Ciprofloxacin, one of the fluoroquinolones, has been used in CF patients for the last 3 years. This drug has a high in vitro activity against mucoid and nonmucoid strains and favorable pharmacokinetic properties. Emergence of resistance was reported very early, and we have also observed resistant strains. An interesting feature of the patient whose findings are shown in Figure 1 was the "cure" of resistance to broad-spectrum β-lactams by long-term administration of ciprofloxacin. "Cure" of plasmid-mediated resistance in Gram-negative rods by ciprofloxacin has also been noticed.

Emergence of resistance and the persistence of susceptible and nonsusceptible strains show no correlation with the clinical response. Most studies report a temporarily favorable clinical outcome despite the emergence of resistance. It is therefore too simple to ascribe failure to the emergence of resistance. Resistant strains usually disappear within 3 months after therapy is stopped, but we have also observed disappearance of resistance without discontinuing therapy. During a long treatment period with ciprofloxacin, which we normally do not recommend, we sometimes saw resistant strains appearing and then disappearing. During this period, the patient felt clinically well, despite the emergence of resistance, and she was not hospitalized for 11 months. Lung function, measured as vital capacity, showed no change, nor did the hematologic parameters for infection (leukocytosis, shift to the left, BSE). Why a patient suddenly then deteriorates is difficult to understand. We still do not understand the interactions between microorganisms and antibiotics in the lungs of CF patients, which mechanisms of resistance occur in vivo, and why and how antibiotics act or do not act in these damaged tissues.

REFERENCES

11 Mehtar S, Blakemore PH. In vivo effect of ciprofloxacin on plasmid mediated resistance. Presented at the Fifth Mediterranean Congress of Chemotherapy, Cairo, 1986 (abstr S117)

Immunologic Aspects of Cystic Fibrosis

Gerd Döring, Ph. D.; Anne Albus, M. D.; and Niels Høiby, M. D., Ph. D.*

Bacterial infections determine life expectancy in the hereditary disease cystic fibrosis (CF). The dominant pathogens are Staphylococcus aureus and Pseudomonas aeruginosa, which persist in the patient's respiratory tract. Current explanations of the chronology of the infections in the apparently immunocompetent host are based on defective opsonophagocytosis. This may be caused by (1) bacterial exopolysaccharide production, leading to capping of antigenic types; (2) cleavage of immunoglobulin, complement, and surface receptors on immunocompetent cells by host proteases; and (3) a change from opsonic to nonopsonic antibody isotypes. Continuous antigenic stimulation of the immune system leads to local immune complex formation and a high chronic hypersensitivity reaction as well as to temporary immune unresponsiveness. Progressive tissue damage caused by lysosomal enzymes and oxygen radicals from polymorphonuclear leukocytes is thought to be ultimately responsible for respiratory failure and death in CF. Besides antibiotic treatment, anti-inflammatory therapy is therefore currently considered beneficial.

There is at bottom only one genuinely scientific treatment for all diseases and that is to stimulate the phagocytes. Stimulate the phagocytes. Drugs are a delusion.

George Bernard Shaw, 1906

The irony in George Bernard Shaw's "The Doctor's Dilemma" concerning pediatric treatment in medicine is obvious. But is it wrong advice? For cystic fibrosis (CF), the most common genetic disorder affecting the white race, we now would not advise phagocytic stimulation or consider a primary immune defect as a factor in the disease. On the contrary, there is evidence that the immune system is overstimulated due to secondarily acquired bacterial lung infections. The observation that continuous hypersensitivity reactions in response to these infections are critical for the determination of life expectancy in CF has led to promising treatment with drugs that inhibit rather than stimulate the phagocytes. In addition, the patient himself appears to downregulate immune functions in order to avoid the harmful and noxious effects of constant inflammation. A review of the immunology of CF, therefore, must primarily address host-parasite interactions. Immunologic aspects of chronic Staphylococcus aureus and Pseudomonas aeruginosa lung infection will be discussed. The reader is referred to

*Hygiene-Institut, University of Tübingen, Tübingen, Federal Republic of Germany, and †Statens Seruminstitut, Copenhagen, Denmark.
previously published reviews for other aspects of the immunology of this disease.1

Is Bacterial Opsonization Defective?

