

Pseudomonas aeruginosa infection in cystic fibrosis and its management

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Cystic fibrosis is the most prevalent of the fatal inherited diseases in white populations. It is characterised by the triad of chronic pulmonary disease, pancreatic insufficiency, and increased concentrations of electrolytes in sweat. Most patients are treated in specialised centres and at least 40 000 are alive in Europe and the United States. Until recently cystic fibrosis was exclusively a childhood disorder (and indeed most patients are still treated in paediatric clinics), but more aggressive treatment regimens in children has resulted in prolonged survival and an improved quality of life. A median survival of 25–30 years is now common and an understanding of the molecular pathology of cystic fibrosis is likely to lead to further improvements in treatment and survival. Respiratory physicians will therefore be concerned increasingly in the care of adult patients with cystic fibrosis and the management of their chronic lung infections, the most important of which is caused by *Pseudomonas aeruginosa*.

A detailed understanding of the pathogenesis of chronic *P aeruginosa* infection is important for the development of preventive and curative strategies that may prolong and improve the quality of life. A proposed pathogenesis of this infection based on published data is outlined in table 1. *P aeruginosa* infection of the lung may usefully be divided into stages to define the major factors responsible for the progression of infection.¹ The major stages in the course of an infection are acquisition of the bacteria; bacterial adhesion to the surface of the host tissue; bacterial proliferation and invasion, leading to tissue damage and clinical symptoms; recruit-

ment of non-specific and specific immunological defence mechanisms; and, if the patient survives, elimination of the bacteria or establishment of chronic infection.

Acquisition of the bacteria

Patients with cystic fibrosis have various recurrent and chronic lung infections from early in life and most will eventually acquire chronic *P aeruginosa* infection of the lung.² Respiratory syncytial virus infection may predispose to chronic *P aeruginosa* infection, but this is not a feature in most patients.³ Infected patients do not spread *P aeruginosa* to family members not suffering from cystic fibrosis, but siblings with cystic fibrosis often carry the same strain of *P aeruginosa*, indicating cross infection or colonisation from the same environmental source.^{2,4} Studies from holiday camps for patients with cystic fibrosis show that the risk of cross infection is low.⁵ Many patients are treated in specialist centres and larger centres appear to have a higher prevalence of *P aeruginosa* infection than smaller ones.² The role of treatment centres in cross infection is not clear, as cross infection has occurred in some centres but not in others.^{2,6,7} The calculated probability/day/patient of contracting chronic *P aeruginosa* infection was 0.73–1.54% in the Danish Cystic Fibrosis Centre for 1970–80, which is equal to an annual incidence of 10–20%. This is a low level of communicability, but because treatment at the centre is life long this means that nearly all adult patients will develop chronic *P aeruginosa* infection.² Such cross infection has now been prevented by separating non-infected from infected patients in the clinic and wards. When such measures were introduced the annual incidence of new chronic *P aeruginosa* infection was reduced to the “natural background” level of infection, which is of unknown origin and assumed to be 1–2% a year.²

Bacterial adhesion

Whether colonisation of the upper respiratory tract precedes establishment of bronchial infection with *P aeruginosa* is unknown. In animal studies *P aeruginosa* adheres to buccal, nasal turbinate, and tracheobronchial epithelial cells and to mucus.⁸ Three types of adhesion factors have been identified on *P aeruginosa*—

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Table 1 Pathogenesis of chronic *Pseudomonas aeruginosa* infection in cystic fibrosis

Stage of infection	Mechanisms and pathogenesis	Clinical signs
Acquisition	Cross infection Concomitant virus infection	None Acute exacerbation
Attachment	Pili, alginate, haemagglutinin	None
Initial persistent colonisation	Bacterial toxins: elastase, alkaline protease, exotoxins A and S, etc	None or minimal
Chronic infection	Persistence: microcolonies embedded in alginate Tissue damage: immune complexes	Chronic suppurative lung inflammation, progressive loss of lung function
Modifying mechanisms	Cleavage of immune complexes by polymorphonuclear leucocyte elastase, IgG ₂ and IgG ₃ subclass responses to <i>P aeruginosa</i>	Individual clinical course of the infection

pili, alginate, and possibly haemagglutinin.⁸⁻¹⁰ The corresponding receptors on the host cells have been identified as glycolipids and glycoproteins containing lactosyl and sialosyl residues.⁸ Injury to epithelial cells of mucus membranes by trypsin or human leucocyte elastase exposed new receptors for pili, and increased bacterial adhesion.^{8,10} These observations are interesting because such damage may follow inflammatory reactions provoked by viruses or by bacteria, such as *Staphylococcus aureus*, which are common in cystic fibrosis before *P aeruginosa* infection.³

