

Inhibition of cytokine-primed eosinophil chemotaxis by nedocromil sodium

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Background: Eosinophil influx into the lung tissue is considered to be relevant for the pathogenesis of asthma. Various chemotactic factors may be responsible for this influx. Recently it has been demonstrated that the cytokines granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-3 (IL-3), and interleukin-5 (IL-5) are present in the circulation of individuals with allergic asthma. These cytokines have the capacity to modulate chemotactic responses of eosinophils toward platelet-activating factor, formyl-methionyl-leucyl-phenylalanine (FMLP) and neutrophil-activating factor (NAF)/IL-8, but not toward complement fragment C5a (C5a). Here the effect of nedocromil sodium on the chemotactic response of eosinophils from allergic asthmatic individuals and from normal donors preincubated with GM-CSF or IL-3 toward FMLP, NAF/IL-8 was evaluated.

Results: Nedocromil sodium inhibited the chemotactic response toward FMLP and NAF/IL-8 of GM-CSF primed eosinophils approximately 60% (inhibitory concentration of 50% [IC₅₀] ~1 to 10 nmol/L), whereas these responses of IL-3 primed eosinophils was completely inhibited (IC₅₀ ~1 nmol/L).

Conclusions: The chemotactic responses toward C5a were inhibited by nedocromil sodium at higher concentrations than were required in the priming studies (IC₅₀ ~10 to 100 nmol/L). Nedocromil sodium (0.1 µmol/L) was also effective in inhibiting the chemotactic response toward FMLP (10 nmol/L) of eosinophils isolated from the circulation of patients with allergic asthma 3 hours after allergen challenge. These findings might explain in part the antiinflammatory action of nedocromil sodium. (*J ALLERGY CLIN IMMUNOL* 1993;91:802-9.)

Key words: Asthma, chemotaxis, eosinophils, nedocromil sodium

Eosinophils are considered to be important cells in the pathogenesis of allergic asthma.¹⁻⁵ Directed locomotion or chemotaxis of these cells is essential for the infiltration into the lung tissue. Mobilization of these cells is thought to take place via lipid chemotactic factors such as platelet-activating factor (PAF) and leukotriene B₄ (LTB₄) and via protein chemotactic factors such as complement fragment C5a.⁶⁻⁹ N-for-

Abbreviations used

FMLP:	Formyl-methionyl-leucyl-phenylalanine
PAF:	Platelet-activating factor
GM-CSF:	Granulocyte-macrophage colony-stimulating factor
IL-3:	Interleukin-3
IL-5:	Interleukin-5
IL-8:	Interleukin-8 (= NAF)
C5a:	Complement fragment C5a
NAF:	Neutrophil-activating factor (= IL-8)
LTB ₄ :	Leukotriene B ₄
FEV ₁ :	Forced expiratory volume in 1 second
HPF:	High-power field
IC ₅₀ :	Inhibitory concentration of 50%

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myl-methionyl-leucyl-phenylalanine (FMLP) and neutrophil-activating factor (NAF/IL-8) are not acting as chemotaxins for eosinophils when isolated from the blood of healthy individuals.^{6, 7, 10-12}

Recently it could be shown that eosinophilia and eosinophil function are regulated by various cytokines.¹³ In particular, granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-3 (IL-3), interleukin-5 (IL-5), tumor necrosis factor alpha, and eosinophil activating factor modulate eosinophil function.¹⁴⁻¹⁶ These cytokines prolong the survival of human eosinophils *in vitro*.^{17, 18} GM-CSF, IL-3, and IL-5 have been detected in the circulation of individuals with allergic asthma, and lymphocytes from these patients can secrete these cytokines spontaneously as well as after activation.^{19, 20} For IL-5 it could be shown that it acts as a selective chemoattractant for eosinophils, although the activity was very weak compared with that of PAF.^{17, 21}

Recently initiation of a potent chemotactic response of eosinophils from healthy individuals toward the well-established neutrophil chemotaxins FMLP or NAF/IL-8 was demonstrated after preincubation with relatively low concentrations (1 to 10 mol/L) of GM-CSF and IL-3.⁶ Higher concentrations of these cytokines turned out to be chemotactic for eosinophils.⁶ Furthermore, a strong increase in the sensitivity of the PAF-induced chemotaxis was demonstrated after pretreatment of eosinophils with these cytokines.²² This increase in sensitivity for PAF was also found in eosinophils isolated from patients with allergic asthma, reflecting an *in vitro* activated state of these eosinophils.

Nedocromil sodium (Tilade), a novel antiasthma drug with antiinflammatory properties, was previously shown to be effective in inhibiting chemotactic responses from eosinophils of healthy individuals toward PAF and LTB₄.^{11, 23, 24}

In the present study the effects of nedocromil sodium on the chemotactic responses of *in vitro* cytokine-primed eosinophils were investigated. These effects were compared with the action of the drug on chemotaxis of eosinophils from asthmatic patients which proved to be primed *in vivo*.

