

# Correlation of bronchial eosinophil and mast cell activation with bronchial hyperresponsiveness in children with asthma

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*Bronchial hyperresponsiveness in asthma has been associated with increased numbers of eosinophils and mast cells in the bronchial airway. It is unclear if these cells are important in the pathogenesis of hyperresponsiveness, and the role of mast cells has been discounted because they are effectively stabilized by  $\beta$ -adrenergic drugs. Because the pathogenesis of asthma in children may be different from that in adults, and to find out if cellular activation is associated with bronchial reactivity, we studied 17 children with mild to moderately severe chronic asthma who had been treated with intermittent bronchodilator therapy and compared their bronchial responsiveness to histamine with the levels of eosinophil cationic protein and mast cell tryptase in bronchial lavage fluid. The number of eosinophils in lavage fluid was correlated with histamine responsiveness ( $r = -0.444$ ,  $p < 0.05$ ) but not with levels of cationic protein ( $r = 0.33$ ,  $p = NS$ ). Bronchial responsiveness to histamine was highly correlated with mast cell tryptase ( $r = -0.714$ ,  $p < 0.005$ ), but there was no correlation with eosinophil cationic protein ( $r = -0.355$ ,  $p = NS$ ). We conclude that in children with chronic asthma mast cells as well as eosinophils contribute to bronchial hyperresponsiveness. Activated mast cells may play a primary role, possibly by tryptase-induced upregulation of bronchial smooth muscle tone. (J ALLERGY CLIN IMMUNOL 1992;90:609-13.)*

**Key words:** Asthma, eosinophil, mast cells, bronchial hyperresponsiveness, eosinophil cationic protein, mast cell tryptase

Bronchial hyperresponsiveness (BHR) is present in many subjects with chronic asthma and in most who are symptomatic.<sup>1</sup> Current models of asthma stress the importance of eosinophils in the etiology of BHR because they are almost universally present in the bronchi of asthmatic subjects.<sup>2-5</sup> Eosinophil-induced damage to bronchial epithelial cells, exposure of neuronal irritant receptors to excessive stimulation, and release of inflammatory mediators of phospholipid metabolism have been hypothesized as causative factors.<sup>3</sup> At the same time, the release of inflammatory mediators from mast cells has been considered unimportant be-

## Abbreviations used

|                    |                                      |
|--------------------|--------------------------------------|
| BHR:               | Bronchial hyperresponsiveness        |
| ECP:               | Eosinophil cationic protein          |
| EDN:               | Eosinophil-derived neurotoxin        |
| EPO:               | Eosinophil-peroxidase                |
| FEV <sub>1</sub> : | Forced expiratory volume in 1 second |
| MBP:               | Eosinophil major basic protein       |
| MCT:               | Mast cell tryptase                   |
| VIP:               | Vasoactive intestinal peptide        |

cause treatment with  $\beta$ -adrenergic drugs before challenge with inhaled allergen stabilizes mast cells, preventing mediator release.<sup>3</sup> Because there may be differences in the underlying mechanisms leading to BHR and asthma in children compared with adults,<sup>6</sup> we studied a group of children with asthma to find out if activation of eosinophils and mast cells, as indicated by the concentration of eosinophil cationic protein (ECP) and mast cell-derived tryptase (MCT) in bronchial lavage fluid, is correlated with BHR to inhaled histamine.

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## METHODS

We studied 17 children, 6 males and 11 females, 8 to 17 years of age (mean, 11 years), all of whom had a history of asthma from infancy. They all had two or more positive prick skin tests for common inhalant allergens, mostly house dust mites. Subjects were receiving inhaled salbutamol, as required, to provide symptomatic control of their asthma and were categorized as having mild to moderately severe symptoms at the time of study. None had previously received inhaled or oral corticosteroid therapy, and none had taken theophylline, sodium cromoglycate, or antihistamines nor had a respiratory infection during the 4 weeks before study.