S aureus and P aeruginosa are very successful at surviving for long periods in the lungs of CF patients. The main hypotheses to explain the persistence of bacterial lung infections involve defective bacterial opsonophagocytosis and include (1) the change in P aeruginosa from nonmucoid to mucoid variants; (2) inadequate immunologic recognition of S aureus; (3) proteolytic cleavage of opsonizing antibodies, complement components, and cell receptors on phagocytes; and (4) a change from opsonic to nonopsonic antibody isotypes.

Change in P aeruginosa from Nonmucoid to Mucoid Variants

In most CF patients, a change takes place from the initial nonmucoid, lipopolysaccharide (LPS)-containing P aeruginosa strains to mucoid, rough strains that lack the O-poly saccharide side chains on LPS.24 The mucoid nature of the strains is related to the production of exopolysaccharide (alginate), which surrounds the organism in vivo in large amounts.7 Mucoid P aeruginosa requires more specific antibodies than nonmucoid strains in an in vitro opsonophagocytic assay.4 Partial or total loss of LPS and inaccessibility to phagocytes (due to the mucous layer of mucoid P aeruginosa strains) of antibodies bound to the bacterial cell wall may explain the results. Both retrospective and prospective studies that showed polyvalent LPS vaccines to be ineffective in CF patients are consistent with this view, as is the demonstration of high LPS antibody titers in chronically infected CF patients.14 However, no differences in killing of mucoid and non-mucoid P aeruginosa were found between immunized and nonimmunized guinea pigs.13

The demonstration of antibodies specific to mucoid exopolysaccharide in CF patients chronically colonized with P aeruginosa16-18 seems to indicate that these antibodies are not protective either.19 Interestingly, antibodies to mucoid exopolysaccharide that enhanced opsonophagocytic P aeruginosa killing in vitro were found in older CF patients not colonized with P aeruginosa.18 Although this phenomenon remained unexplained, it raised the possibility that a vaccine made with mucoid exopolysaccharide could prevent P aeruginosa colonization in CF patients.

Mucoid P aeruginosa strains are serum sensitive,4.50-52 and killing may occur via antibody-independent activation of the alternative pathway of complement.53 Indeed, chronic P aeruginosa infection has been found to be associated with inadequate local complement levels.25 Although actual levels have not been reported, C3c, C4, and C5 in CF sputa have been described as low.54 Decreased complement levels are not due to a primary defect in the patients, but most likely to proteolytic inactivation and enhanced binding to soluble immune complexes, as will be discussed below.

Inadequate Immunologic Recognition of S aureus

As was recently shown, S aureus strains isolated from CF patients are encapsulated, with a predominance of capsular type 8. Despite chronic S aureus colonization, only 10 percent of CF patients responded with elevated antibody levels to the capsular polysaccharides types 5 and 8.55 A similarly poor antibody response was noted to cell wall components of S aureus in colonized CF patients.25-27 Generally, immune response to S aureus antigens in patients with severe staphylococcal disease is low and not very different from that seen in normal persons of comparable age.28 This raises the possibility that repeated infections since early infancy may have maintained a sufficiently large antigenic mass to result in immunologic unresponsiveness.29 On the other hand, recurrent S aureus infections are seen in patients with high antibody titers to a variety of S aureus antigens,56 including the capsule,57 which suggests the presence of virulence factors, such as S aureus exoproteases or protein A,58 that inactivate specific antibodies. Released protein A, a T-cell-independent, B-cell mitogen,59 may induce polyclonal antibody production and nonspecifically bind immunoglobulin and subsequently also complement,60 thereby impairing phagocytosis and reducing opsonizing complement levels.61,62 However, complexed protein A, released or at the bacterial cell surface, has not yet been detected in the CF lung. Antibody triggering by bacterial mitogens63 may substantially contribute to hypergamma-globulinemia in infected CF patients.64

