The effect of inhaled sodium cromoglycate on cellular infiltration into the bronchial mucosa and the expression of adhesion molecules in asthmatics

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ABSTRACT: There is no direct evidence of the anti-inflammatory effect of inhaled sodium cromoglycate (SCG). To investigate whether inhaled SCG has any effect on cellular infiltration into the bronchial mucosa and the expression of adhesion molecules in patients with asthma, biopsies of the bronchial mucosa were taken from nine patients with atopic bronchial asthma before and after treatment with inhaled SCG (8 mg·day\(^{-1}\)) from a metered-dose inhaler (MDI).

Eosinophils were stained with anti-EG2, neutrophils with anti-NP57, mast cells with anti-AA1, T-lymphocytes with anti-CD4, CD8 and CD3, and macrophages with anti-CD68. Intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), endothelial leucocyte adhesion molecule-1 (ELAM-1) and P-selectin were stained at the same time as adhesion molecules expressed in vascular endothelium. The intensity of ICAM-1 expression in the bronchial epithelium was also evaluated.

The numbers of eosinophils, mast cells, T-lymphocytes and macrophages were significantly reduced as a result of SCG administration, and the expression of ICAM-1, VCAM-1 and ELAM-1 was also significantly inhibited. A significant correlation was found between ICAM-1 expression and T-lymphocytes and between VCAM-1 expression and eosinophils.

It was concluded that sodium cromoglycate does have an effect on the infiltration of the bronchial mucosa by inflammatory cells and also on the expression of adhesion molecules.

for their asthma, and four patients also used oral theophylline. The dosage of oral theophylline was maintained constant throughout the trial. No drug other than inhaled salbutamol as required was taken during the trial. All subjects demonstrated a 20% or greater increase in forced expiratory volume in one second (FEV₁) or peak expiratory flow rate (PEFR) after the inhalation of a standard dose of a β₂-agonist. All patients had allergic asthma, which was diagnosed on the basis of a positive personal or familial history of atopy, positive skin-prick tests to house dust, house dust mite or other inhaled allergen, or a positive radioallergosorbent test (RAST) (Class 2 or greater) to an inhaled allergen.

**Study design**

The trial was of open design. Following a 2 week baseline observation period, asthmatic patients underwent flexible bronchoscopy and bronchial biopsy before and after 12 weeks of treatment with inhaled sodium cromoglycate (Fujisawa Pharmaceuticals, Tokyo, Japan), at a dose of 2 mg (2×1 mg) q.i.d. Patients kept a daily record of SCG inhalation, β₂-agonist inhalation, and theophylline usage in their patient diary.

Throughout the baseline and treatment periods, the patients recorded the severity of attacks of asthma, the presence or absence of productive cough, the amount of sputum produced, and effect of the asthma on daily activities and on sleep, using the criteria of asthma severity recommended by the Japanese Society of Allergology [10]. Patients also measured PEFR twice daily using an Assess peak flow meter (HealthScan, Cedar Grove, NJ, USA).

**Scoring of symptoms**

Five symptom scores were recorded daily on the diary card: 1) the severity of asthma attacks using a graded scale, severe=9, moderate=6 and mild=3; 2) cough, present=1 and absent=0; 3) sputum, large=2 and small=1; 4) the effect of asthma on daily activities, daily activities severely restricted because of asthma=18, daily activities moderately restricted because of asthma=12, daily activities only slightly restricted because of asthma=6, and no restriction of daily activity=0; 5) the effect of asthma at night, unable to sleep because of asthma=9, woke 1–2 times during the night=3, and slept well all night=0.

**Functional assessment**

Biopsies were taken at the end of the 2 week baseline and after 12 weeks of treatment with sodium cromoglycate. Lung function tests were carried out 1 week before the biopsies were taken. The measurement of airway responsiveness followed the method of Takushima et al. [11]. This uses an Astograph Direct Writing Recorder (Chest Co., Tokyo, Japan), measuring dose-response curves of respiratory resistance (Rs) during continuous inhalation of methacholine at stepwise incremental concentrations. Methacholine hydrochloride in isotonic saline was gradually increased to 49, 98, 195, 390, 781, 1,563, 3,125, 6,250, 12,500 and 25,000 µg·mL⁻¹. Baseline values were measured after the inhalation of a solution of isotonic saline, then followed after 1 min with increasing concentrations of methacholine hydrochloride. The cumulative dose that had been administered at the point where the reciprocal of the Rs decreased linearly was used as the measure of bronchial reactivity (Dₘᵢₙ). Dₘᵢₙ was scaled by a unit equal to 1 min of a 1.0 mg·mL⁻¹ aerosol inhalation of methacholine.

