

The effect of nedocromil sodium on human airway epithelial cell-induced eosinophil chemotaxis and adherence to human endothelial cell *in vitro*

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ABSTRACT: Although some studies have shown that long-term treatment of asthmatics with nedocromil sodium can reduce airway hyperresponsiveness and improve symptoms and lung function, the mechanisms underlying its effects are not well understood.

We have investigated the effect of nedocromil sodium on eosinophil chemotaxis, eosinophil adherence to human endothelial cells and release of soluble intercellular adhesion molecule-1 (sICAM-1) from endothelial cells, induced by conditioned medium collected from cultured human bronchial epithelial cells.

Conditioned medium significantly increased eosinophil chemotaxis from a baseline median value of 2.1 (range 1.9–4.5) cells·high power field⁻¹ (HPF) to 10.5 (range 7.8–12.3) cells·HPF⁻¹ ($p < 0.05$). Similarly, conditioned medium significantly increased eosinophil adherence to endothelial cells from a baseline value of 9 (range 8–12)% to 23 (range 21–30)% ($p < 0.05$). Nedocromil sodium, at 10⁻⁵ M concentration, significantly attenuated the eosinophil chemotaxis and adherence induced by conditioned medium. Conditioned medium also significantly increased the release of sICAM-1 from endothelial cells, from a baseline value of 11.5 (range 8.1–15.4) pg·μg⁻¹ protein to 67.6 (range 55.6–73.5) pg·μg⁻¹ protein ($p < 0.05$). This was significantly attenuated by anti-tumour necrosis factor-α (TNF-α), anti-interleukin-1β (IL-1β) and 10⁻⁵ M nedocromil sodium.

These findings suggest that human bronchial epithelial cell-derived mediators may potentiate eosinophil activity, and that this can be modulated by nedocromil sodium, suggesting a possible mechanism underlying its anti-inflammatory effect.

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Several studies have suggested that eosinophils are the chief effector cells in the pathogenesis of bronchial asthma [1–5]. Despite increasing evidence of an effector role for eosinophils in asthma, the mechanisms underlying the activation and recruitment of these cells into the airways are not clear. Recent studies have demonstrated that cultured human bronchial epithelial cells are capable of expressing and releasing several proinflammatory cytokines, including interleukin (IL)-1β, IL-8, granulocyte/macrophage colony-stimulating factor (GM-CSF), tumour necrosis factor-α (TNF-α), and the factor regulated on activation, normal T-cell expressed and secreted (RANTES) [6–9], which directly or indirectly influence the activity of eosinophils. Studies by Cox *et al.* [10], have demonstrated that survival of eosinophils is also prolonged by incubation with conditioned medium from human bronchial epithelial cell cultures. Recent studies from our laboratory have demonstrated that conditioned medium from human bronchial epithelial cells significantly enhanced the chemotaxis of eosinophils, *in vitro*, and that this effect was significantly attenuated by addition

of neutralizing antibodies to IL-8, GM-CSF and RANTES [11]. Additionally, our studies demonstrated that the epithelial conditioned medium significantly enhanced the adhesion of eosinophils to cultured human endothelial cells, *in vitro*, and that this effect was significantly attenuated by treatment of the endothelial cells with neutralizing antibodies against IL-1β, TNF-α, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin [11].

Since asthma is conceived as a chronic inflammatory disorder of the airways, in which many inflammatory cells, including mast cells, eosinophils and T-lymphocytes, may play a role in the development of recurrent episodes of wheezing, breathlessness, chest tightness and cough [12], modern treatment favours the use of anti-inflammatory medications rather than bronchodilators. Studies of nedocromil sodium, an anti-inflammatory drug, have shown that this agent reduces airway hyperresponsiveness and improves symptoms and lung function in asthmatics, when compared to placebo [13, 14]. Studies in animals have demonstrated that nedocromil sodium decreases

the migration of eosinophils into the airways and the accompanying hyperresponsiveness induced by repeated antigen challenge [15, 16]. Immunopathological studies from our laboratory, of the effect of nedocromil sodium and albuterol on bronchial inflammation in asthma, have demonstrated that nedocromil sodium decreased the numbers of total and activated eosinophils in bronchial mucosal biopsy tissues, compared with baseline [17]. In contrast, the numbers of total and activated eosinophils was increased after treatment with albuterol. Although there were no significant differences in the numbers of total or activated eosinophils between either active treatment group and placebo, the differences in eosinophil numbers between the nedocromil sodium-treated group and the albuterol-treated group were significant.

