

33. Weisbart RH, Golde DW, Gasson JC. Biosynthetic human GM-CSF modulates the number and affinity of neutrophil formyl-met-leu-phe receptors. *J Immunol* 1986;137:3584-7.
34. Bruijnzeel PLB, Warringa RAJ, Kok PTM. Inhibition of platelet-activating-factor and zymosan-activated-serum induced chemotaxis and cytosolic free calcium mobilization of human neutrophils by nedocromil sodium, BN 52021 and sodium cromoglycate. *Br J Pharmacol* 1989;97:1251-7.
35. Zigmond SH, Slonczewski JL, Wilde MW, Carson M. Polymorphonuclear leukocyte migration is insensitive to lowered cytoplasmic calcium levels. *Cell Motil Cytoskeleton* 1988;9:184-9.

Inhibition by nedocromil sodium of recombinant human interleukin-5-induced lung hyperresponsiveness to platelet-activating factor in actively sensitized guinea pigs

Marina Pretolani, PhD, Jean Lefort, BSc, and B. Boris Vargaftig, MD
Paris, France

Background: The intratracheal instillation of recombinant human interleukin-5 (rhIL-5) into isolated lungs from sensitized and antigen-boosted guinea pigs is followed by enhanced responses to platelet-activating factor (PAF).

Methods: Modulation by nedocromil sodium of rhIL-5-dependent hyperresponsiveness to PAF was investigated. Perfused lungs from guinea pigs actively sensitized to ovalbumin were injected intratracheally with 300 ng rhIL-5 followed by PAF (10 and 100 ng intraarterially).

Results: No inhibition of rhIL-5-induced increased bronchoconstriction and release of thromboxane (TX)₂ and histamine by PAF was observed when nedocromil sodium (10 μ mol/L) was perfused into the lungs. In contrast, when guinea pigs were treated for 2 days (30 mg/kg twice a day), eosinophil recruitment into the bronchoalveolar lavage fluid and hyperresponsiveness to PAF were markedly reduced (100% [$p < 0.001$]; around 70% [$p < 0.05$] and around 90% [$p < 0.001$] inhibition for bronchoconstriction, TXB₂ and histamine release, respectively).

Conclusion: Our results indicate that nedocromil sodium prevents the rhIL-5-induced lung hyperresponsiveness to PAF and the eosinophil migration into the airways only when it is administered in vivo. It is thus suggested that nedocromil sodium interferes with the development of airway inflammation by inhibiting the recruitment in the lung of a target on which PAF and IL-5 interact. (*J ALLERGY CLIN IMMUNOL* 1993;91:809-16.)

Key words: Interleukin-5, platelet-activating factor, nedocromil sodium, lung hyperresponsiveness, guinea pig, eosinophils, bronchoalveolar lavage

From Unité de Pharmacologie Cellulaire Unité Associée Institut Pasteur/INSERM no. 285, 25, rue du Dr. Roux, 75015, Paris, France

Received for publication April 10, 1992.

Revised Sept. 22, 1992.

Accepted for publication Oct. 14, 1992.

Reprint requests: Marina Pretolani, PhD, Unité de Pharmacologie Cellulaire Unité Associée Institut Pasteur/INSERM no. 285, 25, rue du Dr. Roux, 75015, Paris, France.

Copyright © 1993 Mosby-Year Book, Inc.

0091-6749/93 \$1.00 + .10 1/1/43496

The late phase reaction of allergic asthma is associated with airway inflammation, activation of resident lung cells, and migration into the airways of inflammatory cells, including eosinophils, neutrophils, and lymphocytes.¹ These cells may contribute to the amplification and to the perpetuation of the disease, particularly on repeated exposures to the allergen, by releasing cytotoxic enzymes and proinflammatory mediators. As suggested recently, T lymphocytes may play a major role in airway inflammation

Abbreviations used

IL:	Interleukin
rhIL-5:	Recombinant human IL-5
GM-CSF:	Granulocyte-macrophage colony-stimulating factor
PAF:	Platelet-activating factor
TX:	Thromboxane
PG:	Prostaglandin
BAL:	Bronchoalveolar lavage
BSA:	Bovine serum albumin

and in the consequent bronchial hyperreactivity,^{2, 3} because they secrete cytokines in response to antigen. These cytokines include interleukin (IL)-5, IL-3, and granulocyte-macrophage colony stimulating factor (GM-CSF), which induce eosinophil proliferation and differentiation from their bone marrow precursors and prime target cells for enhanced response to different mediators.⁴⁻⁹ In addition, an increased messenger RNA expression for the IL-5, IL-3, and GM-CSF protein was detected in cells obtained by bronchoalveolar lavage (BAL) of asthmatic subjects, as compared with healthy individuals.¹⁰

We have recently shown that the intratracheal administration of recombinant human IL-5 (rhIL-5) to isolated perfused lungs from guinea pigs actively sensitized to ovalbumin triggers a marked dose-dependent hyperresponsiveness to the subsequent intraarterial injection of platelet-activating factor (PAF).¹¹ This phenomenon, which is characterized by an enhanced bronchoconstriction and an increased release of thromboxane (TX)A₂ and histamine into the effluent, is very marked in lungs from guinea pigs killed 2 or 7 days after the booster injection of the antigen and not so in those from nonimmunized animals. The kinetics of the development of lung hyperresponsiveness to PAF induced by rhIL-5 and that of eosinophil migration into the airways were similar. Indeed, the number of eosinophils in the BAL fluid was significantly higher in guinea pigs used 2 or 7 days after the booster injection of the antigen as compared with nonboosted or nonsensitized animals. These results suggest that priming by rhIL-5 of a specific target recruited into the airways after repeated exposures to the antigen is followed by enhanced lung sensitivity to the bronchoconstrictor and secretory effects of PAF. This target may be the eosinophil, since its *in vitro* priming by IL-5 leads to increased chemotaxis and release of enzymes and proinflammatory mediators in response to various agonists, including PAF.^{12, 13} Because the booster injection of antigen is the critical step for the development of hyperresponsiveness to PAF induced

by rhIL-5 and, concomitantly, for the migration of eosinophils into the airways, we hypothesized that an antiallergic drug might inhibit these phenomena when administered at the time of and after the booster injection of the antigen.

