

Nedocromil sodium and airway inflammation in vivo and in vitro

Jagdish L. Devalia, PhD, Csaba Rusznak, MD, Muntasir M. Abdelaziz, MRCP, and Robert J. Davies, FRCP London, United Kingdom

We conducted a series of studies investigating the antiinflammatory effects of nedocromil sodium, with particular reference to its effects on human bronchial epithelial cells and eosinophils in vitro and on eosinophils in vivo. Nedocromil sodium produced a dose-related inhibition of ozone-induced IL-8 release from human bronchial epithelial cells and also attenuated the release of granulocyte macrophage colony-stimulating factor, tumor necrosis factor- α , and soluble intercellular adhesion molecule 1. The culture medium from human bronchial epithelial cell cultures, containing the proinflammatory cytokines IL-8, granulocyte macrophage colony-stimulating factor, "regulated on activation, normal T expressed and secreted," IL-1 β , and tumor necrosis factor- α , increased eosinophil chemotaxis and eosinophil adhesion to cultured human endothelial cells. The chemotaxis and increased adhesion were blocked in the presence of nedocromil sodium. The drug also abrogated the epithelial cell dysfunction (assessed as ciliary beat frequency) induced by the presence of activated eosinophils and blocked the release of eosinophil cationic protein from the eosinophils. We also conducted a double-blind placebo-controlled study of the effects of regular albuterol 200 μ g or nedocromil sodium 4 mg, both given four times daily for 16 weeks, on inflammatory cell numbers in bronchial biopsy and bronchoalveolar lavage samples. Assessed in terms of total and activated eosinophils in biopsy samples, inflammation decreased with nedocromil sodium and was significantly different from a deterioration with albuterol, although neither of these changes was significantly different from that with placebo treatment. Levels of eosinophil cationic protein in bronchoalveolar lavage samples showed a similar trend. (J Allergy Clin Immunol 1996;98:S51-7.)

Key words: Asthma, epithelial cell function, activated eosinophils, eosinophil adhesion, eosinophil chemotaxis

Nedocromil sodium is a pyranoquinolone derivative that has been shown to inhibit the activation of several inflammatory cell types, including eosinophils, monocytes, macrophages, platelets, and mast cells in vitro.¹⁻⁵ In vivo study of nedocromil sodium has demonstrated that this drug prevents anaphylactic bronchoconstriction and bronchial hyperreactivity and attenuates the increases in vascular permeability and cellular infiltration of the epithelium.⁶ More recent work has demonstrated that nedocromil sodium blocks the chloride channel in several cell types, including mast cells, epithelial cells, and neurons.⁷ This work has suggested that, because influx of chloride ions into the mast cell may be a prerequisite for calcium influx

Abbreviations used

BAL:	Bronchoalveolar lavage
cAMP:	Cyclic adenosine monophosphate
CM:	Conditioned medium
ECP:	Eosinophil cationic protein
EG1:	Total eosinophils
EG2:	Activated eosinophils
GM-CSF:	Granulocyte macrophage colony-stimulating factor
HPF:	High-power field
O ₃ :	Ozone
ppb:	Parts per billion
RANTES:	Regulated on activation, normal T expressed and secreted
sICAM-1:	Soluble intercellular adhesion molecule 1
TNF- α :	Tumor necrosis factor- α

From the Department of Asthma and Allergic Respiratory Diseases, St. Bartholomew's and the Royal London School of Medicine and Dentistry, The London Chest Hospital.

Reprint requests: Robert J. Davies, FRCP, The London Chest Hospital, Bonner Rd., London E2 9LX, United Kingdom.

Copyright © 1996 by Mosby-Year Book, Inc.

0091-6749/96 \$5.00 + 0 1/0/76636

and subsequent activation of mast cells, blockade of the chloride ion channel by nedocromil sodium may also prevent mast cell activation. Recent

