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Involvement of prostaglandins in the down-regulation of allergic plasma leakage observed in rats undergoing pleural eosinophilia

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1 Recent evidence has implicated eosinophils in the inhibition of allergen-induced rat pleurisy, but the mechanism of this negative modulation is not completely understood. This study was undertaken in order to define the potential role of prostaglandins in this phenomenon.

2 Wistar rats were actively sensitized by subcutaneous injection of a mixture of ovalbumin and $Al(OH)_3$ and challenged with an intrapleural (i.pl.) injection of ovalbumin (12 μg/cavity) 14 days later.

3 Analysis of the pleural fluid effluent revealed a massive mast cell degranulation and plasma protein extravasation 4 h post-challenge. We confirmed that concurrently with selective pleural fluid eosinophilia caused by platelet-activating factor (PAF), the pleural cavity became hyporesponsive to allergen-induced protein exudation and to the parallel reduction in the number of intact mast cells.

4 These hyporesponsive animals presented a significant augmentation in the pleural effluent level of prostaglandin E_2 (PGE_2), which increased with increasing numbers of eosinophils in the pleural cavity. Furthermore, pretreatment with either indomethacin or aspirin failed to modify allergen-induced exudation but reversed the exudatory hyporesponsiveness associated with eosinophil recruitment.

5 Allergic exudation was clearly down-regulated by the following pretreatments: (i) PGE_2 (10 μg/cavity, i.pl.) plus rolipram (40 μg/cavity, i.pl.), (ii) misoprostol (200 $\mu g\ kg^{-1}$, p.o.) or (iii) dibutyryl cyclic AMP (80 μg/cavity, i.pl.).

6 We conclude that prostaglandins may be implicated in the eosinophil-mediated inhibition of allergic pleurisy, probably acting via cyclic AMP signalling pathway activation.

Keywords: Eosinophil; prostaglandins; allergy; inflammation; pleurisy

Introduction

A wide spectrum of human diseases ranging from parasitic infestations to allergy are accompanied by tissue eosinophil infiltration (for reviews see Gleich *et al.*, 1993; Kroegel *et al.*, 1994). This considerable but circumstantial evidence has implicated eosinophils as the major effector cells in these pathologies. In fact, activated eosinophils are able to release several pro-inflammatory agents, including specific cationic proteins, cytokines, lipid mediators and reactive oxygen species, which have been widely associated with the pathomechanisms of allergic diseases (for reviews see Gleich *et al.*, 1993; Weller, 1993). Several recent studies, however, have demonstrated that eosinophil accumulation is not necessarily accompanied by tissue lesion or hyperresponsiveness (Gibson *et al.*, 1989; Dent *et al.*, 1990; Heuer *et al.*, 1994). Moreover, there is evidence of blockade of eosinophilia under conditions where these parameters remain unaltered (Elwood *et al.*, 1992; Milne & Piper, 1994; 1995).

It is noteworthy that eosinophils were mainly regarded as immunomodulatory cells from the sixties to the seventies (for review see Goetzl *et al.*, 1979). This rather outdated concept has been more recently supported by experiments in which guinea-pigs and mice infected with *Toxocara canis* developed airway eosinophilia and tracheal hyporeactivity (Buijs *et al.*, 1995a, b). The downward shift coincided with an increased concentration of prostaglandin E_2 (PGE_2) in the bronchoalveolar lavage fluid and was prevented by cyclo-oxygenase blockade (Buijs *et al.*, 1995b). According to Cook *et al.* (1988), there is also an inverse relationship between tissue eosinophilia and glycogen-induced neutrophil recruitment into the peritoneal cavity of rats infected with *Mesocestoide corti*. Additional evidence for the anti-inflammatory role of eosinophils comes from studies carried out in this laboratory, showing that allergen-induced pleurisy is markedly inhibited in rats which had the pleural eosinophil background selectively increased by chemoattractants, including eosinophil chemotactic factor of anaphylaxis (ECF-A), platelet-activating factor (PAF), lipopolysaccharide (LPS) or LPS-PW (Martins *et al.*, 1993; Bandeira-Melo *et al.*, 1995).