Nedocromil sodium is an antiallergic drug that reduces airway inflammation. Even though its precise mode of action has not been identified yet, evidence indicates that nedocromil sodium inhibits the *in vitro* mobilization and activation of inflammatory cells, including eosinophils.¹⁴ Furthermore, in various experimental situations eosinophil migration into the lung tissue and the accompanying hyperresponsiveness that follow allergen exposure of immunized animals are markedly decreased by nedocromil sodium.¹⁵⁻¹⁹ These findings led us to investigate the potential protective effect of nedocromil sodium against enhanced bronchoconstriction and mediator release induced by rhIL-5 after PAF administration to isolated lungs from actively sensitized guinea pigs.

In the present study we demonstrate that a 2-day treatment of guinea pigs with nedocromil sodium, the day of the booster injection of the antigen and the day after, inhibited *in vitro* lung hyperresponsiveness to PAF induced by rhIL-5. In contrast, nedocromil sodium was ineffective when added directly to perfusion medium on the day of the experiment. In addition, nedocromil sodium significantly inhibited the increased number of eosinophils recovered in the BAL fluid from immunized and boosted guinea pigs. These results suggest that protection by nedocromil sodium of lung hyperresponsiveness to PAF induced by rhIL-5 in actively sensitized and boosted guinea pigs involves inhibition of the lung invasion by eosinophils.

METHODS

Sensitization procedures

Male Hartley guinea pigs (400 to 600 gm) were actively sensitized by two subcutaneous injections at a 2-week interval of 0.5 ml of 0.9% NaCl (saline solution) containing 10 µg of ovalbumin dispersed in 1 mg Al(OH)₃. In a separate set of experiments, guinea pigs received the first sensitizing injection of ovalbumin, but they were treated with the adjuvant alone, that is, 1 mg Al(OH)₃ on the day of the booster injection. In both cases the animals were used 2 days after the second injection.

When the effect of nedocromil sodium was investigated, the drug was administered subcutaneously on the day of the booster injection and the day after, twice a day, at a dose of 30 mg/kg. Control animals were treated with vehicle alone, that is, saline.

Lung perfusion experiments

Guinea pigs were anesthetized with sodium pentobarbitone (30 mg/kg, intraperitoneally), tracheas were cannulated, and the animals' lungs were ventilated (60

strokes/min; 1 ml/100 gm body weight) with Palmer miniature respiratory pumps (Bioscience, Sheerness, U.K.). A thoracotomy was performed, and the lungs were removed as described.²⁰ Each organ was placed in a plastic chamber, immediately ventilated (60 strokes/min; 1 ml/100 gm body weight), and perfused via the pulmonary artery (10 ml/min, 37° C) with a gassed (95% O₂/5% CO₂) Krebs solution containing 0.25% (wt/vol) bovine serum albumin (BSA) supplemented or not with 10 µmol/L nedocromil sodium. The changes in resistance to inflation were continuously recorded, as described previously.²⁰ After a 10-minute equilibration period, 300 ng of rhIL-5 were injected intratracheally in a volume of 100 µl. In control experiments the lungs received 100 µl saline solution as the vehicle. Ten minutes after the injection of rhIL-5 or of saline solution, PAF doses (10 and 100 ng in 100 µl saline solution containing 0.25% BSA) were sequentially administered into the pulmonary artery, at 10-minute intervals.

Bronchoconstrictions were measured as the area of the tracing above the basal resistance to inflation for 10 minutes. Results are expressed as percent of bronchoconstriction compared with a value of 100% obtained by clamping the ventilation system. One-milliliter fractions of the lung effluent were collected before and during the first 6 minutes after the injection of the various agonists for the determination of TXB₂ and histamine, as described elsewhere.^{21, 22}

Measurement of TXB₂ 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 minutes to allow the conversion of TXA₂ into its stable metabolite, TXB₂. The radioimmunoassay was performed as described earlier.²³ The monoclonal antibody used was less than 0.02% cross-reactive with prostaglandin (PG)D₂, PGE₂, PGF_{2α}, 6-keto-PGF_{1α} and arachidonic acid. The threshold sensitivity of the assay was approximately 2 pg of immunoreactive TXB₂ in 100 µl sample.