**Biopsy of bronchial mucosa**

On the day of the bronchoscopy and the taking of bronchial biopsies, subjects fasted from 09:00 h. All oral and inhaled drugs were withheld from the evening of the previous day. Premedication was provided by intramuscular injection of 0.5 mg of atropine sulphate and 15 mg pentazocine. When the throat had been anaesthetized by spraying with 4% lidocaine, the subjects inhaled 200 µg of salbutamol sulphate to prevent bronchoconstriction. A bronchoscope (BF type 20; Olympus, Tokyo, Japan) was inserted through the mouth and pharynx, and the trachea and bronchi were anaesthetized with 2% lidocaine. Biopsy forceps (FB15C; Olympus) were then used to collect two biopsies, the first from the branch between the right upper lobar branch and right principal bronchus, and the second from the opening of the right central lobar branch. At the second bronchoscopy, tissue samples were taken from the same site as on the first occasion. On each occasion, the biopsy specimen showing the least damage was selected from the specimens taken from the two sites.

**Tissue fixing and staining**

The tissue obtained was mounted in ornithine carbamyl transferase (OCT) compound (Miles Co., Naperville, IL, USA), frozen rapidly in dry ice-acetone and kept in a deep freeze at -70°C. Haematoxylin and eosin (H&E) stained specimens were prepared from all tissues. Continuous sections of 4 µm thickness were prepared and mounted on slides, air-dried for 30 min and fixed for 15 min in cold acetone (-20°C). The tissue sections were washed five times with phosphate-buffered saline (PBS) for 5 min, and then treated for 30 min with 10% normal porcine serum.

The following monoclonal antibodies were then added and allowed to react for 60 min: for activated eosinophils, EG2 (Pharmacia, Uppsala, Sweden), which stained eosinophil cationic protein (ECP); for neutrophils, NP57 (Dako Ltd, High Wycombe, UK), which stained human neutrophil elastase (HNE); for mast cells, AA1 (Dako, Ltd), which stained human mast cell tryptase; for T-lymphocytes, CD3, CD4 and CD8 (Becton Dickinson, Cowley, UK); and for macrophages, CD68 (Dako, Ltd). In addition, monoclonal antibodies were used against the following adhesion molecules: intercellular adhesion molecule-1 (ICAM-1) (British Biotech., Abingdon, UK); vascular cell adhesion molecule-1 (VCAM-1) (Inmunotech, S.A., Marseille, France); endothelial leucocyte adhesion molecule-1 (ELAM-1) (Seika-gaku, Tokyo, Japan); and P-selectin (Novocastra Ltd, Newcastle, UK).
After being washed with PBS, peroxidase-labelled antimouse immunoglobulin G (IgG) was allowed to react for 1 h at room temperature as the secondary antibody was then washed. NaN₃ (65 mg·dL⁻¹) was added in order to prevent nonspecific reaction due to endogenous peroxidase in the eosinophils and neutrophils. Subsequently, 3,3’-diaminobenzidine (DAB) 4HCl was allowed to react for 5 min to develop the colour. Finally, the nuclei were stained with methylene green and, after being washed in running water, dried with alcohol and treated with xylol, the specimens were examined. Staining omitting the primary antibody was used as the negative control.

**Counting of positively-stained cells**

Tissue specimens in a good state of preservation were selected from the biopsy specimens taken from the two sites. The specimens were all coded and examined using a wide-field microscope (BH2; Olympus) at a magnification of ×400. The specimens were all coded to blind the observer and then randomized. Cell counting was undertaken according to the method of Đukanović and co-workers [12, 13]. The observer counted the number of cells in five different fields and calculated the number of cells per field. The number of positively-stained cells was counted only in intact lamina propria in the submucosal area, and epithelial, glandular and vascular tissue was excluded. The tissues measured were traced with extraction apparatus, and a two-dimensional digital program (NEC Co., Tokyo, Japan) was used to measure the area, so that finally the number of cells per square millimetre could be recorded.