Similar to results from studies concerning eosinophil function, research on the role of prostaglandins in inflammation has been divided into two lines of thought. One believes that prostaglandins have proinflammatory effects in that they induce vascular dilatation and synergize with other chemical mediators in order to produce plasma leakage and pain (Ferreira, 1972; Wedmore & Williams, 1981). The other supposes that prostaglandins are anti-inflammatory in that they inhibit the production of leukotriene B_4 (LTB_4) by neutrophils (Ham *et al.*, 1983) and macrophages (Christman *et al.*, 1993), the production of lymphokines (Goodwin & Ceuppens, 1983) and a number of other leukocyte functions including mast cell activation (Hogaboam, 1993), transendothelial migration of T cells (Oppenheimer-Marks *et al.*, 1994) and cytotoxicity (Goodwin & Ceuppens, 1983). Consistently, Raud (1990) has shown that PGE_2 inhibited allergic inflammatory responses in the hamster cheek pouch model via inhibition of mediator release. Interestingly, early studies have provided evidence that following immunological activation human eosinophils could inhibit histamine release *in vitro* via a prostaglandin-mediated mechanism (Hubsher, 1975a, b). Since there is indeed the possibility that, under particular conditions, eosinophils could play a modulator role mediated by prostaglandins, the current study was undertaken in order to investigate the potential involvement of these lipid mediators in the eosinophilia-related down-regulation of plasma leakage evoked by allergen in actively sensitized rats.

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Methods

Animals

Wistar rats of either sex and weighing 150 to 200 g, from the Oswaldo Cruz Foundation Breeding Unit (Rio de Janeiro, Brazil), were used.

Allergic pleurisy in actively sensitized rats

Ether anaesthetized rats were actively sensitized by means of a dorsal subcutaneous injection of a mixture containing 50 μg of ovalbumin and 5 mg of $Al(OH)_3$, in a final volume of 200 μl. Fourteen days later, the sensitized and non-sensitized animals received an i.pl. injection of sterile 0.9% NaCl (saline) or ovalbumin (12 μg/cavity) which was dissolved in saline immediately before use. All i.pl. injections were performed during ether anaesthesia in a final volume of 100 μl with a 27.5-gauge needle adjusted to be 3 mm in length. Four hours after pleural stimulation, the animals were killed with terminal ether anaesthesia, and the thoracic cavity was rinsed with 3 ml of saline containing heparin (10 u ml^{-1}). Pleural washing was collected and its volume measured with a graduated syringe. These volumes were further taken into consideration when total protein and cell numbers in the pleural cavity were estimated. As observed previously (Lima *et al.*, 1991; Martins *et al.*, 1993), the pleural effluent from non-sensitized animals injected with ovalbumin or saline, and sensitized animals injected with saline share the same protein content and cellularity (data not shown).

Pleural exudation under an eosinophilic pleurisy

Eosinophil infiltration in the rat pleural cavity before triggering allergic pleural exudation was obtained through pleural stimulation with one or four successive daily injections of PAF (1 μg/cavity, 100 μl) or its vehicle. Confirming previous findings, this procedure induced a selective and progressive pleural fluid eosinophilia 24 h after the last PAF challenge (Martins *et al.*, 1993).

Treatments

Indomethacin (2 mg kg^{-1}, i.p.), aspirin (200 mg kg^{-1}, i.p.) and misoprostol (200 μg kg^{-1}, p.o.) were given 60 min before antigen challenge. PGE_2 (1–20 μg/cavity), dibutyryl adenosine 3′ : 5′-cyclic monophosphate (cyclic AMP) (80 μg/cavity) and rolipram (40 μg/cavity) were injected intrapleurally, the first two being administered 5 min and the last 60 min before antigen challenge. In some experiments, a combined pretreatment of PGE_2 (10 μg/cavity) and rolipram (40 μg/cavity) was also performed. All the solutions were prepared immediately before use and, except indomethacin and PGE_2, dissolved in saline. Indomethacin was initially dissolved in 0.1 N NaOH and sterile saline, buffered with Tris and neutralised with 0.1 N HCl. PGE_2 was dissolved in ethanol and further diluted with saline.

Measurement of total protein exuded

Analysis of the plasma protein leakage was performed 4 h after injection of allergen. The fluid collected from the pleural cavity was centrifuged (1300 *g*) for 10 min and the protein content of the supernatant quantified in a spectrophotometer (540 nm) by the Biuret technique (Gornall *et al.*, 1949).

Analysis of cells

Total leukocyte and mast cell counts were performed in Neubauer chamber by means of an optical microscope after dilution of the pleural fluid with Türk solution (2% acetic acid) and toluidine blue dye (Mota, 1966), respectively. Differential analysis was made in cytospin preparations stained with May-Grünwald-Giemsa dye under an oil immersion objective. A minimum of 100 cells was counted in each effluent sample.