A histamine bronchial challenge test was performed as previously described.<sup>7</sup> Salbutamol inhalations were withheld for at least 8 hours before the test, and baseline spirometry was performed. Repeated inhalations of histamine acid phosphate were given, increasing in concentration from 0.032 mg/ml to 8 mg/ml. The concentration resulting in a 20% decrease in forced expiratory volume in 1 second ( $FEV_1$ ) was determined by linear interpolation on the log dose response curve ( $PC_{20}H$ ).

Broncholavage was performed 1 week after histamine challenge, except in one subject in whom lavage was performed the next day and one 14 days later. The children were admitted to the day care unit, premedicated with atropine and inhaled salbutamol, and sedated with intravenous diazepam or midazolam, the final dose was titrated to provide light sedation only. After application of topical analgesia (1% xylocaine or 5% cocaine) to the nasal mucosa, the pediatric bronchoscope (Olympus BF 3C10; Olympus Corp., Lake Success, N.Y.) was passed transnasally and the tip placed in the right middle lobe main stem bronchus or medial or lateral segmental bronchi in larger subjects. Twenty milliliters of warm saline solution were injected through the bronchoscope port and immediately suctioned out into a specimen trap. This was repeated twice for a total of 60 ml, with an average of 25 ml pooled lavage fluid being retrieved.

The fluid was immediately placed on ice followed by rapid transfer to the laboratory. After gentle centrifugation at 4° C the supernatant fluid was removed and frozen at -70° C for later use. The cells were resuspended in 1 ml of saline and counted. They were then cytocentrifuged onto slides for staining with Wright stain, esterase and metachromatic stains and differential counts as previously described.<sup>6</sup> The results were expressed as the number of cells per milliliter of lavage fluid.

Activation of eosinophils was assessed by measuring ECP in lavage fluid that had been freshly thawed, in a double antibody radioimmunoassay (Pharmacia Canada, Inc., Baie d'Urfe, Quebec), with results expressed as micrograms per liter lavage fluid. The assay is sensitive to 2 µg/L, has a within-assay coefficient of variation of 7.9% to 10.9% and between assay of 6.6% to 12%, and is specific for ECP. Activation of mast cells was assessed by measuring MCT in a single antibody radioimmunoassay (Pharmacia), sensitive to 0.5 units/L (corresponding to 0.5 µg/L of purified protein). Results were expressed as units per liter of lavage fluid. The within-assay coefficient of variation is 2% to

2.8%, between assays 2.6% to 3.4%, and the assay is specific for mast cell tryptase.

The study was approved by the Clinical Screening Committee for Research and Other Studies Involving Human Subjects, University of British Columbia, and by the Research Review Committee, B.C. Children's Hospital. Written informed consent was obtained from parents/guardians before inclusion of the children in the study. Data were analyzed by logarithmic or exponential regression, as appropriate, with computations of the coefficient of correlation ( $r$ ).<sup>8</sup>

## RESULTS

In all subjects the  $FEV_1$  was greater than 75% of predicted value for height, age, and gender and remained so after a control inhalation of saline diluent. In most patients  $PC_{20}H$  had also been evaluated 1 to 3 months before study. There was never more than a single dilution difference from  $PC_{20}H$  at the time of study. Results of the analysis of lavage fluid are shown in Table I. Elimination of the data from the two subjects in whom bronchoscopy was performed at either 1 day or 2 weeks after histamine challenge only marginally changed the mean values of  $PC_{20}H$  (0.11 mg/ml), cell counts (total  $173 \times 10^3$ /ml, macrophages 150, neutrophils 5.7, lymphocytes 4.8, eosinophils 12.1), ECP (23 µg/L) and MCT (2.9 units/L), and they were therefore included in the analysis.

Enumeration of basomastoid cells with use of metachromatic stains was not successful. Despite the identification of sporadic cells, the numbers were so small as to be uncountable.