**Evaluation of adhesion molecule intensity**

The intensity of the expression of the adhesion molecules, ICAM-1, VCAM-1, ELAM-1 and P-selectin on vascular endothelial cells and of ICAM-1 on bronchial epithelium was evaluated "blind" using a graded scale, by two independent observers, using a semiquantitative method similar to that described by Messadi et al. [14] and Leung et al. [15]. The following scale was used: 2 = strong expression, 1 = weak expression; 0 = no expression. Rare differences were reconciled by consultation between the observers.

**Statistical analyses**

A mean symptom score was calculated from the daily mean of the sum of the five symptoms both for the 2 weeks of the baseline period and for the last 2 weeks of the treatment period. The mean daily PEFR was calculated from the mean of the morning and evening readings, and a mean calculated for the 2 weeks of the baseline and the last 2 weeks of treatment. Diurnal variation of PEFR was calculated from the difference between the morning and evening readings, and expressed as a percentage of the daily mean. The differences between the mean values for FEV1, vital capacity (VC), PEFR, diurnal variation of PEFR, Dmin, and symptom score before and after treatment with sodium Cromoglycate were compared using Student’s 2-tailed paired t-test.

The numbers of each type of inflammatory cell per square millimetre of tissue were counted before and after treatment with inhaled sodium cromoglycate. The results were expressed as means±SEM and Wilcoxon matched-paired, signed rank tests were used to compare the differences. Mann-Whitney U-tests were used for the comparison of the intensity of the expression of each adhesion molecule. The relationship between each type of inflammatory cell and the intensity of the adhesion molecules was tested using the Pearson’s correlation coefficient. A p-value of less than 0.05 was taken as representing a significant difference in the tests.

**Patient consent and ethics approval**

The aims of the study were explained carefully to all the subjects and their consent to participate obtained in writing. The study protocol was approved by the Ethics Committee of the Toho University School of Medicine.

**Results**

**Patients**

Nine atopic adult asthmatic patients, mean age 26 yrs (range 20–35 yrs) were recruited into the study. The individual characteristics are presented in table 1. All

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<table>
<thead>
<tr>
<th>Pt. No.</th>
<th>Sex</th>
<th>Age yrs</th>
<th>FEV1 % pred</th>
<th>VC % pred</th>
<th>Dmin units</th>
<th>Serum IgE IU·mL⁻¹</th>
<th>Treatment</th>
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<tr>
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<td>20</td>
<td>90</td>
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<td>M</td>
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<td>62</td>
<td>111</td>
<td>0.15</td>
<td>720</td>
<td>Beta₂, Theo</td>
</tr>
<tr>
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<td>M</td>
<td>26</td>
<td>64</td>
<td>124</td>
<td>0.33</td>
<td>500</td>
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</tr>
<tr>
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<td>M</td>
<td>29</td>
<td>65</td>
<td>106</td>
<td>0.64</td>
<td>762</td>
<td>Beta₂, Theo</td>
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<td>65</td>
<td>110</td>
<td>0.7</td>
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<tr>
<td>±SD</td>
<td>4.5</td>
<td>4.9</td>
<td>4.12</td>
<td>4.250</td>
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</table>

Pt: patient; M: male; F: female; FEV1: forced expiratory volume in one second; VC: vital capacity; % pred: percentage of predicted value; Dmin: dose of methacholine as a measure of bronchial reactivity; units: equal to 1 min of a 1.0 mg·mL⁻¹ aerosol inhalation of methacholine; IU: immunizing unit; IgE: immunoglobulin E; Beta₂: inhaled salbutamol; Theo: theophylline.
patients had evidence of atopy, with raised serum immunoglobulin E (IgE) levels, mean 541 (range 243–925) immunizing units (IU)·mL⁻¹. The mean value for FEV₁ was 65% predicted normal, and for Dmin 0.7 units. All patients were taking inhaled β₂-agonists as the primary treatment for their asthma, and four patients were also taking oral theophyllines. The dosage of theophyllines was kept constant in the four patients taking them, and the plasma level of theophylline was 4.2±2.0 µg·mL⁻¹ in the baseline period and 3.9±2.3 µg·mL⁻¹ after treatment with inhaled sodium cromoglycate. The mean daily use of inhaled β₂-agonists was 5.4 (range 2.4–8.0) puffs·day⁻¹ in the baseline period, and 3.9 (range 0.4–6.4) puffs·day⁻¹ at the end of the treatment period.