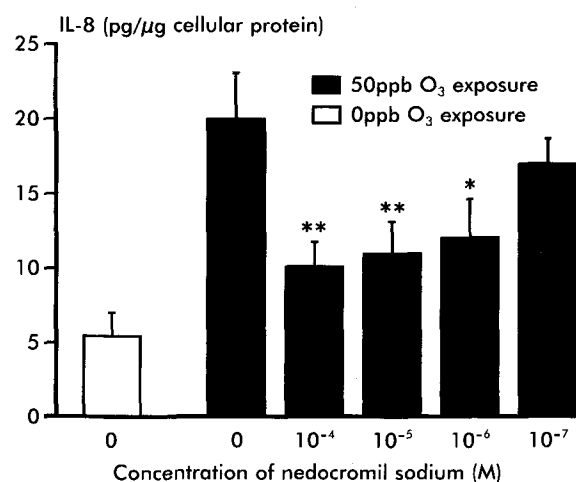


FIG. 1. Effect of nedocromil sodium 10^{-3} to 10^{-7} mol/L on 50 ppb O_3 -induced release of IL-8 by human bronchial epithelial cells in vitro. Results are expressed as means $\pm$ SEM ($n = 6$ minimum at each time point). * $p < 0.05$, ** $p < 0.01$ vs nedocromil sodium 0 mol/L.

findings from our laboratory demonstrated that nedocromil sodium may also influence the activity of human airway epithelial cells, which are thought to play a pivotal role in the development of allergic airway diseases.

EFFECT ON HUMAN BRONCHIAL EPITHELIAL CELL CYTOKINE PRODUCTION

Several studies have demonstrated that airway epithelial cells can synthesize and release proinflammatory mediators, including eicosanoids and cytokines.⁸ In addition, these cells have been shown to express cell adhesion molecules, which play a role in the intertissue trafficking of inflammatory cells.⁹

We have cultured human bronchial and nasal epithelial cells as explant cultures and demonstrated that these cells can express and release IL-1 β , IL-6, IL-8, granulocyte macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor- α (TNF- α), and "regulated on activation, normal T expressed and secreted" (RANTES).^{10,11} More recently, we have investigated the effect of exposure for 6 hours to 0 to 500 parts per billion (ppb) ozone (O_3) on the release of IL-8, GM-CSF, TNF- α , and soluble intercellular adhesion molecule-1 (sICAM-1) from these cells. We demonstrated that O_3 at concentrations of 10 to 100 ppb significantly increased the release of all of these mediators.¹² Maximal release of 20.16 ± 2.56 pg IL-8/ μ g cellular protein was observed after exposure to 50 ppb O_3 , compared with 5.64 ± 0.58

pg IL-8/ μ g cellular protein released from control cells exposed to 5% carbon dioxide in air ($p < 0.05$). Similarly, 50 ppb O_3 led to maximal release of 0.20 ± 0.04 pg GM-CSF/ μ g cellular protein, compared with 0.04 ± 0.03 pg GM-CSF/ μ g cellular protein on exposure to air ($p < 0.05$). Release of TNF- α and sICAM-1 were found to be significantly increased after exposure to an even lower concentration, 10 ppb, of O_3 (TNF- α 0.1 ± 0.01 pg/ μ g cellular protein [$p < 0.05$] and sICAM-1 27.80 ± 6.76 pg/ μ g cellular protein [$p < 0.01$]) when compared with levels on exposure to air (TNF- α 0.06 ± 0.001 pg/ μ g cellular protein and sICAM-1 4.53 ± 0.45 pg/ μ g cellular protein).

We tested whether nedocromil sodium was capable of inhibiting the induced release of IL-8, GM-CSF, TNF- α , and sICAM-1. We first investigated the effect of a range of doses of nedocromil sodium on 50 ppb O_3 -induced release of IL-8 from bronchial epithelial cells. Nedocromil sodium exhibited a dose-dependent effect and inhibited the release of IL-8 maximally at concentrations between 10^{-4} and 10^{-5} mol/L after 6 hours' exposure (Fig. 1). Subsequently, we investigated the effect of nedocromil sodium 10^{-5} mol/L on O_3 -induced release of other cytokines and found that this agent also attenuated the release of GM-CSF, TNF- α , and sICAM-1 (Fig. 2). Our results are in accordance with those of Marini et al.¹³ and Vittori et al.,¹⁴ who have also found that nedocromil sodium 10^{-5} mol/L is optimally active in inhibiting IL-1 β -induced release of GM-CSF and IL-8 from human bronchial epithelial cells in vitro.

These studies suggest that nedocromil sodium may exert its antiinflammatory action at least in part by modulating the synthesis or release of proinflammatory cytokines from human bronchial epithelial cells.