Prostaglandin measurement

Pleural exudates were collected with 1 ml of saline containing heparin (10 u ml^{-1}) and indomethacin (50 μg ml^{-1}) to prevent further production of PGE_2. The pleural fluid samples were centrifuged at 1300 *g* for 10 min at 0°C and the supernatants were added with methanol (200 μl). PGE_2 in the pleural supernatant was extracted with SEP-PAK C18 cartridges (Waters Associates, Milford, U.S.A.) as described previously (Kioymiya & Oh-ishi, 1985). PGE_2 levels were measured with an ELISA kit according to the manufacturer's instructions (Neogen Co., Lexington, U.S.A.).

Drugs

PAF (1-*O*-hexadecyl-2-acetyl-sn-glyceryl-3-phosphorylcholine) was purchased from Bachem (Bubendorf, Switzerland), and sodium heparin from CEME (Brasília, Brazil), Indomethacin, dibutyryl cyclic AMP and PGE_2 were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.) and ovalbumin from Biochemika Fluka (Buchs, Switzerland). Rolipram was synthesized at the Institut de Recherche Jouveinal (Paris, France). Aspirin was from Synthelabo France Laboratories (Paris, France). Misoprostol (Cytotec tablets) was a gift from BIOLAB (São Paulo, Brazil).

Statistical analysis

Data are presented as means ± s.e.mean and statistically analysed by means of analysis of variance (ANOVA) followed by the Newman-Keuls-Student's test. The correlation between number of eosinophils and the total protein content in the pleural fluid was determined by using least squares linear regression and analysed by Student's *t* test. Differences were considered to be statistically significant when $P<0.05$.

Results

Inverse correlation between numbers of eosinophils and allergic protein exudation

The i.pl. injection of allergen into actively sensitized rats increased 8 to 10 fold the basal plasma leakage in the pleural cavity as attested by the total protein content of the pleural washing 4 h post-challenge. In non-sensitized rats, ovalbumin (12 μg/cavity) yielded 5.9 ± 0.7 mg/cavity (mean ± s.e.mean, $n=8$) while in sensitized ones the value was 47.2 ± 4.0 mg/cavity ($n=8$) ($P<0.001$). At this time, both groups shared the same eosinophil counts. The values were $0.9 \pm 0.1 \times 10^6$ cells/cavity (mean ± s.e.mean, $n=8$) and $0.9 \pm 0.2 \times 10^6$ cells/cavity ($n=8$) for non-sensitized and sensitized animals, respectively.

Confirming previous findings (Martins *et al.*, 1993; Bandeira-Melo *et al.*, 1995), concurrently with the selective eosinophil infiltration evoked by PAF, injected 24 h previously, the pleural cavity became hyporesponsive to allergen challenge. In fact, for individual animals, an inverse relationship between eosinophil numbers and allergen-induced protein exudation was clearly observed ($r=0.81$, $P<0.0001$) (Figure 1). The magnitude of exudation reduced from 47.2 ± 4.0 mg/cavity (mean ± s.e.mean, $n=8$), in vehicle-treated animals, to 20 ± 4.0 mg/cavity ($P<0.01$) and 10 ± 4.0 mg/cavity ($P<0.01$) following 1 and 4 successive PAF daily injections, respectively. Table 1 shows that in naive rats no protein exudation was noted 24 h after 1 or 4 PAF daily injections, under conditions where eosinophil numbers were about 3 and 5 fold increased, respectively.

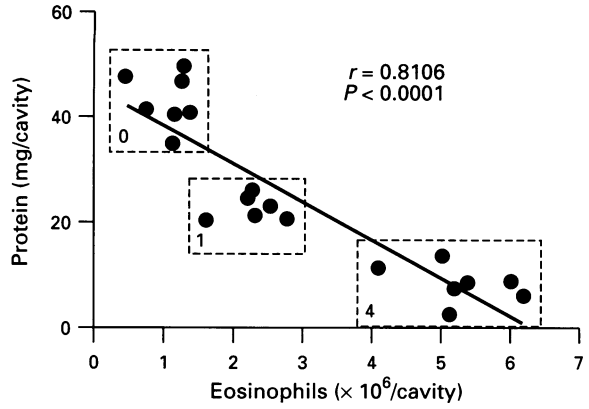

Figure 1 Relationship between the exudatory response and eosinophil number in the pleural cavity of sensitized rats challenged with allergen 24 h after one vehicle stimulation (insert = 0), one PAF stimulation (insert = 1) or four daily PAF stimulations (insert = 4).