METHODS AND MATERIAL

Patients

All individuals with allergic asthma participating in this study met The American Thoracic Society definition of asthma, including episodic airway obstruction and hyperresponsiveness to bronchoprovocation with histamine. Three men and three women with ages ranging between 20 and 45 years participated in the study. All patients had positive skin test reaction to allergens and bronchial hyperreactivity to histamine (PC₂₀ hist ≤ 4 mg/ml) Table I. Medication was stopped 10 days before the study except for β-sympathomimetics (salbutamol-inhaler), which were stopped 24 hours before the patients entered the study. All

TABLE I. Lung parameters and reaction pattern after bronchoprovocation of patients with allergic asthma used in the study

No. patient	EAR	ΔFEV ₁ (%)	EO% (0)	EO% (3)	LAR
1	+	43	5.2	2.9	+
2	+	31	5	3.2	+
3	+	42	1.9	2.6	+
4	+	39	4.3	2.1	+
5	+	39	3.4	1.8	+
6	+	32	5.1	3.5	-

Allergic asthmatic individuals provoked with allergen as described in Material and Methods section. Changes in FEV₁ measured and correlated with occurrence of an early asthmatic reaction (EAR). Eosinophil counts (% of white blood cells) in peripheral blood determined before (0) and 3 hours after provocation (3). Occurrence of a late phase reaction (LAR) measured by symptom scores and change in FEV₁ in which a minimum fall of 15% 6 hours after provocation was accepted as a criterion for a LAR. EO, Eosinophil.

participating individuals gave their informed consent. The study was approved by the hospital's ethics committee.

Bronchial provocation test

Airway reactivity to histamine was determined by a standard bronchoprovocation technique as described by Juniper et al.²⁵ The bronchial provocation with allergen was performed by 2-minutes inhalations (tidal breathing) with 10-minute intervals. The allergen-provocation was preceded by an initial aerosol of buffer (2.5 mg/ml NaHCO₃ + 5 mg/ml NaCl with 0.03% human serum albumin and 0.5% phenol wt/vol) to which none of the patients showed a fall in forced expiratory volume in 1 second (FEV₁) or peak expiratory flow (PEF). Increasing concentrations of antigen aerosol were delivered through a nebulizer (model 646; De Vilbiss Co, Somerset, Pa). Antigen was diluted from stock solutions (10,000 biological units/ml). The dilution of allergen used for the first inhalation was calculated from the skin prick titration test and the preantigen PC₂₀ for histamine.²⁶ Inhalations were performed by stepwise doubling of the dose of antigen until the FEV₁ fell at least 20% from baseline values.

Cell isolation

Eosinophils were isolated from the buffy coat of 500 ml of human blood anticoagulated with 0.4% (wt/vol) trisodium citrate (pH 7.4) exactly as described before.¹⁰ The FMLP treatment applied in this method does not influence eosinophil function as is extensively documented elsewhere.^{6, 10, 27, 28} The purity of eosinophils was always more than 85%, and the recovery ranged from 40% to 60%. Contaminating cells were mostly neutrophils. The viability of the eosinophils was always more than 98%.

Control experiments and experiments with eosinophils from patients with allergic asthma were carried out with highly purified eosinophils isolated from the granulocyte

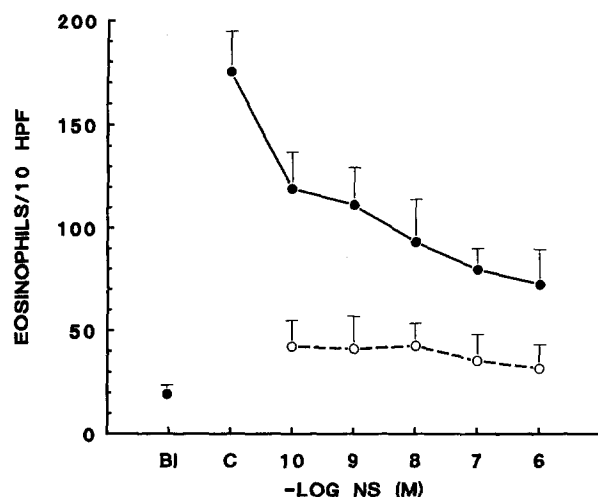


FIG. 1. Effect of nedocromil sodium on random migration (open circles) and on human recombinant C5a-induced chemotaxis (closed circles) of human eosinophils derived from the circulation of healthy volunteers. Geys' buffer (BL) was used as a control (C); optimal chemotactic C5a concentration (10 nmol/L). Number of cells per 10 HPFs was counted; $n = 9$, eosinophil purity was $93\% \pm 2\%$; the dose-response curve of nedocromil sodium was significantly different from the C5a control (ANOVA: $p < 0.0025$), whereas the random migration was not significantly inhibited by nedocromil sodium.

