

Protective effect of nedocromil sodium on the interleukin-1–induced production of interleukin-8 in human bronchial epithelial cells

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Cellular constituents of the bronchial mucosa may participate in the recruitment of polymorphonuclear leukocytes to the inflamed airways through the generation of the neutrophil chemotactic peptide, interleukin-8 (IL-8). One important aspect of this interaction could be represented by the ability of pulmonary monokines, particularly IL-1, to induce gene expression of IL-8 in bronchial epithelial cells. In this study, we have evaluated the constitutive and IL-1–induced production of IL-8 by cultured human bronchial epithelial cells and examined the ability of the anti-inflammatory drug, nedocromil sodium, to inhibit the cytokine synthesis and release. Northern blot analysis demonstrated that IL-1 β –treated human bronchial epithelial cells expressed appreciable levels of IL-8 mRNA during a period of 8 hours. This finding was associated with an increased release of immunoreactive IL-8 to the culture media, as assessed by specific ELISA. The bronchial epithelial cell–derived IL-8 demonstrated specific biologic activity, since the supernatants of stimulated bronchial epithelial cells possessed neutrophil chemotactic activity that could be inhibited by an antibody against human IL-8. Nedocromil sodium reduced the IL-1–induced release of IL-8 in a dose-dependent manner, but concentrations of the compounds, up to 10⁻⁵ mol/L, did not affect the constitutive production of this cytokine. (J ALLERGY CLIN IMMUNOL 1992;90:76-84)

Key words: Interleukin-8, airway epithelium, inflammation

The infiltration of airway mucosa with PMNs can occur in a number of inflammatory airway diseases, particularly in chronic bronchitis and asthma.¹⁻⁶ One of the factors presumed to be responsible for the presence of PMNs at the tissue site is the local generation of chemotactic agents that direct PMN migration from the vascular compartment to the bronchial mucosa along established chemotactic gradients. Indeed, many chemotactic factors have been isolated from the lung⁷⁻⁹ and from airway-resident cells, particularly hu-

Abbreviations used

PMN:	Polymorphonuclear leukocyte
IL:	Interleukin
HBSS:	Hanks' balanced salt solution
fMLP:	Formyl-methionyl-leucyl-phenylalanine
CA:	Chemotactic activity
NCA:	Neutrophil chemotactic activity
SSC:	Saline-sodium citrate buffer
mRNA:	Messenger ribonucleic acid
MAb:	Monoclonal antibody

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man alveolar macrophages,¹⁰⁻¹³ airway epithelial cells,¹⁴⁻¹⁶ and human bronchial epithelial cells exposed to toxic stimuli,¹⁷ suggesting that pulmonary macrophages and bronchial epithelial cells may participate in mediating the recruitment of PMNs to the airway mucosa in some pathologic conditions.

A polypeptide with NCA, IL-8, has been recently cloned^{18, 19} and isolated from a number of cells, including monocytes,^{13, 19, 20} endothelial cells,^{21, 22} fibroblasts,²³ synovial cells,²⁴ and a pulmonary epithelial

cell line.¹⁶ Monocytes and endothelial cells appear to produce IL-8 in response to endogenous and exogenous stimuli, whereas a host-generated stimulus, such as IL-1, is required for the production of fibroblast and epithelial cell-derived IL-8.¹⁶ This latter phenomenon has been indicated as an example of cytokine networking because the function of one cell population is dependent on mediators synthesized by a neighboring cell.¹⁶ A similar interaction has been already demonstrated to occur in vivo and in vitro in human airways in which the airway mucosa is in contact with the environment and can provide, in some pathologic conditions, the exogenous or the endogenous stimulus for monocyte-derived cytokines.⁶ These monokines, particularly IL-1, may then act to stimulate bronchial epithelium to produce additional effector molecules^{6, 25} that are involved in the local activation of inflammatory cells.^{6, 26}

In a previous study,²⁷ we have demonstrated that human bronchial epithelial cells can be induced to express IL-8 gene and protein in culture when they are stimulated with IL-1 β , indicating one additional effector activity that bronchial epithelial cells may have in airway inflammatory processes. In the study reported in this article, we have evaluated the ability of the anti-inflammatory drug, nedocromil sodium (Tilade; Fisons plc, Loughborough, England),²⁸ to prevent the IL-1-induced up regulation of IL-8 synthesis and release in cultured human bronchial epithelial cells.