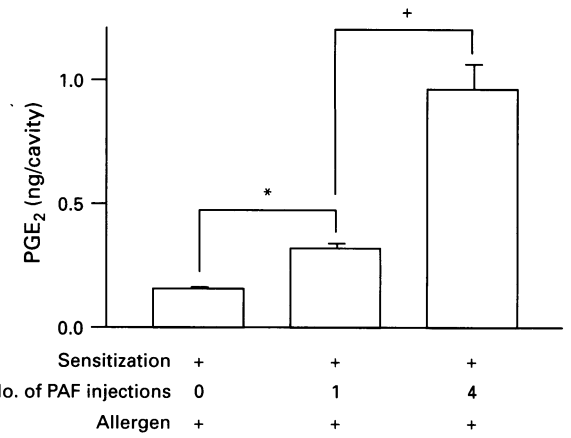

Figure 2 Effect of 1 or 4 PAF prestimulation on PGE_2 levels in the exudate of allergen-induced pleurisy at 4 h. The measurements were done by enzyme immunoassay after the extraction from pleural fluids as described in Methods. The values are expressed as means with s.e.mean from six animals. $^*P<0.05$, $^+P<0.01$.

Table 1 Protein extravasation and eosinophil influx elicited by 1 or 4 successive daily i.pl. injections of PAF (1 μg/cavity) or its vehicle[a]

Stimulation	*N. of daily injections*	*Protein (mg/cavity)*	*Eosinophils* ($\times 10^6$/cavity)
Vehicle	1	5.5 ± 0.5	0.9 ± 0.3
	4	6.6 ± 0.5	1.2 ± 0.3
PAF	1	4.0 ± 0.2	3.0 ± 0.3*
	4	5.1 ± 0.5	5.8 ± 0.5$^+$

[a]The analysis was performed 24 h after the last PAF challenge. Each value represents the mean ± s.e.mean from at least 8 animals. *Statistically significant ($P<0.05$) as compared to the vehicle-treated group. $^+$Statistically significant ($P<0.05$) as compared to animals injected with PAF once.

Table 2 Effect of indomethacin (2 mg kg^{-1}, i.p.) or aspirin (200 mg kg^{-1}, i.p.) on exudation induced by allergen in sensitized rats[a]

Condition	n	*Protein (mg/cavity)*
Non-sensitized	16	5.0 ± 0.4
Sensitized	16	45.4 ± 5.0*
+ Indomethacin	8	40.6 ± 3.7
+ Aspirin	8	42.7 ± 2.8

[a]Drugs were administered 60 min before allergen challenge. Each value represents the mean ± s.e.mean from at least eight animals. All groups were challenged with ovalbumin (12 μg/cavity). Statistically significance difference ($P<0.001$) is indicated by an asterisk.

Measurement of PGE_2 in the pleural effluent

As illustrated in Figure 2, following allergen challenge the effluent level of PGE_2 in sensitized animals was 0.16 ± 0.01 ng/cavity (mean ± s.e.mean, $n=6$). Nevertheless, the values increased to 0.32 ± 0.02 ng/cavity ($n=6$) ($P<0.05$) and 0.96 ± 0.14 ng/cavity ($n=6$) ($P<0.01$) in those sensitized rats which had received 1 or 4 PAF pre-stimulations, respectively, before allergen challenge. Groups of non sensitized rats receiving zero, 1 or 4 PAF pre-stimulations were also assayed but their PGE_2 levels were below the low range of the standard curve (0.1 ng ml^{-1}).

Effect of cyclo-oxygenase blockade on the hyporesponsiveness to allergen that parallels eosinophil infiltration

As illustrated in Table 2, intraperitoneal pretreatment with either indomethacin (2 mg kg^{-1}) or aspirin (200 mg kg^{-1}) 1 h before challenge failed to alter allergen-induced protein exudation. As shown in Figure 3, these treatments also failed to modify the pleural eosinophil enrichment evoked by PAF, but they did restore the exudatory response to allergen which had been down-regulated in PAF-pretreated rats.

