Circulating Immune Complexes in Patients with Cystic Fibrosis

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Circulating immune complexes (CICs) were measured in the sera of clinically stable and acutely infected patients with cystic fibrosis (CF). Twenty CF patients were studied when clinically stable; an additional 18 patients were studied during an acute exacerbation of pulmonary infection as evidenced by fever, tachypnea, increased white blood cell count, increased sputum production, and acute chest x-ray film changes. Three methods for determining CICs were employed: polyethylene glycol precipitation, a C1q solid phase assay, and the Raji cell radioimmune assay. Ten of 20 clinically stable CF patients had one or two positive assays for CICs; two of 20 had two positive assays. In contrast, 16 of 18 acutely infected CF patients had a positive CIC test, and 12 of these were positive with two or three of the assays employed. Serum C3 and C4 concentrations and total hemolytic complement activity did not correlate with the presence of CICs in either patient group. These findings suggest that immune complex formation may mediate some of the tissue damage characteristic of CF, although this usually does not involve intravascular complement activation.

Although the primary pathogenetic event in cystic fibrosis is unknown, the pulmonary pathology appears to be associated with chronic infection. Previous investigations suggest that systemic specific and nonspecific immune responses are normal in these patients. On the contrary, cystic fibrosis patients often have elevated serum immunoglobulin concentrations and high levels of circulating antibodies to their infecting organisms.

Circulating immune complexes (CICs) are associated with a variety of chronic diseases characterized by immunologic hyperreactivity and are known to mediate tissue damage. The purposes of this study were to determine the presence of CICs in clinically stable and acutely infected patients with cystic fibrosis and to evaluate and compare these assays for CICs in these patients.

SUBJECTS AND METHODS

Serum for immune complex and complement determinations

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Detection of CICs by Polyethylene Glycol (PEG) Precipitation

This method of CIC determination depends on the differential solubility of CICs and other serum proteins. To 0.1 ml of each test serum was added 0.1 ml of a cold 8 percent PEG solution in phosphate buffered saline (PBS) (final concentration of PEG, 4 percent). This mixture was incubated for one hour at 4°C. The precipitate was washed once with cold 4 percent PEG in PBS, and the washed precipitate was solubilized in 0.1 ml of PBS. The concentrations of IgG in an aliquot of the original test serum and in the solubilized PEG precipitate were then measured with kinetic nephelometry (Beckman Immunochemistry System, Beckman Instruments, Fullerton, Calif.). The percentage of IgG precipitated in 4 percent PEG was calculated with the formula:

\[
\% \text{ IgG precipitated} = \frac{\text{IgG in solubilized PEG precipitate} \times 100}{\text{IgG in test serum}}
\]
Detection of CICs by the Raji Cell Radioimmune Assay (Raji-RIA)

Raji cells were cultured in Eagle's minimal essential media (MEM), with cell viability determined by trypan blue exclusion. Human sera were assayed for CICs as follows: 2 × 10⁶ Raji cells in 500 μg MEM were reacted with 25 μg of a 1:4 dilution of test serum. After an incubation period of 45 minutes at 37°C with periodic shaking, the cells were washed three times with MEM and then reacted for 30 minutes at 4°C with an optimum amount of 1:2 dilution of 125I goat antihuman IgG in MEM containing 1 percent bovine serum albumin (BSA) (1 percent MEM-BSA). The cells were then washed three times with 1 percent MEM-BSA, and the radioactivity of the cell pellets was determined by counting in a gamma counter. The amount of uptake was then referred to a standard curve of radioactive antibody uptake by cells incubated with 25 μg of a 1:4 dilution of normal human sera (NHS), or a pool of NHS as a source of complement, to which various amounts of aggregated human IgG (AHG) were added. The mixture of NHS to which increasing amounts of AHG were added was preincubated for 30 minutes at 37°C before being added to the Raji cells. The amount of CICs in the sera tested was expressed as μg of AHG-equivalent per ml of serum.¹⁹

Detection of CICs by Clq Solid Phase Assay (Clq-SPA)

The method of Clq-SPA was a modification of the method described by Hay et al.¹³ Clq was isolated from NHS by the method of Yonematsu and Stroud,¹⁴ with the substitution of relative salt concentrations of 0.4 M and 0.78 M for the first and second precipitation steps, respectively. The purity of the isolated Clq was confirmed by immunoelectrophoresis using rabbit antiserum to whole human sera and to human Clq.

