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Effect of human recombinant interleukin-5 on *in vitro* responsiveness to PAF of lung from actively sensitized guinea-pigs

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1 The intra-tracheal (i.t.) administration of human recombinant interleukin-5 (rhIL-5; 100 or 300 ng) to isolated perfused lungs from guinea-pigs actively sensitized to ovalbumin induced an increased bronchoconstriction and release of thromboxane A_2 (TXA_2) and histamine into the lung effluent following the subsequent (10 min) intra-arterial injection of platelet-activating factor (PAF). Lung responses to 5-hydroxytryptamine were unaffected by rhIL-5.

2 Hyperresponsiveness to PAF was observed when the lungs were obtained from guinea-pigs used 2 or 7 days after a booster injection of the antigen and, to a lower extent, when they were from animals sensitized by a single antigen administration. By contrast, rhIL-5 did not modify the responses to PAF of lungs from passively sensitized or from adjuvant-treated guinea-pigs, suggesting that immunological stimulation is required to allow the expression of synergism between rhIL-5 and PAF.

3 Guinea-pigs killed 2 and 7 days after the booster injection of the antigen exhibited a marked increase in the number of eosinophils in the bronchoalveolar lavage fluid (BAL), as compared to non-sensitized animals.

4 Our results demonstrate that rhIL-5 and PAF act synergistically to induce enhanced bronchoconstriction and mediator release exclusively when lungs are obtained from guinea-pigs sensitized once to ovalbumin and then boosted. Since recruitment of eosinophils into the airways and the development of hyperresponsiveness to PAF are concomitant, it is suggested that eosinophils are the target cells for interaction between rhIL-5 and PAF.

Keywords: Interleukin-5; lung hyperresponsiveness; PAF; isolated lung; eosinophils; bronchoalveolar lavage

Introduction

Non-specific bronchial hyperreactivity, an increased sensitivity to inhaled irritants which characterizes some forms of intrinsic or allergic asthma and lung inflammation are related (Boushey *et al.*, 1980). Inflammation is thought to follow the activation of resident cells which triggers the migration of eosinophils, neutrophils and lymphocytes into the lung tissue (Djukanovic *et al.*, 1990). These invading cells may in turn release cytotoxic enzymes and pro-inflammatory mediators which contribute to the amplification and perpetuation of the disease.

We have previously demonstrated that isolated lungs from actively sensitized guinea-pigs exhibit a long-lasting bronchopulmonary hyperresponsiveness to various mediators including platelet activating factor (PAF) (Pretolani *et al.*, 1988). This phenomenon, which is expressed as a reduced threshold for bronchoconstriction and an enhanced release of the secondary mediators thromboxane A_2 (TXA_2), leukotriene C_4 (LTC_4), prostacyclin and histamine, is triggered by the booster injection of the antigen. Acting as an immune provocation, the booster injection may start changes in the reactivity of resident cells and/or promote lung invasion by inflammatory cells. In confirmation, the development of lung hyperresponsiveness is accompanied by an increased number of eosinophils in the bronchoalveolar lavage fluid (BAL) (Pretolani *et al.*, 1990), as demonstrated in other experimental situations (Kallos & Kallos, 1984; Hutson *et al.*, 1988; Coyle *et al.*, 1989).

More recently, it became apparent that cytokines, secreted by T cells in response to antigen stimulation, may play a role in pulmonary eosinophilia and lung inflammation and in the consequent hyperresponsiveness (Gonzalez *et al.*, 1987; Frew *et al.*, 1990), although the precise relationship between these phenomena is not yet established. The main cytokines involved are interleukin-5 (IL-5), IL-3 and granulocyte-macrophage colony stimulating factor (GM-CSF), which support eosinophil proliferation and differentiation from their bone marrow precursors (Coffman *et al.*, 1988; Miyajima *et al.*, 1988) and prime target cells to express enhanced responses to exogenous stimuli (Silberstein *et al.*, 1986; Dahinden *et al.*, 1989; Bischoff *et al.*, 1990a,b). However, administration of very large doses of rhIL-3, rhGM-CSF or tumour necrosis factor-α to non-sensitized guinea-pigs induces an increased eosinophilia in the BAL (Kings *et al.*, 1990; Sanjar *et al.*, 1990), but does not lead to lung hyperresponsiveness. These results indicate that eosinophilia *per se* does not necessarily correlate to hyperresponsiveness.

In the present study we show that the exposure of guinea-pigs to antigen results in lung eosinophilia and in a small increase in bronchoconstriction and mediator release in response to PAF. However, *in vitro* administration of rhIL-5 to lungs prior to challenge with PAF, leads to a marked increase in these parameters. This suggests that priming of a pulmonary target cell, probably the eosinophil, by rhIL-5 is followed by an increased mediator release from other cell types, which in turn accounts for the enhanced bronchoconstriction.

Methods

Sensitization procedures

Male Hartley guinea-pigs (400–600 g) were actively sensitized by two s.c. injections, 2 weeks apart, of 0.5 ml of 0.9% NaCl (saline) containing 10 μg of ovalbumin dispersed in 1 mg $Al(OH)_3$. The animals were used either 2 or 7 days after the

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second injection (booster injection). This procedure promotes the production of high titers of specific homocytotropic antibodies (Ovary, 1964), estimated to be approximately 1/800 by passive cutaneous anaphylaxis in serum from animals killed 7 days after the booster injection of antigen (Pretolani *et al.*, 1988). In a separate set of experiments, guinea-pigs received the first sensitizing injection of ovalbumin, but on the day of the booster injection, they were injected with the adjuvant alone, i.e. 1 mg $Al(OH)_3$, to be used 2 days thereafter. In control experiments, guinea-pigs were injected twice, 2 weeks apart, with 1 mg $Al(OH)_3$ alone and killed 2 or 7 days after the second injection.

