IgE-dependent activation of alveolar macrophages augments neurally mediated contraction of small airways

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1 We studied the effect of immunologically activated pulmonary alveolar macrophages (PAM) on functions of canine bronchiolar smooth muscle under isometric conditions *in vitro*.

2 PAM, stimulated with monoclonal anti-dinitrophenyl (DNP) IgE antibody and DNP-human serum albumin (DNP-HSA), augmented the contractile responses of bronchioles to electrical field stimulation, whereas PAM treated with IgE antibody alone had no effect.

3 In contrast, the contractile responses to exogenously administered acetylcholine were not influenced by immunologically activated PAM.

4 The PAM-induced increase in the contractile responses to field stimulation was inhibited by pretreatment of PAM with indomethacin and by addition of the thromboxane A₂ (TxA₂) receptor antagonist SQ 29548.

5 The release of TxA₂ from PAM was increased by anti-DNP IgE and DNP-HSA, an effect that was prevented by indomethacin.

6 These results suggest that PAM may play a role in the development of antigen-induced hyperreactivity of small airways through an IgE-dependent release of TxA₂ which potentiates prejunctionally the parasympathetic component of bronchiolar smooth muscle tone.

Keywords: Asthma; allergy; cholinergic nerve; thromboxane A₂; airway hyperreactivity

Introduction

Pulmonary alveolar macrophages (PAM) reside predominantly in the alveoli and small airways on top of epithelial cells, but are also present at the air surface interface of relatively large airways (Brain *et al.*, 1984). In normal human beings as well as patients with asthma, PAM have been shown to constitute more than 90% of the cells recovered from bronchoalveolar lavage and approximately 40% of the cells recovered from lavage of isolated bronchial segments (Eschenbacher & Gravelyn, 1987; Joseph *et al.*, 1983). Because of this strategic location and their pronounced phagocytic capabilities, PAM play an important role not only in the first line of the lung defence mechanism but also in the induction of allergic reactions by encountering inhaled particles and allergens (Fels & Cohn, 1986).

Among several cells contributing individually and in concert to the development and maintenance of airway hyperresponsiveness that is a characteristic feature of asthma (Boushey *et al.*, 1980), interest has recently been focused on PAM as one of the potential candidates for the following reasons: (1) PAM possess functional Fc receptors for binding of IgE (Spiegelberg, 1984); (2) activation of PAM by IgE and specific allergens, or IgE and anti-IgE, elicits the synthesis and release of a wide variety of biochemically different substances (Rankin *et al.*, 1982; Balter *et al.*, 1988); and (3) some of these mediators have a putative role in asthmatic syndromes by modulating humoral and cellular components of airway inflammation (Henderson, 1987).

On the other hand, it has been shown that augmentation of parasympathetic components regulating airway calibre may be involved in airway hyperresponsiveness produced by allergen challenge (Gold *et al.*, 1972; Mitchell *et al.*, 1986). Therefore, we have used canine isolated bronchiolar segments, to determine whether the immunological activation of PAM alters contractility of small airways in response to cholinergic stimulation.

Methods

Preparation of pulmonary alveolar macrophages

Mongrel dogs of either sex (22–31 kg) were anaesthetized with intravenous sodium pentobarbitone (30 mg kg⁻¹) and bronchoalveolar lavage was carried out with 20 × 50 ml cold phosphate-buffered saline. The lavage fluid was filtered through 4 layers of gauze and centrifuged for 20 min at 400 *g*. The cell pellets were washed three times with Ca²⁺-Mg²⁺-free Hanks' balanced salt solution. PAM were separated from epithelial cells by density-gradient centrifugation (Shaw & Anderson, 1984) and resuspended in Krebs-Henseleit (KH) solution of the following composition (mm): NaCl 118, KCl 5.9, CaCl₂ 2.5, MgSO₄ 1.2, NaH₂PO₄ 1.2, NaHCO₃ 25.5 and glucose 5.6 to produce a cell count of 5 × 10⁷ ml⁻¹. Approximately 95% of the cells in this preparation were PAM as determined morphologically by Wright-Giemsa staining, and viability was greater than 90% as assessed by Trypan blue exclusion.