Additionally, studies from our laboratory have demonstrated that nedocromil sodium blocks the release of eosinophil cationic protein (ECP) from eosinophils and inhibits eosinophil-induced attenuation of ciliary activity of epithelial cells *in vitro* [18]. Indeed, others have also demonstrated that nedocromil sodium can influence the activity of several inflammatory cell types, including mast cells, mononuclear cells, platelets and eosinophils [19–21], and have suggested that the clinical improvement observed in the treatment of bronchial asthma with this drug could be due to its anti-inflammatory effects.

We have hypothesized that in bronchial asthma, anti-inflammatory agents, such as nedocromil sodium, exert their effects by modulating the activity of epithelial cell-derived proinflammatory mediators which influence the activity of eosinophils. To test this hypothesis, we have cultured human bronchial epithelial cells to confluence and studied the effect of nedocromil sodium on eosinophil chemotaxis and adhesion to endothelial cells, modulated by conditioned medium from these cultures. Additionally, we have studied the effect of conditioned medium on the release of soluble intercellular adhesion molecule-1 (sICAM-1) from cultured human endothelial cells, and the modulation of this release by nedocromil sodium.

Materials and methods

All chemicals and reagents were of tissue culture grade and, unless otherwise stated, were obtained from the Sigma Chemical Co. (Poole, UK). Anti-IL-1 β and anti-TNF- α neutralizing antibodies and enzyme-linked immunosorbent assay (ELISA) kits used for the measurement of cytokines and sICAM-1 were purchased from R&D Systems Europe Ltd (Abingdon, UK).

Isolation and culture of bronchial epithelial cells

Bronchial tissue was obtained from nonallergic patients undergoing surgery for lung cancer at St Bartholomew's Hospital, London, and used immediately for culture of epithelial cells by a modified explant cell culture technique developed in our laboratory [22]. Briefly, the epithelium was dissected away from the underlying lamina propria and cut into pieces approximately 1 mm³ in size. Following three gentle washings with fresh prewarmed and aerated sterile medium 199, 2–3 sections were

explanted into each culture dish. All explants were incubated in 2 mL culture medium 199, supplemented with 2.5% Nu-serum (Hyclone, Cramlington, UK), antibiotics and a variety of growth factors (insulin, hydrocortisone, glutamine, transferrin, epidermal growth factor; Universal Biologicals, London, UK), at 37°C in a 5% CO₂ in air atmosphere. The culture medium was replaced every other day and the explants were observed for cell outgrowth, until the outgrowing cells had grown to confluence, usually after 2–3 weeks.

Prior to further experimentation, 12 fully confluent cultures were washed and incubated with serum-free medium 199 for 24 h. At the end of this incubation, the medium was collected from each culture, pooled, and then used as conditioned medium for investigations of eosinophil chemotaxis and adherence to cultured human endothelial cells.

Culture of endothelial cells

Human endothelial cell cultures were established from a spontaneously transformed cell line (ECV304, passage number 126; European Collection of Animal Cell Cultures, Porton Down, UK), as described previously [11]. Endothelial cells were seeded at a cell density of 2×10^5 cells-well⁻¹, in 15 mm diameter 24-well Multiwell™ cell culture plates (Becton Dickinson Ltd, Oxford, UK), and incubated at 37°C in a 5% CO₂ in air atmosphere, in 0.4 mL culture medium 199 supplemented with 10% foetal calf serum (FCS), antibiotics and a variety of growth factors, including insulin, glutamine, hydrocortisone and transferrin (Universal Biologicals, London, UK). The cells were allowed to adhere to the culture plates for 2–4 days, and the culture medium was then replaced every other day, until the cells had grown to confluence.