In another series of experiments, guinea-pigs were passively sensitized with 1 ml of homologous serum obtained from actively sensitized animals and used after 10 days (Lagente *et al.*, 1987).

Lung perfusion experiments

Guinea-pigs were anaesthetized with sodium pentobarbitone (30 mg kg^{-1}, i.p.), tracheae were cannulated, and the animals were ventilated (60 strokes min^{-1}; 1 ml 100 g^{-1} body weight) with Palmer miniature respiratory pumps. A thoracotomy was performed, and the lungs were removed, as described (Lefort *et al.*, 1984). Each organ was placed in a plastic chamber, immediately ventilated (60 strokes min^{-1}, 1 ml 100 g^{-1} body weight) and perfused via the pulmonary artery (10 ml min^{-1}, 37°C) with gassed (95% O_2 5% CO_2) Krebs solution containing 0.25% (w/v) bovine serum albumin (BSA). The changes in resistance to inflation were continuously recorded. After a 10 min equilibration period, 100 or 300 ng rhIL-5 were injected intratracheally (i.t.) in a volume of 100 µl. In control experiments, the lungs received the vehicle of rhIL-5, i.e. 100 µl saline. Ten min after the injection of rhIL-5 or of saline, PAF (0.01, or 1 and 10 ng in 100 µl saline containing 0.25% BSA) or 5-hydroxytryptamine (5-HT, 10 to 3000 ng in 100 µl saline) were successively administered into the pulmonary artery (i.a.), at 10 min intervals for PAF, or every 5 min, for 5-HT.

In a separate series of experiments, lungs from actively sensitized guinea-pigs used 2 days after the booster injection of the antigen were injected i.t. with 100 µg lipopolysaccharide (LPS) and then challenged with 1 and 10 ng PAF, as described above.

Bronchoconstriction was estimated from airways resistance tracings, measured as the area under the curve during a 10 min period. Results are expressed as a percentage of the maximal value obtained by clamping the tracheal cannula. Bronchoconstriction to 5-HT was calculated as % of the peak-intensity of the response.

One ml fractions of the lung effluent were collected before and during the first 6 min after the injection of the various agonists for the determination of TXB_2 and histamine, as described (Sors *et al.*, 1978; Lebel, 1983).

Measurement of thromboxane B_2 and histamine in the lung effluent

Before storage at −20°C, aliquots (1 ml) of the lung perfusate were maintained at room temperature for 30 min to allow the conversion of TXA_2 into its stable metabolite, TXB_2 which approaches 100% under these experimental conditions (Charpentier *et al.*, 1986). The radioimmunoassay for TXB_2 was performed as described (Pretolani *et al.*, 1988), using a monoclonal antibody with less than 0.2% cross-reactivity with prostaglandin D_2 (PGD_2), PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$ and archidonic acid. The sensitivity of the assay was approximately 2 pg of immunoreactive TXB_2 in 100 µl sample.

For the histamine assay, 1 ml of each fraction was mixed with 1 ml of a 0.8 N solution of perchloric acid, centrifuged for 10 min (1,200 *g* at 4°C), and supernatants were stored at 4°C. Histamine content of supernatants was measured by an automatic spectro-fluorometric assay method (Lebel, 1983).

Harvest of bronchoalveolar cells

Four groups of animals were used: (i) non sensitized, (ii) actively sensitized non-boosted, and actively sensitized guinea-pigs killed either (iii) 2, or (iv) 7 days after the booster injection of the antigen. The animals were anaesthetized as above and bronchoalveolar cells were collected in 10 successive lavages with 5 ml aliquots of sterile saline at room temperature injected and recovered through a polyethylene tracheal cannula. The lavage fluid was stored on ice and total cell counts made. The suspension was then diluted to reach a final concentration of 1.5×10^5 cell ml^{-1} and, after cytocentrifugation, differential cell counts were performed after staining with May-Grünwald Giemsa dye. Since the yield of the injected fluid was equivalent in all groups of animals (around 90%), the results are expressed as a concentration of each cell population ml^{-1}.

Materials

The composition of the Krebs solution was (in mM): NaCl 118, KCl 4.7, $CaCl_2 \cdot 2H_2O$ 2.5, $MgSO_4 \cdot 7H_2O$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 5.6. Suppliers of the reagents were as follows: rhIL-5 (Mony's International Imports, Beverly Hills, CA, U.S.A.); PAF (1-O-hexadecyl-2-acetyl-sn-glycero-3-phosphocholine), BSA fraction V, bovine γ-globulins fraction II, TXB_2, 5-HT (serotonin) (Sigma, St. Louis, MO, U.S.A.); $Al(OH)_3$, perchloric acid, polyethyleneglycol 6000 (Merck, Darmstadt, Germany); pentobarbitone (sodium pentobarbital, Clin-Midy, Montpellier, France); chicken ovalbumin (Miles, Napterville, IL, U.S.A.); LPS W *E. coli* 0.55:B5 (Difco Laboratories, Detroit, MI, U.S.A.); the antibody and radiolabelled ligand for radioimmunoassay of TXB_2 were from the URIA, Institut Pasteur/INSERM U 207, Paris, France.