**Clinical variables and functional examination**

The results of pulmonary function tests, bronchial reactivity and symptom scores for the baseline period and the last 2 weeks of treatment, together with the results of the statistical tests, are summarized in table 2. All variables showed a significant improvement after treatment with inhaled sodium cromoglycate.

**Degree of infiltration by type of inflammatory cell**

After treatment with inhaled sodium cromoglycate, the cell numbers in the lamina propria of the bronchial mucosa declined as follows: eosinophils from 28.2±4.7 to 6.6±2.7 mm⁻²; mast cells from 18.0±3.8 to 7.4±3.9 mm⁻²; CD4⁺ T-cells from 55.7±5.9 to 15.4±3.3 mm⁻²; CD8⁺ T-cells from 45.7±4.1 to 27.3±8.9 mm⁻²; CD3⁺ T-cells from 84.9±6.8 to 39.3±4.7 mm⁻²; and macrophages from 65.2±4.8 to 45.3±4.3 mm⁻². All of these changes were statistically significant. Neutrophils fell from 28.7±5.3 to 24.7±4.7 mm⁻² (NS). The changes are presented graphically in figures 1 and 2. Figure 3a and b illustrates changes in the numbers of eosinophils from a single patient.

**Staining intensity of adhesion molecules**

The use of inhaled sodium cromoglycate significantly inhibited the staining intensity of ICAM-1 in the bronchial epithelium and vascular endothelium (table 3). The staining intensity of VCAM-1 and ELAM-1 was also significantly inhibited. There was no significant difference in the intensity of P-selectin (table 3). Figure 3c and d illustrates tissue specimens from a subject in whom the staining intensity for ICAM-1 went from 2+ to zero.

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### Table 2. - Indices of asthma severity measured pre- and post-treatment

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pretreatment</th>
<th>Post-treatment</th>
<th>Mean difference</th>
<th>95% CL</th>
<th>p-value</th>
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<tbody>
<tr>
<td>FEV₁ % pred</td>
<td>65±49</td>
<td>75±48</td>
<td>9.3±4.6</td>
<td>5.8–12.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VC % pred</td>
<td>110±12</td>
<td>117±12</td>
<td>7.3±6.6</td>
<td>2.2–12.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Dmin units</td>
<td>0.7±0.7</td>
<td>1.3±1.1</td>
<td>0.6±0.4</td>
<td>0.26–0.89</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diurnal variation %</td>
<td>22±4</td>
<td>10±2</td>
<td>-11.6±4.5</td>
<td>8.12–15.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total mean symptom score</td>
<td>3.8±1.5</td>
<td>1.2±0.7</td>
<td>-2.6±1.7</td>
<td>-1.7–3.42</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are expressed as mean±sd. Symptom score is daily mean of sum of five symptoms. PEFR: peak expiratory flow rate; 95% CL: 95% confidence limit. For further definitions see legend to table 1.
Relationship between type of inflammatory cell and intensity of adhesion molecules

The coefficient of variation (CV) for repeat counting by a single observer for the immunostains were as follows: EG2 6%; NP57 4%; AA1 7%; CD4 8%; CD8 9%; CD3 11%; and CD68 9%. A significant positive correlation was found between CD4+ (r=0.83; p<0.005), CD8+ (r=0.72; p<0.025) and CD3+ (r=0.86; p<0.005) T-cells and the expression of ICAM-1 in bronchial epithelium; and between CD4+ (r=0.76; p<0.005) and CD3+ (r=0.72; p<0.025) T-cells and the expression of ICAM-1 in vascular epithelium. A significant positive correlation was also found between EG2+ eosinophils and the expression of VCAM-1 (r= 0.87; p<0.005), but none was found between the other types of inflammatory cell and adhesion molecules.

Discussion

This is the first study of the effect of inhaled sodium cromoglycate on the inflammatory cell infiltrate and

<table>
<thead>
<tr>
<th>Intensity of staining</th>
<th>ICAM-1 Epithelium</th>
<th>ICAM-1 Endothelium</th>
<th>VCAM-1</th>
<th>ELAM-1</th>
<th>P-selectin</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
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<td>Post</td>
<td>Pre</td>
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<td>0</td>
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<td>5</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>NS</td>
</tr>
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</table>

The values presented are the absolute number of patients. ICAM-1: intercellular adhesion molecule-1; VCAM-1: vascular cell adhesion molecule-1; ELAM-1: endothelial leucocyte adhesion molecule-1; NS: nonsignificant.
adhesion molecule expression in the bronchial mucosa of patients with mild-to-moderate allergic asthma. No group of subjects receiving placebo was included because of constraints imposed by our Ethics Committee, who considered that a placebo group was not justified. This and the lack of blinding weakens the study and means that the results have to be interpreted with some caution. Consequently, we cannot be certain that the improvement in asthma and the anti-inflammatory effects are due solely to the effects of sodium cromoglycate.