For the histamine assay, 1 ml of each collected fraction was mixed with 1 ml of an 0.8N perchloric acid solution. After centrifugation for 10 minutes at 1200 g and at 4° C, the supernatants were stored at 4° C. An automatic spectrofluorometric assay for histamine was performed according to a previously published method.²²

Harvest of bronchoalveolar cells

Three groups of animals were used: (1) sensitized and nonboosted guinea pigs; actively sensitized and boosted guinea pigs killed 2 days after the booster injection of the antigen and treated subcutaneously with (2) saline solution; or with (3) 30 mg/kg nedocromil sodium. The animals were anesthetized as above, and bronchoalveolar cells were collected in 10 successive lavages with use of 5 ml aliquots of sterile saline solution at room temperature injected and recovered through a polyethylene tracheal cannula. The lavage fluid was stored on ice, and total cell counts were performed. The suspension was then diluted to reach a final concentration of 1.5×10^5 cell/ml, and after cytocentrifugation, dif-

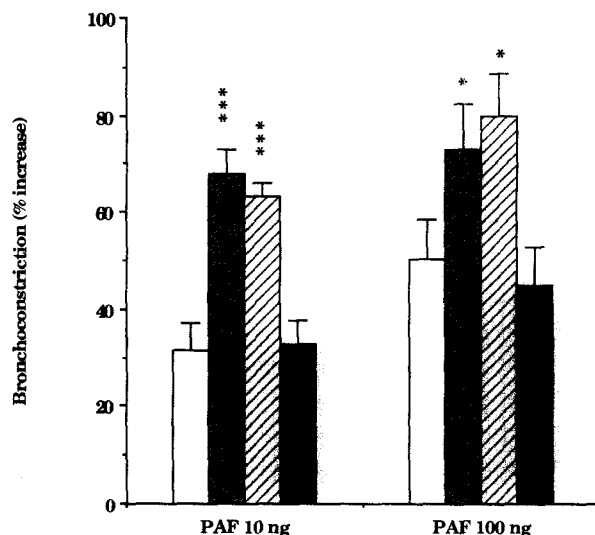


FIG. 1. Bronchoconstriction induced by intraarterial administration of 10 and 100 ng PAF into isolated guinea pig lungs injected intratracheally 10 minutes before either with 100 µl saline solution (vehicle, *open columns*) or with 300 ng rhIL-5 (*heavily hatched columns*). Lungs obtained from actively sensitized animals used 2 days after a booster injection of antigen. Nedocromil sodium (10 µmol/L) added to perfusion medium (*hatched columns*) or administered subcutaneously for 2 days, twice a day, at the dose of 30 mg/kg (*solid columns*). Bronchoconstriction expressed as percent of area of tracing measured over basal resistance to inflation for 10 minutes. Results expressed as mean ± SEM of 6 to 9 experiments. **p* < 0.05 ****p* < 0.001.

ferential cell counts were performed after staining with May-Grünwald-Giemsa dye. Because the yield of the injected fluid was equivalent in all groups of animals (approximately 95%), the results are expressed as concentration of each cell population per milliliter.

Materials

The composition of the Krebs solution was (in mmol/L): NaCl, 118; KCl, 4.7; CaCl₂ · 2H₂O, 2.5; MgSO₄ · 7H₂O, 1.2; KH₂PO₄, 1.2; NaHCO₃, 25 and glucose, 5.6. The reagents used were the following: rhIL-5 (Mony's International Imports, Beverly Hills, Calif.); PAF (1-O-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine), BSA fraction V, bovine γ-globulins fraction II, TXB₂ (Sigma Chemical Co., St. Louis, Mo.); Al(OH)₃, perchloric acid, polyethyleneglycol 6000 (Merck, Darmstadt, Germany); pentobarbitone (sodium pentobarbital; Clin-Midy, Montpellier, France); chicken ovalbumin (Miles, Naperville, Ill.); the antibody and radiolabeled ligand for radioimmunoassay of TXB₂ were from the UR1A, Institut Pasteur/INSERM U 207, Paris, France. Nedocromil sodium (kindly provided by Dr. A. Norris, Fisons plc, Loughborough, U.K.) was dissolved in saline solution at a concentration of 75 mg/ml and further diluted in saline solution.

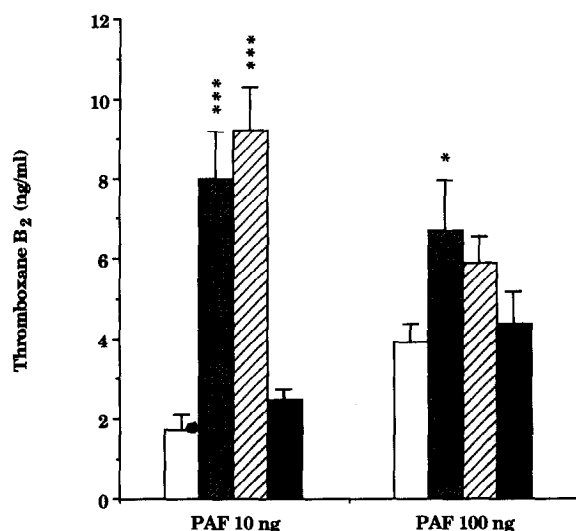


FIG. 2. TXB₂ release induced by intraarterial administration of 10 and 100 ng PAF into isolated guinea pig lungs injected intratracheally 10 minutes before either with 100 μ l saline solution (vehicle, *open columns*) or with 300 ng rhIL-5 (*heavily hatched columns*). Lungs obtained from actively sensitized animals used 2 days after a booster injection of antigen. Nedocromil sodium (10 μ mol/L) added to perfusion medium (*hatched columns*), or administered subcutaneously for 2 days, twice a day, at the dose of 30 mg/kg (*solid columns*). TXB₂ contents determined by radioimmunoassay in fractions of lung effluent collected before and during first 6 minutes after successive injections of PAF. Background value for TXB₂ release was 0.75 ± 0.20 ng/ml; not modified either by the presence of nedocromil sodium in buffer or by its in vivo administration. Data expressed as means $\pm$ SEM of 6 to 9 experiments). * $p < 0.05$ *** $p < 0.001$.