We have confirmed that the allergen challenge led to an intense mast cell degranulation reducing the number of intact mast cells in the control pleural effluent from 780 ± 50 × 10^3 cells per cavity (mean ± s.e.mean, $n=8$) to 30 ± 10 × 10^3 cells per cavity ($n=8$) ($P<0.001$). As shown in Figure 4, the massive mast cell degranulation was slightly but consistently impaired in rats undergoing eosinophil accumulation. On the other hand, this protective effect was completely prevented by pretreatment with either indomethacin (Figure 4a) or aspirin (Figure 4b). It is noteworthy that these treatments *per se* did not affect the pleural resident mast cell population. The values in naive, indomethacin and aspirin pretreated rats were 635 ± 67 × 10^3 intact mast cells per cavity (mean ± s.e.mean, $n=8$), 599 ± 43 × 10^3 intact mast cells per cavity ($n=8$) and 674 ± 39 × 10^3 intact mast cells per cavity ($n=8$), respectively.

Effect of treatment with E-series prostaglandins or dibutyryl cyclic AMP on allergen-induced pleural protein exudation

Figure 5 shows that the pleural exudatory response induced by allergen in actively sensitized rats was markedly reduced by the oral pretreatment with misoprostol (200 μg kg^{-1}), a synthetic and stable PGE_1 analogue. The effect of misoprostol was not prevented by pretreatment with indomethacin (2 mg kg^{-1}, i.p.), as illustrated in Figure 5. We further observed that i.pl. injection of PGE_2 (1–20 μg/cavity) performed 5 min before allergen challenge did not affect allergic pleural protein leakage (data not shown). In contrast, by combining PGE_2 (10 μg/cavity) with an ineffective dose of the phosphodiesterase inhibitor rolipram (40 μg/cavity), a significant reduction of allergen-induced exudation was observed (Figure 6).

The possibility that the inhibitory effect of E-series prostaglandin treatment was mediated via alterations in intracellular levels of cyclic AMP was considered. Table 3 shows that i.pl. pretreatment with the permeable and stable analogue dibutyryl cyclic AMP (80 μg/cavity) 5 min before allergen challenge indeed reduced pleural protein exudation in about 46% ($P<0.05$).

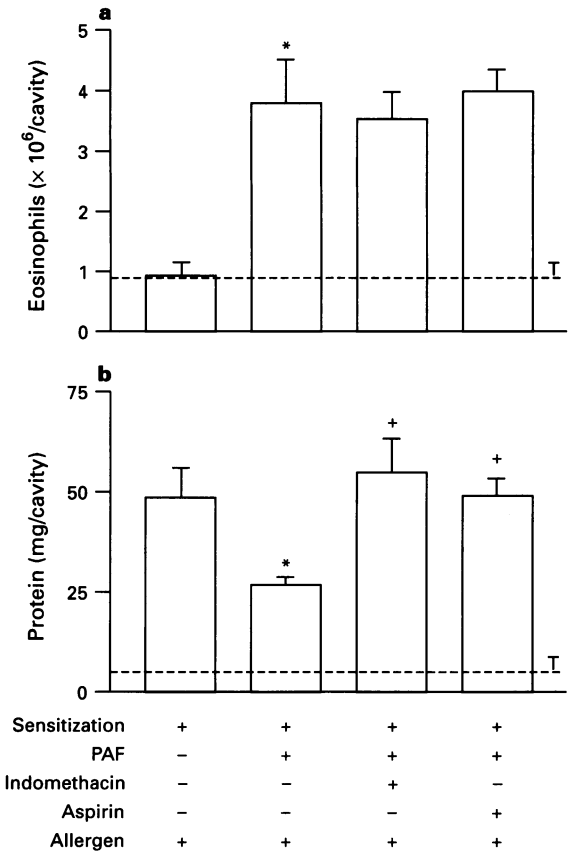

Figure 3 Reversal effect by either indomethacin or aspirin treatment on suppression of allergen-induced protein exudation during eosinophilia caused by PAF in sensitized rats. Both cyclo-oxygenase inhibitors were administered 1 h before i.pl. injection of allergen. PAF was administered 24 h before allergenic challenge. Results are expressed as the mean ± s.e.mean from at least eight animals. Dotted line represents the background values for eosinophils (a) and total protein (b) in naive rats. $^{*}P<0.01$ compared with group prestimulated with PAF vehicle. $^{+}P<0.01$ compared with PAF-prestimulated rats.