Isolation and purification of human eosinophils

Eosinophils were isolated from human blood as described previously [11, 18]. Forty millilitres of heparinized blood were mixed with 10 mL of 6% dextran (molecular weight (MW) 100,000–200,000) in 0.15 M saline, and allowed to sediment at room temperature for 40 min. The leucocyte-rich fraction (buffy layer) was aspirated gently and washed twice by centrifugation at 500 \times g for 10 min and resuspension in 15 mL buffered sterile Hank's balanced salt solution, pH 7.4. The final washed cell pellet was resuspended in 1.5 mL isotonic Percoll solution (density 1.07 g·mL⁻¹), supplemented with 10% FCS, and carefully loaded onto a discontinuous density Percoll gradient at densities between 1.085–1.105 g·mL⁻¹. The Percoll gradient was centrifuged at 1,600 \times g for 20 min at 20°C and, at the end of centrifugation, 0.5 mL fractions of the gradient were aspirated from the bottom upwards. A small aliquot of each fraction was stained using Kimura's stain [23], and all fractions containing >95% eosinophils were pooled, made up to a final volume of 15 mL with sterile medium 199, and then centrifuged at 500 \times g for 10 min. The contaminating red blood cells in the suspension were lysed by incubation in ice-cold 0.87% ammonium chloride solution for 10 min.

Following gentle washing by centrifugation at $500\times g$ for 10 min and resuspension in sterile medium twice, the final washed cell pellet was resuspended in 1.5 mL sterile culture medium and the cells were stained with Kimura's stain and trypan blue to determine the purity and viability, respectively, of the eosinophil cell preparation. Only eosinophil suspensions of $>95\%$ viability and $>95\%$ purity were used in further investigations.

Effect of nedocromil sodium on epithelial cell-induced chemotaxis of eosinophils in vitro

Eosinophil chemotaxis was studied using a modified Boyden chamber technique as described previously [11]. Sets of six chambers each were set up to investigate different experimental conditions. Half a millilitre of either medium 199, conditioned medium, or conditioned medium containing 10^{-7} , 10^{-6} or 10^{-5} M nedocromil sodium, was placed in the lower compartment of each chamber, and incubated for 90 min at 37°C in the presence of 25×10^3 eosinophils in the upper compartment, separated by an $8\ \mu\text{m}$ pore size microporous polycarbonate membrane. At the end of incubation, the membrane was removed, fixed in absolute alcohol for 5 min and then washed under running tap water for 1 min. The membrane was stained with Chromotrope R for 5 min. The stained membrane was dehydrated in absolute alcohol for 5 min, cleared in CNP 30 reagent (BDH Laboratory Supplies, Lutterworth, UK), and then mounted in Styrolite™ mounting medium. The membrane was immediately examined by light microscopy, and the number of eosinophils coming through to the other side of the membrane was counted in 10 random high power fields (HPF) at $\times 40$ magnification. All slides were read by two independent observers blinded to the experimental conditions, and the chemotactic activity was expressed as the mean number of cells/HPF $^{-1}$.

Effect of nedocromil sodium on epithelial cell-induced eosinophil adherence to human endothelial cells in vitro

Eosinophil adherence was investigated by estimating the number of cells adhering to human endothelial cell cultures, according to the technique described previously [11].

Confluent endothelial cell cultures were washed gently with prewarmed medium 199, and then sets of six separate cultures each were incubated with 0.5 mL of either medium 199, conditioned medium, or conditioned medium containing 10^{-7} , 10^{-6} or 10^{-5} M nedocromil sodium, for 6 h at 37°C in a 5% CO_2 in air atmosphere. Following this incubation, the endothelial cell cultures were washed three times with 1 mL aliquots of fresh medium 199, and each culture was incubated for a further 30 min in the presence of 0.1×10^6 eosinophils, at 37°C in a 5% CO_2 in air atmosphere. At the end of this incubation, the nonadherent eosinophils were removed by thoroughly washing the endothelial cell culture several times with 1 mL aliquots of fresh prewarmed medium 199. The washed endothelial culture was then incubated for 2 min at room temperature, in 0.5 mL aliquots of a solution made up of 2 mM tetramethyl benzidine (TMB) and 0.1% (w/v) cetyltrimethylammonium bromide (CTAB),

in 0.1 M sodium acetate buffer, pH 4.2; and then for a further 2 min in the presence of 0.7 mM hydrogen peroxide. The peroxidase reaction was stopped by the addition of 0.5 mL of 4 N acetic acid, containing 10 mM sodium azide. The optical density of the reaction mix was then determined by measuring the absorbance at 620 nm, in a BioTek model EL340 automated microplate reader (Luminar Technology, Southampton, UK). The number of eosinophils adhering to the endothelial cells was calculated from a calibration curve prepared using cell suspensions containing known numbers of eosinophils. All results were expressed as the percentage of total cells adhering.