EFFECT ON EOSINOPHIL CHEMOTAXIS AND ADHESION IN VITRO

Several studies have demonstrated that the activation of inflammatory cells, including cell adhesion and migration (chemotaxis), is modulated by specific cytokines. Shute¹⁵ has recently demonstrated that IL-8, which is generally regarded as a potent chemoattractant for neutrophils, also acts as a potent eosinophil chemoattractant in the presence of secretory IgA but not IgA. Indeed, a study in our laboratory has demonstrated that GM-CSF also potentiates the activity of IL-8 as a potent eosinophil chemoattractant.¹⁶

To investigate the biologic relevance of the IL-8, GM-CSF, RANTES, IL-1 β , and TNF- α synthe-

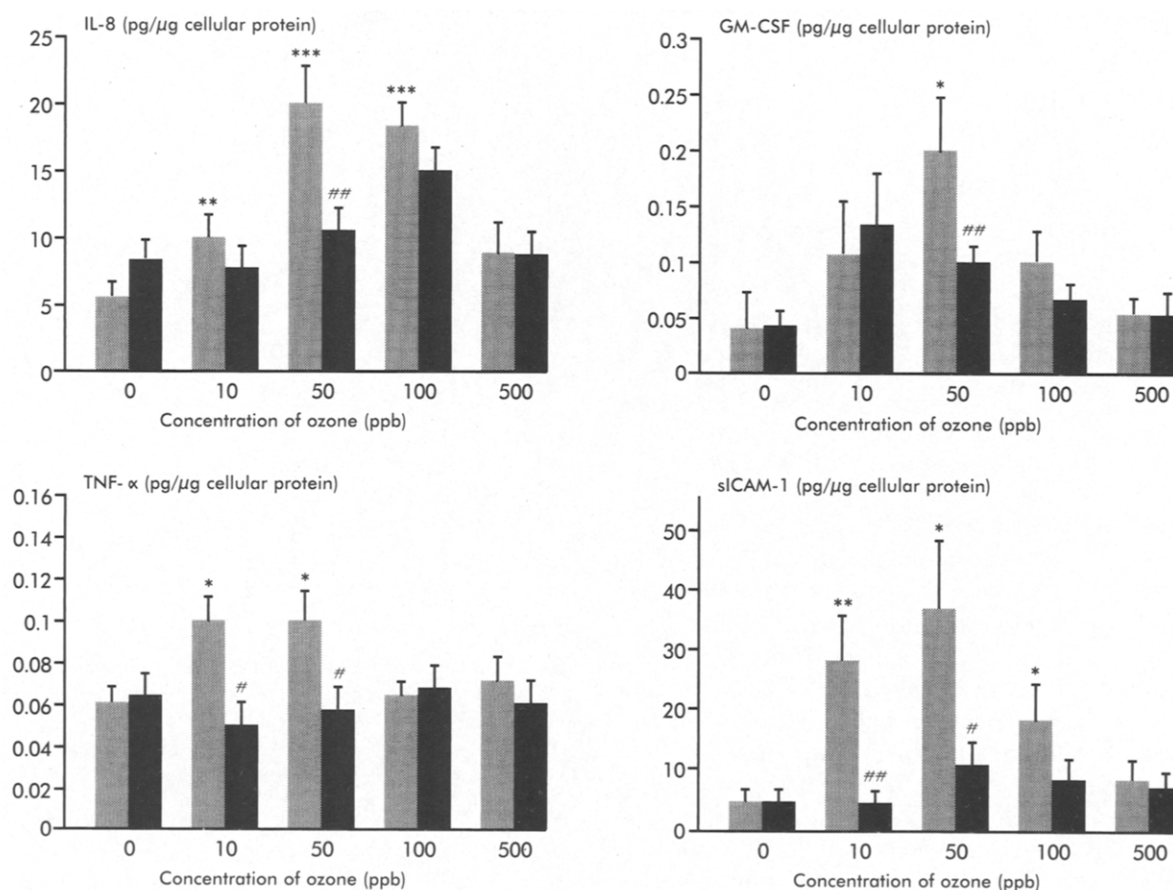


FIG. 2. Effect of nedocromil sodium 10^{-5} mol/L on O_3 -induced synthesis of IL-8, GM-CSF, TNF- α , and sICAM-1 by human bronchial epithelial cells in vitro. Results are expressed as means $\pm$ SEM ($n = 6$ minimum at each time point). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs 0 ppb O_3 ; # $p < 0.05$, ## $p < 0.01$ vs nedocromil sodium 0 mol/L.