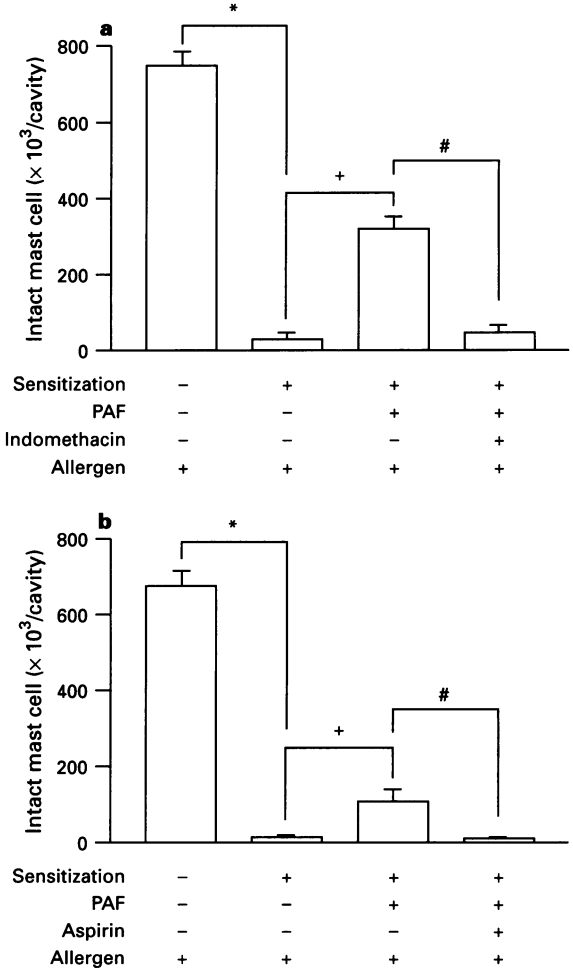

Figure 4 Effect of indomethacin (a) and aspirin (b) on the eosinophilia-related prevention of intact mast cell count decrease evoked by allergen. The number of intact mast cells was evaluated as described in Methods and expressed as the mean ± s.e.mean from at least eight animals. $^{*}P<0.001$, $^{+}P<0.05$, $^{\#}P<0.01$.

Discussion

This study provides evidence for the involvement of PGE_2 in the eosinophil-mediated down-regulation of the allergic exudatory response triggered by allergen in actively sensitized rats. We found that the refractoriness to allergen noted 24 h post-PAF challenge is (i) well correlated with the magnitude of eosinophil infiltration, (ii) associated with increased levels of PGE_2 dissolved in the pleural fluid, (iii) overcome by cyclo-oxygenase blockers and (iv) imitated by pretreatment with either the PGE_1 analogue misoprostol or by a combination of PGE_2 plus rolipram.

There have been two lines of thought concerning the eosinophil function in allergy. From the sixties to the late seventies eosinophils were regarded as immunomodulatory cells mainly in the context of the immediate hypersensitivity reaction. This down-regulating role is illustrated, for instance, by studies showing that eosinophils can release immunomodulatory substances and specific enzymes able to exert a negative control on the function of pivotal cell targets and to neutralise their inflammatory products, respectively (for review see Goetzl *et al.*, 1979). More recently, however, the eosinophilic reaction has been identified as a key inflammatory alteration in the pathogenesis of several allergic diseases including asthma (Gleich *et al.*, 1993; Kroegel *et al.*, 1994). One of the important observations supporting this concept is that the treatment of monkeys with anti-ICAM-1 monoclonal antibody clearly inhibited allergen-induced lung eosinophil influx and also prevented airway hyperresponsiveness (Wegner *et al.*, 1990).

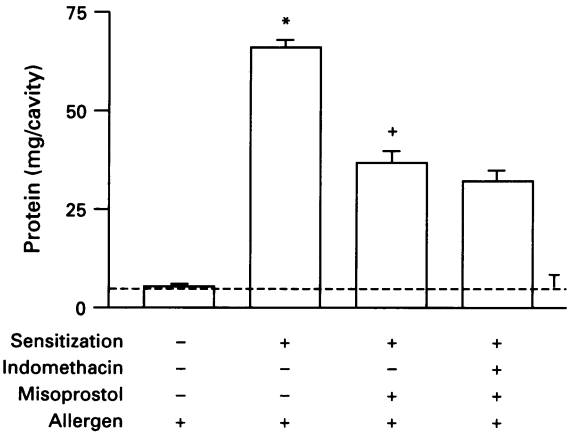

Figure 5 Lack of effect of indomethacin ($2\,mg\,kg^{-1}$, i.p.) on the reduction by misoprostol ($200\,\mu g\,kg^{-1}$, p.o.) of allergen-induced protein exudation. Indomethacin and misoprostol were administered 60 min before i.pl. injection of allergen. Results are expressed as the mean ± s.e.mean from at least eight animals. Dotted line represents the background values for total protein in naive rats. $^{*}P<0.001$ compared with non-sensitized group. $^{+}P<0.01$ compared with allergen-challenged sensitized group.