Preparation of bronchiolar smooth muscle

After the completion of bronchoalveolar lavage, both lungs were rapidly removed and immersed in oxygenated KH solution. Bronchioles (tenth to thirteenth generation) with an outer diameter of 1.0–2.0 mm were dissected from the lung and cut helically (45°-pitch) to obtain thin bronchiolar strips (2 mm wide and 20 mm long).

Tissue strips were then mounted in glass chambers filled with 4 ml of KH solution maintained at 37°C and at pH 7.4, bubbled constantly with a mixture of 95% O₂:5% CO₂, and were connected to strain gauges (TB-652T; Nihon Kohden, Tokyo, Japan) for continuous recording of isometric tension (pen recorder WT-685G; Nihon Kohden). Each organ bath was fitted with two rectangular platinum electrodes (4 × 30 mm) placed alongside the tissue for transmural electrical field stimulation (biphasic pulse; pulse width, 0.5 ms; supramaximal voltage, 20 V for 20 s). After 2 h of equilibration, changing the bathing solution every 15 min, resting tension was adjusted so that contractile responses to field stimulation

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were maximal, from preliminary studies maximal responses occurred at 500 mg resting tension. The β -adrenoceptor antagonist propranolol (10^{-6} M) was present in the chamber throughout the experiments to avoid a β -adrenergic component of neurally mediated contraction of canine airway smooth muscle.

IgE challenge

After incubating suspensions of PAM with affinity-purified mouse monoclonal hybridoma anti-dinitrophenyl (DNP) IgE antibody (final IgE concentration $10 \mu\text{g ml}^{-1}$, Sigma, St. Louis, Missouri, U.S.A.) for 20 min at 37°C , PAM (10^7 cells ml^{-1}) were added to organ chambers containing canine bronchiolar smooth muscle, and the chambers were then continuously perfused with DNP-human serum albumin (DNP-HSA, 100 ng ml^{-1} , Sigma). This procedure did not alter resting tension of the tissues.

To assess whether IgE immune complexes affect contractile responses of bronchiolar smooth muscle to field stimulation, we obtained responses at increasing impulse frequencies (0.5 to 50 Hz) before addition of PAM and again at 5 min after starting the DNP-HSA perfusion. Control experiments were carried out to examine the effect of PAM incubated with anti-DNP IgE antibody alone on the responses to field stimulation. Frequency-response relationships were linear and regression analysis used to assess the frequency that produced 50% of the maximal contraction (ES_{50}).

The effect of varying density of PAM was assessed by measuring contractions produced by field stimulation (5 Hz) before and 5 min after addition of PAM ($0.3\text{--}30 \times 10^6$ cells ml^{-1}) and after 5 min of DNP-HSA perfusion. The number of PAM required to produce a half-maximal effect was also determined.

To determine whether the site of action of stimulated PAM on bronchiolar smooth muscle was prejunctional or postjunctional, the effect of IgE challenge on the contractile responses to acetylcholine (10^{-9} to 10^{-3} M) was assessed with the same time sequence for the measurements of the responses to field stimulation. Acetylcholine was added cumulatively either at 5 min intervals or 2 min after a steady-state response was achieved.

The possible contributions of arachidonic acid metabolites released from PAM were investigated by adding the following compounds to PAM suspensions 15 min before anti-DNP IgE: the lipoxygenase inhibitor nordihydroguaiaretic acid (NDGA, 10^{-5} M), the cyclo-oxygenase inhibitor indomethacin (3×10^{-6} M) and the thromboxane (Tx) A_2 receptor antagonist (Ogletree *et al.*, 1985) SQ 29548 (10^{-8} to 10^{-5} M). PAM (10^{-7} cells ml^{-1}) treated as described were then stimulated with anti-DNP IgE antibody and DNP-HSA and the effect on the contractile responses of bronchioles to field stimulation (5 Hz) assessed in the usual way.