Proteolytic Cleavage of Opsonizing Antibodies, Complement Components, and Cell Receptors on Phagocytes

Opsonophagocytosis depends on intact opsonic immunoglobulins that bind to cell receptors on phagocytes via the Fc part of the immunoglobulin or via attached complement.66-68 Proteolytic cleavage of immunoglobulin resulting in F(ab) or Fab and Fc fragments significantly inhibits uptake of immune complexes by phagocytes.7 Immunoglobulin fragments have been detected in CF sputa and intact local IgG was reported to be only 18 percent of the total IgG.7 It is now generally believed that the proteolytic activity responsible for significant fragmentation is elastase from polymorphonuclear leukocytes (PMNs),69-71 since it can be abolished in vitro by adding serine proteinase inhibitors but not metalloproteinase inhibitors.72-74 Furthermore, lysosomal PMN proteinase have been shown to cleave purified IgG,75,76 IgM,77 and IgG or IgA immune complexes.78 The occurrence of IgG cleavage products correlates with the proteolytic activity of PMN elastase in these sputa79 (Döring G, Patheiger U, unpublished observations). PMN elastase has also been shown to cleave the central complement component of the classic and alternative pathway C3 as well as C5.80 Theoretically, bacterial proteinases such as P aeruginosa alkaline protease and elastase may also be responsible for IgG cleavage.81-83 However, this mechanism of pathogenicity is most probably restricted to the beginning of chronic infection, when specific antibodies to the proteinases are missing.84 Unequivocal demonstration of free P aeruginosa proteinases during the course of infection is lacking in CF. However, specific antibodies to alkaline protease and elastase isolated from CF patients neutralize enzymatic activities in vitro,85 and both antigens have been detected in immune complexes isolated from CF sputa.86 A third means whereby PMN elastase may be involved in the impairment of opsonophagocytosis has emerged with the recent finding that PMN elastase cleaves the complement

Pulmonary Infection and Antibiotic Treatment in Cystic Fibrosis

Downloaded From: http://journal.publications.chestnet.org/pdftoaccess.ashx?url=/data/journals/chest/21582/ on 06/21/2017
receptor of C3b (CR1) on human PMNs in vitro\textsuperscript{40} and that PMNs isolated from CF sputa reveal low CR1 expression, suggesting ongoing cleavage in vivo.\textsuperscript{41} Inflammation in the CF lung may therefore impair local phagocytosis of \textit{P. aeruginosa} and \textit{S. aureus} at the level of opsonizing immunoglobulins, complement, and cell receptors on phagocytes.

\textit{Change from Opsonic to Nonopsonic Antibody Isotypes}

Circulating antibodies are intact in CF patients due to high levels of endogenous, active protease inhibitors.\textsuperscript{1,41} However, CF serum inhibits phagocytosis of autologous \textit{P. aeruginosa} strains.\textsuperscript{54,55} This inhibition is restricted to \textit{P. aeruginosa} and alveolar macrophages and is not seen with other bacteria or PMNs.\textsuperscript{55,56} This phenomenon was first attributed to so-called blocking antibodies, a hypothesis that subsequently gained support from findings that affinity-purified IgG antibodies from CF sera specific for \textit{P. aeruginosa} LPS showed the same inhibitory effect.\textsuperscript{27} Determinations of IgG subclass levels in patient sera gave conflicting results,\textsuperscript{12,55-58} as did determinations of LPS-specific IgG subclasses. Elevated levels of the nonopsonizing subclasses IgG2,\textsuperscript{14} IgG4\textsuperscript{60} or IgG2 and IgG4,\textsuperscript{61} and lack of the opsonizing subclass IgG1,\textsuperscript{14}\textsuperscript{59} were thought to be responsible for the inhibitory effect. In the light of findings that it is mainly LPS-deficient, mucoid \textit{P. aeruginosa} strains\textsuperscript{62} as well as encapsulated \textit{S. aureus} strains\textsuperscript{63} that are present in CF sputa, an evaluation of IgG subclasses against mucoid and capsular polysaccharide merits further attention. This is especially important since the IgG2 subclass, normally the primary antibody to carbohydrates,\textsuperscript{62} has been varyingly reported to be decreased\textsuperscript{57,58} and increased.\textsuperscript{14} The normal IgG1 response to protein antigens such as tetanus toxoid\textsuperscript{64} is intact in cystic CF patients.\textsuperscript{65} Interestingly, all IgG subclasses against \textit{P. aeruginosa} alkaline protease and elastase in 50 CF patients revealed significantly elevated levels (Tesch W, Döring G, unpublished findings).