The assay was performed by incubating 50 μl of test serum with 100 μl 0.2 m ethylenediamine tetracetic acid (EDTA), pH 7.5, for 30 minutes at 37°C. Sixty microliters of the EDTA-treated serum was added to Clq-coated tubes in 1.0 ml PBS. Tests were performed in triplicate. The tubes were incubated for 60 minutes at 37°C and then for 30 hours at 4°C. At the end of the incubation period, the tubes were washed three times with PBS. Approximately 2.5 μg of 125I goat antihuman IgG antibody was added to each tube in 1 ml of 1 percent BSA-PBS, and incubated for 60 minutes at 37°C and 30 minutes at 4°C. Tubes were washed three times in PBS, and radioactivity bound to the tubes was counted in a gamma counter. The results were expressed as percentage of counts bound to the tubes.

Determination of C3 and C4 Concentrations and Total Hemolytic Complement C1q Activity

Serum complement components C3 and C4 were measured with standard radial immunodiffusion plates (Meloy Laboratories, Springfield, Va). Total hemolytic complement activity (CH₅₀) was determined with a standard assay employing hemolysin-sensitized sheep red blood cells.¹⁵

Statistical Analysis

Comparisons were made between normal and CF groups and between the two CF groups using analysis of variance and independent t tests. Correlations of positive results with the three CIC assays were statistically tested using χ² analysis.

RESULTS

Results of the PEG precipitation assays for CICs in 17 normal control subjects, 17 clinically stable cystic fibrosis patients, and 16 cystic fibrosis patients during acute exacerbations are illustrated in Figure 1. The mean percentage of IgG precipitated from the sera of cystic fibrosis patients, clinically stable and acutely infected, is significantly greater than that from control sera.

Positivity for CICs using the PEG precipitation assay was arbitrarily defined as a test result greater than 2 SD above the mean value for concurrently performed controls. Tables 1 and 2 detail the results of the assays for CICs in individual cystic fibrosis patients. Seven of 17 clinically stable cystic fibrosis patients had CICs by the PEG precipitation method; 12 of 16 acutely infected cystic fibrosis patients had positive tests. Mean total serum IgG concentrations did not differ between clinically stable and acutely infected patients, and percent IgG precipitated did not correlate with serum IgG concentrations in either group.

Raji cell-RIA for CICs were performed on the sera from 16 clinically stable cystic fibrosis patients and concurrently on the sera of ten healthy adult subjects. Similarly, 18 acutely infected cystic fibrosis patients were studied, again concurrently with ten control subjects. Figure 2 illustrates the results of these tests. The mean μg AHG-equivalent/ml found in the sera of clinically stable cystic fibrosis patients did not differ significantly from that noted in control sera. However, cystic fibrosis patients

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Table 1— Assays for Circulating Immune Complexes and Serum C3, C4, and Hemolytic Complement (CH50) Levels in Clinically Stable Patients with Cystic Fibrosis*  

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Number of assays positive: 7  
Number of assays performed: 17

Normal values and ranges: 55-120 20-50 105-220

*Assays for circulating immune complexes: PEG = polyethylene glycol precipitation assay; Raji = Raji cell radioimmune assay; Clq = Clq solid phase assay; and nd = not done.

During acute infectious exacerbation, there were significantly greater μg AHC-equivalent/ml in their sera than in control subjects.