Release of eicosanoids

Among arachidonic acid metabolites released from PAM, TxA_2 and prostaglandin (PG) $\text{F}_{2\alpha}$ are known to potentiate the electrical field stimulation-induced contraction of canine airway (Leff *et al.*, 1985; Tamaoki *et al.*, 1987a). Thus, to determine whether IgE immune complexes stimulated the release of these eicosanoids from PAM, aliquots ($100 \mu\text{l}$) taken from the medium before and 5 min after IgE challenge were analysed in duplicate for TxB_2 , a stable metabolite of TxA_2 and prostaglandin $\text{F}_{2\alpha}$ ($\text{PGF}_{2\alpha}$) by radioimmunoassay (New England Nuclear, Boston, MA, U.S.A.) as described previously (Tamaoki *et al.*, 1987b).

Drugs

The following drugs were used: ($\pm$)-propranolol hydrochloride, acetylcholine chloride, NDGA, indomethacin (Sigma), and $[1\text{S}-[1\alpha,2\beta(5\text{Z}),3\beta,4\alpha]]-7-[3-[[2-[(\text{phenylamino})\text{carbonyl}]\text{hydrazino}]\text{methyl}]-7\text{-oxabicyclo}[2.2.1]\text{hept-2-yl}]-5-$

heptenoic acid (SQ 29548, a gift from The Squibb Institute for Medical Research, Princeton, NJ, U.S.A.).

Statistics

Results are expressed as means $\pm$ s.e. Statistical analysis was performed by analysis of variance or the Newman-Keuls multiple comparison test, and a P value of less than 0.05 was considered significant.

Results

PAM stimulated with anti-DNP IgE antibody and DNP-HSA had no effect on the resting tone of canine bronchiolar smooth muscle, but potentiated the contractile responses to field stimulation at all frequencies (Figure 1). Thus, the frequency-response curves were displaced to the left, so that the ES_{50} value decreased from 1.9 ± 0.5 to 0.7 ± 0.2 Hz ($P < 0.01$, $n = 8$). On the other hand, PAM treated with anti-DNP IgE antibody alone did not significantly alter the contractile responses of bronchiolar smooth muscle to field stimulation. The potentiation of bronchiolar contraction was dependent on the number of PAM added, the maximal increase from the baseline response being $34.0 \pm 5.5\%$ ($P < 0.001$, $n = 9$) and 50% of this maximum occurred 1.8×10^6 cells ml^{-1} (Figure 2). Addition of IgE antibody alone to PAM at a concentration of up to 3×10^7 cells ml^{-1} was without effect.

To determine the site and mechanism of action of immunologically stimulated PAM in the vagal motor pathway, the effect of PAM on the contractile responses to exogenously

Figure 1 Effect of pulmonary alveolar macrophages (PAM) on contractile responses of canine bronchiolar smooth muscle to electrical field stimulation (EFS) at increasing impulse frequency. After obtaining baseline responses to EFS ($\circ$), PAM (10^{-7} cells ml^{-1}) stimulated with monoclonal anti-DNP IgE antibody and then DNP-HSA (PAM + challenge, a) or those incubated with anti-DNP IgE antibody alone (PAM + Ab alone, b) were added, and the measurements were repeated ($\bullet$). Data are expressed as percentage of maximum baseline responses. Each point represents mean of $n = 8$; vertical bars show s.e.mean.

Figure 2 Dose-dependent effect of pulmonary alveolar macrophages stimulated with anti-DNP IgE antibody and then DNP-HSA (solid columns) or those incubated with anti-DNP IgE antibody alone (stippled columns) on contractile responses of bronchioles to electrical field stimulation at 5 Hz. Values are expressed as a percentage of baseline responses obtained before macrophage administration. Data are means of $n = 9$ for each column; vertical bars show s.e.mean. * $P < 0.05$; ** $P < 0.01$, significantly different from baseline responses.

administered acetylcholine was examined. In contrast to the effect on the contractions induced by field stimulation, acetylcholine-induced contractions of bronchioles were not affected by either PAM stimulated with IgE or PAM incubated with IgE antibody alone (Figure 3).

The increase in the contractile responses to PAM stimulated with IgE challenge was not influenced by pretreatment

Figure 3 Effect of pulmonary alveolar macrophages (PAM) on contractile responses of canine bronchiolar smooth muscle to acetylcholine. After obtaining baseline responses to acetylcholine (○), PAM (10^7 cells ml^{-1}) stimulated with anti-DNP IgE antibody and then DNP-HSA (PAM + challenge, a) or those incubated with anti-DNP IgE antibody alone (PAM + Ab alone, b) were added, and the measurements were repeated (●). Data are expressed as percentage of maximum baseline responses. Each point represents mean of $n = 8$; vertical bars show s.e.mean.