\textbf{Immune Complexes and Tissue Destruction}

The hypothesis that immune complexes resulting from infection are indirectly responsible for lung tissue destruction in CF was originally presented more than a decade ago by Höiby and colleagues.\textsuperscript{66-68} Since then, many investigators have reported high antibody titers to a large number of \textit{P. aeruginosa} antigens\textsuperscript{61-64,68-70} and the presence of immune complexes\textsuperscript{11-13,68-70} in sera and sputa of infected CF patients. Furthermore, \textit{P. aeruginosa} antigens have been detected in immune complexes.\textsuperscript{39,68-71} However, conflicting results have been obtained in attempts to correlate levels of antibodies and immune complexes with the patients’ clinical states.\textsuperscript{66,68,71-73} Proteolytic cleavage of immune complexes probably accounts for failure to detect immune complexes and hence lack of correlation.\textsuperscript{74}

The sequelae of immune complex-mediated (type III) hypersensitivity reactions in CF are not essentially different from those in classic immune complex diseases.\textsuperscript{75} However, the strict limitation of bacterial infection to the lung\textsuperscript{67} and the peculiarity of the bacterial antigen\textsuperscript{76} distinguish CF from other immune complex diseases. The microcolonies built up by the mucoid exopolysaccharide,\textsuperscript{77} in which huge numbers of \textit{P. aeruginosa} multiply, are far larger than a single PMN, and “frustrated phagocytosis”\textsuperscript{78,79} is thought to contribute significantly to PMN enzyme release. It is intriguing to speculate that such a microcolony, distributed focally in a damaged area of the airways, is one huge immune complex that cannot be engulfed because of its size. As a consequence, local inflammation is severe. In advanced states of chronic lung infection in CF, more than 99 percent of the cells are PMNs, which may reach cell numbers of about 10\textsuperscript{7}.\textsuperscript{10,11,12} Mean values of about 100 μg of PMN elastase and 138 μg of myeloperoxidase per milliliter of sputum have been found in 15 CF patients.\textsuperscript{43} These enzyme levels correlate well with the total enzyme content of 10\textsuperscript{10} PMNs,\textsuperscript{44} indicating that PMNs in the infected lungs of CF patients are highly activated. About 90 percent of endogenous α,-proteinase inhibitor is locally inactivated by PMN elastase\textsuperscript{44} and probably also by oxidative attack.\textsuperscript{79} A considerable imbalance between proteases and proteinase inhibitors is therefore present in the inflamed lungs of these patients.\textsuperscript{44-50} Apart from oxidative damage,\textsuperscript{44} this imbalance is thought to be the primary cause of lung tissue destruction in CF.\textsuperscript{44} Evidence has been presented for elastin cleavage products in urine, and active elastolysis in the lungs of infected CF patients which correlated with the severity of lung disease.\textsuperscript{43}