sized by and released from the airway epithelial cell cultures in our laboratory, we collected culture medium from confluent human bronchial epithelial cell cultures (24 hours, conditioned medium) and investigated its effect on eosinophil chemotaxis and adhesion to cultured human endothelial cells. Our studies demonstrated that eosinophil chemotaxis, as assessed by the Boyden chamber technique, was significantly increased, from a baseline median value of 2.1 cells/high-power field (HPF) (range 1.9 to 4.5 cells/HPF) for medium 199 only to 10.5 cells/HPF (range 7.8 to 12.3 cells/HPF; $p < 0.05$) for conditioned medium. Addition of nedocromil sodium 10^{-5} mol/L to the conditioned medium blocked the eosinophil-chemotactic effect of the conditioned medium (3.6 cells/HPF [range 2.2 to 4.1 cells/HPF]; $p < 0.05$). Similarly, assessment of the percentage of total eosinophils adhering to cultured endothelial cells, preincubated in the presence of conditioned medium for 6 hours,

demonstrated that eosinophil adhesion was also significantly increased, from a baseline value of 9.3% (range 7.5% to 12.2%) for medium 199 to 23.2% (range 21.3% to 30.5%; $p < 0.05$) for conditioned medium. As was the case for chemotaxis, the addition of nedocromil sodium 10^{-5} mol/L to the conditioned medium blocked this increase in eosinophil adhesion (10.0% adherent cells [range 7.5% to 13.7%]; $p < 0.05$) (Fig. 3).

These studies suggest that products derived from human bronchial epithelial cells, almost certainly cytokines, probably play a role in the recruitment of eosinophils and that nedocromil sodium can block this effect.

EFFECT ON EOSINOPHIL-INDUCED NASAL EPITHELIAL CELL DYSFUNCTION

Studies, particularly those by Motojima et al.¹⁷ and Frigas et al.,¹⁸ have suggested that the eosinophil is the chief cell effecting epithelial damage in

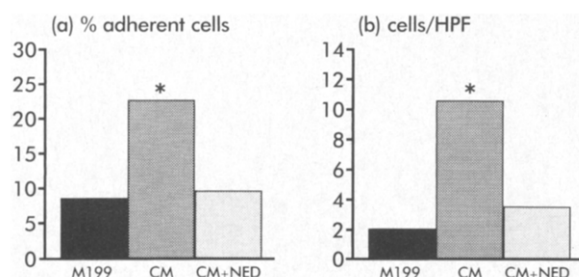


FIG. 3. Effect of conditioned medium from human bronchial epithelial cells alone (CM) or with nedocromil sodium 10^{-5} mol/L (CM+NED) on (A) eosinophil adhesion and (B) eosinophil chemotaxis. * $p < 0.05$ vs medium 199 (M199).

bronchial asthma.^{3, 17-19} In view of the increasing evidence for the detrimental effects of "activated" eosinophils and their products on the airway epithelium, we investigated the effect of activated or nonactivated human eosinophils on the ciliary beat frequency of human nasal epithelial cells and the influence of nedocromil sodium 10^{-5} mol/L in vitro.²⁰ We found that eosinophils stimulated by opsonized latex beads and phorbol-12-myristate-13-acetate significantly attenuated the ciliary beat frequency of the epithelial cells after 2 and 15 hours, respectively, when compared with nonactivated eosinophils. Analysis of eosinophil cationic protein (ECP) released into the culture medium after incubation for 24 hours revealed that, of the two activating agents, only opsonized latex beads induced the release of significantly greater amounts of ECP from eosinophils. Nedocromil sodium 10^{-5} mol/L abrogated the attenuation of ciliary beat frequency induced by phorbol-12-myristate-13-acetate- and by opsonized latex bead-stimulated eosinophils and also blocked the release of ECP from the eosinophils (Fig. 4).

These data suggest that activated eosinophils are capable of causing airway epithelial cell dysfunction and that nedocromil sodium can inhibit eosinophil-mediated effects on epithelial cells.