Data analysis

The results are expressed as means ± s.e.mean of the indicated number of experiments. Statistical significance was assessed by two-way analysis of variance (ANOVA). If significance was determined, comparisons were made by Student's *t* test for unpaired values. A value of $P \leqslant 0.05$ was considered significant.

Results

Effect of rhIL-5 on bronchial resistance to inflation and mediator release

The i.t. instillation of 100 µl saline or 300 ng rhIL-5 into isolated lungs from adjuvant-treated guinea-pigs or from actively sensitized animals used either 16 days after the first sensitizing injection, or 2 or 7 days after the booster injection of the antigen, induced a slight bronchoconstriction ranging between 6.5 ± 3.3% and 12.9 ± 4.4% ($n = 5-6$ per group) and no statistically significant difference was observed between the groups. Similarly, TXB_2 and histamine release following the i.t. administration of 100 µl saline or 300 ng rhIL-5 ranged between 0.8 ± 0.2 and 2.1 ± 0.9 ng ml^{-1} ($n = 5-6$ per group) and 1.4 ± 0.4 ng ml^{-1} and 6.0 ± 1.6 ng ml^{-1} ($n = 5-6$ per group), respectively. Again, no statistically significant difference was observed between the groups.

Effect of rhIL-5 on PAF-induced bronchoconstriction

The i.a. injection of 10 ng PAF into lungs from adjuvant-treated guinea-pigs 10 min after *in vitro* i.t. instillation of 100 µl saline or 300 ng rhIL-5 induced a slight bronchoconstriction (approximately 10% of the maximum), whereas

no response followed 1 ng of the agonist (Figure 1a). Administration of 1 and 10 ng PAF to lungs from single-sensitized (non-boosted) (Figure 1b) or from passively sensitized guinea-pigs induced a moderate and dose-dependent bronchoconstriction (Table 1). As previously shown (Pretolani *et al.*, 1988), the response to 1 ng PAF was significantly enhanced in lungs from actively sensitized guinea-pigs killed 2 or 7 days after the booster injection of the antigen (Figure 1c and d). Such an enhanced response was not observed following the subsequent administration of 10 ng of PAF. Lungs from adjuvant-treated (Figure 1a) or from passively sensitized (Table 1) guinea-pigs instilled i.t. with 300 ng rhIL-5, responded to PAF as intensively as vehicle-treated preparations. By contrast, a slight increase in the extent of bronchoconstriction by 1 ng of PAF was observed in lungs from single sensitized animals, i.e., those which received no booster injection of the antigen (Figure 1b). This increased response to PAF after rhIL-5 administration was more pronounced when the lungs were obtained from guinea-pigs which had been boosted with antigen and were used 2 or 7 days thereafter (Figure 1c and d). Under these conditions, no further increase in the bronchoconstrictor response following challenge of the organs with 10 ng PAF was noted (Figure 1c).

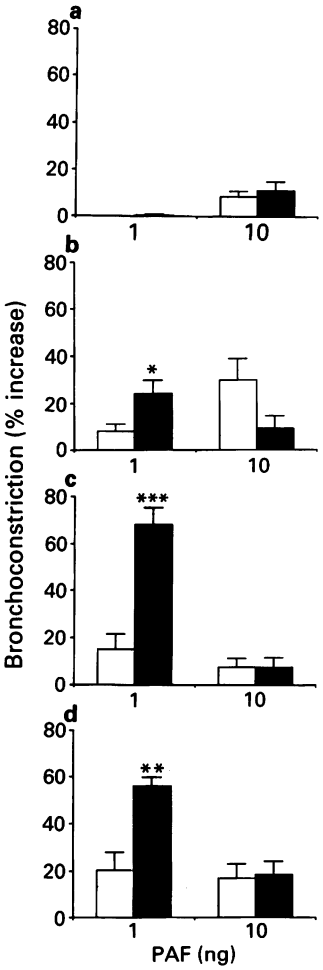

Figure 1 Bronchoconstriction induced by the i.a. administration of 1 and 10 ng PAF into guinea-pig isolated lungs 10 min after an i.t. injection of 100 µl saline (open columns) or 300 ng rhIL-5 (solid columns). Lungs were obtained from adjuvant-treated guinea-pigs (a), or from actively sensitized animals used either 16 days after the first sensitizing injection (b), or 2 (c) or 7 (d) days after the booster injection of the antigen. Bronchoconstrictions were calculated as areas under the curve of increased airways resistance measured for 10 min. Results (mean with s.e.mean of 5–6 experiments) are expressed as % maximal bronchoconstriction obtained by clamping the tracheal cannula.
$^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$.

The instillation of 100–300 ng of rhIL-5 to lungs from actively sensitized guinea-pigs used 2 days after the booster injection of antigen induced a dose-related increase in bronchoconstriction in response to 1 ng of PAF (Figure 2a, left panel). By contrast, when 300 ng of rhIL-5 was instilled, the bronchoconstrictor response to 1 ng of PAF was so intense as to desensitize the lungs to a subsequent administration of 10 ng of PAF (Figure 2a, right panel). Such a desensitization was also observed with respect to TXB_2 and histamine release (see below).