METHODS

Isolation of human bronchial epithelial cells and generation of conditioned media

Bronchial epithelial cells were isolated from the grossly normal bronchi of 13 patients, undergoing surgery for peripheral lung cancer, by enzymatic digestion, as previously described.^{17, 25, 29} Nonepithelial cells were eliminated by repeated "panning"³⁰ with the MAbs against the surface markers of monocytes, lymphocytes, and granulocytes,^{17, 25, 29} such that >99% of the final cell population contained cytokeratin filaments, as assessed by immunospecific labeling.^{17, 29} The bronchial epithelial cells were finally resuspended in MCDB-153 medium supplemented with epidermal growth factor (25 ng/ml), transferrin (5 μ g/ml), insulin (5 μ g/ml), 1% pituitary extract, penicillin (100 U/ml), streptomycin (100 μ g/ml) and 0.1% fetal calf serum, and plated in collagen-coated 24-well culture plates at the density of 10^5 cells per well. Cells were incubated for 5 days at 37° C, in 5% CO₂ and 100% humidity. Under these conditions, cells grew to confluence.³¹ The monolayers were then trypsinized and replaced on culture wells, split again at 80% confluence, and used between the third and the fourth passage.

At this time, the confluent cultures were washed repeatedly with HBSS, and during the experiments discussed be-

low, the medium was replaced by medium 199 with antibiotics and without any other supplement to prevent any further growth of the cells or changes in their functional state not caused by the tested stimuli.

Duplicate cultures from each donor were used for cell characterization with immunospecific labeling for epithelial cells (MAb against cytokeratin, DAKO-CK1, Dakopatts, Glostrup, Denmark), monocytes (MAb against CD14, OKM14, Ortho Diagnostic System, Milan, Italy), T cells (MAb against CD2, OKT11, Ortho Diagnostic System), and fibroblasts (rabbit antiserum against vimentin, Amersham Int., Amersham, Buckinghamshire, U.K.).^{17, 25, 29, 31} Only the cultures containing pure epithelial cell populations were used for subsequent experiments.

Stimulation of bronchial epithelial cells by IL-1 β

Different concentrations of recombinant human IL-1 β (Genzyme Corp., Boston, Mass.) or the diluent alone (HBSS) as control were added to the serum- and hormone-free medium for 4 or 8 hours. Conditioned media were then harvested for further analysis, and the epithelial cells were counted and used for evaluation of IL-8 mRNA.

All culture reagents were purchased from GIBCO Europe (Paisley, Scotland) and were free of endotoxin contamination, as assessed by Limulus test.

Protective effect of nedocromil sodium

Parallel epithelial cell cultures from each donor were preincubated for various periods of time with nedocromil sodium, in the concentration range 10^{-7} to 10^{-9} mol/L (final concentration) or the diluent only (sterile HBSS).

Then, the cultures were reincubated for 8 hours with or without recombinant human IL-1 β and nedocromil sodium, added singly or in combination. The compound, applied as pure substance, was dissolved in HBSS just before use at the concentration of 10^{-3} mol/L, and the solutions containing different concentrations of nedocromil sodium were prepared by diluting the stock solution with the serum- and hormone-free culture medium.

Northern blot analysis

For cytoplasmic RNA extraction, equal numbers of pooled bronchial epithelial cells (3×10^6 cells from each donor), cultured for 8 hours in absence of any treatment, or with the addition of 5 ng/ml of IL-1 β and 10^{-5} mol/L of nedocromil sodium, singly or in combination (1.5×10^7 total cells for each experimental condition), were treated with guanidine isothiocyanate, as previously described.^{32, 33} The total RNA extracted from the cells of each donor under each experimental condition was stored in ethanol at -20° C. At the end of the study, the RNA samples obtained under the same conditions from the cells of five donors were pooled for Northern blot analysis.

Fifteen micrograms of total RNA were analyzed by electrophoresis through 1% agarose-formaldehyde in the presence of ethidium bromide (Sigma Chemical Co., St. Louis, Mo.) followed by capillary transfer to Gene Screen Plus sheets (New England Nuclear, Boston, Mass.).

The plasmid containing a human IL-8 cDNA clone¹⁹ was nick translated with ³²P-dCTP (5000 Ci/mmol; Amersham Int.).

Membranes were hybridized in 50% formamide (Merck, Rahway, N.Y.) with 10% dextran sulfate (Sigma Chemical Co.) and washed twice with 2 × SSC and once with 1 × SSC, 0.15 mol/L of sodium chloride and 0.015 mol/L of sodium citrate at room temperature for 10 minutes, and then twice with 2 × SSC, 1% sodium dodecyl sulfate (Merck) at 60° C for 30 minutes, and finally twice with 0.1% SSC at room temperature for 30 minutes.

