Decrased C4 Complement Component Serum Levels Correlate With the Degree of Emphysema in Patients With Chronic Bronchitis*

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Patients with COPD who fulfill the diagnostic criteria of chronic bronchitis have been shown to exhibit lower serum levels of complement components C3 and C4 than healthy subjects, and this may indicate sustained complement activation as a result of recurrent respiratory tract infections. Since activation of complement leads to influx of inflammatory cells into the lung parenchyma with subsequent release of elastases and oxidants that cause damage to elastic lung tissue, we postulated that there might be a quantitative relationship between complement consumption and degree of elastic tissue destruction. In this study, we tried to investigate possible correlations between serum levels of C3 and C4 and degree of emphysema among patients with COPD of the bronchitic type. We studied 20 patients with chronic bronchitis aged 68 ± 1 years (mean ± SEM) without significant fluctuations of serum C3 and C4 levels over a 3-month period by performing detailed lung function tests, recording of emphysema score in chest radiogram, and the incidence of infective exacerbations during the past 3 years. Measured C3 and C4 serum levels were 124 ± 9 and 28.5 ± 2 mg/dL, respectively, lower than the respective levels in control subjects (141 ± 3 and 39 ± 2 mg/dL, respectively). Significant correlations were observed between levels of C4 and (1) incidence of respiratory tract infections during the past 3 years (r = -0.747, p < 0.001), (2) radiologic emphysema score (r = -0.936, p < 0.001), and (3) various functional indexes, such as midexpiratory flow rate, percent of predicted (r = 0.629, p < 0.01), forced expiratory flow rate at 50% of vital capacity, percent of predicted (r = 0.606, p < 0.01), residual volume/total lung capacity ratio (r = -0.615, p < 0.01), and the exponential constant of static pressure-volume curve (r = -0.606, p < 0.01). These results suggest that patients with chronic bronchitis with the lowest levels of C4 are those experiencing more frequent respiratory infections, tend to have more signs indicative of emphysema in their chest radiograph, have a more prominent small airways dysfunction and gas trapping, and present a greater defect in lung elastic recoil.

(CHEST 1997; 112:341-47)

Key words: chronic bronchitis; complement; lung function; pulmonary emphysema

Abbreviations: C1, C2, C3, C4, C3a, C3b, C5a, C6, C7, C567 = complement components; FEF 25-75% = midexpiratory flow rate; FRC = functional residual capacity; Kst = exponential constant of static pressure-volume curve; PA = posteroanterior; Raw = airways resistance; RV = residual volume; TLC = total lung capacity

The complement system, which is an extremely complex group of serum proteins present in low concentrations in normal serum, consists of various components. Being a part of the humoral defense, it is activated by the formation of antigen-antibody complexes in the classic pathway. However, C3 component may be activated by bacterial and fungal mucopolysaccharides through the alternative pathway, which bypasses the components C1, C2, and C4.¹

Activation of complement pathways, in response to bacterial or viral respiratory infection, induces influx of macrophages and neutrophils through chemotactically active fragments of complement proteins (C3a, C5a, C567).² Activation and thus consumption of complement can be inferred by the presence of low levels of specific complement components, such as C3 or C4.² Aggregation of inflammatory cells acting as phagocytes in distal airways and pulmonary microvasculature in response to chemotactic com-
plement components results in the release of elastases and oxygen radicals that are known to play a significant role in the pathogenesis of pulmonary emphysema.3,4

We therefore hypothesized that in patients with chronic bronchitis, sustained activation of complement pathways due to recurrent respiratory tract infections, evidenced by the presence of low levels of serum C3 and C4, may bear a quantitative relationship to the extent of elastic tissue damage and hence the "degree" of emphysema. Our objective in this study was to investigate possible correlations between serum complement levels and various radiologic and functional indexes that may reflect the degree of emphysema in patients with chronic bronchitis.