preparation containing neutrophils and eosinophils as described by Hansel et al.²⁹ In brief, immunomagnetic beads (Dynal Beads; Dynal A.S., Oslo, Norway) were coated with a monoclonal antibody against CD16 (CLB FcR-gran 1). These coated beads were coincubated at 4°C for 20 minutes with the granulocyte preparation (10^7 cells/ml) in a ratio 1:4 (cells/beads). The neutrophils were subsequently removed by a magnetic particle concentrator (MPCTH-1; Dynal A.S.). After this procedure, the purity of eosinophils was always more than 95%.

Chemotaxis assay

Chemotaxis was measured with a modified Boyden chamber assay with use of a 48-well microchemotaxis chamber (Neuroprobe, Cabin John, Md.). Chemotaxins or Geys' buffer (30 μl) were placed in the lower compartments. Two filters (cellulose nitrate) were placed between the lower and the upper compartment. The lower filter had a pore width of $0.45\text{ }\mu\text{m}$ (type HA; Millipore Corp., Bedford, Mass.) and the upper filter had a pore width of $8\text{ }\mu\text{m}$ (SM 113; Sartorius AG, Göttingen, Germany). Before use the filters had been soaked in Geys' buffer. Purified eosinophils preincubated for 30 minutes 37°C with the various cytokines or Geys' were placed in the upper compartments (25 μl of 5×10^6 cells/ml). Concentration ranges of the chemotactic agents NAF/IL-8, FMLP, C5a, and the cytokines GM-CSF and IL-3 were tested with the buffer as a control.^{6, 11} At optimal concentrations of these chemotactic agents, dose-response curves with nedocromil sodium were performed. The cells were preincubated for 30 minutes at 37°C with

TABLE II. Chemotactic responses of eosinophils from healthy individuals toward various chemotaxins and IC_{50} values of nedocromil sodium on these responses

Chemotaxin	Eosinophils/10 HPFs	IC_{50} values of nedocromil sodium ($\mu\text{mol/L}$)
Buffer	20 ± 6	NS
C5a (10 nmol/L)	186 ± 29	0.1
PAF (1 $\mu\text{mol/L}$)	90 ± 15	1
LTB ₄ (1 $\mu\text{mol/L}$)	76 ± 16	0.1

Comparison of chemotactic responses toward various chemotaxins (in rank order of potency) of eosinophils derived from the circulation of healthy individuals. IC_{50} values (in micromolar) of nedocromil sodium on these responses are presented (right part). For further experimental data and statistical analysis see also ref. 11 and the legends in the figures.

NS, No significant inhibition by nedocromil sodium.

the drug before the chemotaxis assay was carried out.¹¹ After this preincubation period the viability of the cells was always greater than 95% (trypan blue exclusion). The chemotaxis chambers were subsequently incubated for 2.5 hours at 37°C . Thereafter the filters were removed, fixed in butanol/ethanol (20%/80%, vol/vol) for 10 minutes, and stained with Weigert solution (composition: 1% vol/vol hematoxylin in ethanol mixed with a 70 mmol/L acidic FeCl_3 solution in a 1:1 ratio).

The filters were dehydrated with ethanol, made transparent with xylene, and fixed upside down. The number of cells per 10 high-power fields (HPFs) was determined with light microscopy (magnification $\times 400$). In this way the number of cells that had passed the upper filter was determined.

Material

Recombinant human complement factor C5a and *N*-FMLP were purchased from Sigma Chemical Co. (St. Louis, Mo.). Human recombinant complement C5a was stored at -70°C until use. Ficoll-Paque and Percoll were obtained from Pharmacia A. B. (Uppsala, Sweden). All other materials were reagent grade. The experiments were carried out in Geys' balanced salt solution supplemented with 1.5 mmol/L CaCl_2 , 1 mmol/L MgCl_2 , heparin (10 IU/ml), 5 mmol/L glucose, and 1.0% human serum albumin (wt/vol). Recombinant human GM-CSF (2.5×10^8 U/mg) and IL-3 (10^8 U/mg) were purchased from Genzyme (Cytokine Research Products, Boston, Mass.). NAF (NAP-1/IL-8; 10 $\mu\text{g/ml}$) was derived from British Biotechnology Limited, (Oxford, U.K.). Stock solutions of the cytokines were prepared in phosphate-buffered saline supplemented with 0.5% purified human serum albumin and were stored at -70°C until use. Nedocromil sodium was provided by Fisons plc (Loughborough, Leicestershire, U.K.). Human blood was obtained from healthy volunteers at the Red Cross Blood bank Foundation (Utrecht).