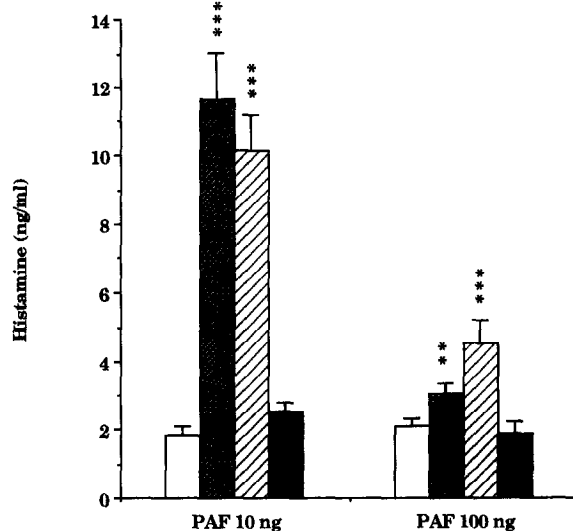


FIG. 3. Histamine release induced by intraarterial administration of 10 and 100 ng PAF into isolated guinea pig lungs injected intratracheally 10 minutes before either with 100 μ l saline solution (vehicle, *open columns*) or with 300 ng rhIL-5 (*heavily hatched columns*). Lungs obtained from actively sensitized animals used 2 days after a booster injection of antigen. Nedocromil sodium (10 μ mol/L) added to the perfusion medium (*hatched columns*), or administered subcutaneously for 2 days, twice a day, at the dose of 30 mg/kg (*solid columns*). Histamine contents determined by a spectrofluorometric method in fractions of lung effluent collected before and during first 6 minutes after successive injections of PAF. Background value for histamine release was 1.5 ± 0.2 ng/ml; modified neither by the presence of nedocromil sodium in the buffer nor by its in vivo administration. Data expressed as mean $\pm$ SEM of 6 to 9 experiments). * $p < 0.05$ *** $p < 0.001$.

Data analysis

The results are expressed as means $\pm$ SEM 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 $p \leq 0.05$ was considered significant.

RESULTS

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

The intratracheal instillation of 100 μ l saline solution or 300 ng rhIL-5 into isolated lungs from actively sensitized animals, used 2 days after the booster injection of the antigen, induced low levels of bronchoconstriction of $7.3\% \pm 1.9\%$ and $7.2\% \pm 3.4\%$, respectively ($n = 6$ to 9). Similarly, the intratracheal administration of 100 μ l saline or 300 ng rhIL-5 was followed by the release of low amounts of TXB₂ and histamine in the effluent (0.8 ± 0.2 and 2.1 ± 0.9 ng/ml TXB₂; $n = 6$ to 9 per group and 1.7 ± 0.4 ng/ml and 2.5 ± 0.3 ng/ml histamine; $n = 6$ to 9

per group, respectively), no statistically significant difference being observed between the groups. Finally, the responses to saline solution or to rhIL-5 in terms of bronchoconstriction and mediator release of lungs from guinea pigs treated in vivo or perfused in vitro with the drug also were not modified (not shown).

Effect of nedocromil sodium on increased bronchoconstriction induced by rhIL-5 in response to PAF

The intraarterial injection of 10 and 100 ng PAF into lungs from actively sensitized guinea pigs 10 minutes after the in vitro intratracheal instillation of 100 μ l saline solution induced dose-dependent bronchoconstriction (Fig. 1). When PAF was preceded by the intratracheal instillation of 300 ng rhIL-5, a marked and significant increase in the extent of bronchoconstriction was observed (Fig. 1). Bronchoconstriction was not affected when the perfusion medium was supplemented with nedocromil sodium (10 μ mol/L) and the lungs were challenged with rhIL-5