Similar findings were obtained following treatment with an anti-IL-5 antibody (TRFK-5) (Mauser *et al.*, 1995) reinforcing the interpretation that there is indeed a causal link between

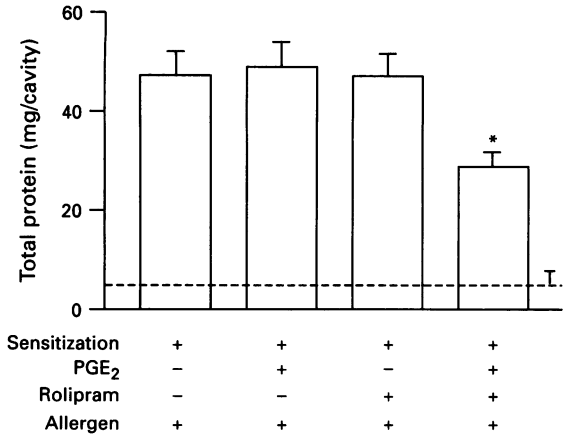

Figure 6 Effect of PGE_2 (10 μg/cavity) and rolipram (40 μg/cavity), alone or in combination, on allergen-induced protein exudation. PGE_2 and rolipram were administered i.pl., 5 and 60 min before allergen challenge, respectively. Results are expressed as the mean ± s.e.mean from at least eight animals. Dotted line represents the background values for total protein in naive rats. Statistically significant difference ($P<0.05$) is indicated by an asterisk.

Table 3 Effect of dibutyryl cyclic AMP (80 μg/cavity) on exudation induced by allergen in sensitized rats[a]

Condition	*Protein (mg/cavity)*	*% inhibition*
Non-sensitized	5.03 ± 0.4	–
Sensitized	70.4 ± 5.8*	–
+ dibutyryl cyclic AMP	40.16 ± 4.7^{+}	46.3

[a]Dibutyryl cyclic AMP was injected 5 min before allergen-challenge. Each value represents the mean ± s.e.mean from 8 animals. All groups were challenged with ovalbumin (12 μg/cavity). *Statistically significant ($P<0.001$) as compared to the non-sensitized group. $^{+}$Statistically significant ($P<0.05$) as compared to the actively sensitized group.

eosinophilia and airway hyperreactivity. However, the evidence that another antibody to interleukin-5 (IL-5) (NC-17) (Nagai *et al.*, 1993) or a soluble IL-5 receptor (Yamaguchi *et al.*, 1994) suppresses allergen-induced bronchoalveolar lavage fluid (BALF) eosinophilia with little effect on bronchial hyperreactivity opposes this concept, and indicates that the functional role of eosinophils in allergy is still a matter of debate.

We have recently shown that allergen-induced pleural exudation is selectively down-regulated in sensitized rats undergoing a PAF-induced pleural eosinophil accumulation. This inhibitory effect is not specifically associated with PAF since other eosinophil chemoattractants, including ECF-a, LPS or a soluble eosinophilotactic factor generated during LPS pleurisy, also had the same action. Moreover, chemical blockade of the increase in eosinophils clearly restored the sensitivity to allergen challenge, suggesting that the refractoriness is not associated with a particular chemoatractant but seems to be related to the eosinophilia it produces (Martins *et al.*, 1993; Bandeira-Melo *et al.*, 1995). The present study aimed to clarify the potential mechanism of action implicated in this immunomodulatory phenomenon. With a model of actively sensitized rats, we confirmed that concurrently with a local eosinophilic response triggered by PAF 24 h previously, the pleural cavity became hyporesponsive to antigen challenge. In fact, the magnitude of eosinophil infiltration and allergen-evoked exudation were shown to be inversely correlated ($r=0.81$ and $P<0.0001$). In addition, we found that rats which were experiencing a pleural eosinophilia and had become concomitantly hyporesponsive to allergen, also presented an increase in the concentration of the cyclo-oxygenase product PGE_2 in their pleural fluid, as compared to normal responders. There was about a 2 fold increase in PGE_2 levels of animals pretreated with one PAF injection and a 6 fold elevation in animals given 4 PAF injections where there was almost complete inhibition of allergen-induced plasma protein leakage. Since we did not detect PGE_2 in the pleural effluent of non-sensitized animals undergoing PAF-induced eosinophil accumulation, it is assumed that both eosinophil enrichment and allergen challenge are required to evoke the generation of PGE_2. The potential of eosinophils to generate PGE_2 is well-established concept (Foegh *et al.*, 1986; Giembycz *et al.*, 1990; Kroegel & Matthys, 1993). Moreover, early studies have shown that eosinophil-derived E-series prostaglandins following immunological provocation can prevent antigen-induced histamine release *in vitro* (Hubsher, 1975a, b). Therefore it seems to be reasonable to assume that allergen-induced exudation could be attenuated by PGE_2 generated during the ongoing pleural eosinophilia.