The membranes were exposed to autoradiographic films at -80° C with intensifying screens. RNA loading and transfer were checked by ultraviolet examination of filters.

Assay of immunoreactive IL-8

The contents of immunoreactive IL-8 in the supernatants of unstimulated or IL-1β-exposed bronchial epithelial cells, cultured in the presence or in absence of nedocromil sodium, were assayed at specific time points in triplicate by ELISA. We used a double-ligand method,²⁷ similar to that published by other groups,^{16, 34, 35} with a rabbit anti-IL-8 antiserum as the capturing antibody and a biotinylated goat anti-IL-8 antibody as the revealing antibody.^{27, 36}

The polyclonal antihuman IL-8 antiserum was produced by immunization of rabbits with recombinant IL-8 in multiple intradermal sites with complete Freund's adjuvant. This IL-8 antiserum and the goat anti-IL-8 antibody reacted with recombinant IL-8 in Western blot analysis and failed to cross-react with connective tissue-activating peptide III and platelet factor 4.^{27, 36}

In this assay, recombinant human IL-8 (Genzyme Co.) was used as standard at one-half log dilutions. Fresh culture medium was used to dilute the standard IL-8 and as blank. The assay consistently detected IL-8 concentrations >0.35 ng/ml in a linear fashion, with an intra- and interassay variability of 6.9% and 12.6%, respectively.

The results were normalized for the number of cells in culture and expressed as nanograms of immunoreactive IL-8 per milliliter of conditioned medium per 10⁶ cells. This normalization was possible because the experiments were performed with culture medium without any growth factor, and the cells did not grow during the period of incubation, as previously observed in similar experimental conditions.^{25, 27}

CA of epithelial cell-conditioned media

Human neutrophils were obtained from the peripheral blood of six healthy donors by Ficoll-Hypaque density-gradient centrifugation, followed by sedimentation in 5% dextran in 0.9% saline (Sigma Chemical Co.). Neutrophils were separated from erythrocytes by hypotonic lysis and then suspended in HBSS at 2 × 10⁶ cells per milliliter with >95% viability (trypan blue exclusion method).

Chemotaxis was performed as follows: 225 μl of diluted (1:2) epithelial cell supernatants, 10⁻⁷ mol/L of fMLP (Sigma Chemical Co.), or recombinant human IL-8 (10 and 100 ng/ml), or culture medium alone, incubated in absence of cells, was seeded in duplicate bottom wells of blind-well

chemotaxis chambers. A 3 μm pore-sized polycarbonate filter (polyvinylpyrrolidone free, Nucleopore, Milan, Italy) was placed in the assembly, and 333 μl of neutrophil suspension was pipetted into each of the top wells. Chemotaxis chamber assemblies were incubated at 37° C in humidified air and 5% CO₂ for 2 hours. The filters were removed, fixed in methanol, and stained with 2% toluidine blue (Sigma Chemical Co.). Neutrophils that had migrated through the bottom of each filter were counted in 10 high-power fields. (Original magnification ×1000.) Chemotactic activity was expressed as the percent of the positive control (10⁻⁷ mol/L of fMLP).

In neutralization studies, the conditioned media obtained from epithelial cell cultures exposed to 5 ng/ml of IL-1β for 8 hours were incubated with 1:50 or 1:100 dilution of either control (rabbit preimmune serum) or neutralizing rabbit antihuman IL-8 antiserum for 30 minutes at 37° C and then assayed for CA. Recombinant human IL-8 was used as positive control.

Statistical analysis

Data are expressed as mean ± SEM. Groups of data were evaluated by the nonparametric equivalent of analysis of variance. Data that appeared significantly different were compared by a multiple-comparison test (Newman-Keuls' test). Significance was accepted if *p* values were <0.05.

RESULTS

CA of human bronchial epithelial cell-derived supernatants

Confluent monolayers of human bronchial epithelial cells were stimulated at time 0 with 0.5 or 5 ng/ml of recombinant human IL-1 and assessed for the dose- and time-dependent generation of CA. As reported in Fig. 1, IL-1β-treated epithelial cells yielded significant neutrophil CA. This effect was dose and time dependent. The CA observed 8 hours after the addition of 0.5 and 5 ng/ml of IL-1β was, respectively, 18.9% ± 4.6% and 39.2% ± 8.3% fMLP control. The CA of 10 and 100 ng/ml of recombinant human IL-8 is also reported in Fig. 1 as percent fMLP control for comparison.

Unstimulated bronchial epithelial cells generated only modest CA that was 6.1% ± 3.7% fMLP control at 8 hours (Fig. 1). IL-1 alone did not exert any CA for neutrophils.