Statistical analysis

All data are presented as means $\pm$ SE. Statistical analysis was performed with Complete Statistical System (CSS) by Statsoft, Tulsa, Okla., with use of analysis of variance (ANOVA) for dose-response curves or Student's *t* test for paired observations; *p* values < 0.05 were considered significant.

RESULTS

The effect of nedocromil sodium on the chemotactic responses of human eosinophils toward complement fragment C5a

Complement fragment C5a turned out to be a potent chemotaxin for human eosinophils derived from the circulation of healthy volunteers, with an optimal chemotactic concentration of 10 nmol/L. At this optimal chemotactic concentration the influence of dose ranges of nedocromil sodium was evaluated (Fig. 1). Nedocromil sodium caused a 60% inhibition of maximal C5a-induced chemotaxis at a drug concentration of approximately 0.1 μ mol/L. This effect of nedocromil sodium is specific for stimulated migration, because random migration of eosinophils is not inhibited by a dose range of nedocromil sodium (Fig. 1).

The effect on nedocromil sodium on the chemotactic responses of unprimed and cytokine-primed human eosinophils toward different chemotaxins

In the next series of experiments we evaluated the inhibition of nedocromil sodium on different chemotactic responses. In Table II, inhibitory concentration of 50% (IC₅₀) values of nedocromil sodium for the various eosinophil chemotaxins at optimal concentration are compared. The IC₅₀ values for C5a, PAF, and LTB₄ turned out to be in the same range.

In an earlier article we have shown that FMLP and NAF/IL-8 are chemotaxins for healthy human eosinophils only after priming with cytokines.⁶ In the next series of experiments the sensitivity of cytokine-primed eosinophil chemotaxis was studied. Human eosinophils derived from the circulation of healthy volunteers were preincubated with optimal priming concentrations of GM-CSF and IL-3 (10 pmol/L and 100 pmol/L, respectively) or in Geys' buffer for 30 minutes at 37°C.⁶ These primed eosinophils were subsequently incubated with dose ranges of nedocromil sodium for 30 minutes (optimal time) at 37°C before transfer to the upper compartment of the Boyden chamber. Thereafter chemotactic responses toward optimal FMLP and NAF/IL-8 concentrations were measured (Methods and Material).

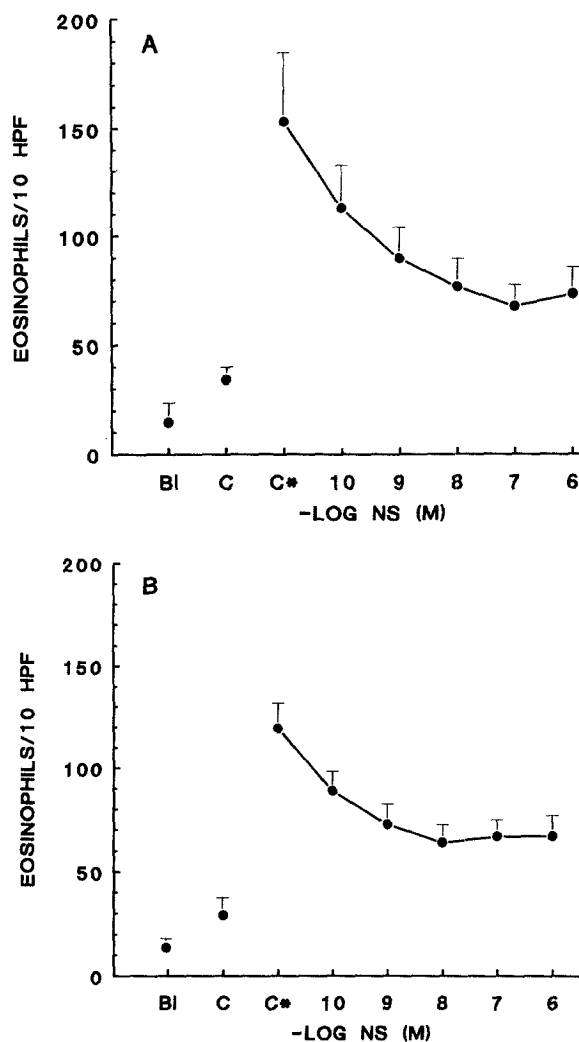


FIG. 2. Effect of nedocromil sodium on FMLP (A) and NAF/IL-8-induced (B) eosinophil chemotaxis. Eosinophils derived from healthy donors were preincubated for 30 minutes at 37°C with either an optimal priming concentration of GM-CSF (10 pmol/L) (C*) or Geys' buffer (C). Hereafter, chemotaxis toward FMLP and NAF/IL-8 was studied in the presence of different concentrations of nedocromil sodium with Geys' buffer as a control (BL). Dose-response curve of nedocromil sodium significantly different from FMLP control (ANOVA, *p* < 0.025 ; *n* = 10) and NAF/IL-8 control (ANOVA, *p* < 0.001 ; *n* = 10). The purity of the eosinophils was 92% $\pm$ 4%.