In a separate series of experiments, the administration of 0.01 ng PAF to lungs from guinea-pigs killed 2 days after the booster injection of the antigen, also induced a moderate bronchoconstriction, which was markedly increased when preceded by the i.t. instillation of 300 ng of rhIL-5. Indeed, bronchoconstrictions amounting to 3.3 ± 1.1% and 40.2 ± 5.9% ($n = 5–6$, $P<0.001$) were measured in vehicle- and rhIL-5-treated lungs, respectively.

Effect of rhIL-5 on PAF-induced mediator release

No release of TXB_2 or histamine above basal values was detected following the administration of 1 and 10 ng PAF to lungs from adjuvant-injected guinea-pigs, irrespective of prior instillation of either rhIL-5 or its vehicle (Figures 3a and 4a). Higher amounts of TXB_2 were released after injection of 1 ng PAF to lungs obtained from actively sensitized guinea-pigs killed at various intervals following the booster injection of antigen, as compared to those from adjuvant-treated or actively sensitized and non-boosted guinea-pigs (Figure 3). Following the injection of 300 ng rhIL-5, the production of TXB_2 in response to 1 ng PAF was markedly increased in lungs from guinea-pigs used 2 days after the booster injection of antigen (Figure 3c) and, to a lesser extent, in those from animals used after 7 days (Figure 3d). In both groups, the increased TXB_2 production was also observed after the injection of 10 ng PAF, although the difference was less marked (Figure 3c and d).

As previously described (Pretolani *et al.*, 1988), histamine was secreted by PAF-stimulated lungs in amounts above basal values only when the organs were obtained from guinea-pigs used 2 or 7 days after the booster injection of antigen (Figure 4). Under these conditions, the administration of 300 ng rhIL-5 significantly increased histamine release in re-

Table 1 Bronchoconstriction and release of thromboxane B_2 (TXB_2) and of histamine induced by the i.a. injection of 1 and 10 ng of PAF 10 min after the i.t. administration of 100 µl saline (vehicle) or 300 ng rhIL-5 into isolated lungs of passively sensitized guinea-pigs

	Vehicle		*rhIL-5 (300 ng)*	
PAF (ng)	1	10	1	10
Bronchoconstriction (%)	3.2 ± 1.6	16.9 ± 3.1	3.0 ± 1.9	14.2 ± 3.0
Thromboxane B_2 (ng ml^{-1})	0.7 ± 0.3	3.3 ± 1.4	0.8 ± 0.3	3.2 ± 0.6
Histamine (ng ml^{-1})	1.3 ± 0.3	2.4 ± 0.2	1.3 ± 0.1	2.9 ± 0.1

Bronchoconstriction was calculated and expressed as described in the legend of Figure 1. Thromboxane B_2 and histamine release were measured in the lung effluent over a 6 min period following the injection of PAF. Results are expressed in ng ml^{-1} (mean ± s.e.mean of 5–6 experiments).

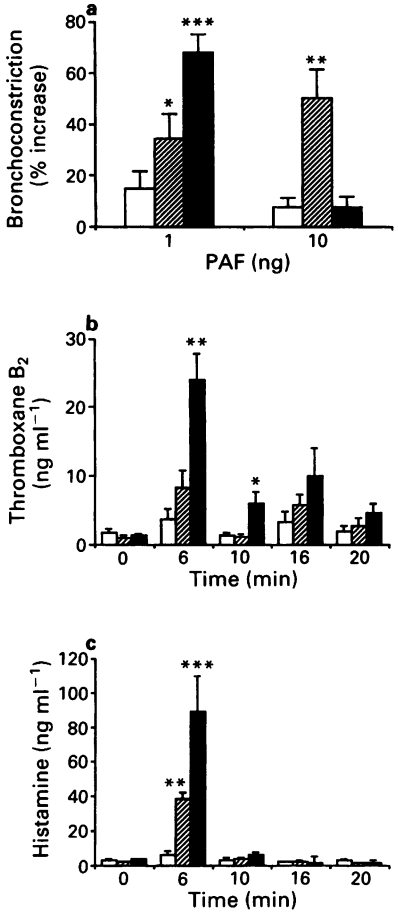

Figure 2 Bronchoconstriction (a) and kinetics of the release of thromboxane B_2 (TXB_2) (b) and of histamine (c) induced by the i.a. injection of 1 and 10 ng PAF 10 min after the i.t. administration of 100 µl saline (open columns), or 100 ng (hatched columns) or 300 ng (solid columns) rhIL-5 into isolated lungs from actively sensitized guinea-pigs used 2 days after the booster injection of antigen. Bronchoconstriction was calculated as described in the legend of Figure 1. Release of TXB_2 and histamine were measured in 1 ml fractions of lung effluent collected before and during the first 6 min after injection of 1 and 10 ng PAF. Results are expressed as mean with s.e.mean (vertical bars) of 5 experiments.
*$P<0.05$; **$P<0.01$; ***$P<0.001$.

