Relationship of the Ventilatory Response to Cotton Bract Extract and the Cells and Proteins of the Lung*

W. W. Merrill, M.D., F.C.C.P.; M. Buck, Ph.D.; J. A. D. Cooper, Jr., M.D., F.C.C.P.; and E. N. Schachter, M.D., F.C.C.P.

Many healthy subjects who have had no exposure to cotton textile dusts will experience significant reductions in expired flow rate following an inhalational challenge with an aqueous extract of cotton bracts (CBE). Differences noted among individuals in the magnitude of the bronchial response to a standardized preparation of CBE suggest variable airway reactivity. The mechanism of this response and the reasons for its variability among these naive subjects are unknown. We have studied this problem by performing bronchoalveolar lavage on 13 volunteer subjects with no history of textile dust exposure. Two to three months later, a bronchial provocation with aqueous CBE was performed by an investigator blinded to the lavage results. Subjects with greater than 20 percent drop in flow rate at 40 percent of vital capacity during a partial forced expiration (MEF 40 percent [P]) following CBE had a reduction in total recoverable alveolar macrophages, with a resultant increase in the percentage of recoverable lymphocytes. The magnitude of response (MEF 40 percent [P]) correlated directly with the measured lymphocyte percentage (r = 0.69 p less than 0.01) and inversely with the total numbers of recovered cells.

Bysinosis is a disease of cotton and other textile workers characterized by the development of airway obstruction. In its early phase, reversible bronchospasm accompanies such symptoms as shortness of breath and chest tightness. The acute subjective and objective findings of byssinosis can be reproduced in healthy volunteers never before exposed to cotton dust. Aqueous extracts of cotton bracts when aerosolized and inhaled by normal volunteers result in airway responses ranging from significant bronchospasm to essentially no change in lung function. The intrapulmonary factors which control this response are not known. However, recent studies suggest that the release of mediators is related to this reaction. We have shown that the intradermal injection of a purified low molecular weight extract of cotton bracts gave evidence of mast cell degranulation. Sequential dermal biopsy specimens showed a late phase response characterized by polymorphonuclear leukocyte (three hours) and mononuclear cell (24 hour) infiltrates.

A number of investigations suggest that airway cells may be involved in airway hyperreactivity. It has been demonstrated that alveolar macrophages may release mediators capable of eliciting bronchospasm. Lymphocytes, although themselves not capable of releasing spasmogenic mediators, have been indirectly implicated in the pathogenesis of immediate hypersensitivity. For this reason, we examined the composition of luminal lower respiratory tract washings in subjects who subsequently had their response to CBE measured six to 12 weeks following the lavage procedure. The relationship between bronchoalveolar lavage cellular and protein characteristics and the subsequent airway response to CBE form the basis of this report.

METHODS

Subjects and Technique of Bronchoalveolar Lavage (BAL)

Young normal volunteers were recruited from the New Haven area. All specifically denied industrial exposure to textile dusts, a history of asthma, or recent (two months) history of viral respiratory infections. A chest roentgenogram and results of pulmonary function tests (see below) were within normal limits in all subjects. Bronchoalveolar lavage was performed as previously described. Briefly, the nose and upper airway were anesthetized with topical 4 percent lidocaine, and the bronchoscope was passed transnasally or orally. Minimal amounts of lidocaine (1 percent) were used endobronchially to suppress cough. The bronchoscope was advanced into a subsegment of the lingula until it reached maximum penetration. Aliquots of sterile 0.9 percent saline (50 ml each) were alternately instilled and aspirated until 300 ml had been instilled.

