Bronchial Hyperresponsiveness in Asthmatic Children*  
Correlation with Macrophages and Eosinophils in Broncholavage Fluid

Alexander C. Ferguson, M.B., Ch.B.; and Frances W. M. Wong, B.Sc.

Bronchial responsiveness assessed by histamine bronchial challenge testing in 22 children with chronic stable asthma was compared with the number of inflammatory cells per milliliter of broncholavage fluid obtained by fiberoptic bronchoscopy. Hyperresponsiveness was closely correlated with increased counts of eosinophils and macrophages and with the ratio of eosinophils to macrophages. There was no correlation of neutrophil or lymphocyte counts with bronchial hyperresponsiveness and none of the cell types was correlated with airway obstruction. Our findings support the hypotheses that macrophages may be important in the development of bronchial hyperresponsiveness in children with asthma and that they may modulate bronchial responsiveness both directly and by recruitment of eosinophils. 

(Chest 1989; 96:988-91)

Bronchial hyperresponsiveness to nonspecific stimuli is a common characteristic of asthma and is closely associated with the severity of asthmatic symptoms.14 Inhalation of a single dose of aerosolized allergen results in both an immediate onset asthmatic reaction and a late-onset asthmatic reaction which lasts up to 24 h and is associated with the development of bronchial hyperresponsiveness that may persist for days or weeks before resolving.56 The late-onset reaction is associated with infiltration of the bronchial mucosa and lumen by inflammatory cells including eosinophils, neutrophils and T-helper lymphocytes,711 and with the release of proinflammatory mediator substances from mast cells situated in the bronchial mucosa, though this is not universally accepted.6 In patients with chronic asthma, in whom bronchial inflammation is also found, it is likely that the inflammatory reaction contributes to the development of bronchial hyperresponsiveness, and in adults, bronchial eosinophils and mast cells have been correlated with responsiveness to inhaled histamine and methacholine1213 but the processes involved are poorly understood.16 

Asthma affects as many as 7 percent of children in North America,17 is more often associated with atopy and viral infections than in adults, and may have a different response to therapy and outcome suggesting that there are subtle differences in the underlying pathophysiology in children. To determine if bronchial hyperresponsiveness is quantitatively associated with specific inflammatory cell populations in children as well as adults, we studied a group of children with asthma and compared the numbers of cells in broncholavage fluid with airway responsiveness to histamine.

METHODS

There were 22 children, ten boys and 12 girls aged from 6 to 16 years (mean, 10.7 years). All had a history of chronic asthma since infancy with recurring cough, wheeze and shortness of breath responsive to bronchodilator therapy. Each subject had two or more positive skin tests for common inhalant allergens, usually house dust mites. All the children were clinically stable using inhaled salbutamol at the time of study; and none had ever used steroid medications or received immunotherapy. None had received theophylline, sodium cromoglicate or antihistamines nor had they developed a respiratory infection during the four weeks prior to the study period. Written informed parental consent was obtained for all subjects and the study protocol was approved by the Research Review Committee, B.C. Children's Hospital and by the Clinical Screening Committee for Research and Other Studies Involving Human Subjects, University of British Columbia. Histamine bronchial challenge tests were performed according to our clinic protocol as previously published.48 All tests were performed between 9:30 am and noon. Salbutamol was withheld for at least 8 h and baseline spirometry was evaluated. Each subject had an FEV1 greater than 75 percent of the predicted value for height, age and gender. Control inhalations were given using saline diluent followed by repeated inhalations of histamine acid phosphate in concentrations from 0.032 to 8 mg/ml until the PC20 histamine was recorded.

Broncholavage was performed within 1 to 14 days of the histamine challenge test, in most subjects one week later. Subjects were admitted to the Day Care Unit and premedicated with atropine and salbutamol, with or without light sedation with meperidine and diazepam. A pediatric fiberoptic bronchoscope (Olympus BF3C10) was passed via the nose with stepwise applications of topical anesthesia and the tip placed in the right middle lobe bronchus in

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*From the Department of Pediatrics, University of British Columbia, Vancouver, British Columbia, Canada. 
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Reprint requests: Dr. Ferguson. Children's Hospital, 4480 Oak Street, Vancouver, B.C., Canada V6H 3V4