A test was considered positive for CICs by the Raji-RIA when the result was greater than 2 SD above the mean value for concurrent control sera. Of the 18 clinically stable cystic fibrosis patient tests, four were positive (Table 1). In contrast, during acute infection, 12 of 18 cystic fibrosis patients had CICs by this technique (Table 2).

CICs as determined with the Clq-SPA were measured in 16 stable cystic fibrosis patients and concurrently in ten control subjects. Similarly, 13 acutely infected cystic fibrosis patients and ten control subjects

![Graph showing results of Raji cell radioimmune assays for circulating immune complexes in clinically stable CF patients compared with concurrent normal adult control subjects, and in CF patients during acute infectious exacerbations compared with concurrent normal adult control subjects.](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21251/ on 06/13/2017)

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Table 2—Assays for Circulating Immune Complexes and Serum C3, C4, and Hemolytic Complement (C50) Levels in Acutely Infected Patients with Cystic Fibrosis*

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<th>Patient No.</th>
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Number of assays positive | 12 | 12 | 7
Number of assays performed | 16 | 18 | 18
Normal values and ranges | - | - | 55-120 | 20-50 | 105-220

*Assays for circulating immune complexes: PEG = polyethylene glycol precipitation assay; Raji = Raji cell radioimmune assay; Clq = Clq solid phase assay; and nd = not done.

current control subjects were tested. As shown in Figure 3, there is no difference in the mean percentage of radioactivity bound to the solid phase between either group of cystic fibrosis patients and the respective control subjects.

A test was considered positive for CICs by the Clq-SPA when the result was greater than 2 SD above the mean value for concurrent control sera. Only one of 16 clinically stable cystic fibrosis patients had CICs by this technique (Table 1); seven of 18 acutely infected cystic fibrosis patients had CICs with this assay.

Ten of 20 clinically stable cystic fibrosis patients had one or more positive assays for CICs, and two of 20 had two assays positive. In contrast, 16 of 18 acutely infected cystic fibrosis patients had a positive CIC test, and 12 of these were positive with two or three of the assays employed.

The χ² analyses of the results of the PEG precipitation, Raji cell radioimmune, and Clq-SPA assays failed to reveal positive correlations when any two of these assays for CIC detection were compared.

Clinically stable and acutely infected cystic fibrosis patients did not differ in their serum C3 and C4 concentrations and total hemolytic complement activities (Fig 4). These levels generally were within the range of normal, although one acutely infected cystic fibrosis patient had very low total hemolytic complement activity. Further, there was no association between the presence of CICs and low serum complement levels, and elevated complement levels were noted in sera with CICs by two or three assays.

**DISCUSSION**

Systemic immune responses in patients with cystic fibrosis are normal or hyperreactive, and immunologic hyperreactivity is commonly noted in disease states with CICs. That CICs may mediate the tissue damage in cystic fibrosis was suggested in 1975 when McFarlane et al18 described deposits of...
immunoglobulin and complement in a variety of tissues from cystic fibrosis patients, including lung, liver, pancreas, and thymus, but not kidney.

Strunk et al. reported serum complement component C3 and C4 depression during viral lower respiratory tract infection in cystic fibrosis. Those authors postulated that antigen-antibody complex activation of complement occurred, but they did not test for CICs.

Using a complement consumption assay, Schisttz et al. found CICs in cystic fibrosis patients, and those infected with Pseudomonas had a much higher incidence of CICs than those not so infected. Also in this study, immunofluorescent staining of skin biopsy specimens revealed granular deposits of IgM at the dermal-epidermal junction in 17 of 21 cystic fibrosis patients, although this finding appeared unrelated to Pseudomonas infection. In an extension of this work, immune complexes were detected with complement consumption and solid phase rheumatoid factor binding assays in sputum from Pseudomonas-infected cystic fibrosis patients.