NEDOCROMIL SODIUM AND BRONCHIAL INFLAMMATION IN ASTHMA

To investigate whether long-term nedocromil sodium treatment has beneficial effects on bronchial inflammation in vivo, we investigated the effect of treatment with nedocromil sodium for 16 weeks and compared the results with the effect of treatment with placebo or albuterol for a similar period in groups of persons with asthma. Adult nonsmoking patients with mild to moderate

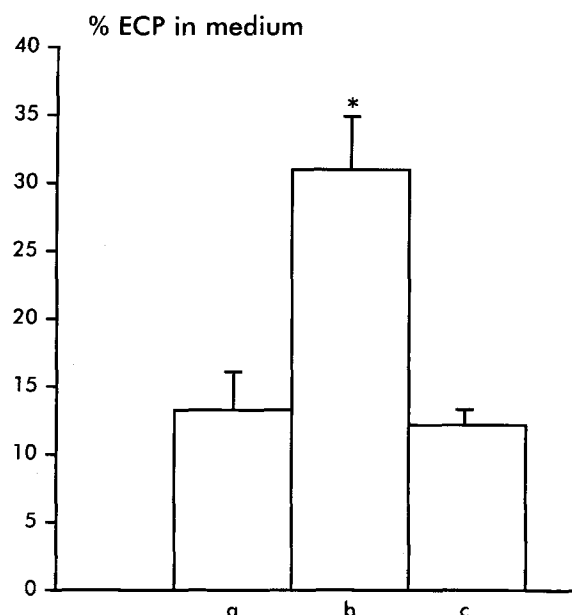


FIG. 4. Release of ECP into culture medium from human airway epithelial cell cultures after 24-hour incubation and exposure to (A) eosinophils ($n = 7$), (B) eosinophils plus latex beads ($n = 9$), and (C) eosinophils plus latex beads plus nedocromil sodium 10^{-5} mol/L ($n = 7$). * $p < 0.05$ vs nonactivated eosinophils.

asthma were randomly assigned to receive nedocromil sodium 4 mg, albuterol 200 μ g, or placebo four times daily for 16 weeks in a double-blind, double-dummy protocol. Before and after treatment, patients underwent histamine bronchial provocation followed by fiberoptic bronchoscopy to obtain bronchoalveolar lavage (BAL) and bronchial mucosal biopsy samples. BAL and biopsy samples were analyzed for any changes in inflammatory cells and mediators, with particular emphasis on eosinophils. These studies demonstrated that, compared with baseline, the numbers of total eosinophils (EG1) (Fig. 5) and activated eosinophils (EG2) (Fig. 6), expressed as the number of cells per square millimeter of bronchial biopsy tissue, decreased after treatment with nedocromil sodium (pretreatment: EG1 = 152.2 ± 42.5 cells/mm² and EG2 = 143.8 ± 30.8 cells/mm²; posttreatment: EG1 = 115.4 ± 35.1 cells/mm² and EG2 = 104.9 ± 31.6 cells/mm²) and that they increased after treatment with albuterol (pretreatment: EG1 = 129.3 ± 28.0 cells/mm² and EG2 = 127.5 ± 30.2 cells/mm²; posttreatment: EG1 = 238.0 ± 55.0 cells/mm² and EG2 = 211.4 ± 50.4 cells/mm²).

Although the changes between the active treatment groups were significantly different ($p < 0.05$),

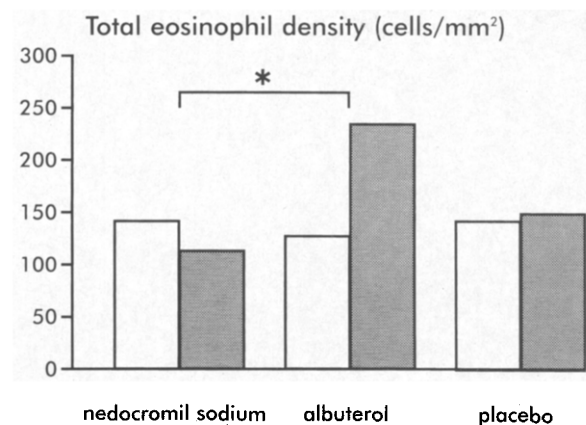


FIG. 5. Mean total eosinophil (EG1) density expressed as cells per square millimeter of bronchial submucosal tissue section, before (biopsy 1, open bars) and after 16 weeks of treatment (biopsy 2, solid bars) with nedocromil sodium ($n = 9$), albuterol ($n = 11$), or placebo ($n = 12$). * $p < 0.05$, nedocromil sodium-treated group vs albuterol-treated group for difference from pretreatment to post-treatment.