The effect of nedocromil sodium on FMLP and NAF/IL-8-induced chemotaxis of GM-CSF-primed eosinophils is shown in Fig. 2, A and B. Nedocromil sodium was able to inhibit responses toward both tested chemotaxins in a similar fashion, resulting in IC₅₀ values of approximately 1 nmol/L.

The inhibition of FMLP and NAF/IL-8-induced chemotaxis of IL-3-primed eosinophils by concentration ranges of nedocromil sodium is shown in Fig.

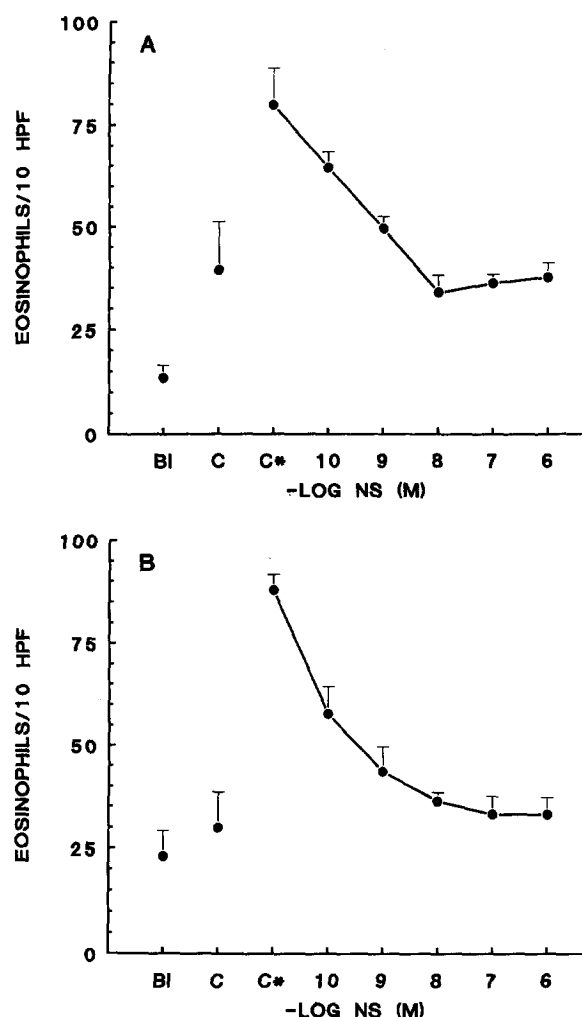


FIG. 3. Effect of nedocromil sodium on FMLP (A) and NAF/IL-8-induced (B) eosinophil chemotaxis. Eosinophils derived from healthy donors were preincubated for 30 minutes at 37° C with either an optimal priming concentration of IL-3 (100 pmol/L) (C*) or Geys' buffer (C). Hereafter, chemotaxis toward FMLP and NAF/IL-8 was studied in the presence of different concentrations of nedocromil sodium with Geys' buffer as a control (BL). The dose-response curve of nedocromil sodium was significantly different from the FMLP control (ANOVA, $p < 10^{-6}$; $n = 6$) and NAF control (ANOVA, $p < 10^{-6}$; $n = 6$). The purity of the eosinophils was $87\% \pm 4\%$.

3, A and B. Nedocromil sodium inhibited the chemotactic response to both chemotaxins with an IC_{50} value of approximately 1 nmol/L, and with higher concentrations of nedocromil sodium, complete inhibition of chemotaxis was achieved.

In a series of control experiments, it was investigated whether the time point of nedocromil addition was important. Therefore eosinophils were preincubated with the drug before cytokines were added. The results of these experiments showed that the time point

of drug addition does not influence the inhibitory activity of the drug (Table III).

The effect of nedocromil sodium on FMLP-induced chemotaxis of eosinophils from patients with allergic asthma

Human eosinophils isolated from blood of individuals with allergic asthma (Methods and Material) are in contrast to eosinophils derived from healthy donors responsive to FMLP in a chemotactic assay (manuscript submitted for publication). This chemotactic response is optimal at 10 nmol/L, FMLP, and an enhancement of the response occurs in eosinophils isolated from the blood of individuals with allergic asthma after allergen challenge. For this reason we have isolated eosinophils from the circulation of patients with allergic asthma before and 3 hours after allergen challenge with the appropriate allergen. These eosinophils were incubated with 100 nmol/L nedocromil sodium, and subsequently chemotactic responses were measured toward 10 nmol/L FMLP. Table IV shows that these eosinophils elicit an initial chemotactic response to FMLP, and that this response is significantly increased in eosinophils isolated 3 hours after allergen challenge (Table IV and manuscript submitted for publication). Nedocromil sodium significantly inhibited chemotactic responses toward FMLP of cells isolated before and after allergen challenge.