The activity of different dilutions of supernatants of bronchial epithelial cells was evaluated in a previous study²⁷ with similar experimental design, and checkerboard analysis demonstrated the true chemotactic effects of those supernatants.

Inhibition of epithelial cell-derived CA by rabbit antihuman IL-8 antibody

To determine the relative contribution of IL-8 to epithelial cell-derived NCA, 8-hour conditioned me-

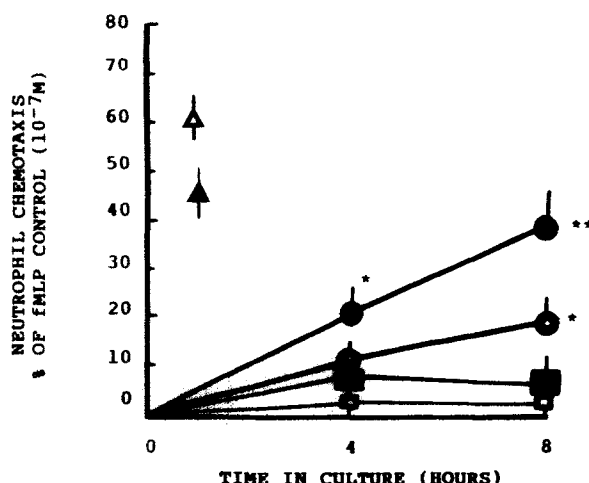


FIG. 1. NCA of the supernatants of human bronchial epithelial cells (1:2 dilution) cultured for 4 and 8 hours in presence of 0.5 (○) or 5 (●) ng/ml of IL-1 β . Activity of supernatants of unstimulated epithelial cell cultures (■); nonspecific activity of the culture medium alone, incubated in absence of cells (□). Neutrophil chemotaxis on the ordinate, expressed as a percent of fMLP (10^{-7} mol/L) positive control; activity of 10 (▲) and 100 (Δ) ng/ml of recombinant human IL-8, reported for comparison. Mean values $\pm$ SEM; N = 8; * p < 0.05; ** p < 0.025 versus effect of supernatants from unstimulated epithelial cells.

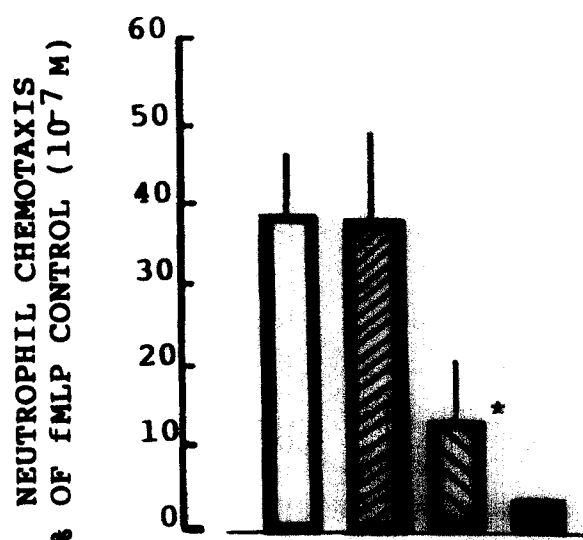


FIG. 2. Inhibition of CA of stimulated epithelial cell-conditioned media by preincubation with rabbit antibody against human IL-8. Supernatants of bronchial epithelial cells exposed for 8 hours to 5 ng/ml of IL-1 β were preincubated with monospecific antibody (1:50) (▨) or with rabbit preimmune serum (1:50) (▧) as control, then tested for CA (1:2 dilution); activity of same supernatants without preincubation (□); activity of supernatants from epithelial cells cultured for 8 hours in absence of IL-1 (■); mean values $\pm$ SEM; N = 5; * p < 0.05 versus (□).

dia were harvested from bronchial epithelial cells stimulated with 5 ng/ml of IL-1 β and preincubated with control rabbit serum or polyclonal rabbit anti-human IL-8 antibody. As indicated in Fig. 2, the CA of the conditioned media from IL-1 β -treated bronchial epithelial cells was reduced by >50% in the presence of antihuman IL-8 antibody (p < 0.05), whereas control antiserum did not exert any effect.

Effect of nedocromil sodium on the CA epithelial cell-conditioned media

In five experiments, we tested the ability of nedocromil sodium to inhibit the CA of the supernatants of bronchial epithelial cells exposed to IL-1. To do this test, three different concentrations of the compound or the diluent alone was added to the supernatants of epithelial cells stimulated with 5 ng/ml of IL-1 β for 8 hours, and chemotaxis was evaluated again, as described above. As indicated in Fig. 3, concentrations of nedocromil sodium up to 10^{-5} mol/L did not significantly affect the CA of epithelial cell-conditioned media.