Processing of Lavage Fluid

Recovered fluid was filtered through two layers of gauze mesh to remove gross mucus particles and centrifuged (4°C, 500 g, 10 minutes) to pellet cellular elements. The soluble phase of the fluid was concentrated 20-fold by positive pressure ultrafiltration and stored at −70°C until analyzed. The cellular elements were washed once in modified Hanks solution (Ca++ and Mg++ free), and resuspended in modified Hanks for cytocentrifuge counts and viability by trypan blue dye exclusion. The cell population was adjusted to 10⁶ cells/ml and 100 μL of this suspension were pipetted into the cassette of a cytocentrifuge and applied to glass slides by centrifugal force (450 rpm, 5 minutes). Wright's stain was applied to

*From the Research Service, West Haven VAMC, West Haven, and the Pulmonary Section, Department of Medicine, Yale University School of Medicine, New Haven, CT.

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Reprint requests: Dr. Merrill, PO Box 3333, 333 Cedar Street, New Haven, Connecticut 06510.
air-dried preps, and the percentage of cell types present was
determined by differential counting of 500 cells.

*Analyses of Lavage Proteins*

Frozen, concentrated lavage fluid was thawed, and the protein
congestion was estimated by the method of Lowry et al.\(^{11}\)
Albumin concentration was determined by a competitive enzyme-
linked immunoassay method developed in this laboratory\(^{12}\) and
adapted from the monograph of Voller et al.\(^{13}\) Immunoglobulin A
(IgA) concentration was measured by single radial immunodiffusion
in agar plates calibrated with 11s IgA purified from human co-
lostrum.'\(^{14}\) Immunoglobulin G concentration was assessed by a
commercially-available immunofluorimetric method.'\(^{15}\)

*Bract Preparation and Aerosolization*

Aqueous extracts of cotton bracts (collected from Texas cotton
fields just before harvest) were prepared with a buffer.'\(^{16}\) The clear
brown supernatant obtained following centrifugation of the portion
of the bracts that could be squeezed through cheese-cloth was
sterilized by passage through 0.45 micron filter. A single large
quantity of bract extract was prepared in this manner at the onset of
these experiments. This extract was freeze dried for storage pur-
poses and reconstituted to 15 mg dry wt/ml sterile water for
individual challenges. This approach was used to assure delivery of
similar concentrations of the active agent with each challenge. Each
subject inhaled the extract by tidal breathing for a ten-minute
period. Five milliliters were aerosolized using a nebulizer attached
to the subject by a J valve. Reservoir tubing was attached to the valve
to assure uniform concentration. The aerosol nebulizer was powered
by compressed air under a constant pressure of 20 psi. Under such
circumstances, the output of the nebulizer is constant, and the
particle size delivered has been measured to be less than 1 micron in
diameter, assuring delivery of aerosol beyond the large airways.'\(^{17}\)

*Pulmonary Function Testing*

Pulmonary function was measured using partial and maximal
expiratory flow-volume (PEFV and MEFV) curves.'\(^{18}\) A pneumotach
integrator system was used, and flow was plotted against volume.'\(^{19}\)
The maneuver performed consisted of inspiring initially to approx-
imately 70 percent of vital capacity and then exhaling forcefully to
residual volume. The subject next inhaled to total lung capacity and
once again exhaled forcefully to residual volume. These maneuvers

![Graph](image-url)
generated PEFV and MEFV curves. Subjects performed partial/maximal curves several times during the initial testing to ensure reproducibility. A one-second timer permitted us to identify forced expiratory volume in one second. From these data, forced vital capacity and peak flow (PF) were also determined. Maximal expiratory flows on both MEFV and PEFV curves were measured at 60 percent of the control vital capacity below total lung capacity (MEF 40 percent and MEF 40 percent [P], respectively). Three tests were performed at each time point, and the mean value for each physiologic factor was recorded.

Responders to CBE were defined as subjects displaying a maximal decrease in MEF 40 percent (P) of 20 percent or more. This value was selected because it is a very sensitive marker of alterations in airway caliber and because of its ability to identify consistently changes in lung function.

Analysis of Data

Data were stored and analyzed using software. Mean differences between sets of continuous variables were compared by two-tailed Student's t-test. Relationships between data points were assessed by Pearson's correlation coefficient.

