Phagocytosis and Cutaneous Delayed Hypersensitivity in Patients with Chronic Obstructive Pulmonary Disease*

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The objective of this study was to determine if the phagocytic and intracellular killing capacity of peripheral granulocytes or an expression of cellular-mediated immunity, delayed cutaneous reactivity, as measurements of native and acquired immunity, might be risk factors associated with chronic obstructive pulmonary disease (COPD). Over 100 patients with a value for their forced expiratory volume in one second (FEV₁) less than or equal to 70 percent of normal were carefully matched with healthy participants having an FEV₁ greater than or equal to 86 percent of normal, and together they served as the study group. Phagocytosis and intracellular killing were normal in patients with COPD; however, these patients demonstrated a significant impairment in the ability of their peripheral leukocytes to reduce nitroblue tetrazolium. The delayed-hypersensitivity response rate and the degree of reactivity were similar in the two groups, except for the patients with COPD having a significantly greater degree of reactivity to Monilia albicans extract ("canadin.") This finding is thought to be a consequence of reduced mucociliary clearance rather than a risk factor. The significance of decreased resting and stimulated cells' reduction of nitroblue tetrazolium in patients with COPD is not clear.

Chronic obstructive pulmonary disease (COPD) or combinations of bronchitis and emphysema have been considered multifactorial in etiology. Putative exogenous causes associated with a high incidence of disease are cigarette smoking, urban air pollution, certain dusty occupations, and socioeconomic status. While all of these factors have had varying degrees of support from both laboratory and epidemiologic studies, very little is known about host factors. The most dramatic demonstration of an important host factor is the high degree of correlation of certain α1-antitrypsin phenotypes and related deficient blood levels in up to 5 percent of patients with bronchitis and emphysema.

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This study was approved by the Mayo Clinic Committee on Human Experimentation, and all participants gave their voluntary consent after having been informed of the details of the investigation.

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SUBJECTS

In an effort to investigate other potential host factors which might play a role in these multifactorial diseases, we identified over 100 subjects in a rural or nonmetropolitan area comprising 12 counties in southeastern Minnesota. The subjects were chosen on the basis of having a forced expiratory volume in one second (FEV₁) less than or equal to 70 percent of normal.1 The age range was 45 through 59 years. Each index case was matched on the basis of age, sex, occupation, and lifelong smoking history with a person whose FEV₁ was at least 86 percent of the predicted normal. Most of these latter participants were patients who had a dental extraction or traumatic fracture treated sometime in the past, and all were healthy. Subjects whose FEV₁ measured between 71 and 86 percent of normal were classified as being in an indeterminate category and were excluded from the study. Some patients who carried a diagnosis of chronic bronchitis or asthmatic bronchitis who were invited to enter the study as likely index cases actually had an FEV₁ of greater than 86 percent of the predicted normal. Since they were not invited to participate in the study as potential controls and did have, at least by history or their physician's judgment, a diagnosis of pulmonary disease, they could not be considered in the normal control population but were placed in a "special control" category. Groups studied include 113 index cases and 114 normal controls; from these two groups, 100 matched pairs and the 36 "special controls" who had a diagnosis of a chronic bronchial condition but who had clearly normal flow rates were chosen. Each participant underwent a detailed respiratory and familial questionnaire, cardiopulmonary physical examination, and a number of laboratory tests, including those described later. Further details on the match-
ing of patients and their characteristics are to be presented elsewhere.

The subject of this report is a comparison of these two major groups in respect to peripheral leukocytic function as measured by an opsonocytophagic test, nitroblue tetrazolium reduction, and cutaneous delayed hypersensitivity reactions to common microbial antigens.

METHODS

Skin-test antigens were administered in the amount of 0.1 ml given intradermally to raise a distinct bleb and included the following: mumps (Eli Lilly and Co.) undiluted, 2 complement fixing units; purified protein derivative of liquid tuberculin (PPD) in polysorbate (Tween; Connaught Laboratories, Ltd.), 5 tuberculin units; streptokinase-streptodornase (Variodase; American Cyanamid Co.), adjusted to 50 units of streptokinase in 0.1 ml; Monilia albicans extract ("canadin," Holister-Stier Laboratories) diluted 1:100 in sterile normal saline solution and Dermatophytin (Hollister-Stier Laboratories) diluted 1:20 in sterile normal saline solution and control normal saline solution. Skin induration was measured by calipers at 48 hours after injection. Diameters of indurated areas were measured at 90° angles. The average induration less that of the saline control (if any) was recorded for each antigen. A modified nitroblue-tetrazolium dye-reduction test, originally described by Baehner and Nathan, was performed with the polymorphonuclear neutrophilic granulocytes. The cell suspension was diluted to a final concentration of 2.0 \( \times 10^9 \) polymorphonuclear neutrophilic granulocytes per milliliter. Optical density of the pyridine-extracted dye was determined in a spectrophotometer (Spectronic 20; Bausch and Lomb, Inc.) at 515 nm against a pyridine blank. "Resting" and latex-phagocytosing values were determined, and the differences were calculated.