Evaluation of IL-8 release from bronchial epithelial cells

To evaluate the pattern of release of IL-8 from human bronchial epithelial cells on exposure to IL-1 β ,

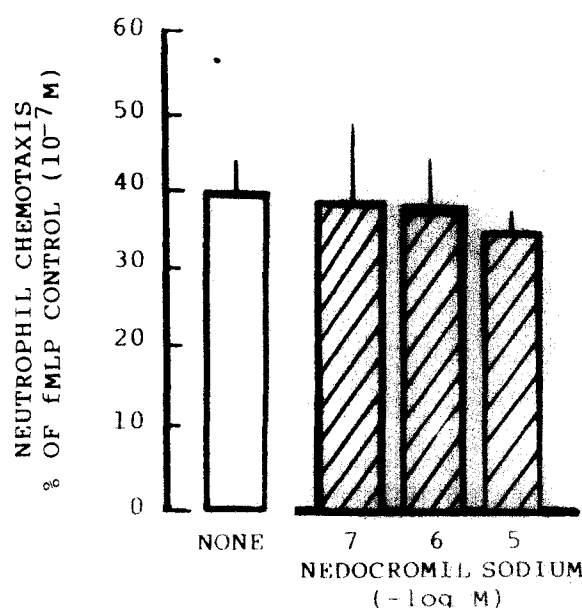


FIG. 3. Effect of nedocromil sodium on CA activity of supernatants of human bronchial epithelial cells exposed for 8 hours to 5 ng/ml of IL-1 β . Supernatants were 50% diluted with diluent of nedocromil sodium (NONE) or with different solutions of the compound to obtain final concentrations (▨), then assayed. Neutrophil chemotaxis on ordinate, expressed as a percent of fMLP (10^{-7} mol/L) positive control; mean values $\pm$ SEM; N = 5.

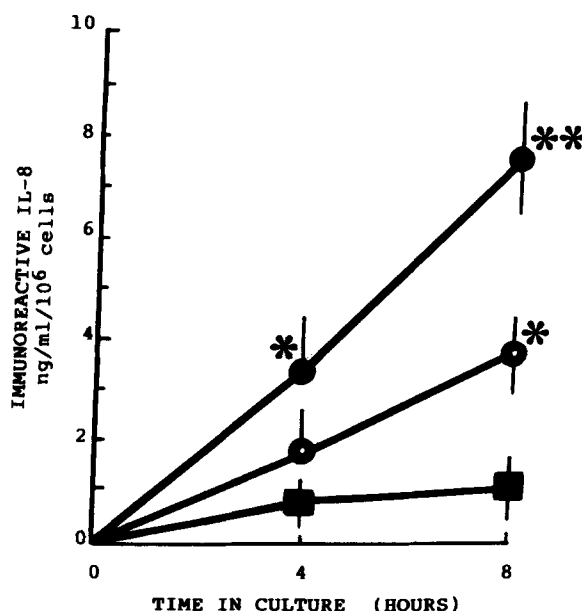


FIG. 4. Contents of immunoreactive IL-8 in supernatants of bronchial epithelial cells cultured for 4 and 8 hours in presence of 0.5 (○) or 5 (●) ng/ml of IL-1 β by ELISA. Release of immunoreactive IL-8 in unstimulated epithelial cell cultures is also reported (■); mean nanograms per milliliter per 10^6 cells $\pm$ SEM; N = 8; * p < 0.05; ** p < 0.025 versus supernatants from unstimulated cells.

epithelial cells were stimulated with two different concentrations of this cytokine during a period of 8 hours, and conditioned media were assessed for immunoreactive IL-8 by ELISA at specific time points. The supernatants from unstimulated epithelial cell cultures were also assayed in the same manner.

The dose- and time-dependent generation of antigenic IL-8 during IL-1 β exposure is illustrated in Fig. 4. IL-1-induced IL-8 was higher than the constitutive release, both at 4 and 8 hours, and the induction was dose dependent. At 8 hours, the amounts of immunoreactive IL-8 in supernatants of cells stimulated with 0.5 and 5 ng/ml of IL-1 were, respectively, 3.8 ± 0.75 and 7.92 ± 1.21 ng/ml/ 10^6 cells.