Initial persistent colonisation

P aeruginosa produces many toxins that in animal experiments cause tissue damage and enhance bacterial persistence (table 1).^{11,12} The interaction of the proteolytic enzymes alkaline protease and elastase with human defence mechanisms have been extensively investigated.¹³

Antiproteases such as α_1 antitrypsin and α_2 macroglobulin are present in the circulation, but α_1 antitrypsin is non-functioning in airways secretions and α_2 macroglobulin is not present.¹⁴ Pseudomonal elastase cleaves human transferrin, but not lactoferrin, releasing iron needed for bacterial growth.¹⁵ This is probably why *P aeruginosa* does not initially express iron regulated outer membrane proteins in the lungs of patients with cystic fibrosis.¹⁶ Later, when antibody levels against the proteases rise, iron regulated proteins are expressed by *P aeruginosa*.¹⁶ Pseudomonal proteases inhibit the function of phagocytes and natural killer and T lymphocytes by cleaving the CD4 molecule on T helper cells, inhibiting interleukin-1 and interleukin-2 activity, cleaving immunoglobulins, and inactivating complement components.¹³ The proteases of *P aeruginosa* are present in the lungs of patients

with cystic fibrosis; when neutralising antibodies develop, however, free protease activity cannot be detected, though proteases are present in immune complexes.¹⁷ It is therefore not surprising that antibodies to the proteases develop later than antibodies to other antigens of *P aeruginosa*. They are usually not found until 9–12 months after the onset of chronic infection.¹⁸

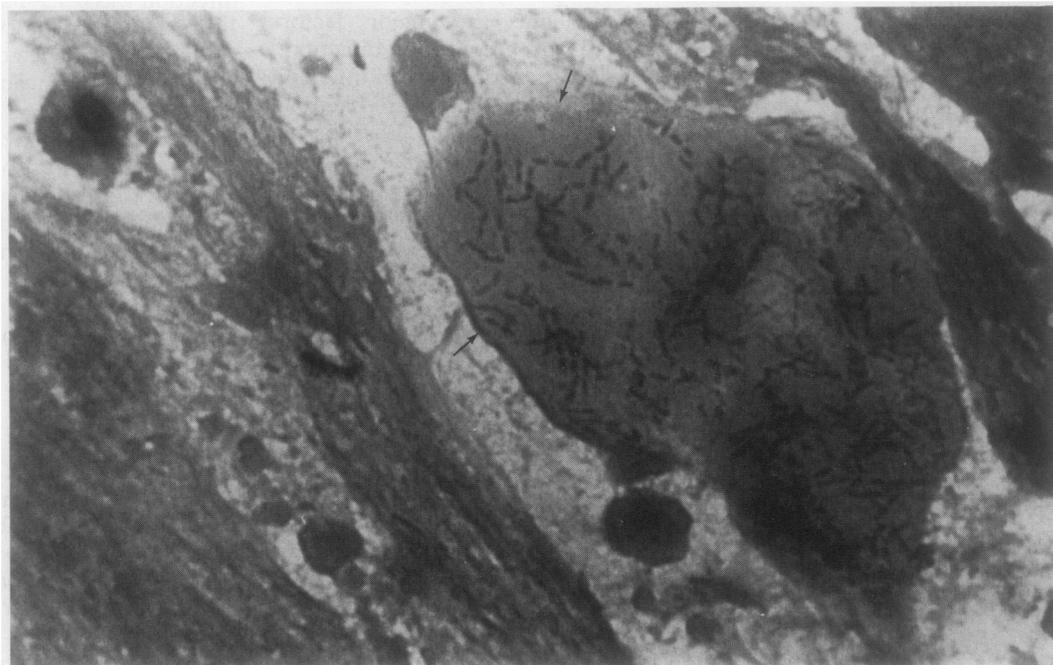
The proteases of *P aeruginosa*, and possibly toxins such as exotoxin A and S, may be important in the establishment of the initial colonisation by preventing the host from mounting a local immune response and inhibiting non-specific defence mechanisms.^{11,12,13}