Before and during the study, all nine patients were being treated with inhaled β₂-agonists as required, and four of them with oral theophyllines at a fixed dose. Both these classes of drugs have been reported to affect inflammatory cells [8, 16, 17]. In the four patients who were taking theophyllines, the dose was maintained constant throughout and although the serum theophylline levels fell slightly this was not significant. The effects seen in these four subjects did not differ from the other five, and we therefore conclude that the theophylline is unlikely to have been responsible for the changes observed. All nine patients were using inhaled salbutamol as required for the treatment of their asthma before and during the study. After the addition of inhaled sodium cromoglycate they were able to reduce their dose of inhaled salbutamol. It is possible that this reduction accounted for some of the changes seen.

Manolitsas et al. [18] used regular inhaled albuterol in addition to “as required” inhaled albuterol in their study of the anti-inflammatory effects of inhaled nedocromil sodium and placebo. The albuterol-treated group showed an increase (nonsignificant compared to placebo) in the EG2+ eosinophils but no change in mast cells or lymphocytes. In the study by Laitinén et al. [8], which was a comparison of budesonide, terbutaline and placebo, the group treated with regular terbutaline showed a comparison of budesonide, terbutaline and placebo. The albuterol-treated group showed an increase (nonsignificant compared to placebo) in the EG2+ eosinophils but no change in mast cells or lymphocytes. In the study by Laitinén et al. [8], which was a comparison of budesonide, terbutaline and placebo, the group treated with regular terbutaline showed a mean (nonsignificant) increase in eosinophils, a nonsignificant reduction in mast cells, and a significant reduction in lymphocytes. In an open study, by Dukanović and co-workers [13], similar to the present study but with inhaled beclomethasone, 7 out of 9 patients discontinued inhaled β₂-agonists completely; the possibility that this might have contributed to the anti-inflammatory changes was considered and rejected.

Similar reductions in the numbers of eosinophils in bronchoalveolar lavage (BAL) fluid after treatment with inhaled sodium cromoglycate have been reported by Draz et al. [19]. In their study, there was also a reduction in inhaled salbutamol usage in the sodium cromoglycate-treated group and an increase in the placebo group. Changes in eosinophil numbers in the placebo group were not consistent, so that it seems unlikely that these changes were related to alteration in the doses of salbutamol. In the present study, the reduction in inhaled salbutamol dosage was only possible due to the addition of inhaled sodium cromoglycate, and it is possible that the changes observed resulted from the combined effect of the reduction of one drug and the addition of the other. On balance, we conclude that the anti-inflammatory effects seen were primarily attributable to the addition of inhaled sodium cromoglycate.

Nedocromil sodium is an alternative anti-inflammatory drug to sodium cromoglycate. It is considered to have similar pharmacological effects but to be more potent. Manolitsas et al. [18] compared inhaled nedocromil sodium with inhaled albuterol and placebo, in a study in which they took bronchial biopsies before and after 16 weeks of treatment. Compared to baseline values, treatment with nedocromil sodium resulted in a significant reduction in EG2+ eosinophils but the changes were not significantly different to placebo. There were no changes in the numbers of mast cells or in T-lymphocyte subtypes. Because of differences in technique, it is not possible to make a direct comparison of the effects seen in the study by Manolitsas et al. [18] with those in the present study. The effects of nedocromil sodium would appear to be rather less. The supposedly greater potency of nedocromil sodium is based upon in vitro experiments and bronchial challenge studies; these experiments may not be relevant in clinical usage or in in vivo biopsy changes in asthmatic patients.

In allergic inflammatory conditions, including asthma, various types of cell adhesion molecule are thought to be responsible for the selectivity of inflammatory cell movement in and through the tissues from the blood vessels [6]. The adhesion molecules investigated in the present study were ELAM-1, VCAM-1, ICAM-1 and P-selectin. Upregulation of ELAM-1 has been shown in asthmatic patients at 6 h after antigen challenge [20], and Gundell et al. [21] found a correlation between the increased expression of ELAM-1 and neutrophil infiltration in the airways of nonhuman primates showing delayed-type bronchoconstriction. However, there is very little evidence of increased neutrophils in biopsies from the bronchial mucosa of asthmatic patients [22, 23]. The present study, likewise, failed to indicate any change in the number of neutrophils before and after using sodium cromoglycate and, although the expression of ELAM-1 was significantly inhibited by sodium cromoglycate, no relationship could be found between changes in the expression of ELAM-1 and changes in the numbers of neutrophils.