RESULTS

The study population consisted of 13 young (mean age 22 years) healthy volunteers. All denied any significant respiratory illnesses. Three subjects were smokers; however, their exposure to cigarette smoke was slight, ranging from one to five pack years. Bronchial lavage instillate and recovery volumes were similar to those we have reported previously. Following administration of CBE by nebulizer, the maximal change in MEF 40 percent (P) varied from an 8 percent improvement to a 43 percent decrement. The maximum fall in the flow occurred from 60 to 90 minutes following inhalation challenge. Five subjects experienced a 20 percent or greater reduction in MEF 40 percent (P), while eight (including all three smokers) did not. The percentage fall in FEV₁, although smaller in magnitude (range 4 percent increase to 10 percent decrease), paralleled the change in MF 40 percent (P) (Fig 1).

Analyses of lavage fluids from these volunteers demonstrated a range of values for cells and proteins similar to those we have noted previously in healthy subjects. Comparison of protein concentrations in responders and nonresponders (Table 1) revealed no significant differences (p greater than 0.1 for all comparisons). By contrast, the analysis of the cellular elements recovered (Table 2) revealed definite differences. Subjects with the greatest decline in MEF 40 percent (P) had fewer cells recovered by lavage than those who failed to respond to CBE. Furthermore, there was a significant alteration in the percentage composition of the recovered lavage cell population. The lymphocyte percentage was increased among the lavage cells of subjects who had significant declines in flow rate compared to cell populations of those who did not respond to bract (Fig 2). The percentage of macrophages was correspondingly decreased in bract responders.

Although the separation of subjects into responder and nonresponder groups defines important physiologic differences in inhalation challenge studies, analysis of lung function data for changes in MEF 40 percent (P) and FEV₁ did not reveal a bimodal distribution for these variables. Data for cell populations were also continuously distributed. When we compared the proportion of lymphocytes seen in BAL to an individual's degree of bronchospasm, there was an inverse correlation between the percentages of lymphocytes in lavage cell populations and the fall in MEF 40 percent (P) (Fig 3). Data for fall in FEV₁ showed similar results (r = 0.695, p less than 0.01). A significant inverse relationship was also seen between the total cell recovery or the percentage of macrophages and drops in lung function (Table 3).

![Figure 2](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21554/) Percentage of lymphocytes in responder and nonresponder groups. The percentage of lymphocytes (ordinate) among cells recovered during lavage of the lower respiratory tract of responders and nonresponders to nebulization of aqueous cotton bract extract. Open circles refer to nonsmokers and smokers are designated by closed circles.

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**Table 1—Comparison of Protein Concentrations**

<table>
<thead>
<tr>
<th>Group*</th>
<th>(n)</th>
<th>Alb/TP†</th>
<th>IgG/Alb†</th>
<th>IgA/Alb†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonresponders</td>
<td>(8)</td>
<td>0.56 ± 0.03</td>
<td>0.12 ± 0.01</td>
<td>0.23 ± 0.03</td>
</tr>
<tr>
<td>Responders</td>
<td>(5)</td>
<td>0.46 ± 0.05</td>
<td>0.16 ± 0.02</td>
<td>0.33 ± 0.12</td>
</tr>
</tbody>
</table>

*Responders are defined by a greater than 20 percent decline in MEF 40 percent (P) following bract nebulization.
†Ratios of albumin to total protein, IgG to albumin, IgA to albumin in microgram/microgram.

**Table 2—Analysis of Recovered Bronchoalveolar Cells**

<table>
<thead>
<tr>
<th>Group*</th>
<th>(n)</th>
<th>TNC†</th>
<th>% Am†</th>
<th>% Ly†</th>
<th>% PMN†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonresponders</td>
<td>(8)</td>
<td>50±7.7</td>
<td>97±9</td>
<td>2.8±7</td>
<td>0.3±0.5</td>
</tr>
<tr>
<td>Responders</td>
<td>(5)</td>
<td>89±2.4</td>
<td>97±1.7</td>
<td>0.7±0.5</td>
<td></td>
</tr>
</tbody>
</table>