Moss and Lewiston found CICs in 51 percent of cystic fibrosis patients tested during acute exacerbations. The percentage of patients positive for CICs was not affected by the presence of infection, although the number of CICs per milliliter was higher in infected than in uninfected patients.

![Figure 3](image-url) Results of C1q solid phase assays for circulating immune complexes in clinically stable CF patients compared with concurrent normal adult control subjects, and in CF patients during acute infectious exacerbations compared with concurrent normal adult control subjects.

![Figure 4](image-url) Serum CH₅₀ activities and C3 and C4 concentrations in acutely infected CF patients (I) compared with those of clinically stable CF patients (S). Vertical rectangles indicate normal ranges for the respective test results. Horizontal bars and numbers indicate mean group values. Open circles = no circulating immune complexes (CICs) detected with any method. Triangles = CICs detected with one assay. Closed circles = CICs detected with two or three of the assays employed.
tions or routine clinic visits. Those investigators used a Clq binding assay and discerned no correlation between a positive test for CICs and Pseudomonas colonization or antibody response, and no evidence of in vivo complement activation. Recently, Berdischewski et al21 demonstrated CICs in 18 of 20 hospitalized CF patients with a PEG precipitation assay.21 Analysis of the precipitated complexes revealed enrichment of antibodies against Pseudomonas lipopolysaccharide.

In the present investigation, three techniques were employed to detect CICs. These studies confirm a high incidence of CICs in clinically stable cystic fibrosis patients; and patients during acute infectious exacerbation have an even greater incidence of CICs. That these assays correlate poorly with one another has been noted previously,8 and has been attributed to the different immunochromehical or physical properties of CICs in various disorders. The PEG precipitation method as used in these studies depends on the differential solubility in 4 percent polyethylene glycol solution between aggregated and native IgG. That positive PEG assays for CICs did not reflect nonspecific aggregation is suggested by the lack of correlation between individual PEG assay results and serum IgG concentrations. The Raji cell-RIA detects CICs by quantifying immune complexes which have already activated complement and which bind to Raji cell C3b and C3a receptors. This assay is very sensitive and appears to be selective for immune complexes in the 11S to 19S range. The Clq-SPA is based on binding of Clq to antigen-complexed IgG, IgG, IgG, and IgM. It is more sensitive to immune complexes 9S to 11S in size and it does not detect complexes composed of noncomplement binding immunoglobulin, such as IgG, and IgA. These three assays detect different families of CICs that might not be detected if only one assay were used, and the Clq-SPA appears less sensitive than the PEG precipitation assay or the Raji cell-RIA in detecting CICs in stable and acutely infected patients with cystic fibrosis.

Depression of C3, C4 or CH50 levels did not correlate with the presence of CICs. In addition, serum from clinically stable and acutely infected cystic fibrosis patients generate normal chemotactic responses, a complement-dependent function, when activated with zymosan.22 Normal levels of specific complement proteins and total hemolytic complement activities may falsely suggest lack of complement activation if synthetic rates of these components are increased. Further, specific assays for detection of activation products or turnover studies would be required to rule out complement involve-

ment in cystic fibrosis. However, the present studies do not support intravascular complement activation in the pathogenesis of this disorder.

Although there may be a subset of patients with immune complexes who get sick more frequently, the present studies suggest that clinically stable and acutely infected CF patients are discrete populations. A prospective study of the development of CICs in CF patients will be necessary to clarify the role of CICs in this disorder. The detection of CICs in a high proportion of acutely infected cystic fibrosis patients and the previously noted finding of immunoglobulin and complement deposits in the lungs of these patients suggest that immune complex formation mediates at least some of the tissue damage characteristic of cystic fibrosis.

ACKNOWLEDGMENT: We gratefully acknowledge the expert technical assistance of Rebecca Sakai, Carol Nye, the Childrens Hospital of Los Angeles Blood Bank under the supervision of Jacque Dellinger, and the excellent manuscript preparation of Gert Coldewey.

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