The expression of ICAM-1 both on bronchial epithelium and vascular endothelium was significantly inhibited by administration of sodium cromoglycate. Saito et al. [24] found a significant correlation between the distribution and staining intensity of ICAM-1 in the mucosa of the inferior nasal concha in patients with nasal allergy, and the distribution and degree of infiltration of T-lymphocytes, especially CD4+ cells. As the staining intensity of ICAM-1 on bronchial epithelium showed a positive correlation with CD4+, CD8+ and CD3+ T-cells, and the staining intensity of ICAM-1 on vascular endothelium with CD4+ and CD3+ T-cells, it is suggested that one way in which T-cell infiltration into the bronchial mucosa was inhibited following the administration of sodium cromoglycate was by a reduction in the expression of ICAM-1.

The expression of VCAM-1 was significantly inhibited by the administration of sodium cromoglycate. The analysis showed a significant positive correlation between the intensity of VCAM-1 and EG2+ eosinophils. VCAM-1 expression and its binding to the integrin ligand, very late activation antigen-4 (VLA-4) [25], has attracted attention as a mechanism to explain cellular infiltration in eosinophil- and T-lymphocyte-dominant allergic inflammation. VLA-4 does not appear in neutrophils [26]. Nakajima et al. [27] used mice challenged...
with ovalbumin (OA) sensitizing antigen, and compared a group pretreated with anti-VCAM-1 antibody or anti-VLA-4 antibody and a group pretreated with anti-ICAM-1 antibody or anti-lymphocyte function-associated antigen-1 (LFA-1) antibody. They reported that eosinophils in the airways were significantly inhibited in the former.

Our findings suggest, therefore, that the inhibition of T-lymphocyte infiltration into the airways following administration of sodium cromoglycate involved the inhibition of ICAM-1 on bronchial epithelial cells, and that the reduction in the infiltration of eosinophils involved the inhibition of VCAM-1 on vascular epithelial cells. However, as the expression of adhesion molecules was evaluated using a semiquantitative method, the interpretation of these relationships must be regarded with some degree of caution. We showed a significant reduction in mast cells but no relationship was found between the change in the number of mast cells and the expression of adhesion molecules. It is possible that this reduction is mediated by another mechanism.

The increased expression of adhesion molecules after antigen exposure is probably mediated by specific cytokines, particularly tumour necrosis factor-α (TNF-α), interleukin (IL)-1 and IL-4 [28, 29]. The source of these cytokines is likely to be mast cells or activated macrophages in the bronchial mucosa. Mast cells from allergic individuals show strong immunostaining for a range of cytokines, specifically TNF-α, IL-4, IL-5 and IL-6 [30]. Sodium cromoglycate has been shown to inhibit TNF-α activity in antigen-stimulated mast cells [31], and in skin mast cells exposed to ultraviolet B irradiation [32]. It is, therefore, possible that the anti-inflammatory effects of the drug are related to its ability to reduce the release of inflammatory mediators and cytokines both from sensitized mast cells and possibly from other cells, such as macrophages or epithelial cells.

Detailed studies by electron microscopy [23, 33] have demonstrated that the number of macrophages increases in the bronchial epithelium and mucosa of patients with asthma. Activated macrophages produce many mediators and cytokines. The adhesion of mononuclear cells in vitro is dependent upon interactions with ELAM-1 and VCAM-1 [34]. The finding that sodium cromoglycate decreased the number of macrophages may be due to its inhibition of the expression of these adhesion molecules.

In conclusion, this is the first prospective study to demonstrate the anti-inflammatory effects of inhaled sodium cromoglycate in symptomatic patients with asthma by showing a significant reduction in the relevant inflammatory cells, with an associated improvement in the clinical indices of the disease. It has also provided some clues as to the likely mechanisms of the anti-inflammatory effects by correlating the reduction in the inflammatory cells with downregulation of the relevant adhesion molecules.

Acknowledgement: The authors thank A. Edwards for revising the manuscript.

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