In this assay, eosinophils show more than 10 times higher peroxidase activity than neutrophils on a per cell basis, thus ruling out any significant interference by the occasional contaminating neutrophils ($<5.0\%$ of total cells).

Effect of nedocromil sodium on epithelial cell-induced release of sICAM-1 from human endothelial cells in vitro

Confluent endothelial cell cultures were washed gently, as above, and then sets of six cultures each were incubated in the presence of: 1) medium 199; 2) conditioned medium; 3) conditioned medium containing 10^{-7} , 10^{-6} or 10^{-5} M nedocromil sodium; and 4) conditioned medium containing $50\ \mu\text{g}\cdot\text{mL}^{-1}$ anti-IL-1 β or anti-TNF- α , for 24 h at 37°C in a 5% CO_2 in air atmosphere. At the end of this incubation, the medium was collected from each culture and stored by freezing, prior to analysis of the total sICAM-1. The total amount of sICAM-1 both in the endothelial cell cultures incubated in the presence of bronchial epithelial cell conditioned medium and the conditioned medium itself was measured using commercially available ELISA kits (R&D Systems Europe Ltd, Abingdon, UK). The amount of sICAM-1 released specifically from the endothelial cells was calculated by subtracting the amount of sICAM-1 present in the conditioned medium from the total.

Statistical analysis

All results were tested for normality and then expressed as median values with the range. The significance of any differences in median values was compared using the Mann-Whitney test and all p-values of less than 0.05 were considered to be significant.

Results

Analysis of the cytokines in conditioned medium demonstrated that epithelial cells constitutively released 12.8 (range 3.5–29.7) $\text{pg}\cdot\text{mL}^{-1}$ IL-1 β , 75.5 (range 60.3–80.8) $\text{ng}\cdot\text{mL}^{-1}$ IL-8, 61.5 (range 19.4–97.3) $\text{pg}\cdot\text{mL}^{-1}$ TNF- α , 310 (range 208–597) $\text{pg}\cdot\text{mL}^{-1}$ GM-CSF, and 29.0 (range 18.0–44.0) $\text{pg}\cdot\text{mL}^{-1}$ RANTES, which are comparable to the concentrations of these cytokines found in our previous studies [11].

Analysis of the chemotactic response of eosinophils towards conditioned medium from cultured human bronchial epithelial cells demonstrated that this was significantly

increased from a median value of 2.1 (range 1.9–4.5) eosinophils·HPF⁻¹ for medium 199, to 10.5 (range 7.8–12.3) eosinophils·HPF⁻¹ ($p < 0.05$; fig. 1). Nedocromil sodium acted in a dose-dependent manner and attenuated the eosinophil chemotaxis induced by conditioned medium at 10^{-5} and 10^{-6} M, but not significantly at 10^{-7} M. The eosinophil chemotaxis induced by conditioned medium was significantly attenuated to 7.7 (range 5.5–9.5) eosinophils·HPF⁻¹ ($p < 0.05$) and 3.6 (range 2.2–4.1) eosinophils·HPF⁻¹ ($p < 0.05$) by 10^{-6} and 10^{-5} M nedocromil sodium, respectively. Addition of 10^{-5} M nedocromil sodium to control medium 199 did not alter the chemotaxis of eosinophils (fig. 1).

Assessment of the percentage of total eosinophils adhering to cultured endothelial cells, preincubated in the presence of conditioned medium for 6 h, demonstrated that eosinophil adhesion was also significantly increased from a baseline value of 9 (range 8–12)% for Medium 199, to 23 (range 21–30)% ($p < 0.05$) for conditioned medium (fig. 2). As for eosinophil chemotaxis, the increase in eosinophil adherence to endothelial cells induced by

