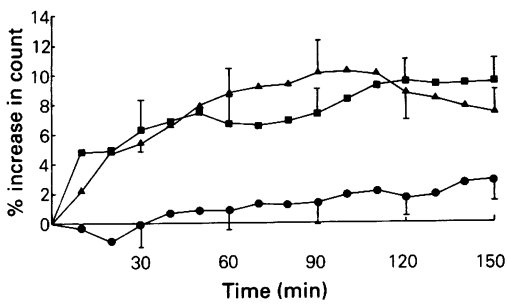

Figure 5 Time course of thoracic accumulation of ^{111}indium oxine labelled platelets following prior exposure to an aerosol of endotoxin (50 μg ml^{-1}) in the presence of Na_2HCO_3 (n = 15, ■); indomethacin (1 mg kg i.v., n = 5, ▲) or indomethacin (5 mg kg i.v., n = 7, ●). Results are expressed as mean percentage increase in thoracic counts. Vertical lines represent s.e.mean.

Effect of heparin and ZK 36374

In animals pretreated with heparin (100 units kg^{-1}, i.v.) or ZK 36374 (25 μg kg^{-1}, i.v.) there was a significant inhibition of pulmonary platelet recruitment induced by LPS (50 μg) (Figure 6 and Table 2).

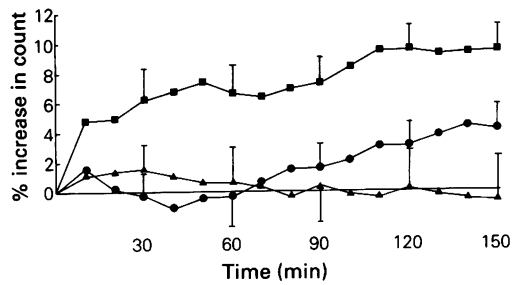

Figure 6 Time course of thoracic accumulation of ^{111}indium oxine labelled platelets following prior exposure to an aerosol of endotoxin (50 μg ml^{-1}, n = 15, ■) in the presence of ZK 36374 (25 μg kg^{-1}, n = 7, ●) or heparin (100 μ kg^{-1}, n = 5, ▲). Results are expressed as mean percentage increase in thoracic counts. Vertical lines represent s.e.mean.

Discussion

These experiments demonstrate that exposure of guinea-pigs to an aerosol of LPS leads to a progressive accumulation of platelets into the thoracic region. These data are complementary to the observations that have identified platelets in the pulmonary circulation (Lantz *et al.*, 1985) and bronchoalveolar lavage fluid of guinea-pigs exposed to LPS aerosols (Beijer & Rylander, unpublished data). This LPS-induced pulmonary platelet recruitment would appear to be secondary to the generation of Paf since the platelet accumulation can be abrogated by three different classes of selective Paf antagonists; the Paf analogue, CV-3988 (Terashita *et al.*, 1983), the gingkolide BN 52021 (Nunez *et al.*, 1986) and the triazolobenzodiazepine, brotizolam (Casals-Stenzel & Weber,

1987). These observations also complement previous experiments demonstrating that i.v. Paf will induce an accumulation of platelets into the lungs of guinea-pigs (Robertson & Page, 1987) and that platelets accumulate in the lungs of primates following intratracheal administration of Paf into the lungs (Arnoux *et al.*, 1985). The pulmonary source of Paf following exposure to LPS is unknown but it could be the alveolar macrophages since LPS exposure in the guinea-pig induced Paf formation in these cells (Rylander & Beijer, 1987).