DISCUSSION

Influx of eosinophils into the lung tissue is considered to be an important process in the pathogenesis of allergic asthma.^{1, 2} Studies on bronchoalveolar lavage fluid and bronchial tissue after allergen challenge revealed the presence of activated eosinophils.³⁰ The influx of these eosinophils is correlated with the late phase asthmatic reaction.³ Moreover, treatment of primates with a monoclonal antibody against ICAM-1 counteracted eosinophil influx in the lung tissue, and with that, the enhancement of bronchial hyperreactivity.⁵ The processes that regulate this eosinophil influx are poorly understood, but adhesion to endothelium, diapedesis, and a subsequent migration of eosinophils in response to chemoattractants released within the lung tissue are important features of this eosinophil influx.

PAF, LTB₄, C5a, and the cytokines GM-CSF, IL-3, and IL-5 are important chemoattractants for eosinophils derived from the circulation of healthy volunteers.^{6-9, 11, 21} In contrast, the well-established neutrophil attractants FMLP and NAF/IL-8 are not chemotactic for these eosinophils.^{6, 7, 10-12} However, when eosinophils from healthy donors are primed with

TABLE III. Effect of time point of nedocromil sodium addition during inhibition of cytokine-primed eosinophil chemotaxis

	FMLP-induced chemotaxis
GM-CSF→FMLP	100
GM-CSF→nedocromil sodium→FMLP	45 ± 6*
Nedocromil sodium→GM-CSF→FMLP	52 ± 4*
IL-3→FMLP	100
IL-3→nedocromil sodium→FMLP	52 ± 7*
Nedocromil sodium→IL-3→FMLP	47 ± 4*

Cells were primed with GM-CSF (10 pmol/L) or IL-3 (100 pmol/L) during 30 minutes at 37° C before chemotaxis in response to FMLP (10 nmol/L) was measured. Nedocromil sodium (0.1 µmol/L) was added 30 minutes before or after priming with the cytokines as indicated with arrows. Values are presented as percentages ± SE of controls of three different experiments. FMLP responses of GM-CSF and IL-3 primed cells were 99 ± 10 cells/10 HPFs and 77 ± 5 cells/10 HPFs, respectively. The eosinophil purity was 85% ± 5%. Values indicated with * differed significantly from controls $p < 0.05$, paired t test.

picomolar concentrations of the cytokines GM-CSF and IL-3, the above-mentioned neutrophil attractants become potent chemotaxins for eosinophils.⁶ Priming of eosinophils of healthy donors with these cytokines causes an increase in the sensitivity of the chemotactic response toward PAF.²² A striking similarity exists between the response to PAF of cytokine-primed eosinophils from healthy donors and the response of eosinophils from the circulation of patients with allergic asthma.²²

In the present study we have evaluated the effects of the antiasthmatic drug nedocromil sodium as a possible antagonist of eosinophil chemotaxis. Our data indicate that chemotactic responses of GM-CSF and IL-3 primed eosinophils toward FMLP and NAF/IL-8 are very sensitive for nedocromil sodium inhibition ($IC_{50} \sim 1$ nmol/L). Moreover, FMLP-induced chemotaxis of eosinophils from individuals with allergic asthma is very sensitive for nedocromil sodium inhibition. This is especially evident for FMLP responses of eosinophils isolated 3 hours after allergen bronchoprovocation. Responses of unprimed eosinophils were less sensitive for nedocromil sodium. C5a-induced chemotaxis (Fig. 1) and chemotaxis induced by zymosan-activated serum, PAF and LTB₄, are inhibited by nedocromil sodium with an IC_{50} 10 to 100 nmol/L (Table I).^{11, 31} These differences are not likely to be caused by the relative potency of the chemoattractant, because relatively weak chemoattractants of unprimed cells, like LTB₄, are inhibited with the same

TABLE IV. Effect of nedocromil sodium on FMLP-induced chemotaxis of eosinophils isolated of patients with allergic asthma

	FMLP response before	FMLP response after
Control	67 ± 27	99 ± 29
Nedocromil sodium (100 nmol/L)	40 ± 15	44 ± 11*