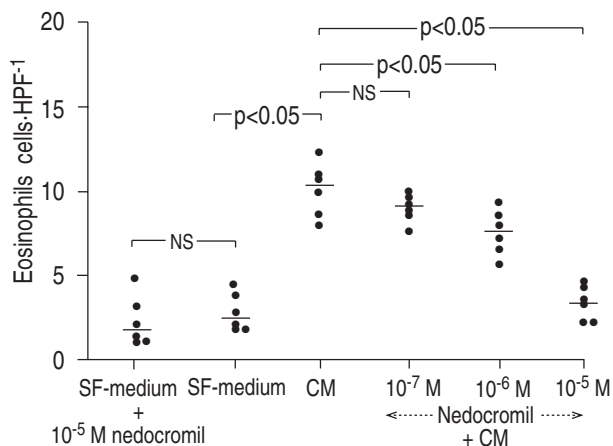


Fig. 1. – The effect of 10^{-7} , 10^{-6} and 10^{-5} M nedocromil sodium on eosinophil chemotaxis induced by conditioned medium (CM). Results are expressed as median (horizontal line) and individual values ($n=6$ for each set of experiments). SF-medium: serum-free medium 199; NS: nonsignificant.

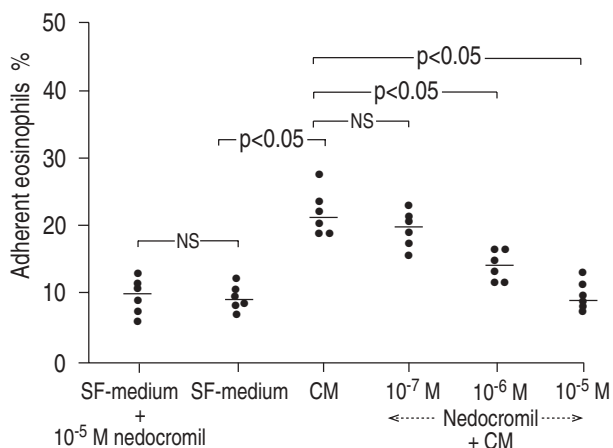


Fig. 2. – The effect of 10^{-7} , 10^{-6} and 10^{-5} M nedocromil sodium on eosinophil adherence to cultured human endothelial cells induced by conditioned medium (CM). Results are expressed as median (horizontal line) and individual values ($n=6$ for each set of experiments). SF-medium: serum-free medium 199; NS: nonsignificant.

conditioned medium was also attenuated by nedocromil sodium in a dose-dependent manner. Although 10^{-7} M nedocromil sodium was not found to significantly alter the eosinophil adherence induced by conditioned medium, 10^{-6} and 10^{-5} M nedocromil sodium significantly attenuated the eosinophil adherence induced by conditioned medium from a median value of 23 (range 21–30)% to 17 (range 15–18)% ($p < 0.05$) and 10 (range 8–14)% ($p < 0.05$), respectively. Addition of 10^{-5} M nedocromil sodium to control medium 199 did not alter eosinophil adherence to endothelial cells (fig. 2).

Figure 3 shows the effect of conditioned medium from cultured human bronchial epithelial cells on the release of sICAM-1 from cultured human endothelial cells. Epithelial conditioned medium significantly increased the release of sICAM-1 from endothelial cells from a median value of 11.5 (range 8.1–15.4) $\text{pg}\cdot\mu\text{g}^{-1}$ protein to 67.6 (range 55.6–73.5) $\text{pg}\cdot\mu\text{g}^{-1}$ protein ($p < 0.05$), over a period of 24 h. Addition of either 50 $\mu\text{g}\cdot\text{mL}^{-1}$ anti-IL-1 β or 50 $\mu\text{g}\cdot\text{mL}^{-1}$ anti-TNF- α neutralizing antibodies to the conditioned medium significantly decreased the release of sICAM-1 from endothelial cells to 39.2 (range 33.0–54.9) $\text{pg}\cdot\mu\text{g}^{-1}$ protein ($p < 0.05$) and 51.8 (range 40.4–59.5) $\text{pg}\cdot\mu\text{g}^{-1}$ protein ($p < 0.05$), respectively. Addition of these neutralizing antibodies in combination produced an additive effect, and attenuated the conditioned medium-induced release of sICAM-1 from endothelial cells even further to 27.8 (range 22.6–43.5) $\text{pg}\cdot\mu\text{g}^{-1}$ protein ($p < 0.05$).

