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Opsonic Defect in Patients with Cystic Fibrosis of the Pancreas

(*Pseudomonas aeruginosa*/IgA deficiency)

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ABSTRACT **Cystic fibrosis of the pancreas is one of the most common inborn errors of metabolism. The high incidence of morbidity and mortality in these patients is primarily due to severe and frequent pulmonary infection. To date, no immune deficiency has been found in cystic fibrosis patients. Their sera contain normal quantities of immunoglobulins and hemolytic complement. In an assay of phagocytosis by alveolar macrophage, six out of nine sera from cystic fibrosis patients failed to support normal phagocytosis of *Pseudomonas aeruginosa*. This deficiency could be corrected by increasing the concentration of serum used in the assay. By contrast, their sera supported normal phagocytosis of Pseudomonas by blood polymorphonuclear leukocytes and continued to support normal phagocytosis when serum dilutions were used. Two patients with severe isolated deficiences of serum immunoglobulin A were found to have a similar defect in the alveolar macrophage assay, but normal phagocytosis by polymorphonuclear leukocytes. It is postulated that cystic fibrosis patients may have a quantitative and (or) functional defect of IgA antibodies, specific for Pseudomonas, and possibly of importance in the pathogenesis of their pulmonary disease.**

Cystic fibrosis of the pancreas (CFP) is one of the most common inborn errors of metabolism. Frequent manifestations of the disease in children include pancreatic malfunction, an inability to secrete hypotonic sweat normally, and recurrent and chronic pulmonary infections leading to progressive pulmonary insufficiency. The high incidence of morbidity and mortality in CFP is primarily due to the pulmonary component of this disease. Impaired mucociliary clearance of pulmonary debris, chronic endobronchial infection, and severe progressive pulmonary insufficiency characterize the pulmonary pathology. Spock *et al.* (1) described a serum factor present in CFP patients which disrupted the normal rhythmic beating of the cilia in explants of rabbit tracheal epithelium. A similar effect was observed when a nasal polyp that had been excised from a CFP patient was exposed to CFP serum. It can be postulated that this inhibitory factor induces ciliary dyskinesis along the patient's respiratory tract epithelium and disrupts the mucociliary transport system. Impaired mucociliary flow would result in the accumulation of secretions, obstruction of small airways, and the establishment of a suitable environment for bacterial growth.

To date, no immune deficiency has been found in children with CFP. They have normal or elevated quantities of serum and secretory immunoglobulins (2–5) and are capable of producing specific antibody in response to bacterial infection (6).

Abbreviations: CFP, cystic fibrosis of the pancreas; PMN, polymorphonuclear leukocytes; HBSS, Hanks' balanced salt solution.

The organisms cultured from the sputum of CFP patients vary considerably, but the significance of *Pseudomonas aeruginosa* as a pathogen has become apparent (7–9). The successful establishment of this organism as a pathogen may be attributed, at least in part, to the suppression of other bacterial species by antibiotic therapy.

The following study was undertaken to examine the capacity of CFP patients to produce serum factors which promote phagocytosis of Pseudomonas. To this end patients' sera were examined in phagocytosis assays that used blood polymorphonuclear leukocytes (PMN) and rabbit alveolar macrophages.

MATERIALS AND METHODS

Selection of patients

Nine children who regularly attend the cystic fibrosis clinic at the University of Minnesota were studied. The age range was from 6 to 15 years. All patients had an abnormal content of sweat electrolytes and had the characteristic pulmonary manifestations of CFP. In addition, 10 patients were studied who had primary deficiences of humoral immunity alone or in association with deficiences of cellular immunity. Their ages ranged from 8 months to 16 years. Seven of the 10 patients were examined prior to initiation of therapy. Control subjects, aged 4–16 years, were nonhospitalized healthy children who had no history of recurrent sinopulmonary infections.

Polymorphonuclear leukocyte function

Phagocytic capacities of human PMN were determined by the Maaløe method (10) with the modifications described by Cohn and Morse (11) and Hirsch and Strauss (12).

Human peripheral leukocytes were prepared by dextran sedimentation of heparinized venous blood. 10 ml of blood containing 1 mg of heparin was mixed with 2 ml of 6% dextran in saline and incubated at room temperature for 1 hour. The plasma—containing leukocytes, platelets, and few erythrocytes—was withdrawn. The leukocytes were sedimented by centrifugation at 200 $\times$ *g* and washed with heparinized saline. The leukocytes were centrifuged, washed again, and resuspended in Hanks' balanced salt solution (HBSS) (Microbiological Assoc., Bethesda, Md.) to give a concentration of 10×10^6 PMN/ml.