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smaller subjects or in the medial or lateral segmental bronchi of the right middle lobe in larger subjects. Three 20-ml warm saline solution washes were instilled with an average of 23 ml being retrieved. The lavage fluid was cytocentrifuged and duplicate slides stained with Wright’s stain. Five hundred to 1,000 cells were counted per slide using an oil immersion lens (magnification, ×1,000) and differential cell counts were expressed as the number per milliliter of lavage fluid retrieved. Eosinophils, neutrophils, small lymphocytes and macrophages could be identified easily by their size, nuclear configuration and characteristic granular staining, and basal, ciliated and mucus epithelial cells were identified by size, nuclear configuration, cilia and cytoplasmic inclusions. Small monocyte/macrophages were differentiated from large lymphocytes by size and nuclear configuration, confirmed for the majority of slides by esterase staining. Mononuclear cells unstained by the esterase technique in this category were very rare. Differential counts were performed with the observer blinded to the corresponding histamine response and then compared to the \(PC_{20}\) histamine for each subject using logarithmic regression analysis.

RESULTS

The mean values and ranges for lavage fluid recovered, \(PC_{20}\) histamine and absolute cell counts are shown in Table 1. The coefficients of correlation for cell counts on duplicate slides by two observers were: eosinophils, 0.97 (p<0.0005); macrophages, 0.95 (p<0.0005); neutrophils, 0.93 (p<0.0005) and lymphocytes, 0.53 (p<0.025). There was a significant inverse correlation between eosinophil counts and \(PC_{20}\) histamine, \(r = -0.68\), p<0.0005, indicating that increased numbers of eosinophils were associated with increased airway responsiveness to histamine (Fig 1). Similarly alveolar macrophage counts were inversely correlated with \(PC_{20}\) histamine, \(r = -0.55\), p=0.006 (Fig 2), but neutrophils, \(r=0.03\), and lymphocytes, \(r=0.0095\), were not correlated with the histamine

Table 1 — Mean Values, Standard Errors and Range of Values in 22 Asthmatic Children

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SE</th>
<th>Range</th>
</tr>
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<tbody>
<tr>
<td>(PC_{20}) histamine (mg/ml)*</td>
<td>0.38 ± 0.068</td>
<td>0.008-8</td>
</tr>
<tr>
<td>Lavage fluid recovered (ml)</td>
<td>23 ± 1.8</td>
<td>10-38</td>
</tr>
<tr>
<td>Total cells recovered (×10⁹)</td>
<td>91 ± 21</td>
<td>14-425</td>
</tr>
<tr>
<td>Total cells (×10⁹/ml)</td>
<td>144 ± 19</td>
<td>20-368</td>
</tr>
<tr>
<td>macrophages</td>
<td>113 ± 15</td>
<td>15-343</td>
</tr>
<tr>
<td>neutrophils</td>
<td>18 ± 10</td>
<td>1.54-196</td>
</tr>
<tr>
<td>lymphocytes</td>
<td>5 ± 0.75</td>
<td>0.19-14</td>
</tr>
<tr>
<td>eosinophils</td>
<td>7.7 ± 2</td>
<td>0.12-44</td>
</tr>
</tbody>
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*Mean \(PC_{20}\) histamine is normalized from log transformed data.

Figure 1. Correlation of eosinophil counts in broncholavage fluid with \(PC_{20}\) histamine.

Figure 2. Correlation of macrophage counts in broncholavage fluid with \(PC_{20}\) histamine.

Figure 3. Correlation of eosinophil counts per 10⁷ macrophages in broncholavage fluid with \(PC_{20}\) histamine.
response. When eosinophil counts were expressed in terms of the ratio of eosinophils to macrophages, a significant inverse correlation with $PC_{20}$ histamine again was noted, $r = -0.61, p = 0.001$ (Fig 3). There was no correlation of the ratios of other cell types with $PC_{20}$ histamine.

Alveolar macrophage counts were inversely correlated with peripheral airway flow rates assessed as the percentage of predicted value of FEF25-75%, $r = -0.39, p = 0.044$, but there was no correlation of the counts of other cell types with FEF25-75%. When 14 subjects who had airway obstruction (FEF25-75% < 70 percent predicted value) were studied separately, no significant correlation of macrophage counts with FEF25-75% was found, $r = 0.08$.