PMN activation in chronic inflammatory states may be regulated by PMN elastase in a feedback mechanism,\textsuperscript{44,45,46} thus considerably reducing the release of dangerous lysosomal enzymes and oxygen radicals. Sputum immune complex levels and PMN elastase activities have been shown to fluctuate, reciprocal changes of the two parameters being observed.\textsuperscript{46} This regulatory mechanism may explain the observation that immune complexes are not always found in CF patients, since immune complex detection depends largely on the presence of the functionally intact Fc portion of the immunoglobulins.\textsuperscript{46} Thus, PMN elastase cleavage may lead to temporary unresponsiveness of PMNs, another possible reason for the chronicity of the infections. Temporary immunologic unresponsiveness may also result from PMN elastase–mediated receptor cleavage on lymphocytes. It was recently shown that PMN elastase cleaves surface receptors on normal human T-cells.\textsuperscript{64} Cleavage of the antigen receptor in particular may have implications for the immunologic status of the host.

Although many questions concerning the immunopathology of CF remain unanswered, considerable progress in the understanding of host-parasite interactions has been achieved. The importance of host factors in the progressive pathologic processes in the respiratory tract of these patients is reflected in the current approach of combining anti-inflammatory therapy with effective antibiotic treatment.\textsuperscript{8} Optimizing therapeutic approaches to bacterial infections will continue to be of the utmost importance even after elucidation of the primary molecular defect.

\section*{References}

4. Hancock REW, Mutharia LM, Chan L, Darveau RP, Speert DP.
Pulmonary Infection and Antibiotic Treatment in Cystic Fibrosis


17 Bryan LE, Kureishi A, Rabin HR. Detection of antibodies to Pseudomonas aeruginosa, alginate extracellular polysaccharide in animals and cystic fibrosis patients by enzyme-linked immunosorbent assay. J Clin Microbiol 1983; 18:276-78


23 Pier GB, Ames P. Mediation of the killing of rough, mucoid isolates of Pseudomonas aeruginosa from patients with cystic fibrosis by the alternative pathway of complement. J Infect Dis 1984; 150:233-238


28 Que PC, Wannemaker LW. Serum antibodies in staphylococcal disease. Pediatrics 1964; 33:63-70


30 Möller G, Landwall F. The polyclonal B-cell activating property of protein A is not due to its interaction with the Fe part of immunoglobulin receptors. Scand J Immunol 1977; 6:357-59


41 Goldstein W, Döring G. Lysosomal enzymes and proteinase inhibitors in the sputum of patients with cystic fibrosis. Am Rev Respir Dis 1986;


44 Folds JD, Prince H, Spitznagel JK. Limited cleavage of human

Downloaded From: http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21582/ on 06/21/2017
immunoglobulins by elastase of human neutrophil polymorphonuclear granulocytes. Lab Invest 1978; 38:313-31


53 Berger M, Sorensen RU, Dearborn DG, Döring G. Complement receptor expression on neutrophils at an inflammatory site, the Pseudomonas-infected lung in cystic fibrosis (unpublished)


58 Pressler T, Mansa B, Jensen T, Pedersen SS, Halsby N, Koch C. Increased IgG2 and IgG3 concentration is associated with advanced Pseudomonas aeruginosa infection and poor pulmonary function in cystic fibrosis. (unpublished)


60 Moss RB, Hsiu Y-P, Lewiston NJ, deLange G. Impaired natural IgG2 antibody response to polysaccharide antigens and decreased Km(1)-A2m(2) allotypes in cystic fibrosis. Pediatr Res 1987; 2:4940


66 Hancock REW, Movat ECA, Speert DP. Quantitation and human identification of antibodies to outer membrane proteins of Pseudomonas aeruginosa in sera of patients with cystic fibrosis. J Infect Dis 1984; 149:220-26