**Materials and Methods**

**Patients—Inclusion Criteria**

Among 45 consecutive patients with established chronic air-flow obstruction (FEV₁/FVC ratio <85% of predicted), we recruited only those patients who fulfilled the following inclusion criteria: (1) age range between 60 and 75 years; (2) smoking history of >30 pack-years (range, 30 to 48 pack-years) but with absence from smoking for at least 5 years prior to the study; (3) history and clinical assessment fulfilling the criteria of chronic bronchitis;5,6 (4) absence of reversibility of the obstruction (<5% change in FEV₁) confirmed by prebronchodilatation and postbronchodilatation spirometry; (5) history of recurrent infective exacerbations of their disease (at least three episodes per year, during the past 3 years), diagnosed by symptoms (increased breathlessness, fever, cough with purulent or mucopurulent expectoration), compatible findings on physical examination, WBC count, spumum Gram's stain and culture, and chest radiograph; (6) stability of their condition (ie, absence of either current or recent clinically obvious infective or other exacerbation) for at least 3 months prior to the study; (7) no evidence of bullous emphysema or emphysema due to an α₁-antitrypsin deficiency or other concomitant lung disease on clinical and radiologic grounds; (8) absence of systemic (systemic lupus erythematosus, rheumatoid arthritis, renal or hepatic disease), allergy, neoplastic disease, or general immunologic disorder that might interfere with complement activation; (9) ability of performing lung function tests acceptably; and (10) lack of significant fluctuations in serum levels of C3 and C4 components measured on three separate occasions within a period of 3 months prior to the initiation of the study.

From the 45 patients who were initially evaluated, we excluded 13 patients due to specific aspects related to the disease entity under study (inclusion criteria 2 to 7), two patients who were far beyond the age range (criterion 1), one patient with nephrotic syndrome (criterion 8), and two patients who refused to participate in the study. From the remaining 27 patients, we finally recruited 20 because seven patients exhibited a variation of more than ±10% in serum C3 and/or C4 level during the three monthly serial measurements.

A group of 20 healthy age- and smoking-matched ex-smokers were also studied in terms of C3 and C4 determination in serum. The study was approved by the Institutional Ethics Committee and a written informed consent was signed by all subjects.

**Determination of C3 and C4 Serum Levels**

A venous blood sample of 10 mL was drawn in midmorning from each subject and serum C3 and C4 levels were determined by radial immunodiffusion (Sanofi Diagnostics; Chaska, Minn) within 2 to 3 h of phlebotomy. The measurements were repeated three times on a monthly basis during the past 3 months prior to the initiation of the study to screen for patients with remarkable (>±10%) fluctuations of complement levels. The mean value of the three repetitive measurements was used in the analysis of the 20 patients who were eventually recruited and of their age- and smoking-matched control subjects.

At the same time, routine blood tests and full biochemical serum screening was done for each subject in both study and control group.

**Chest Radiography—Emphysema Quantification**

Chest radiographs (posteroanterior [PA] and lateral views) were obtained with the patients upright and holding their breath at full inspiration.

The chest radiographs were interpreted blindly and independently by three experienced observers (one radiologist and two chest physicians) who did not know the clinical diagnosis, the functional data, and the purpose of the study, according to a previously described method.7 Signs of overinflation (depression and flattening of hemidiaphragms evaluated on both PA and lateral views and increased retrosternal space) were individually scored from 0 to 3. For the evaluation of lung vascular abnormalities (increased radiocuency, increased branching angles of vessels, loss of normal sinuosity of vessels with loss of side branches, increased sharpness of vessels with reduction in caliber, and widening of the normal peripheral clear zone of the lung), the PA radiograph was divided in four quadrants by the level of the carina. Each quadrant was analyzed for the presence of any of the vascular abnormalities that were assigned an individual score of 0.5. The partial scores from the four quadrants were summed and combined with the overinflation score to yield an emphysema score ranging from 0 to 16. The individual scores from each observer were averaged to yield the emphysema score of each patient.

**Pulmonary Function Tests**

The lung function tests were done inside a 650-L, constant-volume plethysmograph (Autolink; PK Morgan Instruments Inc; Kent, UK) containing a linear pneumotachograph (Fleisch No. 3; Fleisch; Lausanne, Switzerland), two ±2 cm H₂O differential pressure transducers (Validyne Engineering Corp; Northridge, Calif), two ±100 cm H₂O differential pressure transducers (Sanborn; Waltham, Mass), and a solenoid shutter assembly. The body plethysmograph was connected to software (Wyvern Software; PK Morgan Instruments Inc; Andover, Mass) installed in a computer (IBM compatible) and to an eight-channel strip-chart recorder (Hewlett Packard OPT 004; Boston).