no such significant differences were found in eosinophil numbers when either of the active treatment groups was compared with the placebo group. Although not significant, the changes in the concentration of ECP in BAL samples reflected the changes seen in numbers of EG2. No treatment differences were detected for mast cell or lymphocyte numbers. There were no statistical differences between treatment groups for clinical findings, with the exception of evening peak flow, which was significantly increased ($p < 0.05$) in the albuterol group.²¹

These studies suggest that nedocromil sodium has an antiinflammatory role in vivo, albeit less marked than that demonstrated by inhaled corticosteroid treatment,²² consistent with the mechanism of action of these two groups of drugs. Whereas corticosteroids can down-regulate cytokine production in the asthmatic epithelium, reversing inflammation,²³ nedocromil sodium only prevents further inflammation by its blocking effect on the activation of inflammatory cells. On this basis, it is likely that further and more significant effects of nedocromil sodium on inflammation would be detected over a longer time. These studies also indicate that, although it is possible that the increase in eosinophilic inflammation noted in the albuterol-treated group may be a chance finding unrelated to therapy, the observation that EG1 and EG2 in the bronchial mucosa and ECP levels in the BAL fluid of these patients

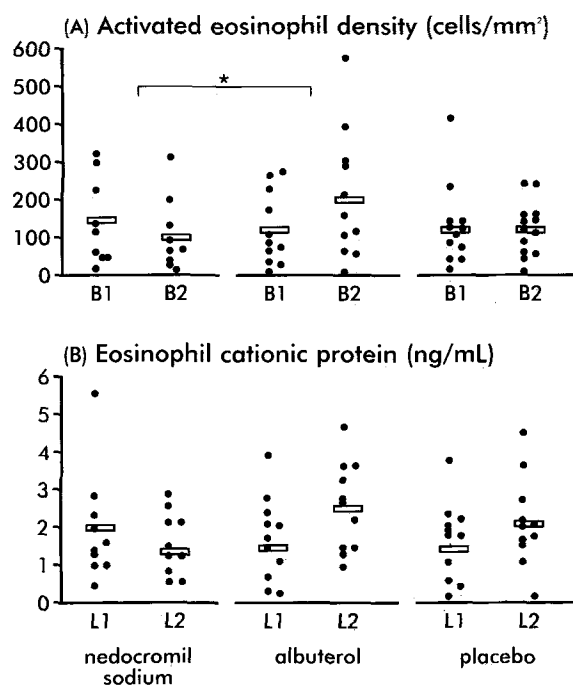


FIG. 6. A, Mean activated eosinophil (EG2) density expressed as cells per square millimeter of bronchial submucosal tissue section, before (biopsy 1, B1) and after 16 weeks of treatment (biopsy 2, B2) with nedocromil sodium ($n = 9$), albuterol ($n = 11$), or placebo ($n = 12$). B, Mean ECP concentration in BAL fluid (nanograms per milliliter) before (lavage 1, L1) and after 16 weeks of treatment (lavage 2, L2). Numbers of paired lavages: nedocromil sodium $n = 10$; albuterol $n = 11$, and placebo $n = 11$. Bars, Mean values. * $p < 0.05$, nedocromil sodium-treated group vs albuterol-treated group for difference from pretreatment to posttreatment.

were consistently increased suggests that this increase in inflammation may indeed be a true effect of the drug. It is possible that bronchodilators (but not antiinflammatory agents), by virtue of their bronchodilator effect, allow greater access of airborne allergens and other irritants into the airways, where they exert detrimental effects on an already inflamed bronchial mucosa. Alternatively, it is possible that agonists may inhibit the antiinflammatory effects of endogenous and exogenous glucocorticosteroids by increasing the interaction between the cyclic adenosine monophosphate (cAMP)-responsive element binding protein and the glucocorticoid receptor complex; this change has an overall effect of preventing glucocorticoid gene transcription as a result of lack of binding of the individual components (cAMP-responsive element binding protein and glucocorticoid receptor complex) to their respective nuclear DNA sequences.²⁴