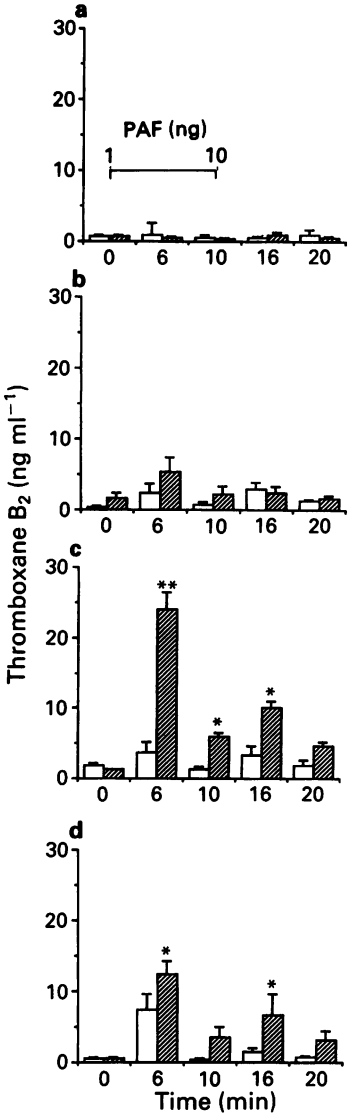

Figure 3 Kinetics of thromboxane B_2 (TXB_2) release induced by the i.a. administration of 1 and 10 ng PAF 10 min after an i.t. injection of 100 µl saline (open columns) or 300 ng rhIL-5 (hatched columns) into guinea-pig isolated lungs. Lungs were obtained from adjuvant-treated guinea-pigs (a), or from actively sensitized animals used either 16 days after the first sensitizing injection (b), or 2 (c) or 7 (d) days after the booster injection of antigen. Results were calculated and expressed as described in the legend of Figure 2 (mean with s.e. mean [vertical bars] of 5–6 experiments).
*$P<0.05$; **$P<0.01$.

sponse to the injection of 1 ng PAF (Figure 4). This increase was more pronounced in lungs from animals killed 2 days after the booster injection of antigen (Figure 4c), as compared to those used 7 days thereafter (Figure 4d). However, no histamine release was noted following the second challenge with 10 ng PAF.

As in case of bronchoconstriction, the effect of rhIL-5 was dose-dependent, since lungs from actively sensitized guinea-pigs used 2 days after the booster injection of the antigen and injected i.t. with 100 ng rhIL-5 also released more TXB_2 and histamine in response to 1 ng PAF, but this increase was less than when 300 ng of the cytokine was used (Figure 2b and c).

The administration of 0.01 ng PAF to lungs from actively sensitized guinea-pigs used 2 days after the booster injection of antigen, induced the release of low quantities of TXB_2 and histamine into the effluent. Pretreatment of the lungs with 300 ng rhIL-5 significantly increased these amounts, since 2.3 ± 1.3 and 8.1 ± 1.3 ng ml^{-1} TXB_2 ($n = 5-7$; $P<0.05$) and 2.3 ± 0.4 and 28.2 ± 4.0 ng ml^{-1} histamine ($n = 5-7$, $P<0.001$) were measured in vehicle- and in rhIL-5-treated lungs, respectively.

Kinetics of rhIL-5-induced lung hyperresponsiveness to PAF

The i.a. injection of 1 ng of PAF 2 min after the i.t. administration of 300 ng rhIL-5 to lungs from guinea-pigs killed 2 days after the booster injection of antigen triggered slight bronchoconstriction and the release of small amounts of TXB_2 and histamine, responses similar to those observed in saline-injected preparations (Figure 5). In contrast, the pulmonary response was increased markedly when PAF was injected 10 min after rhIL-5 and this increase persisted for at least 30 min (Figure 5).

Effect of rhIL-5 on 5-HT-induced bronchoconstriction

Regardless of the previous administration of 300 ng rhIL-5 or of its vehicle, i.a. injections of 5-HT (10 to 3000 ng) to

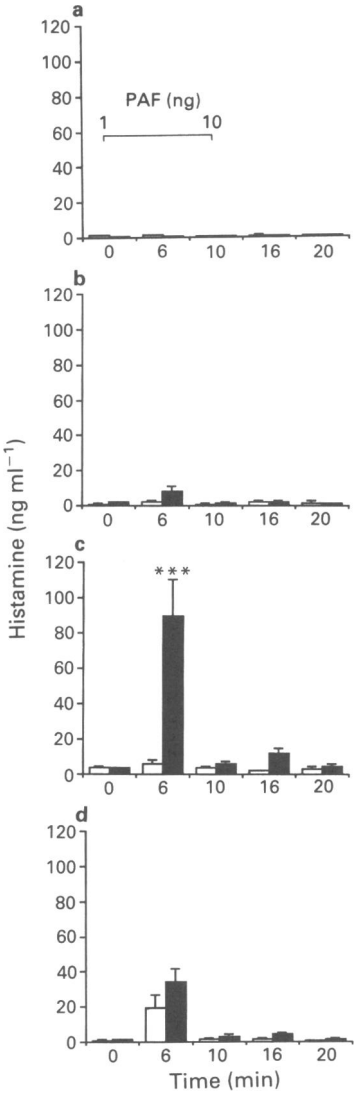

Figure 4 Kinetics of histamine release induced by the i.a. administration of 1 and 10 ng PAF 10 min after an i.t. injection of 100 µl saline (open columns) or 300 ng rhIL-5 (solid columns) into guinea-pig isolated lungs. Lungs were obtained from adjuvant-treated guinea-pigs (a), or from actively sensitized animals used either 16 days after the first sensitizing injection (b), or 2 (c) or 7 (d) days after the booster injection of antigen. Results were calculated and expressed as described in the legend of Figure 2 (mean with s.e.mean [vertical bars] of 5–6 experiments).
***$P<0.01$.