*Groups identical to Table 1.
†Total nucleated cells ×10⁶, percent alveolar macrophages, percent lymphocytes, percent polymorphonuclear leukocytes. Eosinophils and basophils comprised much less than 1 percent of lavage cells and were seen with equal frequency in both groups.
$p$ less than 0.01 by Student's t-test.
$p$ less than 0.05 by Student's t-test.
DISCUSSION

Subjects exposed to cotton textile dust in the workplace are at risk of developing a syndrome characterized by cough and wheeze following dust inhalation. Not all subjects with similar exposure develop clinical symptoms or lung function changes. This variability in the occupational population also exists in healthy subjects never exposed to cotton dust who inhale CBE. Although the precise cause of this syndrome is unclear, substantial evidence suggests that some substance or substances within the dust act either directly or indirectly to cause bronchoconstriction. The mechanism for the variable response of the exposed subject population is unknown.

Our findings suggest that one important component in this variability in airway reactivity to CBE (and by extension reactivity to cotton dust) may relate to differences in bronchoalveolar cell populations. On a separate occasion, six weeks to three months prior to bract administration, BAL was performed in a group of 13 naive subjects. Analysis of the results from these studies has shown that the cellular and protein composition of BAL for this subject population was similar to those noted by us previously in the study of normal volunteers and are also similar to those noted by other investigators.

We have shown significant differences among healthy subjects classified by their sensitivity to CBE when BAL cells were quantitated. The types of cellular alterations were surprising. Subjects with the greatest response to bract extract had the smallest number of cells recovered and an increase in the percentage of lymphocytes present. Moreover, there was a direct and significant correlation between the lymphocyte percentage and equally strong inverse correlations between total cell recovery or percentage of macrophages and the magnitude of fall in flow rate strongly linking these subject characteristics. Although we did not test the bronchial reactivity of these subjects to other stimuli, Schachter and colleagues have shown an association between the reactivity to CBE and that observed following administration of methacholine. By contrast, immunoglobulin and protein analyses of BAL were unrelated to reactor status or degree of bronchospasm following CBE.

The mechanism by which this alteration in respiratory tract cells might predispose to bronchoconstriction is unclear; however, several interpretations are possible. First, the relative increase in lymphocytes may alter the function of other airway cells. Lymphocyte alterations have been noted in both peripheral blood and airways of asthmatics, and also in an animal model of asthma. Furthermore, lymphocytes can exert potent effects on the function of other immunocompetent cells.

A second possibility is that the reduction in lower respiratory tract alveolar macrophages is responsible for the enhanced susceptibility to cotton bracts extract. We know of no definite published mechanism for this type of effect. However, in preliminary experiments, we have detected a factor synthesized by adherent bronchoalveolar cells which inhibits PMN chemotaxis. It is possible that such a factor could inhibit CBE bronchoconstriction responses because we have shown that these responses are accompanied by an increase in bronchoalveolar PMN. A factor which blocks PMN migration might block the bronchoconstriction response.

Finally, it is possible that the reduction in alveolar macrophages is an indication of alveolar macrophage activation. More activated macrophages may be more adherent to other lower respiratory tract cells and may release different mediators than normal resident cells.

It is not immediately clear how our observation concerning reduced airway cells and a relative increase in lymphocytes relates to our finding that intradermal bract extract injection causes evidence of mast cell degranulation. The three subjects studied in that report had insignificant respiratory responses to

![Graph](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21554/)
nebulized bract extract but definite early and late response to intradermal injection. Although mast cells are infrequent among human lavage cell populations, bract extract does cause significant mediator release from dermal mast cells, and it is likely that a respiratory tract cell population is similarly affected by some component of bract.

In summary, we have detected simple differences between normal subject populations who differ with respect to their bronchoconstriction response to cotton bract extract. These differences were confined to bronchoalveolar cells and were characterized by a decrease in recoverable cells and alveolar macrophages and a resultant increase in the percentage of lymphocytes. However, the mechanism by which these alterations exert their effect is obscure.

REFERENCES