Effect of nedocromil sodium on the release of immunoreactive IL-8

Since the mechanisms of action of nedocromil sodium at the cellular level are still unknown, to test the ability of this compound in inhibiting the release of immunoreactive IL-8 from bronchial epithelial cells, we initially evaluated the time required for nedocromil sodium to exert any possible effect. To perform this evaluation, in five preliminary experiments, we incubated parallel cultures of bronchial epithelial cells with 5 ng/ml of IL-1 β for 8 hours and added 10^{-5} mol/L of nedocromil sodium together with IL-1 β or

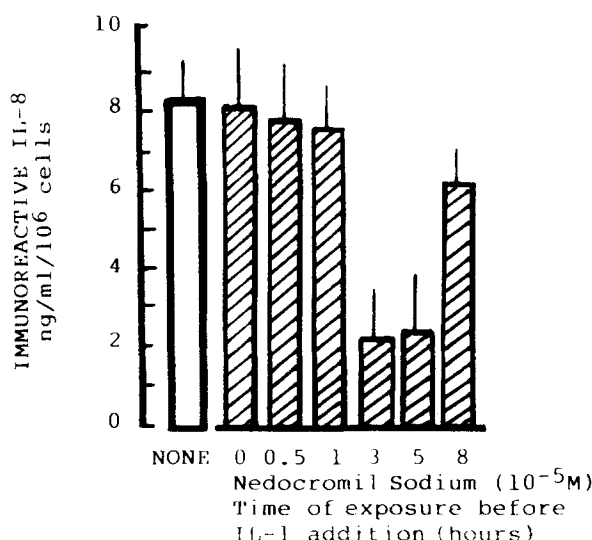


FIG. 5. Effect of nedocromil sodium on release of immunoreactive IL-8 from human bronchial epithelial cells cultured for 8 hours in presence of 5 ng/ml of IL-1 β . Nedocromil sodium (10^{-5} mol/L), added to epithelial cell cultures together with IL-1 β (time 0) or at indicated time points before IL-1 β (▨). Positive control cultures, incubated with IL-1 β alone for 8 hours (□). Contents of immunoreactive IL-8 in supernatants, evaluated by ELISA; mean nanograms per milliliter per 10^6 cells $\pm$ SEM; N = 5.

at different time points before IL-1 β (Fig. 5). By this method, we demonstrated that nedocromil sodium was able to reduce by >50% the release of immunoreactive IL-8 in stimulated epithelial cell cultures and that this effect only occurred when the compound had been added 3 to 5 hours before IL-1 β (Fig. 5).

On the basis of these observations, all the subsequent experiments, concerning the protective effect of nedocromil sodium on the IL-1 β -induced production of IL-8, were performed by adding IL-1 after preincubation of the epithelial cell cultures with the compound, or its diluent, for 4 hours. The results of these latter experiments are reported in Fig. 6, A and B. Nedocromil sodium significantly reduced the release of immunoreactive IL-8 induced by IL-1 β (Fig. 6, A), and this effect was dose dependent (Fig. 6, B). By contrast, concentrations of the compound up to 10^{-5} mol/L did not modify the constitutive release of IL-8 (Fig. 6, A).

Expression of IL-8 mRNA in unstimulated and IL-1 β -exposed bronchial epithelial cells and effect of nedocromil sodium

To evaluate the molecular basis of IL-8 induction by IL-1 β in bronchial epithelial cells and the protective effect of nedocromil sodium, the expression of IL-8 transcripts was examined by Northern blot analysis.