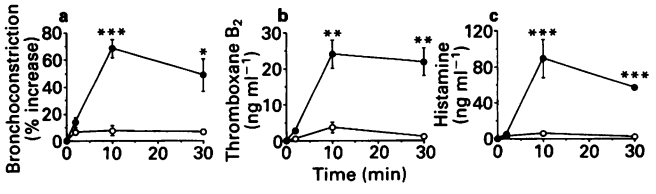

Figure 5 Kinetics of rhIL-5-induced lung hyperresponsiveness to PAF in isolated lungs from actively sensitized guinea-pigs used 2 days after the booster injection of antigen. PAF (1 ng) was administered i.a. at different time intervals (2, 10 and 30 min) after the i.t. injection of 100 µl saline (○) or 300 ng rhIL-5 (●). Bronchoconstriction (a) was calculated as described in the legend to Figure 1. Thromboxane B_2 (b) and histamine release (c) were measured in the lung effluent collected for 6 min after the injection of PAF and results are expressed in ng ml^{-1} (mean with s.e.mean [vertical bars] of 4–6 experiments).
*$P<0.05$; **$P<0.01$; ***$P<0.001$.

lungs from actively sensitized guinea-pigs used 2 days after the booster injection of antigen evoked a similar dose-dependent bronchoconstriction (Figure 6). No significant release of TXB_2 or histamine in response to 5-HT was measured in the effluent of vehicle- or rhIL-5-treated lungs (not shown).

Effect of lipopolysaccharide on PAF-induced bronchoconstriction and mediator release

The possibility that rhIL-5 was contaminated with LPS led us to study whether the lung response to PAF would be modified by LPS alone. The i.t. administration of 100 µg LPS neither induced bronchoconstriction nor mediator release from lungs of actively sensitized guinea-pigs used 2 days after the booster injection of antigen (not shown). In addition, bronchoconstriction and release of TXB_2 and histamine in response to the i.a. injections of 1 and 10 ng PAF were similar in LPS-treated, as compared to saline-injected lungs. Indeed, bronchoconstrictor responses of $15.1 \pm 3.5\%$ and $7.6 \pm 3.7\%$ were measured after 1 and 10 ng PAF, respectively in lungs previously injected i.t. with 100 µl saline, and $13.5 \pm 2.9\%$ and $8.4 \pm 3.0\%$ ($n = 4$; not significant) in LPS-treated lungs. PAF at 1 and 10 ng triggered the release of 3.7 ± 1.4 ng ml^{-1} and 3.3 ± 1.4 ng ml^{-1} TXB_2 after 100 µl saline and of 3.2 ± 0.2 ng ml^{-1} and 3.2 ± 0.6 ng ml^{-1} TXB_2 after the injection of LPS ($n = 4$; not significant). Similarly, 6.1 ± 2.1 ng ml^{-1} and 2.2 ± 0.4 ng ml^{-1} and 5.6 ± 1.4 ng ml^{-1} and 3.0 ± 1.2 ng ml^{-1} histamine ($n = 4$; not significant) were measured in the effluent of saline- and LPS-treated lungs.

Effect of active sensitization on the cell composition of bronchoalveolar lavage fluid

The average number of cells recovered was $3.3 \pm 0.8 \times 10^5$ cells ml^{-1} ($n = 8$), $4.1 \pm 0.4 \times 10^5$ cells ml^{-1} ($n = 10$), $4.9 \pm 0.4 \times 10^5$ cells ml^{-1} ($n = 10$) and $7.1 \pm 0.4 \times 10^5$ cells ml^{-1} ($n = 10$) in BAL from non-immunized, actively sensitized non boosted and actively sensitized guinea-pigs used 2 or 7 days after the booster injection of the antigen, respectively. The number of cells recovered in BAL from actively sensitized guinea-pigs used 7 days after the booster injection of antigen was significantly higher ($P<0.01$) when compared to that evaluated in the other groups of animals. As seen in Figure 7, non boosted or boosted guinea-pigs showed a significant increase in the number of eosinophils in BAL, as compared

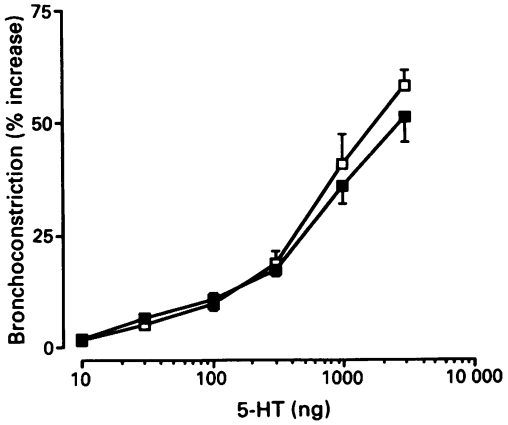

Figure 6 Bronchoconstriction induced by the i.a. administration of 10 to 3000 ng 5-hydroxytryptamine (5-HT) into isolated lungs from actively sensitized guinea-pigs killed 2 days after the booster injection of antigen and pretreated by an i.t. injection of 100 µl saline (□) or 300 ng rhIL-5 (■) 10 min before-hand. Bronchoconstriction was expressed as % of the peak-intensity of the response (mean with s.e.mean [vertical bars] of 4 experiments).