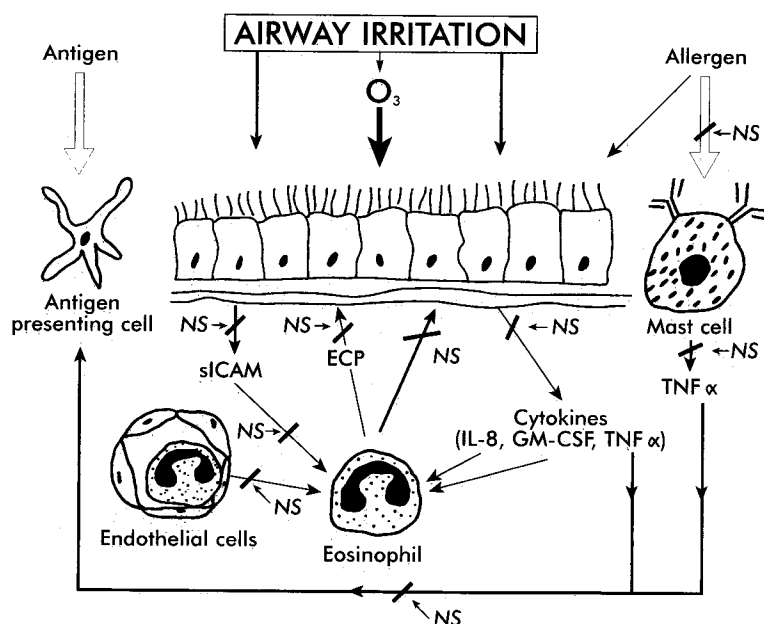


FIG. 7. Putative mode of action of nedocromil sodium (NS).

CONCLUSION

There is increasing evidence to suggest that nedocromil sodium is an effective antiinflammatory and antiallergic agent, because it is capable of attenuating the activation of several cell types—not only mast cells but also macrophages, eosinophils, and epithelial cells, which are central to the development of airway inflammation. Taken together, results from our *in vitro* and *in vivo* studies suggest that this agent may exhibit its antiinflammatory properties by several mechanisms. It is likely that nedocromil sodium has a profound influence on eosinophils by inhibiting the migration of these cells from the blood vessels into the tissues (inhibition of adhesion and chemotaxis) and by directly preventing the activation of these cells (inhibition of cytotoxic protein release and subsequent damage to epithelial tissue). It is likely that the overall inhibiting effects of nedocromil sodium on eosinophils are mediated primarily by down-regulation of the expression of inflammatory cytokines such as IL-1 β , IL-8, GM-CSF, TNF- α , and RANTES (which are known to influence, directly or indirectly, eosinophil adhesion and chemotaxis), as a consequence of its action on the chloride channel; this action results in abrogation of the initial stages of the signal-transduction cascades essential for cytokine gene activation. It is tempting to speculate that in addition to the mast cell membrane-stabilizing effect, which probably explains in part any direct antiallergic properties of

nedocromil sodium, this agent may also exhibit indirect antiallergic properties by blocking the synthesis and release of cytokines such as TNF- α , which are known to be chemokinetic for the antigen-presenting cells in the airway epithelium (Fig. 7). This action would have an overall effect of blocking the migration of the antigen-presenting cells into the lymph nodes, effectively preventing the presentation of allergen to the T lymphocytes and subsequently inhibiting the synthesis of specific IgE by the plasma cells.

REFERENCES

1. Leung KBP, Flint KC, Brostoff J, Hudspeth BN, Johnson NM, Lau HYA, et al. Effects of sodium cromoglycate and nedocromil sodium on histamine secretion from human lung mast cells. *Thorax* 1988;43:756-61.
2. Thorel T, Joseph M, Tsicopoulos A, Tonnel AB, Capron A. Inhibition by nedocromil sodium of IgE-mediated activation of human mononuclear phagocytes and platelets in allergy. *Int Arch Allergy Appl Immunol* 1988;85:232-7.
3. Mattoli S, Mezzetti M, Fasoli A, Allegra L. Nedocromil sodium prevents the release of 15-hydroxyeicosatetraenoic acid from human bronchial epithelial cells exposed to toluene diisocyanate *in vitro*. *Int Arch Allergy Appl Immunol* 1990;92:16-22.
4. Bruijnzeel PLB, Hamelink ML, Kok PTM, Kreukniet JK. 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.
5. Borish L, Williams J, Johnson S, Mascali JJ, Miller R, Rosenwasser LJ. Antiinflammatory effects of nedocromil