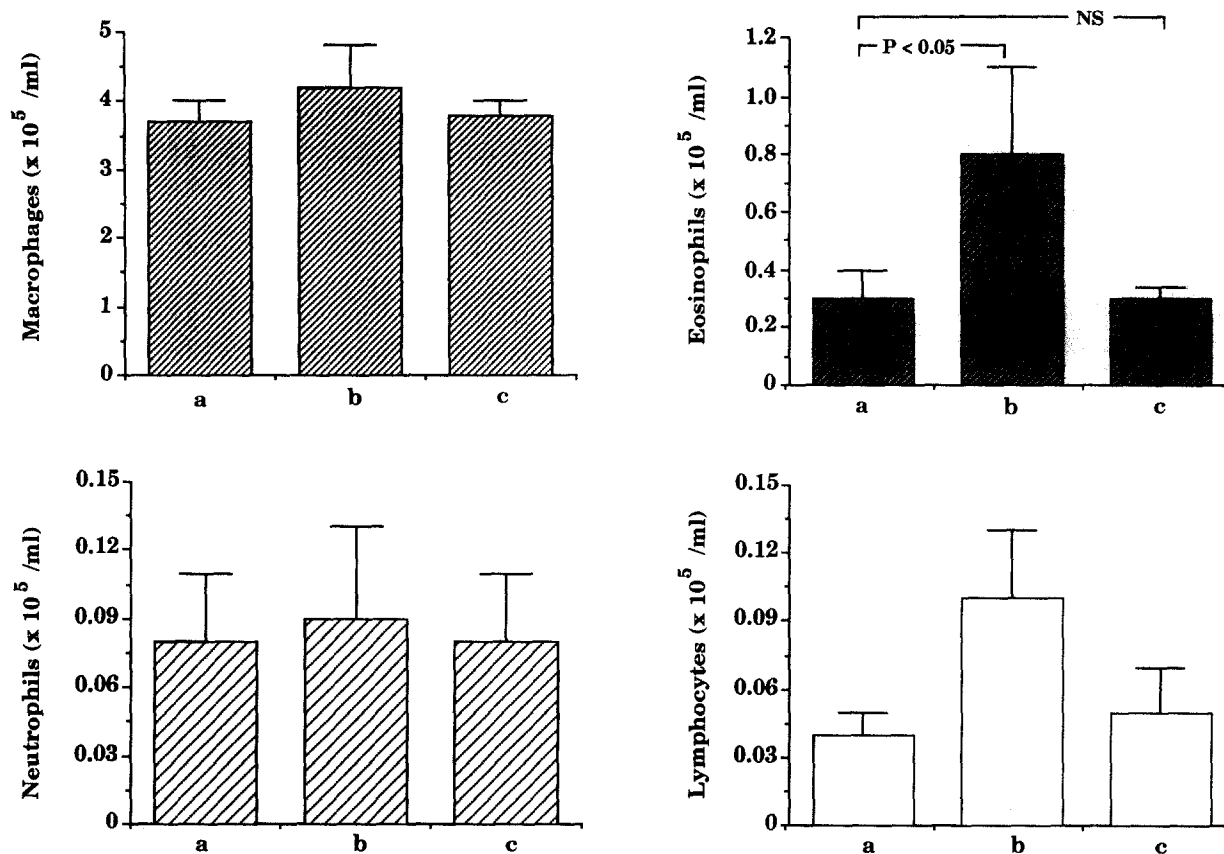


FIG. 4. Cell contents of BAL fluid obtained from actively sensitized, nonboosted guinea pigs (column *a*), or from actively sensitized and boosted animals that had been pretreated either with the vehicle (column *b*), or with nedocromil sodium (60 mg/kg per day given subcutaneously for 2 days (column *c*). Bronchoalveolar cells were collected in 10 successive lavages and differentiated by May-Grünwald-Giemsa stain. Results expressed as number of each cell type/ml. Data are expressed as mean $\pm$ SEM of 6 to 8 experiments.

and PAF (Fig. 1). By contrast, the enhanced bronchoconstriction to 10 and 100 ng PAF observed after the administration of rhIL-5 was significantly inhibited in lungs from guinea pigs treated for 2 days with nedocromil sodium (Fig. 1).

Effect of nedocromil sodium on increased mediator release induced by rhIL-5 in response to PAF

The intraarterial administration of 10 and 100 ng PAF induced a dose-dependent release of TXB₂ into the effluent of saline solution-treated lungs from actively sensitized and boosted guinea pigs (Fig. 2). After the instillation of 300 ng rhIL-5, the production of TXB₂ was significantly increased for both doses of PAF (Fig. 2). When the lungs were perfused in the presence of 10 μ mol/L nedocromil sodium, the amounts of TXB₂ in the effluent were similar to those found in lungs challenged with rhIL-5 and PAF in the absence of the drug (Fig. 2). By contrast, the release of TXB₂ in response to PAF from lungs from sensitized

guinea pigs treated in vivo with nedocromil sodium was markedly inhibited (Fig. 2).

Large amounts of histamine were also measured after the administration of PAF into lungs from sensitized and boosted guinea pigs previously injected with 300 ng rhIL-5 as compared with saline solution-injected preparations (Fig. 3).¹¹ The addition of 10 μ mol/L nedocromil sodium to the perfusion medium did not affect PAF-induced histamine release, but again the drug was effective when administered subcutaneously for 2 days (Fig. 3).

Effect of nedocromil sodium on the cell composition of BAL of actively sensitized guinea pigs

The average number of cells recovered from the various groups of animals was of $4.2 \pm 0.4 \times 10^5$ cells/ml ($n = 7$), $4.8 \pm 0.5 \times 10^5$ cells/ml ($n = 6$), and $4.3 \pm 0.3 \times 10^5$ cells/ml ($n = 8$) in BAL fluid from actively sensitized nonboosted, sensitized guinea pigs used 2 days after the booster injection of

the antigen and treated or not with nedocromil sodium for 2 days, respectively (difference not statistically significant).

As shown in Fig. 4, the number of eosinophils was significantly increased in sensitized and boosted guinea pigs compared with nonboosted animals. Similarly, the number of lymphocytes was higher in BAL fluid from boosted compared with nonboosted animals, even though the difference was not statistically significant. The 2-day treatment with nedocromil sodium suppressed the increased number of eosinophils and lymphocytes found in the BAL fluid (Fig. 4).