Effect of nedocromil sodium (0.1 µmol/L) on chemotactic responses toward FMLP (10 nmol/L) of eosinophils derived from the circulation of individuals with allergic asthma before and after allergen provocation. Eosinophil chemotaxis toward FMLP (10 nmol/L) was measured before ("before") and 3 hours ("after") after bronchoprovocation with allergen. The chemotactic responses are expressed as means ± SE of six different experiments. Eosinophil purities were 99% ± 3% and 91% ± 4% before and after the provocation, respectively. Values indicated with * differed significantly from controls $p < 0.05$, paired t test.

sensitivity compared with relatively potent chemoattractants like C5a (Fig. 1, Table II).^{11, 31}

Nothing is known about the mode of action of nedocromil sodium on human eosinophils. It is likely that the drug acts on a postreceptor event, because the mode of action is not restricted to one chemoattractant (receptor). It is interesting to note that the responses (PAF, IL-8, FMLP, C5a, and LTB₄) that are particularly sensitive for nedocromil sodium are mediated by receptors that belong to a family of chemoattractant receptors characterized by seven transmembrane regions and G-protein linkage.³¹ Therefore the mode of action of nedocromil sodium might be caused by the effect of a common denominator shared by these different receptors. An interesting possibility in this respect are the G-proteins that are coupled to the receptors. This action might then result in a change in number, conformation, affinity, or signal transduction of these receptors. It has been shown that GM-CSF alters the number and affinity of FMLP receptors expressed on neutrophils.^{32, 33} These changes in FMLP receptors can modulate biologic responses.³³ A study on FMLP and NAF/IL-8 receptor expression of eosinophils primed with cytokines and treated with nedocromil sodium is currently under way. It is unlikely that inhibition of nedocromil sodium is caused by changes in Ca²⁺ homeostasis, because nedocromil sodium does not influence PAF and zymosan activated-serum-induced transient rise in cytosolic free Ca²⁺ (34). Moreover, cytokine-primed eosinophil chemotaxis is not sensitive for depletion of extracellular and intracellular Ca²⁺ (manuscript submitted for publication). This situation is comparable with human neu-

trophils.³⁵ Moreover, the mode of action of nedocromil sodium is probably not caused by interference with early priming events, because the degree of inhibition is the same when nedocromil sodium is added before or after the priming period (Table III).

Nevertheless, these data subscribe to an antiinflammatory effect of nedocromil sodium. Recently the cytokines GM-CSF, IL-3 and IL-5 have been demonstrated in the circulation of patients with allergic asthma.²⁰ These cytokines might cause a primed state of eosinophils in the circulation of individuals with allergic asthma.²² Because nedocromil sodium acts as a potent antagonist for the chemotactic responses of these cytokine-primed eosinophils, this might explain in part its antiinflammatory action and effects on bronchoconstriction in allergic asthmatic disease.