LPS is able to activate a number of biological systems including the coagulation system and the complement cascade (Brown & Lachman, 1973). Thus, the ability of heparin to inhibit LPS-induced platelet accumulation may relate to an inhibitory effect on the coagulation system rather than a direct effect of platelet activation. However, LPS will continue to induce inflammatory changes in animals depleted of complement (Rylander & Snella, 1983; Flick *et al.*, 1986). Alternatively, the mechanism of action of heparin in inhibiting LPS-induced platelet recruitment may be due to other effects of this molecule, for example, on the vascular endothelium (Paul *et al.*, 1984), or as a free radical scavenger (McClain & Brestel, 1982). The prostacyclin analogue, ZK 36374, is known to inhibit platelet activation *in vivo* in other circumstances (Van der Giessen *et al.*, 1984) and its action in these experiments suggests that our observations are consistent with the concept that the increase in thoracic platelet numbers reflects platelet activation rather than changes in pulmonary blood flow, a conclusion confirmed by the failure of LPS to induce significant accumulation of radiolabelled erythrocytes.

Indomethacin at a dose shown to inhibit cyclo-oxygenase completely and the subsequent generation of thromboxane A_2 in the guinea-pig (Mallarkey & Smith, 1985) did not significantly inhibit LPS-induced platelet recruitment, suggesting that cyclo-oxygenase metabolites of arachidonic acid are unlikely to play a role in this phenomenon. The ability of indomethacin to inhibit LPS-induced pulmonary platelet recruitment at five fold higher concentrations is therefore unlikely to reflect the contribution of cyclo-oxygenase derived metabolites of arachidonic acid but could be secondary to other properties of this molecule such as inhibition of phospholipase A_2 (Blackwell & Flower, 1983), a necessary enzyme in the biosynthesis of Paf in inflammatory cells (Jouvain-Marche *et al.*, 1984) or to inhibition of the formation of oxygen free radicals. The latter property of indomethacin is certainly a possible explanation for the inhibitory effect of indomethacin at higher concentrations since DMSO was also observed to have a significant effect on endotoxin-induced platelet recruitment, a drug well known for its ability to act as a free radical scavenger (Ganote *et al.*, 1982).

The significance of platelet recruitment to the lungs following exposure to LPS is unknown but it is of interest that platelet recruitment following i.v. Paf is associated with an increased reactivity of the airways (Robertson & Page, 1987). Bronchial hyperreactivity is known to follow exposure of animals (Pauwels *et al.*, 1986; Hutchinson *et al.*, 1983) or man (Haglind *et al.*, 1983) to LPS and it is therefore plausible that the platelets accumulating in the lung contribute to the induction of airway hyperreactivity. Additionally, activated platelets are known to release a number of chemotactic factors for other inflammatory cell types such as neutrophils and mononuclear cells (Duel *et al.*, 1982), both shown to be found in the lung 24 h after exposure to endotoxin (Rylander & Snella, 1983). It is possible therefore that the platelet recruitment observed is the forerunner of more sustained inflammatory changes in the lung.

As platelet activation in man is known to accompany exposure to cotton dusts (Bomski *et al.*, 1971), the present observations suggest that the role of the platelet and Paf in the aetiology of byssinosis should be further investigated.

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References

ARNOUX, B., DENJEAN, A., PAGE, C.P., MORLEY, J. & BENVENISTE, J. (1985). Pulmonary effects of platelet activating factor in a primate are inhibited by ketotifen. *Am. Rev. Resp. Dis.*, **131**, A2.

BARNES, P.J., GRANDORDY, B., PAGE, C.P., RHODEN, K. & ROBERTSON, D. (1987). The effects of platelet activating factor on pulmonary beta-adrenoceptor function. *Br. J. Pharmacol.*, **90**, 709–715.

BEIJER, L., BOTTING, J., OYEKAN, A.O., PAGE, C.P. & RYLANDER, R. (1987). The involvement of platelet activating factor in endotoxin-induced pulmonary platelet recruitment in the guinea-pig. *Br. J. Pharmacol.*, **90**, 118P.

BLACKWELL, G.J. & FLOWER, R.J. (1983). Inhibition of phospholipase. *Br. Med. Bull.*, **39**, 260–264.

BOMSKI, H., OTAWSKI, J. & BOMSKA, H. (1971). Hamatologische und serologische Untersuchungen bei Byssinosesgefahrdeten Arbeitern. *Int. Arch. Arbeitsmed.*, **27**, 309–323.