DISCUSSION

The protective effects of nedocromil sodium in asthma are thought to result from the ability to antagonize airway inflammation and the consequent hyperresponsiveness. Thus nedocromil sodium inhibits *in vitro* anti-IgE-induced histamine release from mast cells,²⁴ eosinophil-dependent basophil degranulation induced by anti-IgG₄,²⁵ and the *in vitro* activation of alveolar macrophages from asthmatic patients.²⁶ *In vivo*, nedocromil sodium prevents anaphylactic bronchoconstriction, increased vascular permeability, cellular infiltration, and bronchial hyperreactivity.²⁷ Recently, studies have focused on the effect of nedocromil sodium on *in vitro* and *in vivo* eosinophil activation and chemoattraction,²⁸⁻³⁰ because it is well established that the presence of these cells in the airways of asthmatic subjects and the degree of their activation correlate well with the severity of the disease.³¹

Here we show that a 2-day administration of nedocromil sodium completely prevents rhIL-5-induced hyperresponsiveness to PAF in isolated lungs from actively sensitized guinea pigs and airway eosinophilia after the repeated exposure of the guinea pigs to ovalbumin. With use of the same model, we had demonstrated previously that the functional and secretory responses to PAF of isolated lungs from the actively sensitized guinea pigs are enhanced.²³ This phenomenon, which is characterized by a reduced threshold for bronchoconstriction and an enhanced release of eicosanoids and histamine in the effluent, started 4 to 5 days after the booster injection of the antigen. Hyperresponsiveness to PAF was prevented when nedocromil sodium was administered daily to the guinea pigs during the interval between the day of the booster injection of antigen and lung collection.¹⁵ More recently we have shown that the responses to PAF are significantly increased by rhIL-5, but only in lungs from actively sensitized and boosted guinea pigs and not in those from nonimmunized an-

imals.¹¹ Because rhIL-5 was administered *in vitro* intratracheally 10 minutes before challenge with PAF, it is likely that those enhanced effects cannot be accounted for by the differentiating and maturing properties of rhIL-5 nor by its ability to induce cell recruitment into the airways. Thus we have postulated that rhIL-5 primes a target cell present in the airways from sensitized and boosted guinea pigs only and that such a priming is followed by an increased responsiveness to PAF administered as a second stimulus. The target of rhIL-5 may be the eosinophil, because their number increased markedly in the BAL fluid from guinea pigs 2 days after the booster injection of antigen,¹¹ as compared with nonimmunized or nonboosted animals. The hypothesis of priming of airway eosinophils by rhIL-5 in our experimental situation is supported by the observation that this cytokine selectively stimulates mature eosinophils,^{32, 33} and by recent results from our laboratory showing an *in vitro* enhancement by rhIL-5 of PAF-induced chemotaxis, superoxide anion production, and calcium flux on guinea pig eosinophils as recognized for human eosinophils.^{12, 34}

In the present study we demonstrate that the addition of nedocromil sodium to the buffer used for lung perfusion fails to affect the rhIL-5-induced enhanced responses to PAF. By contrast, when administered *in vivo* for 2 days, starting from the day of the booster injection, nedocromil sodium suppressed rhIL-5-induced lung hyperresponsiveness to PAF, as well as the eosinophil influx into the airways triggered by the booster injection of antigen. These results suggest that animals treated with nedocromil sodium lack the specific target that interacts with rhIL-5. Nevertheless, because hyperresponsiveness to PAF after rhIL-5 administration is characterized by an increased release of TXB₂ and of histamine in the effluent, it is likely that eosinophils, which do not contain histamine, are not the only cells responsible for this phenomenon. Alveolar macrophages and mast cells, respectively, are the major source of TXB₂ and histamine in the lung. Thus priming by rhIL-5 of pulmonary eosinophils might be followed by the release of mediators that indirectly stimulate other cell types to produce bronchoconstrictor substances such as TXB₂ and histamine.

In addition to eosinophils, IL-5 also activates other cells and thus primes the *in vitro* basophil response to various agonists for increased histamine secretion,^{8, 35} as was the case for the isolated lungs. Among the cells containing histamine, basophils are unique in degranulating after a PAF challenge.³⁶ Because the number of basophils in the bronchoalveolar lumen is increased in patients with asthma,³⁷ a hypothesis regarding their participation in the rhIL-5-induced lung

hyperresponsiveness to PAF in immunized animals should be investigated.

T lymphocytes may also be involved in our experimental situation. Indeed, we have recently shown that the booster injection of antigen to sensitized guinea pigs recruits a large number of T cells, mainly of the CD4⁺ subset, into the bronchial wall.³⁸ It is thus likely that CD4⁺ lymphocytes participate in the development of lung hyperresponsiveness in the guinea pig, directly or by releasing cytokines, which prime the secretory responses of eosinophils. In addition, we have shown here that the enhanced eosinophil counts found in the BAL fluid from boosted guinea pigs were accompanied by increased numbers of lymphocytes. Because this augmentation was also suppressed by nedocromil sodium, it is likely that this drug prevents the antigen-induced recruitment of cells other than eosinophils, possibly accounting for the down-regulation of the lung responses.

Our results showing that a 2-day treatment with nedocromil sodium prevents the rhIL-5-induced lung hyperresponsiveness to PAF in isolated lungs from guinea pigs actively sensitized by repeated exposures to ovalbumin may be relevant to the clinical situation. For example, IL-5 selectively enhanced the oxidative metabolism of granulocytes from patients with atopic dermatitis and allergic rhinitis and not from those from nonatopic subjects,³⁹ as we found for the lungs.¹¹ More recently, the application of rhIL-5 onto the nasal mucosa of atopic patients was shown to trigger a marked recruitment of eosinophils and epithelial cells in the nasal lavage fluid as well as an increase in the amounts of eosinophil cationic protein.⁴⁰ This phenomenon was accompanied by an enhancement of histamine-induced nasal airway resistance, discharge, and sneeze, suggesting that eosinophil cationic protein production, as a result of eosinophil activation by rhIL-5, may be responsible for epithelium injury and subsequent nasal hyperreactivity, a situation similar to our observations. Furthermore, the fact that incubation of human eosinophils with nedocromil sodium inhibited the IL-5-induced increase in survival,⁴¹ leads to the hypothesis that this drug may interfere with the effects of IL-5 on the eosinophil. Finally, recent data have shown that pretreatment of sensitized guinea-pigs or mice with an antibody against IL-5 suppresses antigen-induced eosinophil recruitment in the airways.⁴²⁻⁴⁴ Taken together, our results suggest that antiasthmatic drugs, such as nedocromil sodium, may exert their beneficial effects either by inhibiting eosinophil activation, or, more precisely, by preventing the establishment of interactions between IL-5 and lipid mediators, perhaps through the suppression of recruitment of the target cells.