Figure 4 Effect of pretreatment of pulmonary alveolar macrophages (PAM) with 10^{-5} M nordihydroguaiaretic acid (NDGA) or 3×10^{-6} M indomethacin (Ind) on the increase in contractile responses of bronchioles to electrical field stimulation (5 Hz) produced by PAM (10^7 cells ml^{-1}) stimulated with anti-DNP IgE antibody and DNP-HSA (Challenge). Values are expressed as percentage of baseline responses obtained before administration of PAM. Data are means of $n = 7$; vertical bars show s.e.mean. ** $P < 0.01$, significantly different from responses to Ab alone. †† $P < 0.01$, significantly different from values for challenge.

Figure 5 Dose-dependent effect of the thromboxane receptor antagonist SQ 29548 on the increase in contractile responses of bronchioles to electrical field stimulation (5 Hz) produced by pulmonary alveolar macrophages (PAM, 10^7 cells ml^{-1}) stimulated with anti-DNP IgE antibody and DNP-HSA (■). Values are expressed as percentage of baseline responses obtained before administration of PAM. Data are means of $n = 8$ for each column; vertical bars show s.e.mean. * $P < 0.05$; ** $P < 0.01$, significantly different from values for challenge.

of PAM with NDGA but it was greatly inhibited by pretreatment with indomethacin (Figure 4). Moreover, addition of the TxA_2 receptor antagonist SQ 29548 to the organ bath attenuated the effect of stimulated PAM on bronchiolar smooth muscle in a dose-dependent fashion (Figure 5) but did not alter either the resting tension of the responses to field stimulation. The PAM-induced potentiation of the bronchiolar contractions was abolished by SQ 29548 10^{-5} M ($P < 0.01$, $n = 8$).

Incubation of PAM with anti-DNP IgE, followed by incubation with DNP-HSA, increased the release of cyclooxygenase products from 12 ± 9 to 120 ± 44 pg/ 10^6 cells min^{-1} for $\text{PGF}_{2\alpha}$ ($P < 0.01$, $n = 9$) and 36 ± 10 to 460 ± 85 pg/ 10^6 cells min^{-1} for TxB_2 ($P < 0.001$, $n = 10$). Pretreatment of PAM with indomethacin greatly reduced the stimulated release of these eicosanoids whereas NDGA was without effect (Table 1).

Discussion

Our *in vitro* studies demonstrate that immunologically stimulated PAM potentiate the neurally mediated contraction of small airways in dogs. The exposure of isolated smooth

Table 1 Release of cyclo-oxygenase products from canine alveolar macrophages

	Eicosanoid release (pg/10 ⁶ cells min ⁻¹)				
	PAM alone	Ab	Challenge	Challenge + NDGA	Challenge + indomethacin
PGF _{2α}	12 ± 9	15 ± 8	120 ± 44**	95 ± 28**	10 ± 8††
TxB ₂	36 ± 10	50 ± 12	460 ± 85***	422 ± 94***	22 ± 6†††

Values are means ± s.e. for 8–10 experiments. PAM, pulmonary alveolar macrophages; Ab, IgE antibody; NDGA, nordihydroguaiaretic acid; PGF_{2α}, prostaglandin F_{2α}; TxB₂, thromboxane B₂.

** $P < 0.01$, *** $P < 0.001$, significantly different from corresponding values for PAM alone.

†† $P < 0.01$, ††† $P < 0.001$, significantly different from corresponding values for IgE-challenge.

muscle from canine bronchioles to PAM incubated with monoclonal anti-DNA IgE antibody and the subsequent application of DNP-HSA caused an increase in the contractile response to field stimulation, whereas PAM incubated with IgE antibody alone had no effect. These findings suggest that the hyperresponsiveness of bronchiolar smooth muscle appears to be associated with release of mediators from PAM induced by IgE immune complexes.