The number of eosinophils per milliliter of lavage fluid was correlated with  $PC_{20}H$ ,  $r = -0.444$ ,  $p < 0.05$ , so that larger concentrations of cells were associated with increasing bronchial hyperresponsiveness (Fig. 1). Neither the concentration of ECP nor the absolute amount of ECP in lavage fluid was correlated with  $PC_{20}H$ ,  $r = -0.355$ ,  $p = NS$  (Fig. 2) and  $r = -0.370$ ,  $p = NS$ , respectively, and the concentration of ECP was not correlated with the eosinophil concentration ( $\log \text{cells} \times 10^3/\text{ml}$ ),  $r = 0.330$ ,  $p = NS$ . In contrast, the concentration of tryptase in lavage fluid was highly correlated with  $PC_{20}H$ ,  $r = -0.714$ ,  $p < 0.005$ , (Fig. 3), as was the absolute amount,  $r = -0.662$ ,  $p < 0.025$ , indicating that elevated levels of tryptase were associated with more severe bronchial hyperresponsiveness. There was no statistical evidence of interaction between eosinophil counts or amount of ECP and the amount of MCT in lavage fluid.

## DISCUSSION

It is unlikely that our findings in the lavage fluid were the result of histamine inhalation, since this was

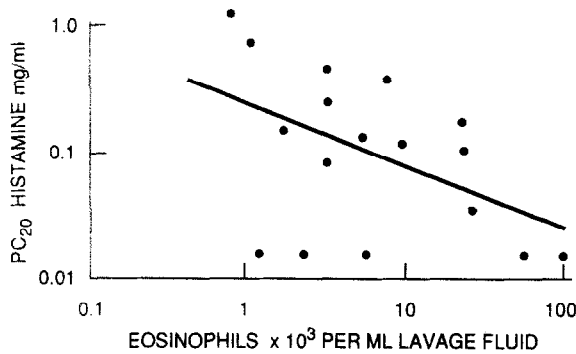


FIG. 1. Correlation of eosinophils in bronchial lavage fluid with PC<sub>20</sub>H; least-square line  $y = 3.78x^{-0.46}$ .

TABLE I. Mean values, standard errors and range of values in 17 children with asthma

|                                         | Mean<br>(SE) | Range        |
|-----------------------------------------|--------------|--------------|
| PC <sub>20</sub> (mg/ml)*               | 0.12         | 0.016 - 1.13 |
| Lavage fluid recovered (ml)             | 25 (1.9)     | 10 - 38      |
| Total cells recovered ( $\times 10^6$ ) | 4.8 (1)      | 0.7 - 15     |
| Total cells ( $\times 10^3$ /ml)        | 176 (26)     | 47 - 420     |
| Macrophages                             | 148 (24)     | 21 - 394     |
| Neutrophils                             | 5.9 (1)      | 1 - 21       |
| Lymphocytes                             | 5.1 (0.9)    | 1 - 8        |
| Eosinophils                             | 16.1 (6)     | 0.9 - 91     |
| ECP ( $\mu$ g/L)                        | 24.8 (6)     | 0.3 - 76     |
| total (ng/lavage)                       | 636 (166)    | 12 - 2118    |
| MCT (units/L)                           | 3.1 (0.6)    | 1 - 8        |
| total (units $\times 10^{-3}$ /lavage)  | 70 (11)      | 31 - 185     |

\*Mean PC<sub>20</sub>H is normalized from log transformed data.

performed in 15 of the 17 children 7 days, and in one 14 days, before bronchial lavage. Histamine has only a transient effect on bronchoconstriction,<sup>9</sup> and inhalation of methacholine, although cumulative in effect, has not been associated with mediator release.<sup>9-11</sup> The infrequent identification of basomastoid cells was probably the result of a combination of factors including the small numbers usually present in lavage fluid,<sup>9, 12-15</sup> the small volumes of infused saline we used and retrieved compared with studies in adults, and the likelihood that the cells we retrieved were from central rather than more peripheral airways where basomastoid cells are more abundant.<sup>16</sup>