80 Goldstein IM. Polymorphonuclear leukocyte lysesomes and tissue injury. Prog Allergy 1976; 20:301-40


82 Kharazi A, Rechnitzer C, Schietz PO, Jensen T, Baek L, Halsby
Discussion

R. van Falth, M.D., Ph.D.*

Host defenses against respiratory infections include non-immunologic mechanisms such as intact barrier-forming mucosal membranes, optimal ventilation, secretion of mucus, and ciliary movement, as well as immunologic mechanisms provided by antibodies (secretory IgA, IgG, and IgM), complement, and phagocytic cells (e.g., granulocytes and alveolar macrophages). Patients with CF frequently suffer from respiratory tract infections, but can handle infections at other sites of the body normally. This indicates that host defense is not generally impaired, unlike that of, for example, patients with hypogammaglobulinemia or agammaglobulinemia, who also have frequent respiratory tract infections.

It is remarkable that respiratory infections in patients with CF are caused mainly by H influenzae, S aureus, and P aeruginosa, whereas the incidence of infections with other bacteria, Mycoplasma pneumoniae, and viruses is not higher in this group than in the general population. Several conditions favor the development of infections by these three species of bacteria in patients with CF. A central factor is the abundant production of abnormal and gelatinous sputum. The abnormal composition of the mucus promotes adherence of bacteria, makes the sputum difficult to cough up, and may even enhance the growth of bacteria via some unknown mechanism.14 Reports on the functioning of the mucociliary transport system in CF patients are conflicting. Both normal ciliary function14 and reduced mucociliary transport14 have been reported. But very little is known about the precise mechanisms that promote infection by H influenzae, S aureus, and P aeruginosa.

With respect to the immunologic host defense mechanism, no specific deficiencies in the serum concentrations of immunoglobulins and specific antibodies to infecting bacteria have been found in CF patients, nor do the respiratory secretions of secretory IgA, IgG, and IgM show any deficiency.11 The functional activity of the classic and alternative pathways of the complement system is normal, although increased, normal, and decreased values have been reported for the concentrations of complement factors in the serum of CF patients.18 It is not known, however, whether sufficient functionally active complement is present on the mucosa of CF patients.

Serum of CF patients has been reported to contain a factor that inhibits phagocytosis of P aeruginosa by alveolar macrophages13-16 but not by peripheral blood monocytes and granulocytes.17 Reports on the nonopsonic and opsonic phagocytosis of nonmucoid and mucoid Pseudomonas strains by blood granulocytes and monocytes are not in complete agreement, but it is generally accepted that the functioning of these cells is not disturbed.

No information is available on the function of alveolar macrophages in CF patients. It is conceivable that the endocytic function of not only these cells, which derive from circulating monocytes,18 but also of granulocytes is impaired. Recent studies in our laboratory have shown that optimally opsonized bacteria of various species (e.g., S pneumoniae and S aureus) are ingested significantly less efficiently by alveolar macrophages than by peritoneal macrophages of normal mice. Furthermore, intracellular killing of ingested bacteria (e.g., S epidermidis) by alveolar macrophages was totally or almost absent, whereas peritoneal macrophages killed these bacteria adequately (Nibbering PH, et al, unpublished data). Since alveolar macrophages contain large amounts of surfactant in their cytoplasm, this substance was thought to inhibit the endocytic functions of alveolar macrophages. We attempted to produce artificial alveolar macrophages by incubating mouse peritoneal macrophages and human blood monocytes with concentrated sheep surfactant. In preliminary studies the endocytic functions (phagocytosis and intracellular killing) of both kinds of cells were found to be impaired with respect to S epidermidis.

Taken together, the results of these experiments indicate that surfactant inhibits the function of macrophages. It is possible that this mechanism also operates—but much more strongly—in CF patients, in whom it inactivates monocytes, and perhaps also granulocytes, that migrate to the respiratory tract and lungs. In CF patients not only surfactant but also substances in the sputum may have a depressive effect on phagocytic cells.

REFERENCES

9 Talamo RL, Stiehm ER, Schwartz RH. Immunological aspects

*Department of Infectious Diseases, University Hospital, Leiden, The Netherlands.