The authors thank Professor B. David and Mrs. A. Leroy (Unité d'Immunoallergie, Institut Pasteur, Paris) for histamine determination and Dr. A. Norris (Fisons plc Pharmaceutical Division, Loughborough, U.K.) for advice and support.

REFERENCES

1. Djukanovic R, Roche R, Wilson JW, et al. Mucosal inflammation and asthma. *Am Rev Respir Dis* 1990;142:434-57.
2. Gonzalez MC, Diaz P, Galleguillos FR, et al. Allergen-induced recruitment of bronchoalveolar helper (OKT4) and suppressor (OKT8) T-cells in asthma. *Am Rev Respir Dis* 1987;136:600-4.
3. Frew AJ, Moqbel R, Azzawi M, et al. Lymphocytes and eosinophils in allergen-induced late-phase asthmatic reactions in the guinea pig. *Am Rev Respir Dis* 1990;141:407-13.
4. Coffman RL, Seymour BWP, Lebman DA, et al. The role of helper T cell products in mouse B cell differentiation and isotype regulation. *Immunol Rev* 1988;102:5-28.
5. Miyajima A, Miyatake S, Schreurs J, et al. Coordinate regulation of immune and inflammatory responses by T cell-derived lymphokines. *FASEB J* 1988;2:2462-73.
6. Silberstein DS, Owen WF, Gasson JC, et al. Enhancement of human eosinophil cytotoxicity and leukotriene synthesis by biosynthetic (recombinant) granulocyte-macrophage colony-stimulating factor. *J Immunol* 1986;137:3290-4.
7. Dahinden CA, Zingg J, Maly FE, de Weck AL. Leukotriene production in human neutrophils primed by recombinant human granulocyte/macrophage colony-stimulating factor and stimulated with the complement component C5a and FMLP as second signals. *J Exp Med* 1988;167:1281-6.
8. Bischoff SC, Brunner T, De Weck AL, Dahinden CA. Interleukin-5 modifies histamine release and leukotriene generation by human basophils in response to diverse agonists. *J Exp Med* 1990;172:1577-82.
9. Bischoff SC, De Weck AL, Dahinden CA. Interleukin-3 and GM-CSF render human basophils responsive to low concentrations of the complement component C3a. *Proc Natl Acad Sci U S A* 1990;87:6813-7.
10. Robinson DS, Hamid Q, Ying S, et al. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992;326:298-304.
11. Pretolani M, Lefort J, Leduc D, Vargaftig BB. Human recombinant interleukin-5 induces in vitro lung hyperresponsiveness to platelet-activating factor in the guinea-pig. *Br J Pharmacol* 1992;106:677-84.
12. Coëffier E, Joseph D, Vargaftig BB. Activation by human recombinant IL-5 of guinea pig eosinophils. Selective priming to PAF and interference of its antagonists. *J Immunol* 1991;147:2595-602.
13. Fujisawa T, Abu-Ghazaleh R, Kita H, Sanderson CJ, Gleich GJ. Regulatory effect of cytokines on eosinophil degranulation. *J Immunol* 1990;144:642-6.
14. Bruijnzeel PL, Warringa RA, Kok PT, Kreukniet J. Inhibition of neutrophil and eosinophil induced chemotaxis by nedocromil sodium and sodium cromoglycate. *Br J Pharmacol* 1990;99:798-802.
15. Pretolani M, Lefort J, Silva P, et al. Protection by nedocromil sodium of active immunization-induced bronchopulmonary alterations in the guinea pig. *Am Rev Respir Dis* 1990;141:1259-65.
16. Hutson PA, Holgate ST, Church MK. Effect of cromonyl sodium and albuterol on early and late phase bronchoconstriction and airway leukocyte infiltration after allergen challenge of