Pseudomonas aerugenosa was obtained from the Diagnostic Laboratory of the University of Minnesota. Organisms were prepared by an overnight incubation in trypticase soy broth in a water-bath shaker at 37°C. The bacteria were collected by centrifugation for 10 min at 1700 $\times$ *g* and resuspended in appropriate dilutions to provide 5–10 $\times$ 10^6 cells/ml in the stationary growth phase. Sera from patients and controls were

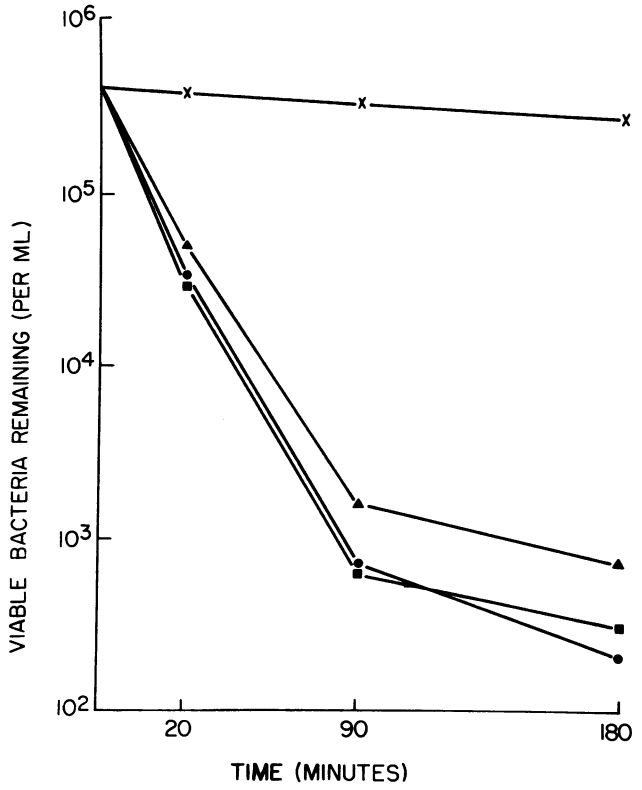

FIG. 1. Viable bacterial counts after incubation for 20, 90, and 180 min with control polymorphonuclear leukocytes (PMN) and serum from (▲) a normal; (■) a patient with an isolated IgA deficiency; (×) a congenital agammaglobulinemic patient; and (●) a cystic fibrosis patient.

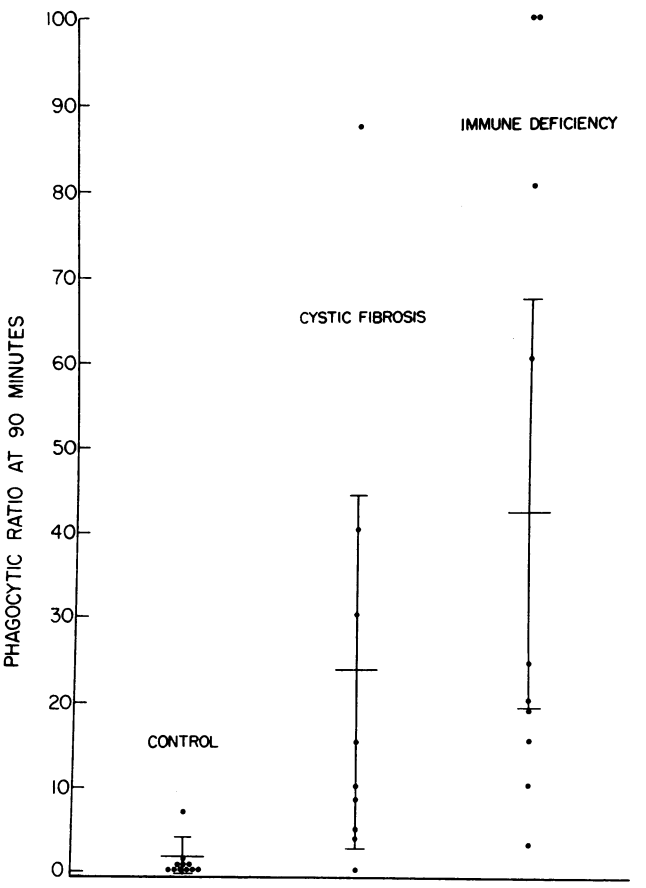

FIG. 2. Phagocytic ratio of alveolar macrophage phagocytosis by normal serum and by sera from patients with cystic fibrosis of the pancreas and primary immunological deficiencies. The phagocytic ratio was calculated as the number of viable extracellular bacteria remaining in the test assay at 90 min divided by the number of viable extracellular bacteria remaining in the control assay.

collected from venous blood and used immediately, then stored in small aliquots at −70°C for future testing.