Addition of 10^{-7} – 10^{-5} M nedocromil sodium to conditioned medium also attenuated the conditioned medium-induced release of sICAM-1 from endothelial cells in a dose-dependent manner (fig. 4). Nedocromil sodium, 10^{-6} and 10^{-5} M, significantly attenuated the conditioned medium-induced release of sICAM-1 from endothelial cells from a median value of 67.6 (range 55.6–73.5) $\text{pg}\cdot\mu\text{g}^{-1}$ protein to 54.1 (range 49.7–56.2) $\text{pg}\cdot\mu\text{g}^{-1}$ protein ($p < 0.05$) and 27.8 (range 25.6–33.8) $\text{pg}\cdot\mu\text{g}^{-1}$ protein ($p < 0.05$), respectively. Nedocromil sodium, 10^{-7} M was not found to significantly alter the release of sICAM-1 from endothelial cells (fig. 4).

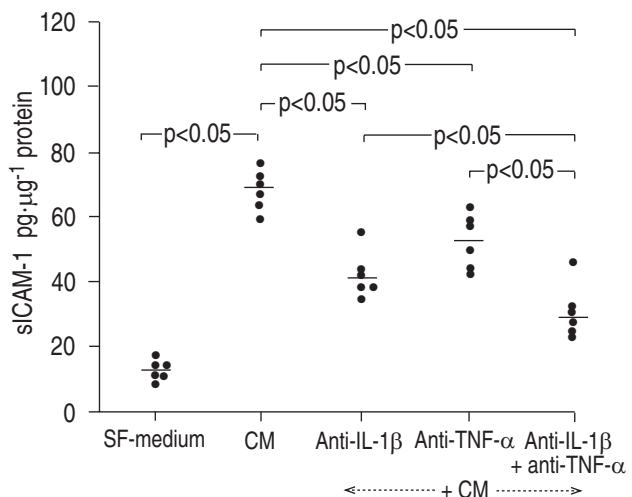


Fig. 3. – The effect of conditioned medium (CM) ± anti-IL-1 β and anti-TNF- α neutralizing antibodies on the release of soluble intercellular adhesion molecule-1 (sICAM-1) from cultured human endothelial cells. Results are expressed as median (horizontal line) and individual values ($n=6$ for each set of experiments). SF-medium: serum-free medium 199; NS: nonsignificant; IL-1 β : interleukin-1 β ; TNF- α : tumour necrosis factor- α .

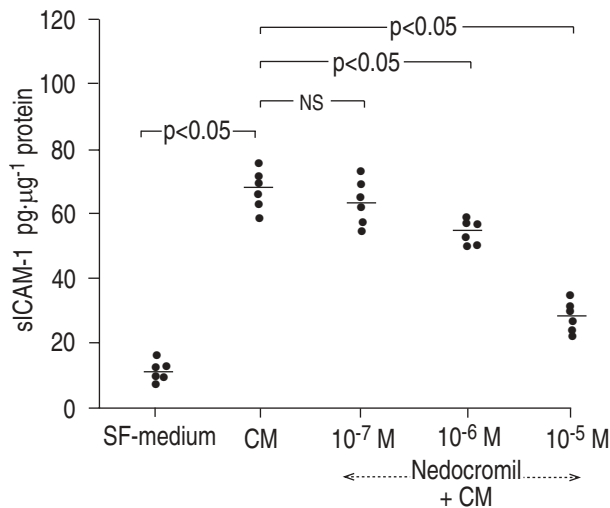


Fig. 4. — The effect of 10^{-7} , 10^{-6} and 10^{-5} M nedocromil sodium on conditioned medium (CM)-induced release of soluble intercellular adhesion molecule-1 (sICAM-1) from cultured human endothelial cells. Results are expressed as median (horizontal line) and individual values ($n=6$ for each set of experiments). SF-medium: serum-free medium 199; ns: nonsignificant.

Discussion

These studies have demonstrated that conditioned medium from human bronchial epithelial cells increases eosinophil chemotaxis and eosinophil adherence to cultured endothelial cells *in vitro*, thus confirming our previous findings [11]. Moreover, these studies have demonstrated that nedocromil sodium attenuates all these epithelial cell-mediated effects. Additionally, these studies have demonstrated that the epithelial cell conditioned medium can significantly increase the release of sICAM-1 from cultured human endothelial cells.