Subjects performed three maximal inspiratory flow-volume loops and the one with the highest sum of FEV₁ and FVC was chosen.

Functional residual capacity (FRC) and airways resistance (Raw) were measured five to six times using the panting technique described by DuBois et al8 at a frequency of 1 Hz and measuring the angles from the shutter-closed and shutter-open loops on the oscilloscope.

Esophageal pressure relative to atmospheric pressure was measured with a balloon (A&E Medical Corp; Farmingdale, NJ)-tipped catheter placed in midesophagus using the technique of Milic-Emili et al.9 The balloon was inflated with 0.8 mL of air

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with a 10-mL glass syringe and the distance from the external nares to the tip of the balloon was kept within the range of 35 to 45 cm in all subjects. Mouth pressure relative to atmospheric pressure was measured with a 3-mm internal diameter needle inserted in the mouthpiece. The needle and the esophageal balloon were connected to the two ±100 cm H2O pressure transducers and flow signal was integrated to volume. Once the FRC and Raw measurements were completed, subjects performed five full expiratory static pressure-volume curves. After inhaling to total lung capacity (TLC), we occluded the subject’s airway and measured transpulmonary pressure (transpulmonary pressure=mouth pressure—esophageal pressure) making these measurements during a stepwise deflation from TLC to FRC. Pressure and volume points were then obtained at the respective plateaus of the two signals that corresponded to zero airflow. We plotted the points from the five static expiratory maneuvers using the software with the capability of exponential fitting of the pressure-volume curve and calculating the exponential shape-constant (Kst) according to Colebatch et al.10

Results were expressed as means±SEM. Differences in C3 and C4 serum levels between the study group and the control subjects were assessed by Student’s t test and the Wilcoxon test for unpaired observations. The strength of association between variables was assessed by Pearson’s linear regression analysis. Significance was set at the 0.05 level of confidence.

RESULTS

Our 20-patient study group (age, 68±1 years; 19 men and one woman) had an incidence of infective exacerbations during the past 3 years equal to 11±0.5 episodes (range, 9 to 15 episodes). Serum levels of C3 and C4 in the study group were 124±9 and 28.5±2 mg/dL, respectively. Control subjects had C3 and C4 levels of 141±3 (higher but not significantly different) and 39±2 (significantly higher, p=0.001) mg/dL, respectively. The mean body weight of our patients was 97±2% of ideal (range, 91 to 115%) and the serum albumin levels were 4.2±0.1 mg/dL (range, 3.8 to 4.9 mg/dL). Serum C3 and C4 levels were not correlated either with the mean body weight (r=0.070 and 0.305, respectively) or with the serum albumin levels (r=0.237 and 0.434, respectively).

Mean values (±SEM) of radiographic emphysema score, dynamic volumes, expiratory flows, static volumes, airway resistance, lung distensibility, and C3, C4 serum levels are presented in Table 1. The correlations (coefficients and p values) between C3, C4 serum levels and the radiologic emphysema score, incidence of infections, and the various functional indexes are shown in Table 2.

The strongest correlations were found between C4 serum levels and the following: (1) the incidence of infections (r=-0.747, p<0.001, Fig 1); (2) the radiologic emphysema score (r=-0.936, p<0.001, Fig 2); (3) the residual volume (RV)/TLC ratio (r=-0.651, p<0.01, Fig 3, top); (4) the Kst (r=-0.606, p<0.01, Fig 3, center); and (5) the midexpiratory flow rate (FEF25-75%) of predicted (r=0.629, p<0.01, Fig 3, bottom).