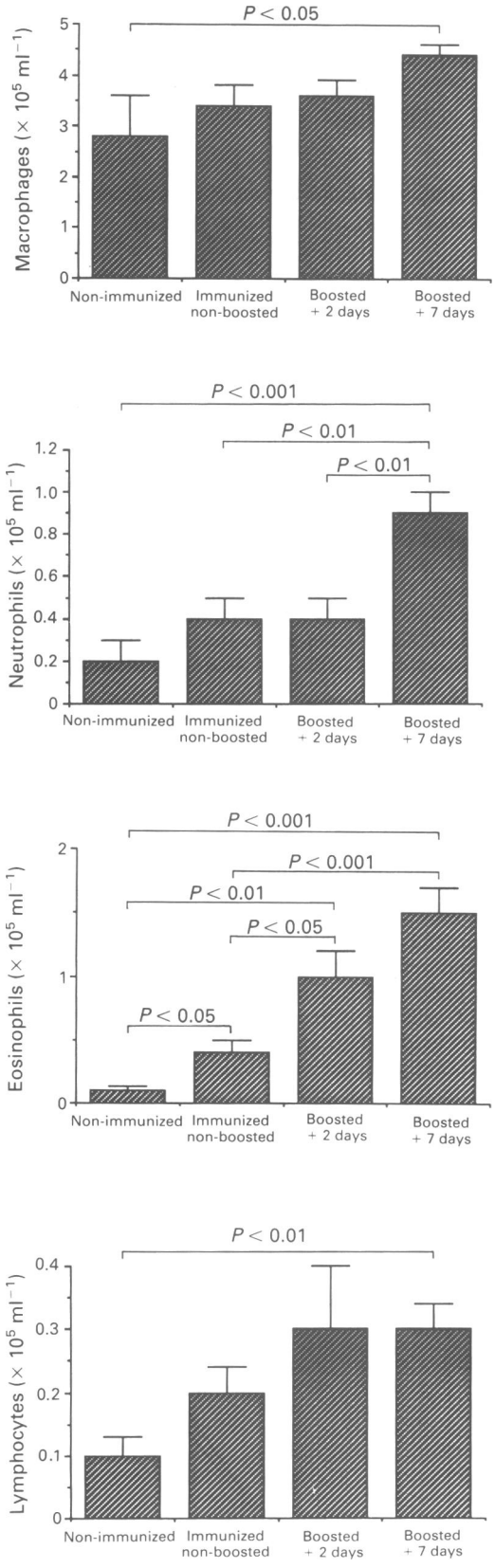

Figure 7 Cell content of bronchoalveolar lavage fluid (BAL) obtained from non-immunized guinea-pigs or from animals actively sensitized by a first s.c. injection of 10 μg ovalbumin in 1 mg of the adjuvant, i.e. $Al(OH)_3$ and used either 2 days after a s.c. injection of the adjuvant alone (group 'Immunized non-boosted'), or 2 or 7 days after a booster injection of antigen (groups 'Boosted + 2 days' and 'Boosted + 7 days', respectively). Bronchoalveolar cells were collected in 10 successive lavages and differentiated by May-Grünwald-Giemsa stain. Results are expressed as concentration of each cell population ml^{-1}. Data are expressed as mean with s.e.mean (vertical bars) of 8–10 experiments.

to non immunized animals. This increase reached a maximum 7 days after the booster injection, even though no significant difference was observed when compared to the animals killed 2 days after the booster injection. A significant increase in the number of the other cell types, i.e., alveolar macrophages, polymorphonuclear neutrophils and lymphocytes was detected only in BAL obtained from guinea-pigs used 7 days after the booster injection of antigen (Figure 7).

Discussion

In this study, the *in vitro* administration of rhIL-5 into lungs from actively sensitized guinea-pigs is shown to trigger a marked time-dependent hyperresponsiveness to PAF. The observation that a human cytokine affects the reactivity of an animal tissue is not an exception, since rhGM-CSF, rhIL-3 and rhIL-5 share antigenic and functional activities with cytokines from other species (Metcalf, 1986; Yamaguchi *et al.*, 1988). Hyperresponsiveness to PAF following i.t. administration of rhIL-5 was intense in lungs obtained from immunized and boosted animals and was present, even though to a much lower extent, in those from guinea-pigs actively sensitized but not boosted. This agrees with our previous results showing that the booster injection of the antigen is followed within a few days by an increased lung responsiveness to PAF (Pretolani *et al.*, 1988). The key role of active sensitization was confirmed by the failure of rhIL-5 to enhance bronchoconstriction and mediator release in response to PAF in lungs from non-immunized or passively sensitized animals. This parallels our previous finding showing that passively sensitized animals do not exhibit lung hyperresponsiveness to PAF (Pretolani *et al.*, 1988), indicating that the presence of homocytotropic antibodies in the lung (Lagente *et al.*, 1987) cannot account alone for the observed modifications of its reactivity.

In the present experiments, rhIL-5 was given i.t., because we assumed that in allergic situations, it is produced by stimulated mast cells (Plaut *et al.*, 1989) and by T lymphocytes (Coffman *et al.*, 1988), located in the airways. T lymphocytes are found in increased numbers in the BAL (this manuscript) and in the bronchial submucosa of sensitized guinea-pigs (Frew *et al.*, 1990; Lapa e Silva *et al.*, 1992). PAF, on the other hand, was given i.a., since this route has allowed us to demonstrate that lungs from sensitized and boosted, as compared to non-boosted guinea-pigs, show an enhanced responsiveness to PAF (Pretolani *et al.*, 1988).