Although anti-IL-1 β and anti-TNF- α neutralizing antibodies exhibited an additive inhibitory effect, when tested in combination, these neutralizing antibodies did not block the release of endothelial sICAM-1 entirely, suggesting that epithelial cell-derived mediators, other than IL-1 β and TNF- α , may also be involved in the regulation of synthesis and/or release of this cell adhesion molecule. Our studies of sICAM-1, however, confirm the findings of other studies, which have demonstrated that this molecule is expressed and released by endothelial cells [24, 25]. Although sICAM-1 has been demonstrated to be present in high concentrations in human serum in many pathological conditions, including bronchial asthma [26, 27], and has also been found to be released by other cell types, including mononuclear leucocytes and epithelial cells [28, 29], its function remains unclear. Structurally, it contains most of the extracellular region of cell surface expressed ICAM-1, and functionally retains the ability to bind lymphocyte function antigen-1 (LFA-1) [30]. Studies by KAKAZU *et al.* [31] demonstrated that the adherence of eosinophils primed with RANTES, to plates coated with sICAM-1, was significantly increased. CHIHARA *et al.* [32] have demonstrated that sICAM-1 augmented eosinophil oxidative metabolism. Collectively these studies suggest that this adhesion molecule probably plays an important role in augmenting eosinophil recruitment and activation, and is likely to exacerbate airway inflammation.

Our finding that nedocromil sodium attenuates epithelial cell-induced eosinophil chemotaxis and adherence to endothelial cells and the release of sICAM-1 from endothelial cells suggests that this drug may exhibit its anti-inflammatory activity by directly or indirectly inhibiting the migration of eosinophils, and possibly other inflammatory cell types, from the vascular compartment into the tissue. These findings are in accordance with the findings of WARRINGA *et al.* [33], who demonstrated that nedocromil sodium blocked IL-8-induced chemotaxis of eosinophils primed with GM-CSF. Similarly, these findings are also in accordance with human [17] and animal [15, 16] studies, which have demonstrated that nedocromil sodium reduces airway eosinophilic infiltration *in vivo*.

Although the anti-inflammatory effect of nedocromil sodium is well-established, the precise mechanisms underlying this effect are not well understood. Studies from our laboratory and those of others have demonstrated that nedocromil sodium can abrogate either ozone-induced or IL-1-induced release of proinflammatory mediators, such as IL-8, GM-CSF, TNF- α and sICAM-1, from human bronchial epithelial cells *in vitro* [34–36], and suggest that this agent may modulate the activity of the inflammatory cells by influencing the synthesis and/or release of mediators which affect these cells.

NORRIS and ALTON [37] have recently reviewed the biochemical aspects of nedocromil sodium activity, and suggested that many effects of this agent may be modulated *via* direct blockage of chloride ion channels, which have a net effect of reducing calcium (Ca^{2+}) influx into the cell. Since initiation of changes in cytosolic Ca^{2+} concentrations is known to play a vital role in the priming of the signal transduction cascades responsible for regulation of gene transcription [38–42], it is possible that nedocromil sodium-induced attenuation in the release of inflammatory mediators [34–36] may primarily be a consequence of the attenuation/inhibition of calcium influx into the cells, which then results in the transcriptional downregulation of the genes encoding these mediators. However, since nedocromil sodium was shown to attenuate the eosinophil chemotaxis and adherence to endothelial cells induced by conditioned medium in the present study, where *de novo* synthesis and release of these inflammatory mediators was not involved, then it is likely that nedocromil sodium-mediated changes in intracellular Ca^{2+} concentrations in eosinophils or endothelial cells may render these cells nonresponsive to the epithelial mediators present in the conditioned medium. It is known that Ca^{2+} influx into cells and intracellular calcium mobilization are essential for chemoattraction and adhesion [43, 44].

Alternatively, it is tempting to speculate that nedocromil sodium may also exert its effects by directly binding either the epithelial cell-derived mediators or their receptors on the target inflammatory cells. Irrespective of the nature of the specific mechanism of action of nedocromil sodium, there is no information so far on the levels of nedocromil sodium *in vivo*. This lack of information has been due, in part, to technical difficulties in measurements as a consequence of the drug being applied topically into the upper and lower airways. Also, since the recommended daily dosage of nedocromil sodium for prophylaxis of asthma is 8–16 mg, the *in vivo*

levels of this drug at site of application are likely to be much higher than the concentration of 10^{-5} M nedocromil sodium investigated as the optimal concentration in these *in vitro* studies.

In summary, these findings complement our previous findings and suggest that nedocromil sodium may influence airways inflammation by modulating the effect of bronchial epithelial cell-derived inflammatory mediators, which influence the migration and activity of inflammatory cells, including eosinophils.

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