BROWN, D.L. & LACHMAN, D.J. (1973). The behaviour of complement and platelets in lethal endotoxin shock in rabbits. *Int. Arch. Allergy appl. Immunol.*, **45**, 193–205.

CAMUSSI, G., PAWLOWSKI, J., TETTA, C., ROFFINELLO, C., ALBERTON, M., BRENTJENS, J. & ANDRES, G. (1983). Acute lung inflammation induced in the rabbit by local instillation of 1-o-octadeyl-2-acetyl-sn-glyceryl-3-phosphorylcholine or of native platelet activating factor. *Am. J. Pathol.*, **112,** 78–88.

CASALS-STENZEL, J. & WEBER, K.H. (1987). Triazolobenzodiazepines: dissociation of their Paf (platelet activating factor) antagonistic and CNS activity. *Br. J. Pharmacol.*, **90,** 139–146.

CHUNG, K.F., AIZAWA, H., LEIKAUF, G.B., UEKI, I.F., EVANS, T.W. & NADEL, J. (1986). Airway hyperresponsiveness induced by platelet activating factor: Role of thromboxane generation. *J. Pharmacol. Exp. Ther.*, **236,** 580–584.

CUSS, F.M., DIXON, C.M. & BARNES, P.J. (1986). Effects of inhaled platelet activating factor on pulmonary function and bronchial responsiveness in man. *Lancet*, **ii,** 189–192.

DUEL, T.F., SENIOR, R.M., HUANG, J.S. & GRIFFIN, G.L. (1982). Chemotaxis of monocytes and neutrophils to platelet-derived growth factor. *J. Clin. Invest.*, **69,** 1046–1049.

FLICK, M.R., HORN, J.K., HOEFFEL, J.M. & GOLDSTEIN, I.R. (1986). Reduction of total hemolytic complement activity with Naja haje Cobra venom factor does not prevent Endotoxin-induced lung injury in sheep. *Am. Rev. Resp. Dis.*, **133,** 62–67.

GANOTE, C.E., SIMMS, M. & SAFAVI, S. (1982). Effects of dimethylsulfoxide (DMSO) on the oxygen paradox in perfused rat hearts. *Am. J. Pathol.*, **109,** 270–276.

HAGLIND, P., BAKE, B. & BELIN, L. (1983). Is mild byssinosis associated with small airways disease. *Eur. J. Resp. Dis.*, **64,** 449–459.

HANDLEY, D.A., VAN VALEN, R.G., MELDEN, M.K., FLURY, S., LEE, M.L. & SAUNDERS, R.N. (1986). Inhibition and reversal of endotoxin-induced, aggregated IgG-induced and PAF-induced hypotension in the rat by SR1 63-072, a PAF receptor antagonist. *Immunopharmacol.*, **12,** 11–16.

HUTCHINSON, A.A., HINSON, J.M., BRIGHAM, K.L. & SNAPPER, J.R. (1983). Effect of endotoxin on airway responsiveness to aerosol histamine in the sheep. *J. Appl. Physiol.*, **54,** 1463–1468.

JOUVAIN-MARCHE, E., NINIO, E., BEURAIN, G. & BENVENISTE, J. (1984). Biosynthesis of Paf-acether (platelet activating factor) VII. Precursors of Paf-acether and acetyl transferase activity in human leukocytes. *J. Immunol.*, **133,** 892–898.

LAGENTE, V., LIDBURY, P., STEEL, G., VARGAFTIG, B.B., WALLACE, J.L. & WHITTLE, B.J.R. (1987). Role of PAF as a mediator of endotoxin-induced gastrointestinal damage. *Br. J. Pharmacol.*, **90,** 114P.

LANTZ, R.C., BIRCH, K., HINTON, D.E. & BURRELL, R. (1985). Morphometric changes of the lung induced by inhaled bacterial endotoxin. *Exp. Mol. Pathol.*, **43,** 305–320.