- nonanesthetized guinea-pigs. *Am Rev Respir Dis* 1988; 138:1157-63.
17. Church MK, Hutson PA, Holgate ST. Effect of nedocromil sodium on early and late phase responses to allergen challenge in the guinea-pig. *Drugs* 1989;37:101-8.
 18. Abraham WM. Effect of nedocromil sodium on antigen-induced airway responses in allergic sheep. *Drugs* 1989;37:78-86.
 19. Schellenberg RR, Ishida K, Thomson RJ. Nedocromil sodium inhibits airway hyperresponsiveness and eosinophilic infiltration induced by repeated antigen challenge in guinea-pigs. *Br J Pharmacol* 1991;103:1842-6.
 20. Lefort J, Rotilio D, Vargaftig BB. The platelet-independent release of thromboxane A₂ by PAF from guinea-pig lungs involves mechanisms distinct from those of leukotrienes. *Br J Pharmacol* 1984;82:565-75.
 21. Sors H, Pradelles P, Dray F. Analytical methods for thromboxane B₂ measurement and validation on radioimmunoassay by gas liquid chromatography-mass spectrometry. *Prostaglandins* 1978;16:277-90.
 22. Lebel B. A high sampling rate automated continuous flow fluorimetric technique for the analysis of nanogram levels of histamine in biological samples. *Analyt Biochem* 1983;133:16.
 23. Pretolani M, Lefort J, Vargaftig BB. Active immunization induces lung hyperresponsiveness in the guinea pig. Pharmacological modulation and triggering role of the booster injection. *Am Rev Respir Dis* 1988;138:1572-8.
 24. Leung KBP, Flint KC, Brostoff J, et al. Sodium cromoglycate and nedocromil sodium on histamine secretion from human lung mast cells. *Thorax* 1988;43:756-61.
 25. Beauvais F, Hieblot C, Benveniste J. Effect of sodium cromoglycate and nedocromil sodium on anti-IgE-induced and anti-IgG4-induced basophil degranulation. *Drugs* 1989;37:4-8.
 26. Damon M, Chavis C, Daures JP, Crastes-de-Paulet A, Michel FB, Godard P. Increased generation of the arachidonic metabolites LTB₄ and 5-HETE by human alveolar macrophages in patients with asthma: effect in vitro of nedocromil sodium. *Eur Respir J* 1989;2:202-9.
 27. Auty RM, Holgate ST. Nedocromil sodium: a review of its antiinflammatory properties and clinical activity in the treatment of asthma. In: Kay AB, ed. *Allergy and asthma. New trends and approaches to therapy*. Cambridge, Massachusetts: Blackwell Scientific Publications, 1990:171-88.
 28. Bruijnzeel PL, Hamelink ML, Kok PT, Kreukniet J. Nedocromil sodium inhibits the A23187- and opsonized zymosan-induced leukotriene formation by human eosinophils but not by human neutrophils. *Br J Pharmacol* 1989;96:631-6.
 29. Moqbel R, Cromwell O, Kay AB. The effect of nedocromil sodium on human eosinophil activation. *Drugs* 1989;37:19-22.
 30. Sedgwick JB, Bjornsdottir U, Geiger KM, Busse WW. Inhibition of eosinophil density change and leukotriene C₄ generation by nedocromil sodium. *J ALLERGY CLIN IMMUNOL* 1992;90:202-9.
 31. Bousquet J, Chanez P, Lacoste JY, et al. Eosinophilic inflammation in asthma. *N Engl J Med* 1990;323:1033-9.
 32. Lopez AL, Sanderson CJ, Gamble JR, Campbell HD, Young IG, Vadas MA. Recombinant human interleukin 5 is a selective activator of human eosinophil function. *J Exp Med* 1988;167:219-25.
 33. Wang JM, Rambaldi A, Biondi A, Guo Chen Z, Sanderson CJ, Mantovani A. Recombinant human interleukin-5 is a selective eosinophil chemoattractant. *Eur J Immunol* 1989;19:701-5.
 34. Sehmi R, Wardlaw AJ, Cromwell O, Kay AB. IL-5 selectively modulates the locomotory responsiveness of eosinophils from normal donors. *International Congresses on ICOI Inflammation*. Rome-Italy: 1991;133 (Abstract).
 35. Hirai K, Yamaguchi M, Misaki Y, et al. Enhancement of human basophil histamine release by interleukin 5. *J Exp Med* 1990;172:1525-8.
 36. Columbo M, Casolaro V, Warner JA, MacGlashan DW, Kagey-Sobotka A, Lichtenstein LM. The mechanism of mediator release from human basophils induced by platelet-activating factor. *J Immunol* 1990;145:3855-61.
 37. Kimura I, Tanizaki Y, Saito K, Takahashi K, Ueda N, Sato S. Appearance of basophils in the sputum of patients with bronchial asthma. *Clin Allergy* 1975;5:95-102.
 38. Lapa e Silva JR, Pretolani M, Bachelet CM, Baker D, Vargaftig BB. Changes in T-lymphocyte subsets in bronchial wall of actively sensitized guinea pigs. *Am Rev Respir Dis* 1992;145:A39.
 39. Kapp A, Zeck-Kapp G. Interleukin-5-induced granulocyte activation in atopic patients. *Br J Dermatol* 1991;125:108-15.
 40. Terada N, Konno A, Tada H, Shirotori K, Ishikawa K, Togawa K. The effect of recombinant human interleukin-5 on eosinophil accumulation and degranulation in human nasal mucosa. *J ALLERGY CLIN IMMUNOL* 1992;90:160-8.
 41. Resler B, Sedgwick JB, Busse WW. Inhibition of interleukin-5 (IL-5) effects on human eosinophils (eos) by nedocromil sodium (ned). *J ALLERGY CLIN IMMUNOL* 1992;89:A362.
 42. Chand N, Harrison JE, Rooney S, et al. Anti-IL-5 monoclonal antibody inhibits allergic late phase bronchial eosinophilia in guinea pigs: a therapeutic approach. *Eur J Pharmacol* 1992;211:121-3.
 43. Gulbenkian AR, Egan RW, Ferndandez X, et al. Interleukin-5 modulates eosinophil accumulation in allergic guinea-pig lung. *Am Rev Respir Dis* 1992;146:263-5.
 44. Nakajima H, Iwamoto I, Tomoe S, et al. CD4⁺ T-lymphocytes and interleukin-5 mediate antigen-induced eosinophil infiltration into the mouse trachea. *Am Rev Respir Dis* 1992;146:374-7.