REFERENCES

- Durham SR, Kay AB. Eosinophils, bronchial hyperreactivity and late phase asthmatic reactions. *Clin Allergy* 1985;15:411-8.
- Metzger WJ, Hunninghake GW, Richerson HD. Late phase asthmatic responses: inquiry to mechanisms and significance. *Clin Rev Allergy* 1985;3:145-65.
- De Monchy JG, Kauffman HF, Venge P, et al. Bronchoalveolar eosinophilia during allergen-induced late phase asthmatic reactions. *Am Rev Respir Dis* 1985;426:373-7.
- Metzger WG, Nugent K, Richerson HB, et al. Methods for bronchoalveolar lavage in asthmatic patients following bronchoprovocation and local antigen challenge. *Chest* 1985;87(1 suppl):16S-19S.
- Wegner CD, Gundel RH, Reilly P, Hayner N, Letts G, Rothlein R. Intercellular adhesion molecule-1 (ICAM-1) in the pathogenesis of asthma. *Science* 1990;247:456-9.
- Warringa RAJ, Koenderman L, Kok PTM, Kreukniet J, Bruijnzeel PLB. Modulation and induction of eosinophil chemotaxis by granulocyte-macrophage colony-stimulating factor and interleukin-3. *Blood* 1991;77:2694-700.
- Altman LC, Hill JS, Hairfield WH, Mullarkey MF. Effects of corticosteroids on eosinophil chemotaxis and adherence. *J Clin Invest* 1981;67:28-36.
- Atkins PC, Wasserman SI. Chemotactic mediators. *Rev Allergy* 1983;1:385-97.
- Wardlaw AJ, Moqbel R, Cromwell O, Kay AB. Platelet-activating factor. A potent chemotactic and chemokinetic factor for human eosinophils. *J Clin Invest* 1986;78:1701-6.
- Koenderman L, Kok PTM, Hamelink ML, Verhoeven AJ, Bruijnzeel PLB. An improved method for the isolation of eosinophilic granulocytes from peripheral blood of normal individuals. *J Leukocyte Biol* 1988;44:79-86.
- Bruijnzeel PLB, Warringa RAJ, Kok PTM, Kreukniet J. Inhibition of neutrophil and eosinophil induced chemotaxis by nedocromil sodium and sodium cromoglycate. *Br J Pharmacol* 1990;99:798-802.
- Leonard EJ, Skeel A, Yoshimura T, Noer K, Kutvirt S, van Epps D. Leukocyte specificity and binding of human neutrophil attractant/activation protein-1. *J Immunol* 1990;144:1323-30.
- Silberstein DS, David JR. The regulation of human eosinophil function by cytokines. *Immunol Today* 1987;8:380-5.
- Thorne KJ, Richardson BA, Taverne J, Williamson DJ, Vadas MA, Butterworth AE. A comparison of eosinophil-activating factor (EAF) with other monokines and lymphokines. *Eur J Immunol* 1988;16:1143-9.
- Owen WR Jr, Rothenberg ME, Silberstein DS, Gasson JC, Stevens RL, Austen KF. Regulation of human eosinophil viability, density, and function by granulocyte-macrophage colony-stimulating factor in the presence of 3T3 fibroblasts. *J Exp Med* 1987;166:129-41.
- Lopez AF, 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;167:219-24.
- Yamaguchi Y, Hayashi Y, Sugama Y, et al. Highly purified murine interleukin-5 (IL-5) stimulates eosinophil function and prolongs in vitro survival. IL-5 as an eosinophil chemotactic factor. *J Exp Med* 1988;167:1737-42.
- Rothenberg ME, Owen WF Jr, Silberstein DS, et al. Human eosinophils have prolonged survival, enhanced functional properties, and become hypodense when exposed to human interleukin-3. *J Clin Invest* 1988;81:1986-92.
- Corrigan CJ, Hartnell A, Kay AB. T lymphocyte activation in acute severe asthma. *Lancet* 1988;1:1129-32.
- Walker C, Virchow JC Jr, Bruijnzeel PLB, Blaser K. T cell subjects and their soluble products regulate eosinophilia in allergic and non-allergic asthma. *J Immunol* 1991;146:1829-36.
- Ming Wang J, Rambaldi A, Biondi A, et al. Recombinant human interleukin 5 is a selective eosinophil chemoattractant. *Eur J Immunol* 1989;19:701-5.
- Warringa RAJ, Mengelers HJJ, Kuijper PHM, Raaijmakers JAM, Bruijnzeel PLB, Koenderman L. In vivo priming of platelet-activating factor-induced eosinophil chemotaxis in allergic asthmatic individuals. *Blood* 1992;79:1836-41.
- Holgate ST. Clinical evaluation of nedocromil sodium in asthma. *Eur J Respir Dis* 1986;69(suppl 147):149-59.
- Auty RM, Holgate ST. Nedocromil sodium: a review of its anti-inflammatory properties and clinical activity in the treatment of asthma. *Allergy and asthma. New trends and approaches to therapy*. Kay AB, ed. London: Blackwell Scientific, 1989:171-88.
- Juniper EF, Frith PA, Dunnett C, Cockcroft DW, Hargreave FE. Reproducibility and comparison of responses to inhaled histamine and metacholine. *Thorax* 1978;33:705-10.
- Cockcroft DW, Ruffin RE, Dolovich J, Hargreave FE. Allergen-induced increase in nonallergic bronchial reactivity. *Clin Allergy* 1977;7:503-13.
- Koenderman L, Tool ATJ, Roos D, Verhoeven AJ. Priming of the respiratory burst in human eosinophils is accompanied by changes in signal transduction. *J Immunol* 1990;145:3883-8.
- Koenderman L, Kuijpers TW, Blom M, Tool ATJ, Roos D, Verhoeven AJ. Characteristics of CR3-mediated aggregation in human eosinophils. Effect of priming by platelet-activating factor. *J ALLERGY CLIN IMMUNOL* 1991;87:947-57.
- Hansel TT, Pound JD, Pilling D, et al. Purification of human blood eosinophils by negative selection using immunomagnetic beads. *J Immunol Methods* 1989;122:97-103.
- Bousquet J, Chanez P, Lacoste JY, et al. Eosinophilic inflammation in asthma. *N Engl J of Med* 1990;323:1033-6.
- Dohlman HG, Thorne J, Caron MG, Lefkowitz R. Model systems for the study of seven-transmembrane-segment receptors. *Annu Rev Biochem* 1991;60:653-88.
- English D, Broxmeyer HE, Gabig TG, Akard LP, Williams DG, Hoffman R. Temporal adaptation of neutrophil oxidative responsiveness to *N*-formyl-methionyl-leucyl-phenylalanine. *J Immunol* 1988;141:2400-6.

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

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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

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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