MALLARKEY, G. & SMITH, G.M. (1985). A comparative study of the involvement of the prostaglandin H_2/thromboxane A_2 pathway in intravascular platelet aggregation in guinea-pigs and rats. *Br. J. Pharmacol.*, **84,** 425–430

MAZZONI, L., MORLEY, J., PAGE, C.P. & SANJAR, S. (1985). Induction of hyperreactivity by platelet activating factor in the guinea-pig. *J. Physiol.*, **365,** 107P.

McCLAIN, E.J. & BRESTEL, E.P. (1982). Heparin and other sulfated polysaccharides as scavengers of superoxide. *J. Allergy Clin. Immunol.*, **69,** 93 (abstract).

MERCHANT, J.A., KUMSDEN, J.C., KILBURN, K.H., O'FALLON, W.M., UJDA, J.R., GERMIRO, V.H. & HAMILTON, J.D. (1973). An industrial study of the biological effects of cotton dust and cigarette smoke exposure. *J. Occup. Med.*, **15,** 212–221.

MORLEY, J., SANJAR, S. & PAGE, C.P. (1984). The platelet in asthma. *Lancet*, **ii,** 1142–1144.

NUNEZ, D., CHIGNARD, M., KORTH, R.E., COVEDIC, J.P., NOREL, X., SPINNEWYN, B., BRAQUET, P. & BENVENISTE, J. *et al.* (1986). Specific inhibition of Paf-acether-induced platelet activation by BN 52021 and comparison with the Paf-acether inhibitors Kadsuresone and CV-3988. *Eur. J. Pharmacol.*, **123,** 197–205.

PAGE, C.P., PAUL, W. & MORLEY, J. (1982). An in-vivo model for studying platelet aggregation and disaggregation. *Thromb. Haemostas.*, **47,** 210–213.

PAUL, W., PAGE, C.P., CUNNINGHAM, F.M. & MORLEY, J. (1984). The plasma protein extravasation response to PAF-acether is independent of platelet accumulation. *Agents & Actions.*, **15,** 80–82.

PAUWELS, R., PELKEMAN, R. & VAN DER STRAETEN, M. (1986). Airway inflammation and non-allergic bronchial responsiveness. *Eur. J. Resp. Dis.*, **68,** Supplement 144, 137–162.

ROBERTSON, D.N. & PAGE, C.P. (1987). Effect of platelet agonists on airway reactivity and intrathoracic platelet accumulation. *Br. J. Pharmacol.*, **92,** 105–111.

RYLANDER, R. & BEIJER, L. (1987). Inhalation of endotoxin stimulates alveolar macrophage production of platelet activating factor. *Am. Rev. Resp. Dis.*, **135,** 83–86.

RYLANDER, R. & SNELLA, M.C. (1983). Endotoxins and the lung: Cellular reactions and risk for disease. *Prog. Allergy*, **33,** 332–344.

TERASHITA, Z.I., TSUSHIMA, S., YOSKIOKA, Y., NOMURA, H., INADA, Y. & NISHIKAWA, K. (1983). CV-3988 – a specific antagonist of platelet activating factor (PAF). *Life Sci.*, **32,** 1975–1982.

TERASHITA, Z.I., IMUA, Y., NISHIKAWA, K. & SURINDA, S. (1985). Is platelet activating factor (PAF) a mediator of endotoxin shock? *Eur. J. Pharmacol.*, **109,** 257–261.

VAN DER GIESSEN, W.J., MOOI, W.J., RUTTEMAN, A.M., BERK, L. & VERDOUW, P. (1984). The effect of the stable protacyclin analogue ZK 36374 on experimental coronary thrombosis in the pig. *Thrombos. Res.*, **36,** 45–51.

VARGAFTIG, B.B., LEFORT, J., WAL, F., CHIGNARD, M. & MEDEIROS, M.C. (1982). Interferance of non-steroidal anti-inflammatory drugs associated to anti-histamine and anti-serotonin agents with the bronchial and platelet effects of platelet activating factor (paf-acether). *Eur. J. Pharmacol.*, **82,** 121–130.

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