Several studies of spontaneous asthma have demonstrated increased cellularity of bronchial lavage fluid, with BHR related to the concentration of eosinophils, macrophages, mast cells, and epithelial cells.<sup>6, 13, 14, 17</sup> Eosinophil degranulation is more prominent in those whose asthma is symptomatic, as judged by levels of

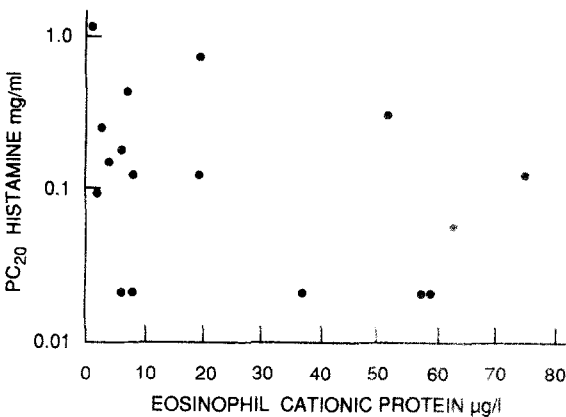


FIG. 2. Correlation of eosinophil cationic protein in bronchial lavage fluid with PC<sub>20</sub>H; least-square line  $y = 0.17e^{-8.48x}$ .

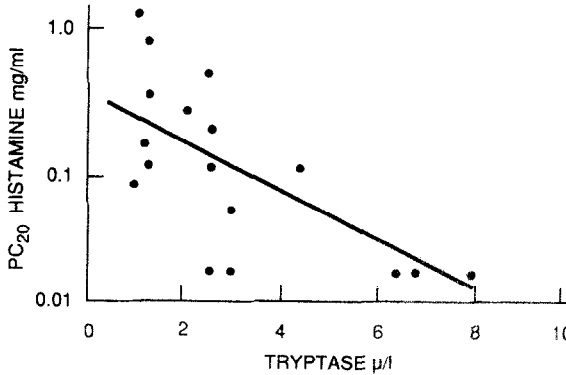


FIG. 3. Correlation of mast cell tryptase in bronchial lavage fluid with PC<sub>20</sub>H; least square-line  $y = 0.42e^{-0.19x}$ .

major basic protein (MBP), suggesting that the contribution of eosinophils is more apparent in those with symptomatic disease.<sup>14</sup> This is consistent with evidence of eosinophil activation at 6 to 12 hours after allergen challenge with increased eosinophil-derived neurotoxin (EDN), eosinophil peroxidase (EPO), and ECP in serum,<sup>18</sup> suggesting eosinophil recruitment during the late-phase asthmatic response, which is associated with the development of BHR.<sup>19</sup> We were, however, unable to find a correlation of ECP levels with PC<sub>20</sub>H in our subjects with chronic asthma, and biopsy studies that demonstrated abundant activated eosinophils in the epithelium and mucosa of patients with atopic asthma found no correlation with PC<sub>20</sub> methacholine.<sup>20</sup> The presence of eosinophils in lavage fluid may not, therefore, presage mediator release, or, alternatively, eosinophil mediators may not cause BHR.

It is also possible that ECP may only partially reflect

eosinophil activation, since stimulation of eosinophils carrying IgE surface antibody with allergen or anti-IgE results in the release of EPO but not ECP.<sup>21</sup> As well, damage to epithelial cells occurs with widely different concentrations of eosinophil-derived mediators, requiring high levels of ECP ( $10^{-6}$  mol/L) and low levels of EPO ( $10^{-8}$  mol/L).<sup>22</sup> ECP levels might therefore underestimate the cytopathic effect of eosinophils and the effects of lipid-derived mediators, which have been implicated in BHR.<sup>3</sup>