Two min after the administration of rhIL-5, no significant increase in the responsiveness to PAF was observed, indicating that a delay of a few minutes is required to allow the diffusion of rhIL-5 throughout the lung tissue or to exert its effect. By contrast, hyperresponsiveness to PAF reached a peak by 10 min and remained at a plateau for at least 30 min, despite the continuous lung perfusion and wash-off of the cytokine. In this case, rhIL-5 can neither operate as a differentiating/maturing substance nor via cell recruitment, since the enhancement of the effects of PAF occurred in the isolated lungs and over a short period of time, i.e. within 10 min.

To verify whether the enhancing effect of rhIL-5 was restricted to PAF, the lungs were challenged with 5-HT, which induces a smooth muscle contraction, attributable mostly to a direct effect. Since the administration of rhIL-5 failed to induce hyperresponsiveness to 5-HT, the smooth muscle is probably not the direct target for rhIL-5. Most likely, active immunization and rhIL-5 promote hyperresponsiveness which is revealed by agonists such as PAF, interacting with inflammatory cells (Braquet *et al.*, 1987).

Among the various cytokines, IL-3, IL-5 and GM-CSF are major candidates for playing a role in the inflammatory component of immediate hypersensitivity reactions. At low concentrations, they increase eosinophil (Fujisawa *et al.*, 1990), basophil (Bischoff *et al.*, 1990a,b) and neutrophil

(Dahinden *et al.*, 1988) response to exogenous stimuli. Interestingly, this increase involves the release of arachidonic acid metabolites including LTC_4, which is produced in larger amounts upon stimulation with various agonists after pretreatment of the cells with these cytokines (Dahinden *et al.*, 1988; 1989; Kurimoto *et al.*, 1989; Bischoff *et al.*, 1990a,b). Since peptido-leukotrienes are potent smooth muscle-contracting agents released during allergic or non-allergic inflammatory reactions (Piper, 1984), a link between the regulatory role of haematopoietic growth factors and lipid mediators appears likely.

Our results on isolated lung preparations raise the question of the target(s) for IL-5 present only in lungs from actively sensitized guinea-pigs and not in those from normal animals. Our previous observation that actively sensitized and boosted guinea-pigs exhibit an increased number of eosinophils in the BAL fluid, as compared to non-immunized animals (Pretolani *et al.*, 1990) and the evidences that IL-5 stimulates selectively mature eosinophils (Lopez *et al.*, 1988; Wang *et al.*, 1989), may explain the increase in lung response to PAF brought about by this cytokine. Indeed, in the present work the number of eosinophils was markedly increased in BAL obtained from guinea-pigs killed after the booster injection of antigen, suggesting that the development of lung hyperresponsiveness to PAF induced by rhIL-5 follows a profile similar to that of eosinophil invasion into the airways. Since a significant ($P<0.05$) augmentation of the number of eosinophils was also observed in BAL from unboosted sensitized guinea-pigs, it is likely that inflammatory mechanisms, which are markedly enhanced by the booster administration of the antigen, are already operative in its absence. This may explain the increased response to PAF after the acute instillation of rhIL-5 which is already observed, although to a lower extent, in lungs from sensitized and non-boosted animals.

A similar increase in the effects of PAF induced by rhIL-5 has been observed in purified peritoneal guinea-pig eosinophils (Coëffier *et al.*, 1991). Indeed, incubation of these cells with this cytokine is followed by an increased PAF-induced chemotactic activity and superoxide anion generation. This phenomenon is restricted to PAF, since no priming of the LTB_4-induced effects by rhIL-5 was observed.

A pulmonary eosinophilia following s.c. or i.p. injections of factors which promote eosinophil differentiation and activation, such as GM-CSF and IL-3, has been demonstrated in normal guinea-pigs (Kings *et al.*, 1990; Sanjar *et al.*, 1990). In those cases, however, eosinophilia was not accompanied by bronchial hyperresponsiveness to PAF or to histamine (Sanjar *et al.*, 1990), indicating that the lung invasion by eosinophils alone does not account for its development. These results and ours do not conflict, since those experiments were performed on non-immunized guinea-pigs which, according to our hypothesis, lack the specific target(s) for IL-5 that are only present in the lungs after the booster injection of the antigen.

Even though eosinophils recruited into the lungs by the booster injection of antigen are a likely target for rhIL-5, other mechanisms may account for the increased responsiveness induced by rhIL-5 observed in the lung from boosted guinea-pigs. Indeed, following a second immune challenge, the release from T lymphocytes of cytokines other than IL-5 may influence the activation of lung cells and thus play a role in the priming effect induced by rhIL-5. In addition, it has been shown that, besides eosinophils, basophils are also a target for IL-5 and may thus participate in the development of lung hyperresponsiveness observed under our conditions. Indeed, rhIL-5 primes human basophils (Bischoff *et al.*, 1990a) which are involved in cutaneous hypersensitivity reaction in the guinea-pig (Golden *et al.*, 1986), a situation that is reminiscent of our observations in lungs.

In conclusion, our data indicate that IL-5 may be implicated in bronchopulmonary allergic reactions in the guinea-pig, particularly in the expression of lung hyperresponsiveness and the underlying airway inflammation. These results should be considered in the light of recent observations (Hamid *et al.*, 1991) showing an increased messenger RNA expression of the IL-5 gene in mucosal bronchial biopsies from asthmatic subjects.

The ability of rhIL-5 to increase the lung response to PAF suggests that an interaction between cytokines and lipid mediators may contribute to the bronchopulmonary hyperresponsiveness that accompanies airway inflammation.

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