The effect of prostaglandin biosynthesis inhibitors on allergic inflammation has been extensively investigated. For instance, intravital microscopy of the hamster cheek pouch revealed that either indomethacin or diclofenac pretreatment significantly increased histamine release, plasma leakage and leukocyte accumulation triggered by antigen. In addition, all these alterations were reversed by PGE_2, giving support to the interpretation that endogenous prostaglandins may indeed function as local regulators of the allergic inflammatory response (Raud *et al.*, 1989). In this study, we examined the effect of inhibition of the cyclo-oxygenase pathway, by both indomethacin and aspirin, in the eosinophilia-related attenuation of the allergic protein exudation. These drugs failed to modify either pleural plasma leakage or the massive mast cell degranulation evoked by antigen challenge in sensitized rats. Nevertheless, they did prevent the pleural hyporeactivity to antigen challenge, under conditions where the concomitant eosinophil infiltration induced by PAF remained unaltered. It is of interest to note that, despite being completely reversed by the pretreatment with either aspirin or indomethacin, the protection upon mast cell population attested by the number of residual intact mast cells recovered from the pleural cavity was very mild, suggesting that inhibition of mediator release via mast cell degranulation alone may not entirely account for the down-regulatory mechanism. However, PGE_2 has been known to modulate immune responses by inhibiting cell targets other than mast cells, including neutrophils (Ham *et al.*, 1983), lymphocytes (Oppenheimer-Marks *et al.*, 1994; Garrone *et al.*, 1994), macrophages (Christman *et al.*, 1993) and endothelial cells (Oppenheimer-Marks *et al.*, 1994). All of them being potentially implicated in the allergic exudatory response.

If the down-regulatory mechanism described here is due to local production of PGE_2, the exogenous administration of this prostanoid would be expected to have a similar supressive effect. Indeed, administered orally misoprostol (200 μg kg^{-1}), a synthetic analogue of PGE_1 , attenuated plasma extravasation induced by allergen in actively sensitized animals in an intensity comparable to that noted during the localized ongoing eosinophilia. Moreover, misoprostol-mediated suppression of the allergic response was not altered by indomethacin, as expected. Misoprostol has been shown to bind specifically to PGE receptors (EP receptors) and to exhibit a comparable pharmalogical profile to prostaglandins (reviewed in Coleman *et al.*, 1994), including the ability to down-regulate immunological events (reviewed in Shield, 1995). We found that PGE_2 (1–20 μg/cavity) administered locally failed to alter the allergic exudatory response, which would be a consequence of its chemical instability and lesser resistance to endogenous enzymatic catabolism when compared to misoprostol (reviewed in Shield, 1995). The concept that PGEs and their analogues increase intracellular cyclic AMP via a receptor-mediated activation of adenylate cyclase is well established (Coleman *et al.*, 1994). Regulation of cyclic AMP levels is also exerted by cyclic nucleotide phosphodiesterases, among which the type IV isoform has been emphasized by its selectivity on cyclic AMP catabolism

and sensitivity to rolipram (recently reviewed in Barnes, 1995). Indeed, the combined administration of PGE_2 plus rolipram significantly inhibited antigen-induced exudation, under conditions where the individual treatments were inactive. This co-operative effect between PGE_2 and rolipram has been observed in other experimental models (Sinha *et al.*, 1995) and is in line with the established interpretation that PGEs-induced inhibition of the allergic response is indeed mediated by cyclic AMP. Accordingly, the intrapleural injection of the permeable analogue dibutyryl cyclic AMP (80 μg/cavity) significantly inhibited the allergen-induced protein exudation, suggesting that endogenous cyclic AMP may indeed play a pivotal role in the hyporesponsiveness to antigen challenge expressed by the eosinophil enriched sites.

In conclusion, our results suggest that PGE_2 is implicated in the eosinophilia-related attenuation of the pleural exudatory response evoked by antigen in actively sensitized rats, by a mechanism probably dependent on the activation of the cyclic AMP signalling pathway.

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