Phagocytic tests were done in 13 × 75 mm Falcon Plastic tubes (Falcon Plastics, Division of Bio-Quest Oxnard). Each tube contained 0.5 ml of leukocyte suspension, 0.1 ml of bacterial suspension, 0.1 ml of serum, and 0.3 ml of HBSS. Each assay included patient's serum and control leukocytes or control serum and control leukocytes.

The tubes for assay of phagocytosis were incubated at 37°C on a Lab Tech Tilter. 0.1-ml aliquots were removed at 20, 90, and 180 min. Viable bacteria were quantitated by a standard dilution-plate technique.

Alveolar macrophage phagocytosis assay

Rabbit alveolar macrophages were obtained by pulmonary lavage according to the method of Myrvik *et al.* (13). A 1.8- to 2.7-kg New Zealand rabbit was anesthetized with intravenous Nembutal and the lungs and trachea were removed from the thoracic cavity. The lungs were distended with 30 ml of sterile HBSS, massaged, and aspirated with gentle suction. Three lung washings were pooled and centrifuged for 5 min at 200 × *g*. The cell pellet was resuspended in HBSS as a 95% pure macrophage population to contain 4 × 10^6 cells/ml. More than 95% of the cells isolated exclude trypan blue.

Phagocytic tests were done in Falcon plastic tubes. Each 13 × 75 mm tube in the phagocytic test contained 0.5 ml of alveolar macrophage suspension, 0.1 ml of a 1:1 dilution of serum in HBSS, 0.1 ml of the suspension of bacteria, and 0.3 ml of HBSS. Each assay included duplicate tests of both test and standardized control sera. The tubes were incubated at 37°C on a Lab Tech Tilter, and 0.1-ml aliquots were removed at 60 and 90 min. Viable bacteria were quantitated by a standard dilution-plate technique.

The phagocytic ratio was calculated as the number of viable extracellular bacteria remaining in the test assay at 90 min divided by the number of viable extracellular bacteria remaining in the control assay.

The method of Mayer (14) was used to determine the serum hemolytic complement activity (C'H50 units).

RESULTS

Fig. 1 shows the results of a typical phagocytosis assay to test the various sera for the promotion of phagocytosis by PMN. Sera from patients with CFP were found to have the same capacity to promote phagocytosis of *Pseudomonas* by PMN as normal control sera. When increasing dilutions of CFP serum were tested, the sera of these patients continued to support phagocytosis of Pseudomonas by PMN as well as or better than control sera of the same dilution. Seven of the ten sera from patients with primary immunological deficiencies failed to promote phagocytosis of Pseudomonas by PMN of normal controls. Two of three patients with immunological deficiencies whose sera promoted normal phagocytosis had

isolated severe deficiencies of IgA. The third had mild hypogammaglobulinemia.

Fig. 2 shows the capacity of the various sera to promote phagocytosis of Pseudomonas by alveolar macrophages. For normal sera, the mean phagocytic ratio was 2.8 ± 1.1 (SE). Normal serum heated at 56°C for 30 min did not promote phagocytosis. Similarly, no phagocytosis occurred when serum was decomplemented by antigen–antibody complexes. Six of the nine sera from CFP patients showed a marked deficiency in the promotion of phagocytosis. Quantitative immunoglobulin determinations showed normal or elevated levels of immunoglobulins and normal total serum hemolytic complement activity. With increasing concentrations of CFP serum, the capacity to promote phagocytosis improved. Preliminary results indicate that approximately four times the concentration of CFP serum is required for normal phagocytosis.

Nine of ten sera from immune deficiency patients failed to promote phagocytosis. Two of these nine patients had isolated deficiences of serum IgA (<6 mg/100 ml), four had severe hypogammaglobulinemia, and three had severe combined dual system immunodeficiencies. The tenth patient, whose serum supported phagocytosis normally, had mild hypogammaglobulinemia and infrequent pulmonary infections.