An opsonocytophagic index test, as previously described, was performed with the following modifications: Hanks' balanced salt solution with 1 percent gelatin was used as the basic medium at pH 7.4 adjusted with 0.154 M NaHCO₃. Buffy-coat leukocytes were pelleted at 150 \( \times g \), washed twice in the Hanks'-gelatin solution, and resuspended in the same medium to a concentration of 10⁷ polymorphonuclear neutrophilic granulocytes per milliliter. Eighteen-hour cultures of Escherichia coli and Staphylococcus aureus propagated in cloxacillin (Bactopen) assay broth were washed twice in sterile normal saline solution and resuspended to a concentration of 5 \( \times 10^9 \) bacteria per milliliter. The final working solution (1 ml) consisted of 0.5 ml of cell suspension (5 \( \times 10^8 \) polymorphonuclear neutrophilic granulocytes) or 0.5 ml of Hanks'-gelatin solution (control), 0.1 ml of bacterial suspension (5 \( \times 10^8 \) bacteria), 0.1 ml of normal pooled serum (five normal donors), and 0.3 ml of Hanks'-gelatin solution. The plastic capped tubes (12 \( \times 75 \) mm) into which the final suspensions were placed were rotated at 7 rpm at 37°C. Samples were taken at 0, 30, 60, and 120 minutes. Test sample aliquots were diluted 1:100 (0 and 30 minutes) and 1:10 (60 and 120 minutes) with sterile water; control samples were all diluted 1:10. Viable bacteria were quantitated by the standard pour-plate technique.

RESULTS

Delayed-hypersensitivity responses in the control and COPD index groups reveal that 4 percent of the normal population was anergic to the four recall antigens, compared to 6 percent of the index COPD group. Except for 9 percent more of the controls responding to two antigens, the index group had more positive skin tests (three, or more than or equal to four) than the controls (Fig 1). Thus, the index group was, on one hand, less responsive in terms of their ability to respond with delayed hypersensitivity and, on the other hand, those who did respond were capable of reacting to a greater number of antigens.

The responses to the individual antigens are presented in Figure 2 and reveal no significant differences in the response rates between the two groups. Indeed, the degree of response as determined by the mean induration, as well as the ranges of induration, are essentially similar for all of the antigens studied, except for M. albicans extract ("canadin"). The median response in the control group for M. albicans extract measured 13.0 mm vs a 17.1-mm response to this antigen in the COPD group, a significant difference whether comparing the median response of the group \((P < 0.01)\) or by the paired \(t\)-test \((P = 0.018)\); however, the "special control" group, while demonstrating an essentially identical response rate of 53 percent (as did the major study groups) had

![Figure 1](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/20979/)

**Figure 1.** Number of delayed-hypersensitivity (DTH) responses to five microbial antigens in control vs COPD subjects.
an even higher and significantly different median induration of 19.0 mm compared to the normal control group. This special control group of 36 patients also differed from the other two groups by having a 94-percent response rate to streptokinase, compared to the 75 percent of both the control and of the index participants, although the degree of streptokinase-induced induration at 48 hours was similar in all three groups.

A radial immunodiffusion assay vs a 1:160 dilution of \textit{M} \textit{albicans} sonicated cell antigen (Hollister-Stier Laboratories) yielded seven positive reactors from the normal control group and four positive reactors from the COPD group. One (6 $\times$ 6 mm of induration) of these seven normal controls and one (15 $\times$ 15 mm of induration) of the latter four patients also had a positive 48-hour delayed cutaneous reaction to \textit{M} \textit{albicans} extract, the remainder of both groups being negative reactors. The only humoral responder in the special control group also had a delayed-hypersensitivity response of 8 mm in diameter.

The results of the opsonocytophagic assays were assessed by the statistical method of Hoffman and Bullock\(^4\) and revealed no differences between the groups. The same conclusion of normal phagocytic activity vs \textit{E} \textit{coli} and \textit{S} \textit{aureus} was substantiated by simple comparison, since there were no unusual or equivocal responses observed in any of the participants.

The nitroblue-tetrazolium dye tests gave a wide range of responses in both groups, which was particularly noticeable in the latex-"stimulated" cultures (Table 1). Although there were several of the patients with COPD who had minimal reduction of nitroblue tetrazolium in their phagocytic cells, it is evident from the median resting (Fig 3) and stimulated levels (Fig 4) that, as a group, those with COPD were significantly less active (Table 1); however, comparison of the change in absorbance (or difference between latex-stimulated and non-stimulated values, the aspect of the test equated with intracellular killing) of the two groups (Fig 5) reveals no significant difference and corroborates the opsonocytophagic assay data. The 36 special controls yielded values almost identical to the 110 normal controls and did not demonstrate the lower levels of resting and latex particle-stimulated cell reduction of nitroblue tetrazolium.