DISCUSSION

Findings arising from this study showed that for patients with chronic bronchitis experiencing recur-

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<th>C3</th>
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<th>p Value</th>
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<td></td>
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<td></td>
<td>FEF75, % of predicted</td>
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<td>RV/TLC</td>
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<td></td>
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*For explanation of abbreviations, see text and Table 1 footnotes. NS=not significant.
bacterial polysaccharides. The activated C3b complement fragment binds to bacteria enabling their attachment to phagocytes (macrophages and neutrophils) through the C3b complement receptors on the surface of these cells. The alternative pathway bypasses the C1, C4, and C2 components and starts with the C3 step. Activation, and thus consumption, of complement can be inferred by low levels of specific complement components, such as C3 and C4.2

It has been documented in previous reports that complement components C3 and C4 are decreased in COPD patients and more so in patients with chronic bronchitis.11,12 Miller et al12 found that the levels of C3 and C4 in 111 COPD (chronic bronchitis and/or emphysema) patients were lower than in 111 healthy age-, sex-, and smoking-matched subjects. Concentrations of C3 and C4 were 123±44 and 30±8 mg/dL for the patients and 142±38 and 34±14 mg/dL for the control subjects, respectively, which is in agreement with our results. The lower C3 and C4 levels in the COPD group in their study were attributed to more frequent and more severe respiratory tract infections in comparison with their control group. However, that study failed to show any association between C3 and C4 levels and frequency or severity of infections within the COPD group. At that particular point, our results were somewhat different, given that we found a significant negative correlation between C4 serum levels and the incidence of infections. That discrepancy may be explained by our smaller number of patients, our

rent respiratory tract infections, measurements of C3 and C4 serum levels fell within the lower normal limits and C4 serum levels differed significantly from those measured in the control subjects. Also, low C4 serum levels correlated with an increased incidence of respiratory infections, a high emphysema score on chest radiograph, an increased RV/TLC ratio and Kst, as well as a reduction in the FEV1 and the late expiratory flow rates.

Complement proteins are part of humoral defense and they have the characteristic of interacting with certain antibody molecules once these have combined with antigen. Complement works as a triggered enzymatic cascade system with the first three components, C1, C3, and C4, circulating in an inactive form as proenzymes. These are converted to their active forms by their predecessors in the cascade. Complement components are not all synthesized by a single type of cell and are all fairly large proteins, C3 being the most abundant. Quantitatively, C3 and C4 comprise approximately two thirds of the complement system. All of the complement components together account for 4 to 5% of the total serum protein, approximately 300 mg/dL. The intestinal epithelium, macrophage, liver, and spleen are the main sources of the components.1

The classic complement pathway is activated by either antibody-coated targets such as microorganisms or antigen-antibody complexes, while the alternative complement pathway is activated directly by

FIGURE 1. A significant negative correlation was found between C4 serum levels and incidence of respiratory tract infections for the past 3 years. Each data point represents a single patient. Four patients with nine infections and a C4 serum level of 32 mg/dL are signified by one data point marked with the asterisk.

FIGURE 2. A very strong correlation was found between C4 serum level and radiologic emphysema score. Each data point represents a single patient. CXR=chest radiograph.
different inclusion criteria, especially concerning the age range, the diagnosis of chronic bronchitis and the recurrence of infections, and our different approach in diagnosing a respiratory tract infection. Miller and coauthors\textsuperscript{12} studied patients who were between 45 and 60 years of age and who had FEV\textsubscript{1} \(\leq\) 70\% of predicted. In addition, they used a self-assessment scoring system in their subjects that was an expanded version of the National Heart, Lung, and Blood Institute standard questionnaire, while in our study, diagnosis of respiratory tract infections was based on an evaluation made by attending chest physicians, clinical examination, laboratory tests, and chest radiographs.