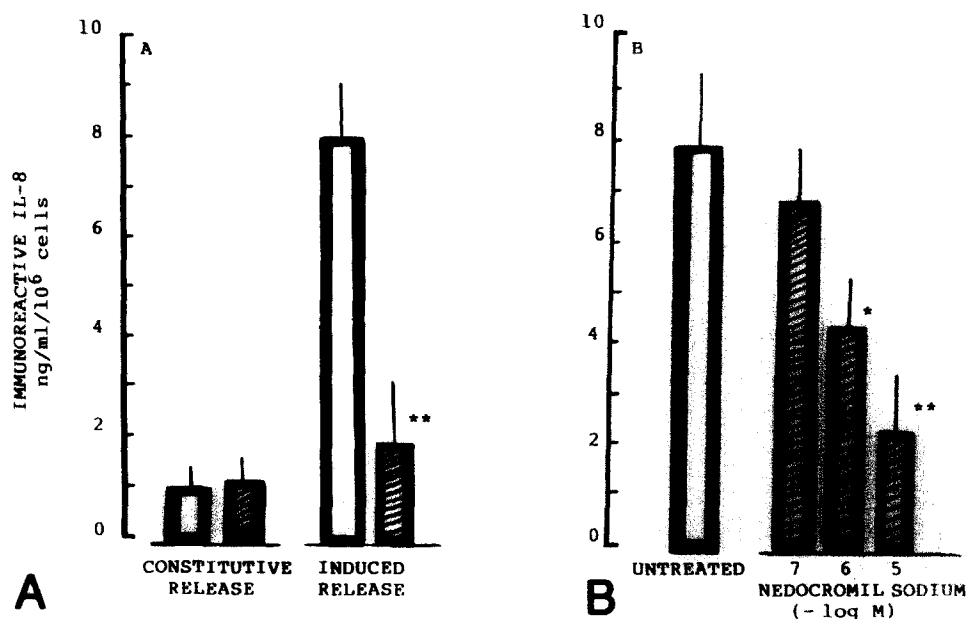


FIG. 6. A, Effect of 10^{-5} mol/L of nedocromil sodium (▨) on release of immunoreactive IL-8 from human bronchial epithelial cells cultured for 8 hours in presence (induced release) or in absence (constitutive release) of 5 ng/ml of IL-1 β (□); ** $p < 0.025$ versus (□); $N = 8$. **B**, Dose-response effect of three increasing concentrations of nedocromil sodium on release of immunoreactive IL-8 from human bronchial epithelial cells cultured for 8 hours in presence of 5 ng/ml IL-1 β ; * $p < 0.05$; ** $p < 0.025$ versus (□); $N = 5$. In all experiments, nedocromil sodium was added 4 hours before IL-1 β (induced release of immunoreactive IL-8) or its diluent (constitutive release of immunoreactive IL-8).

As illustrated in Fig. 7, low but detectable levels of IL-8 mRNA were evident in bronchial epithelial cells cultured for 8 hours in absence of IL-1 and nedocromil sodium. Exposure of the cells to 5 ng/ml of IL-1 β for 8 hours induced a substantial increase in IL-8 transcripts, confirming previous results.²⁷ Preincubation of epithelial cells with 10^{-5} mol/L of nedocromil sodium for 4 hours before IL-1 β addition prevented the increased expression of IL-8 mRNA caused by IL-1.

DISCUSSION

In the mechanisms of airway inflammation, bronchial epithelium has long been considered a target of inflammatory cells devoid of any effector activity.³⁷⁻³⁹ However, recent studies have demonstrated that animal and human bronchial epithelial cells generate inflammatory cell chemoattractants¹⁴⁻¹⁷ and factors that can affect the state of activation of granulocytes and T cells,^{25, 26, 29} and this finding suggests that bronchial epithelial cells may play an important role in initiating inflammatory responses in the airways. Indeed, some of the epithelial cell-derived factors described in the studies referenced above are involved in the pathogenesis of chronic inflammation in asthma,^{6, 26} and their synthesis and release are up reg-

ulated by monokines, particularly IL-1,²⁵ which are also released in increasing amounts in some inflammatory diseases of the airways, including asthma.^{6, 40}

In a previous study,²⁷ we have demonstrated that cultured human bronchial epithelial cells generate CA for neutrophils in the presence of IL-1 β , indicating one more mechanism by which bronchial epithelial cells can modulate the inflammatory responses in the bronchial mucosa and the importance of local interaction with monocytes for this effector activity. This CA of bronchial epithelial cell-conditioned media was associated with the expression of IL-8 gene and with increased release of an IL-8-like substance to the culture media. Furthermore, the CA was significantly inhibited by a monospecific antibody against human IL-8.

IL-8 is a recently described peptide, previously indicated as monocyte-derived neutrophil chemotactic factor¹⁹ and neutrophil-activating peptide-1.¹⁸ It also stimulates neutrophil oxygen-radical generation, degranulation, and leukotriene synthesis,^{41, 42} activates basophils in concert with IL-3,⁴³⁻⁴⁵ and attracts certain subpopulations of T cells.⁴⁶

IL-8 has a relatively long half-life and is resistant to proteolytic enzymatic cleavage compared with that of other chemotactic factors,¹⁸ suggesting that this