- sodium: inhibition of alveolar macrophage function. *Clin Exp Allergy* 1992;22:984-90.
6. Pretolani M, Lefort J, Vargaftig BB. Inhibition by nedocromil sodium of recombinant human interleukin-5-induced lung hyperresponsiveness to platelet-activating factor in actively sensitised guinea pigs. *J Allergy Clin Immunol* 1993;91:809-16.
7. Alton EFWF, Norris AA. Chloride transport and the action of nedocromil sodium and sodium cromoglycate in asthma. *J Allergy Clin Immunol* 1996;98:S102-6.
8. Devalia JL, Davies RJ. Airway epithelium and mediators of inflammation [editorial]. *Respir Med* 1993;87:405-8.
9. Wegner CD, Gundel RH, Rothlein R, Letts G. Expression and probable roles of cell adhesion molecules in lung inflammation. *Chest* 1992;101:34S-9S.
10. Devalia JL, Campbell AM, Sapsford RJ, Rusznak C, Quint D, Godard P, et al. Effect of nitrogen dioxide on synthesis of inflammatory cytokines expressed by human bronchial epithelial cells in vitro. *Am J Respir Cell Mol Biol* 1993;9:271-8.
11. Wang JH, Devalia JL, Xia C, Sapsford RJ, Davies RJ. Expression of RANTES by human bronchial epithelial cells in vitro and in vivo and the effects of corticosteroids. *Am J Respir Cell Mol Biol* 1996;14:27-35.
12. Rusznak C, Devalia JL, Davies RJ. The impact of pollution on allergic disease. *Allergy* 1994;49:21-7.
13. Marini M, Soloperto M, Zheng Y, Mezzetti M, Mattoil S. Protective effect of nedocromil sodium on the IL-1 induced release of GM-CSF from cultured human bronchial epithelial cells. *Pulmon Pharmacol* 1992;5:61-5.
14. Vittori E, Sciacca F, Colotta F, Mantovani A, Mattoil S. Protective effect of nedocromil sodium on the interleukin-1-induced production of interleukin-8 in human bronchial epithelial cells. *J Allergy Clin Immunol* 1992;90:76-84.
15. Shute JK. Interleukin-8 is a potent chemoattractant. *Clin Exp Allergy* 1994;24:203-6.
16. Abdelaziz MM, Devalia JL, Khair OA, Calderon M, Sapsford RJ, Davies RJ. The effect of conditioned medium from cultured human bronchial epithelial cells on eosinophil and neutrophil chemotaxis and adherence in vitro. *Am J Respir Cell Mol Biol* 1995;13:728-37.
17. Motojima S, Frigas E, Loegering DA, Gleich GJ. Toxicity of eosinophil cationic proteins for guinea pig tracheal epithelium in vitro. *Am Rev Respir Dis* 1989;139:801-5.
18. Frigas E, Loegering DA, Gleich GJ. Cytotoxic effects of the guinea pig eosinophil major basic protein on tracheal epithelium. *Lab Invest* 1980;42:35-43.
19. Ayars GH, Altman LC, McManus MM. Injurious effect of the eosinophil peroxidase-hydrogen peroxidase-halide system and major basic protein on human nasal epithelium in vitro. *Am Rev Respir Dis* 1989;140:125-31.
20. Devalia JL, Sapsford RJ, Rusznak C, Davies RJ. The effect of human eosinophils on cultured human nasal epithelial cell activity and the influence of nedocromil sodium in vitro. *Am J Respir Cell Mol Biol* 1992;7:270-7.
21. Manolitsas ND, Wang JH, Devalia JL, Trigg CJ, McAuley AE, Davies RJ. Regular albuterol, nedocromil sodium, and bronchial inflammation in asthma. *Am J Respir Crit Care Med* 1995;151:1925-30.
22. Trigg CJ, Manolitsas ND, Wang JH, Calderon MA, McAuley A, Jordan SE, et al. Placebo-controlled immunopathological study of four months inhaled corticosteroids in asthma. *Am J Respir Crit Care Med* 1994;150:17-22.
23. Wang JH, Trigg CJ, Devalia JL, Jordan S, Davies RJ. Effect of inhaled beclomethasone dipropionate on expression of proinflammatory cytokines and activated eosinophils in the bronchial epithelium of patients with mild asthma. *J Allergy Clin Immunol* 1994;94:1025-34.
24. Peters MJ, Adcock IM, Brown CR, Barnes PJ. β -Agonist inhibition of steroid receptor DNA binding activity in human lung [abstract]. *Am Rev Respir Dis* 1993;147:A772.