In contrast to the effect on responses to field stimulation, stimulated PAM did not alter the contractile responses to exogenously administered acetylcholine. In the presence of propranolol to prevent the β -adrenergic effect of noradrenaline released from sympathetic nerve endings, transmural electrical stimulation, as performed in this study, elicits contraction of canine airway smooth muscle via stimulation of postganglionic vagal nerve fibres with the release of acetylcholine, whereas exogenous acetylcholine is known to act directly on smooth muscle cells (Russell, 1978; Walters *et al.*, 1984). Therefore, the increased responsiveness to field stimulation cannot be attributed to the interaction between PAM-derived products and released acetylcholine, or concomitant changes in smooth muscle function. Rather, the action of mediator(s) released by an IgE-dependent mechanism from PAM is presynaptic.

It has been recognized that PAM possess IgE Fc receptors (Spiegelberg, 1984), which are referred to as type 2 and differ both structurally and functionally from type 1 IgE Fc receptors on mast cells and basophils. Although this type of receptor on PAM is a low-affinity receptor, the number of IgE Fc receptor-positive PAM is known to increase in patients with atopic asthma compared to normal subjects (Melewick *et al.*, 1982). In addition, upon stimulation with IgE and antigen, PAM can release a wide variety of substances that have a putative role in asthma. In the present study, pretreatment of PAM with indomethacin greatly reduced the PAM-induced increase in the contractile responses of bronchioles to EFS, suggesting that cyclo-oxygenase products of arachidonic acid may play a major role in the stimulatory effect of PAM on small airways. Because, among cyclo-oxygenase products released from PAM, TxA₂ and PGF_{2α} are known to potentiate cholinergic neurotransmission in the canine airway (Leff *et al.*, 1985; Tamaoki *et al.*, 1987a), we measured these substances and found that the release of each eicosanoid from PAM was enhanced by anti-DNP IgE antibody and DNP-HSA, and was abolished by indomethacin treatment. Furthermore, we found that the effect of stimulated PAM on the EFS-induced

contraction was inhibited by SQ 29548, a specific antagonist at the TxA₂ receptor (Ogletree *et al.*, 1985), in a dose-dependent manner. Therefore, the potentiation of cholinergic neurotransmission in small airways induced by PAM may be accounted for by TxA₂ released from PAM.

In addition to its direct bronchoconstrictor effect, TxA₂ has been known to have neuromodulatory action on canine airway smooth muscle. We and others have previously shown that the TxA₂-mimetic 11 α ,9 α -epoxymethano-PGH₂ (U46619) (Coleman *et al.*, 1981), at a concentration insufficient to cause muscle contraction, increased the contractile responses to field stimulation but not responses to an exogenous cholinergic agonist (Munoz *et al.*, 1986). In the present study, stimulated PAM at cell densities up to 3×10^7 cells ml⁻¹ did not cause spontaneous contraction of bronchioles, suggesting either that TxA₂ levels in the chamber were subthreshold or bronchodilator prostaglandins such as PGE₂ released concomitantly from PAM overcome TxA₂-induced contraction. The site of action of TxA₂ was prejunctional in the vagal motor pathway, but we did not directly measure the release of acetylcholine. However, Serio & Daniel (1988), using a sucrose gap method, obtained evidence to suggest that TxA₂ does not enhance the initial amount of acetylcholine released *per se*, but prolongs the duration of release or improves propagation of impulses through the muscle.

In conclusion, our present study raises the possibility that PAM play a role in the development of antigen-induced hyperreactivity of small airways through an IgE-dependent release of TxA₂, which prejunctionally augments the parasympathetic component of bronchiolar smooth muscle tone. There is increasing evidence that TxA₂ may be an important mediator in the pathogenesis of airway hyperresponsiveness in animal models (O'Byrne *et al.*, 1985; Chung *et al.*, 1986), but the role of this cyclo-oxygenase product in causing asthma in human subjects is much less clear. Previous studies with thromboxane synthetase inhibitors (Fujimura *et al.*, 1986) and with thromboxane receptor antagonist (Finnerty & Holgate, 1990) have provided conflicting results. Thus, further research needs to be done to extend our observations to human asthma.

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