Respiratory tract infections result in complement activation acting as a host defense mechanism through either the classic or alternative pathway. Activation of complement pathways in response to the presence of bacterial or viral respiratory infection induces a local influx of inflammatory cells such as neutrophils and macrophages through chemotactically active fragments of complement proteins such as C3a and C5a.\textsuperscript{2,3,13} Inflammatory cell recruitment and accumulation serve the purpose of direct phagocytosis and subsequent killing of bacteria or viruses. It has been demonstrated in animal models\textsuperscript{14} that the activation of complement, in response to either intratracheal instillation of chemotactic peptide C5a or IV infusion of cobra venom factor, a known complement activator, results in the aggregation of neutrophils and macrophages into alveoli and pulmonary capillaries. The neutrophil aggregation and activation lead to intrapulmonary capillary sequestration of neutrophils and vascular injury due to the production of toxic oxygen radicals by complement-activated neutrophils that have been quantitated by increases of lung vascular permeability.\textsuperscript{14,15}

From the time of Laennec, the frequent coexistence of chronic bronchitis and centrilobular emphysema has been noted.\textsuperscript{16} Current knowledge on the relationship between chronic bronchitis and centrilobular emphysema suggests that the inflammatory processes on bronchial and bronchiolar wall seem highly likely to be related to the pathogenesis of emphysema.\textsuperscript{17,18} The protease-antiprotease hypothesis is the currently accepted theory for pathogenesis of emphysema\textsuperscript{4} with the neutrophils and alveolar macrophages being responsible for the release of elastases and metalloproteinases during attempted phagocytosis of either deposited foreign materials (eg, smoke condensate) or bacteria.\textsuperscript{19} Released elastolytic enzymes lead to elastolysis and damage of elastic lung tissue.\textsuperscript{19,20} In addition, oxidants derived from neutrophils, macrophages, or cigarette smoke may inactivate protective antiproteases and may interfere with lung matrix repair.

There are several independent reports linking endotoxin administration with complement activation, leukocyte sequestration, and induction of emphysema in animal models. IV administration of

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**Figure 3.** Significant positive correlations are evident between C4 serum levels and RV/TLC ratio (top), Kst (center), and FEF\textsubscript{25-75\%} of predicted (bottom). Each data point represents a single patient.
endotoxin has been shown to activate complement and to cause intravascular pulmonary leukocyte sequestration.\textsuperscript{21,22} Mild emphysema has been induced in animal models either by multiple IV injections of endotoxin\textsuperscript{23,24} or by multiple intratracheal instillations of endotoxin,\textsuperscript{25} and this has been attributed to aggregated leukocytes. It has been postulated that lung elastin damage and emphysema in these experimental models are induced by sequestered leukocytes and consequent release of elastolytic enzymes and that these effects of endotoxin administration may relate to simultaneous complement activation.

In summary, we found that patients with chronic bronchitis experiencing frequent respiratory infective exacerbations have lower levels of C3 and mainly C4 complement components in comparison with control subjects, a finding that implies consumption of these components and therefore a chronic triggering of complement system. Furthermore, the patients with the lowest C4 serum levels tend to present more impaired small airways patency, marked gas trapping, and remarkable loss of elastic recoil, a lung functional profile that is indicative of pulmonary emphysema. These findings are strengthened by a very tight negative correlation found between radiologic emphysema score and C4 serum levels.

However, C3 serum levels did not exhibit any remarkable correlation with the emphysematous profile that one would expect with the hypothesis relating sustained complement activation and degree of emphysema. Nevertheless, we observed that our study patients had lower (though not significantly) levels of C3 compared to control subjects and that these low C3 levels correlated marginally with the incidence of respiratory tract infections, although no correlation was established between C3 and radiologic or functional indexes compatible with emphysema. The C3 component seems to be affected in a more complicated way than the C4 component, given that it is involved in both the classic and alternative complement pathways and it is known to be degraded by other factors as well, such as leukocyte elastase.\textsuperscript{2} This fact may be responsible for deranging the quantitative relationship (proportionality in changes) between degree of emphysema and C3 serum levels, thus hindering the emergence of tighter correlations.

Based on our results, it is very tempting to postulate that chronic complement activation for host defense purposes may be responsible for the extent of centrilobular emphysematous changes encountered in patients with chronic bronchitis via intrapulmonary recruitment and activation of neutrophils and macrophages and subsequent release of elastolytic enzymes. However, the extremely complex aspects of complement activation and interactions and the lack of knowledge regarding the possible impact of emphysema per se on humoral immune responses, including complement, render the establishment of a cause (complement activation) and effect (emphysema) relationship questionable.

\textbf{References}

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