Elevated levels of MCT in lavage fluid are present in adults with atopic asthma both before and after allergen challenge.<sup>23</sup> As well, mast cell counts in bronchial epithelial biopsies were higher in 4 of 12 patients with asthma than in healthy controls, with evidence of swelling and solubilization of granular matrix in the patients with asthma indicating cellular activation, but there was no correlation with PC<sub>20</sub> methacholine.<sup>24</sup> The proinflammatory role of mast cells in asthma has also recently been questioned, because inhalation of  $\beta$ -adrenergic drugs in adults before allergen challenge, which very effectively stabilizes these cells, does not prevent a late-phase asthmatic reaction or the enhancement of BHR.<sup>3</sup> It is therefore surprising that we found a correlation of MCT in lavage fluid with BHR, our subjects all having received salbutamol by inhalation just before the lavage procedure. This may reflect prior secretion by mast cells or some difference in mast cell modulation between children and adults. BHR is associated with spontaneous release of histamine by mast cells in the bronchial lumen,<sup>14</sup> and continuous degranulation may occur in the healthy lung,<sup>14, 25</sup> suggesting a role in the maintenance of smooth muscle tone, which might be upregulated by MCT. The latter is supported by studies in animals in which MCT increased the sensitivity and magnitude of the contractile response of bronchial smooth muscle to histamine and blocked relaxation induced by vasoactive intestinal peptide (VIP),<sup>26</sup> suggesting a regulatory role in nonadrenergic/noncholinergic control of airway tone and a mechanism for BHR in asthma.<sup>27</sup>

Another role for mast cells in asthma may be in controlling bronchial inflammation by release of reparative mediators such as heparin.<sup>28</sup> Since MCT is stored as a tetramer closely bound to heparin and is released with heparin on cellular activation,<sup>29</sup> its presence in lavage fluid could also reflect this function and explain the correlation found in our study with BHR. This hypothesis requires further investigation.

It is evident that activation of both mast cells and eosinophils occurs in asthma and that these cells are important components of the asthmatic inflammatory response. Eosinophils are associated with BHR, but whether they play a causative role remains unclear. Our finding that MCT levels in lavage fluid correlate

with PC<sub>20</sub>H suggests a way in which mast cells may upregulate bronchial smooth muscle tone and contribute to BHR in children with asthma.

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## Increased number of immunoreactive nerve fibers in atopic dermatitis

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*The presence of immunologic markers for neurofilaments, neuropeptides of sensory nerve fibers (Calcitonin gene-related peptide and substance P), for noradrenergic innervation (neuropeptide Y and Tyrosine hydroxylase), and Neuron-specific protein 9.5 was evaluated in frozen tissue sections from normal skin (n = 34) and from skin biopsies manifesting urticaria (n = 6), leukocytoclastic vasculitis (n = 4), systemic lupus erythematosus (n = 23), and atopic dermatitis (n = 40, of which 16 were from lesions induced by epicutaneous atopic allergen patch tests). In some normal skin specimens immunoreactive nerve fibers expressing Neuron-specific protein 9.5 were observed in the epidermis, dermis, and around blood vessels. For the other markers, immunolabeling was mainly observed in the dermis around blood vessels. Neurofilaments, which are scarce in normal skin epidermis, were present in higher density in the epidermis of affected skin in all disease conditions. Biopsies from urticaria and systemic lupus erythematosus showed a decrease in density of fibers immunolabeled for neuropeptides substance P and Calcitonin gene-related peptide and for Neuropeptide Y. In biopsies from skin with atopic dermatitis, an increased density of fibers was observed for all markers except Neuropeptide Y and Tyrosine hydroxylase. In this group, biopsies from positive atopic allergen patch tests showed an enhanced density of fibers labeled by antibody to Neuron-specific protein 9.5 and a lower density in labeling for Tyrosine hydroxylase. The data indicate a potential role of innervation and neuropeptides in dermatoses like atopic dermatitis. (J ALLERGY CLIN IMMUNOL 1992;90:613-22.)*

**Key words:** Atopic dermatitis, dermatoses, inflammation, innervation, neuropeptides, skin

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