\begin{table}[h]
\centering
\begin{tabular}{lccc}
\hline
 & Control & COPD & Special Control \\
\hline
$A_W$ (stimulated) & 0.328 $\pm$ 0.099\textsuperscript{**} & 0.288 $\pm$ 0.098\textsuperscript{**} & 0.316 $\pm$ 0.136 \\
$A_O$ (resting) & 0.113 $\pm$ 0.056\textsuperscript{†} & 0.084 $\pm$ 0.053\textsuperscript{†} & 0.100 $\pm$ 0.059 \\
Change in $A$ & 0.215 $\pm$ 0.084\textsuperscript{‡} & 0.204 $\pm$ 0.076\textsuperscript{‡} & 0.216 $\pm$ 0.058 \\
\hline
\end{tabular}
\caption{Optical Density (Absorbance) of Nitroblue-Tetrazolium Dye Test in Control, COPD, and “Special” Control Subjects\textsuperscript{*}}
\end{table}

\textsuperscript{*}A, Absorbance. Table values are means $\pm$ SD of data from 110 control subjects, 114 COPD subjects, and 36 special control subjects.

\textsuperscript{**}P = 0.0027 for two-tailed value associated with median test; P = 0.0028 for matched-pair $t$-test.

\textsuperscript{†}P = 0.00009 for two-tailed value associated with median test; P = 0.0064 for matched-pair $t$-test.

\textsuperscript{‡}P = 0.304 for two-tailed value associated with median test; P = 0.365 for matched-pair $t$-test.
DISCUSSION

There are some significant differences between the two major groups studied. For example, there is a statistically significant difference between the two groups in the median induration response of delayed hypersensitivity to \textit{M. albicans} extract. This cannot be due to technique, since all skin tests were performed and read by the same person, who was unaware of the participant’s status, a change in antigen, or the time sequence during the course of the study; however, it is also apparently not due to nor causally related to COPD, since the special controls had even greater induration to this antigen but pulmonary function tests placed them in the normal category.

It was tempting to impute to COPD a possible mechanism for enhanced pulmonary sensitization to \textit{Monilia} in subjects with diffuse airway obstruction, known to be associated with impairment of mucociliary clearance; however, it was evident from the small numbers of special controls with even greater reactivity to this ubiquitous antigen that their normal values for FEV$_1$ made this thesis untenable. It has been pointed out by Lourenço et al\textsuperscript{5} that subjects with chronic bronchitis have delayed clearing of experimental aerosol particles. Our participants in the special control group were brought in with the clinical suggestion that they had chronic bronchitis because of coughing and wheezing characteristic of airway irritability. Since their FEV$_1$ values were normal, the special controls could not be placed in our COPD group; however, impairing of mucociliary clearance can occur in bronchitic patients with grossly normal flow rates. Therefore, our special control group would be expected to have values for mucociliary clearance more like the index cases than our controls. Since our controls were matched for smoking, it can also be assumed that there was some degree of delayed clearance of inhaled particles, \textit{ie}, \textit{M. albicans}; however, the incidence of cough and expectoration and particularly wheezing or noisy breathing was significantly lower in our control group than either the index or the special control group. It would, therefore, seem possible to relate the higher incidence of skin reactivity to \textit{M. albicans} to delayed mucociliary clearance and consequent
longer exposure time of the bronchopulmonary epithelium to a particulate antigen. These differences in skin reactivity to \textit{M albicans} were not so great, however, that one could identify individuals with COPD by this method.

The data from the nitroblue-tetrazolium dye tests and opsonocytophagic assays do not indicate that patients with COPD have any impairment of phagocytosis or intracellular killing. The significantly lowered intracellular reduction of nitroblue tetrazolium in both resting and actively phagocytosing peripheral leukocytes, compared to normals, has no parallel of which we are aware in other disease states. It is difficult to postulate a reasonable mechanism to explain why this phenomenon might be a consequence of having COPD. Further study will reveal if our finding has this import or if this apparently depressed ability to reduce nitroblue tetrazolium in the resting cell witnesses a genetic or acquired predisposition of patients who develop COPD. Should it prove to be the latter, nitroblue-tetrazolium dye tests might serve as a screening mechanism for those at risk for this disease; however, there has been little study of the nitroblue-tetrazolium dye test for this kind of purpose, particularly in adults, so it is possible that other diseases or syndromes may yield similar findings. It is worth reemphasizing that our reported observations do not suggest any impairment in the patient with COPD to effect intracellular killing, which is the established rationale for interpreting the nitroblue-tetrazolium dye test.

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REFERENCES


Mechanical Clocks

Mechanical clocks of a sort existed in the 13th century. The mechanism of these clocks was based on a falling weight tied to a rope which was wound around a revolving drum. They had no dials and probably no means of striking, but they could indicate certain hours so that the keeper of the clock, usually a monk, could sound a bell. Automatic striking came a little later and monastery clocks struck the seven canonical hours. The arrival of the main spring has no certain date. Leonardo da Vinci made certain drawings of coiled springs, which may or may not have found their way into spring-driven clocks. If they had not, then the credit for their invention must be given to a Nuremberg locksmith named Peter Henlein, who was making use of them in the 16th century. The mechanical clock reached its maturity by way of the pendulum which gave it accuracy. Galileo understood the principle of the pendulum and was aware of its potentialities, but most of the century had gone before the famous Dutch clockmaker, Christian Huggens, used falling weights to keep a pendulum going.