**DISCUSSION**

Postmortem studies of the bronchi of those with acute severe asthma are characterized by shedding of epithelial cells and the accumulation of large numbers of eosinophils and eosinophilic granular debris in the bronchial lumina, suggesting that eosinophils are of major importance in the bronchial inflammatory reaction. The finding that eosinophil counts in lavage fluid are correlated with histamine reactivity suggests that eosinophils also are important in the development of bronchial hyperresponsiveness, as in adult studies. The correlation of luminal macrophage counts with bronchial responsiveness to histamine, which has not been reported in adults, suggests that macrophages also play a role in the development of bronchial hyperreactivity in children. They are known to be involved in many facets of bronchial inflammation, and may be actively involved in the asthmatic process, but an explanation for the observed correlation in children and not adults is unclear. There are several possible reasons. First, the cells recovered were from small-volume lavages and probably more representative of cells from the central airways rather than from the peripheral airways and alveoli as are recovered in the large-volume lavages used in adults. Second, alveolar macrophages retain a limited ability to replicate which might be more pronounced in children, and they are activated by the T lymphocyte cytokines gamma-interferon and granulocyte-macrophage colony stimulating factor as well as endotoxin, possibly contributing to the enhancement of bronchial reactivity by respiratory viral infections in young children who are not already immune to these viruses. Third, alveolar macrophages carrying low affinity IgE receptors comprise 5 to 10 percent of cells in normal adults and may be increased to 30 percent or more in asthmatic subjects. It is likely that IgE-bearing macrophages are also increased in asthmatic children who are atopic, perhaps resulting in macrophage counts which correlate with bronchial hyperresponsiveness to a greater degree than in adults in whom atopy is less prominent.

Stimulation of alveolar macrophages with aggregated IgE or with specific allergen after preincubation with IgE antibody results in the release of proinflammatory mediators including the granulocyte chemotactic factor leukotriene B$_{4}$ and PAF, the latter having potent inflammatory effects including chemotaxis of eosinophils and greatly enhanced bronchoconstrictor responsiveness to inhaled methacholine. Our finding of a correlation between increasing bronchial hyperreactivity and elevated ratios of eosinophils to macrophages is consistent with the concept of recruitment of eosinophils by luminal macrophages, as are reports that alveolar macrophage chemiluminescence, a marker of their activation, is associated with increased numbers of eosinophils and that alveolar macrophages enhance the respiratory burst of activated eosinophils. Activated eosinophils also are known to release PAF, which might lead to chemotaxis of more eosinophils and a self-perpetuating cycle of inflammation and persisting bronchial hyperreactivity. There is as yet, however, no direct evidence of eosinophil recruitment by alveolar macrophages.

We were unable to demonstrate any correlation of cell counts in the lavage fluid with airway obstruction in contrast to the findings in adults with asthma. This may be because airway obstruction was completely reversible in all of our subjects except ten who had very mild persisting obstruction with a postbronchodilator FEF25-75% between 47 and 66 percent of predicted value, and because small volume washes were used to reflect the cell populations of the more central rather than peripheral airways.

The precise role of broncholavage fluid cells in the development of bronchial hyperresponsiveness remains unclear and it is possible that other complex activities involving inflammatory mediators, neuropeptides, epithelial cell factors or other agents may be of greater consequence. Our data are nevertheless consistent with studies which incriminate alveolar macrophages as well as eosinophils in the inflammatory reaction of asthma. More importantly they strongly suggest that in children, macrophages as well as eosinophils play a role in the generation of bronchial hyperresponsiveness. Finally, since macrophages are known to be activated by a variety of stimuli other than the interaction of IgE with allergen, it is possible that they may also play a role in the development of airway hyperresponsiveness in those with non-IgE-mediated asthma.

**REFERENCES**

1 Cockcroft DW, Killian DN, Mellon JJA, Hargrave FE. Bronchial reactivity to inhaled histamine: a method and clinical survey.
Clin Allergy 1987; 7:235-43
6 O'Byrne PM, Dolovich J, Hargrave FE. Late asthmatic responses. Am Rev Respir Dis 1987; 136:740-51
16 Cockcroft DW. Mechanism of perennial allergenic asthma. Lancet 1983; 2:253-56
18 Murray AB, Ferguson AC. Dust-free bedrooms in the treatment of asthmatic children with house dust or house dust mite allergy: a controlled trial. Pediatrics 1983; 71:418-22