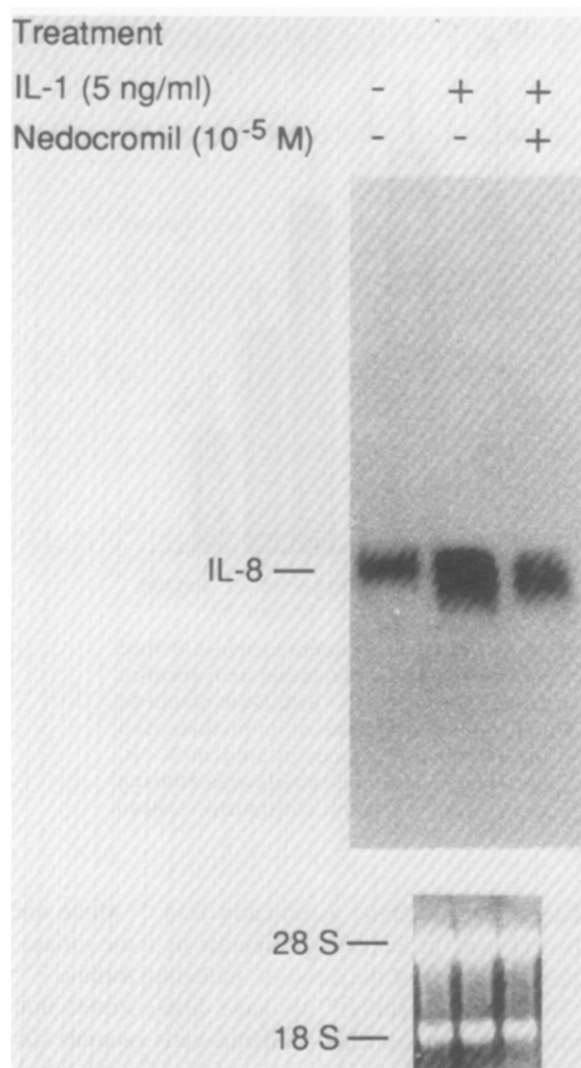


FIG. 7. Expression of IL-8 mRNA in human bronchial epithelial cells cultured for 8 hours in presence or in absence of 5 ng/ml IL-1 β and effect of nedocromil sodium, 10⁻⁵ mol/L. Nedocromil sodium was added to epithelial cell cultures 4 hours before IL-1 β or its diluent; **bottom**, ethidium bromide-stained RNA after blotting on filter.

cytokine may mediate more prolonged inflammatory cell influx.

In the present study, we have confirmed the previous results reported above²⁷ and tested the ability of the anti-inflammatory drug, nedocromil sodium, to abolish the constitutive and the IL-1-induced production of IL-8 in cultured human bronchial epithelial cells.

Nedocromil sodium did not affect the synthesis and release of IL-8 in unstimulated epithelial cell cultures but prevented the up regulation of IL-8 production caused by IL-1 β in a dose-dependent manner. At the concentration of 10 μ mol/L, this compound com-

pletely abolished the increased expression of IL-8 gene and protein induced by IL-1 β .

The effect on IL-8 mRNA expression suggests an action of nedocromil sodium on the signal transduction pathway for IL-1 β that would deserve further evaluation. This compound was effective only when it was added to the epithelial cell cultures 3 to 5 hours before IL-1, indicating that its action may be mediated by one or more substances in cell membrane or cytoplasm that must be synthesized or activated. Interestingly, nedocromil sodium is also effective in inhibiting granulocyte-macrophage colony-stimulating factor release from cultured human bronchial epithelial cells when it is added 4 hours before cell stimulation with IL-1 β ,⁴⁷ and this nonspecific effect on the up regulation of cytokine expression by IL-1 would support the hypothesis that the compound indirectly interferes with the signal transduction pathway for IL-1. The alternative hypothesis that nedocromil sodium can affect IL-1 receptor number or affinity in bronchial epithelial cells²⁵ should also be evaluated in future studies.

Nedocromil sodium has been demonstrated to inhibit in vitro the migration and the activation of a number of cells involved in the inflammatory reaction of bronchial mucosa.⁴⁸⁻⁵³ Since the mediators released from those inflammatory cells are known to contribute to the genesis and persistence of functional and structural alterations in inflamed airways,⁵⁴ the ability of nedocromil sodium to inhibit cell migration and activation may explain the effectiveness of the compound in preventing the acute exacerbation of some inflammatory diseases of the airways, particularly asthma.²⁸

Although nedocromil sodium has been demonstrated to prevent the infiltration of neutrophils in the bronchial mucosa of animals exposed to asthmogenic stimuli,⁵⁵ we were unable to demonstrate a direct effect of this compound on neutrophil chemotaxis in vitro. These results are in keeping with previous preliminary observations⁵⁶ and suggest that nedocromil sodium may prevent neutrophil influx in vivo by inhibiting the release of factors with CA rather than their effect. Our findings indicate that epithelial cell-derived IL-8 may represent one of these factors.

The results of our study add additional information on the mode of action of nedocromil sodium in vitro that may contribute to its effectiveness in vivo. These results could be particularly important since the drug is administered by inhalation and the epithelial cell membranes represent the first point of contact.

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