DISCUSSION

Phagocytosis of bacteria is a complex series of events. This includes the interaction of the bacterium with humoral factors (heat-stable and heat-labile opsonins), attraction and adherence to the phagocyte, and finally, ingestion. Chemical and physical environmental factors greatly influence the outcome of the interaction between a microorganism and the phagocyte. Serum factors, critical in influencing the efficiency of phagocytosis, include specific antibody, complement, and other less well-defined components. These are capable of interacting with the surface of bacteria to change its properties (12, 15). In addition, some serum antibodies (cytophilic antibodies) are capable of "fixing" to cells and influencing the specificity and efficiency of that cell's participation in an immune response (16). The rate and the efficiency of the phagocytic process is also influenced by temperature, pH, and divalent cation concentration of the supporting milieu (17, 18).

Malfunction within this complex and dynamic process of phagocytosis may result in deficiences of phagocytosis and an increased susceptibility to infection.

Recently, a group of clinical disorders characterized by abnormalities of phagocytosis and an increased susceptibility to infection have been described. The patients, as distinct from those with the usual immune deficiency syndromes, appear to have a normal capacity to execute humoral and cellular immune responses, but have a failure of the normal inflammatory process. Since chronic granulomatous disease was first clearly defined as a failure of normal killing of certain bacteria by PMN (19), several additional defects in the normal processing of microorganisms by PMN have been described. These syndromes include opsonic deficiencies (20, 21), complement abnormalities (22), and functional abnormalities of the PMN (23–25). None of the serum defects thus far described has been studied with the alveolar macrophage. If we consider comparative studies of monocytes and neutrophils (26), differences in serum factor requirements might also be expected when alveolar macrophages are compared to PMN.

The alveolar macrophage resides in the lung and there represents a primary defense mechanism against a wide variety of stimuli. The environment of the lung is in many ways unique and differs considerably from the environment of the circulating PMN and the peritoneal macrophage. Furthermore, the alveolar macrophage is metabolically and functionally different from the blood PMN and the peritoneal macrophage (27).

Our studies of the phagocytosis of Pseudomonas by the alveolar macrophage indicate that, as with the PMN, both immunoglobulin and complement are required. Neither cell type effectively phagocytizes Pseudomonas in decomplemented serum or sera from patients with primary immunoglobulin deficiencies.

Sera from CFP patients contain normal quantities of immunoglobulin and hemolytic complement and support normal phagocytosis of Pseudomonas by blood PMN. Furthermore, with increasing dilutions, sera from CFP patients promote phagocytosis as well as or better than normal serum. Sera of two patients with severe deficiencies of serum IgA (<6 mg/100 ml) but normal quantities of IgG and IgM were also capable of promoting phagocytosis of Pseudomonas by PMN from controls. Patients with primary immunodeficiency diseases, on the other hand, showed little or no opsonic activity (28).

In contrast, six of nine sera from CFP patients failed to support phagocytosis of Pseudomonas by rabbit alveolar macrophages. That this failure to support phagocytosis is due to a deficiency and not to an inhibitor is evidenced by the capacity of the abnormal serum to correct the phagocytic defect when the concentration used in the test assay is increased. As was observed with PMN, serum from immunodeficiency patients, heat-inactivated serum, or serum previously treated with antigen–antibody complexes failed to promote phagocytosis. Serum from the IgA-deficient patients also failed to support phagocytosis.

The role of IgA in promoting phagocytosis by alveolar macrophages is unknown. IgA does not ordinarily fix complement (29) and does not appear to be essential for phagocytosis by the PMN. The alveolar macrophage functions in a unique milieu, and is in contact with secretions of which IgA is the primary immunoglobulin. It is possible that the alveolar macrophage has different opsonic requirements, with IgA playing a major role in their capacity to phagocytize Pseudomonas.

The requirement for complement in this assay is, of course, difficult to explain if IgA is the only immunoglobulin involved. IgG and (or) IgM and complement, together with IgA, may be required for completely normal phagocytosis. On the other hand, the requirement for complement appears to be absolute and indicates that a complement-fixing antibody is of primary importance. An analysis of alternative pathways for the activation of the later components of complement may resolve the apparent contradiction. Our studies raise the possibility that the defect observed in CFP patients represents a failure to produce adequate IgA antibodies specific for Pseudomonas and is important in the pathogenesis of the progressive pulmonary disease.

The alveolar macrophage phagocytosis assay described here has revealed that six out of nine sera from CFP fail to support normal phagocytosis of Pseudomonas. Since IgA appears to be important in this assay of phagocytosis, it is postulated

that CFP patients may have a quantitative and (or) functional defect of IgA antibodies specific for Pseudomonas. Experiments are in progress to test this hypothesis.

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