Chronic infection

The course of chronic *P aeruginosa* infection varies greatly in individual patients; some die after only a few years of infection, whereas other patients tolerate the infection for 15–20 years.¹⁹ The reason for this variation is not clear, but interacting mechanisms between the host and bacteria have been described and are active during infection.

Chronic *P aeruginosa* infection in cystic fibrosis is characterised by a pronounced antibody response whereas no increase in cell mediated immunity is detectable.^{1,20,21} High or rapidly rising titres of antibody against *P aeruginosa* are associated with a poor prognosis.²⁰ The antibodies belong to all the major immunoglobulin classes and show specificities for all the major toxins and antigens of *P aeruginosa*, including alginate.^{1,20,22} Specific antibodies to *P aeruginosa* are present in the blood and respiratory secretions of patients with cystic fibrosis.²¹ The antibody response does not lead to the elimination of *P aeruginosa* from the lungs and the function of the antibodies has been reported to be blocking rather than opsonising or lysis

Gram-stained smear of sputum from a cystic fibrosis patient. A mucoid microcolony (arrows) of *Pseudomonas aeruginosa* and some polymorphonuclear leucocytes are seen.



promoting.^{1 20 23-25} The major reason why *P aeruginosa* persists in the lungs appears to be its ability to produce alginate containing microcolonies.²⁶ In the natural state most bacteria are present in a sessile form in biofilms or in microcolonies adhering to surfaces and a few are in the freely motile planktonic form. The microcolony mode of growth protects the bacteria against hostile environments and the same strategy is used by *P aeruginosa* in the lungs of chronically infected patients with cystic fibrosis. Alginate has been shown to inhibit phagocytosis²² and biofilm bacteria to stimulate a lesser oxidative burst response by neutrophils than free swimming bacteria can stimulate.²⁷ In addition, alginate may reduce the action of some antibiotics.²⁸ Although antibodies to alginate are produced in patients with cystic fibrosis, this does not lead to elimination of the chronic infection.^{20 22}

As neutralising antibodies to the toxins of *P aeruginosa* are produced in chronically infected patients, the tissue damage produced by such toxins is difficult to explain.^{1 20} Many reports of immune complexes in the peripheral blood and sputum of chronically colonised patients have appeared, such complexes being associated with poor prognosis.¹ Immune complexes generate a continuous inflammatory response, dominated by the neutrophil, in the lungs of colonised patients and the release of leucocyte proteases, myeloperoxidase, and oxygen radicals is the main mechanisms underlying the lung injury.^{1 14 21 29} The presence or absence of *P aeruginosa* antibodies and immune complexes has been used to construct a clinically relevant staging system for infected patients.³⁰

The two classical ways of preventing infections are vaccination and separation of susceptible and infected patients. Vaccination of non-infected patients with a polyvalent *P aeruginosa* vaccine has been tried without success; there was in fact a trend to more rapid deterioration in the vaccinated patients.³¹ This is not surprising given the immune complex mediated tissue damage that occurs during

chronic *P aeruginosa* infection.¹ As separation of non-infected from infected patients in cystic fibrosis clinics effectively reduced the incidence of new chronic *P aeruginosa* infection in the Danish Cystic Fibrosis Centre² this may be of value in larger cystic fibrosis centres.

Chronic *P aeruginosa* infection in most patients is preceded by a period of intermittent colonisation.³² During this period treatment with colomycin by inhalation may prevent development of chronic infection.³³ Combination of colomycin inhalation with oral ciprofloxacin in patients with early *P aeruginosa* infection is currently being studied in the Danish Cystic Fibrosis Centre, with encouraging early results. The combined effects of preventing cross infection and early intensive treatment of initial *P aeruginosa* colonisation will, we hope, result in a much delayed onset of chronic lung infection in patients with cystic fibrosis.

Management of chronic infection

Chronic *P aeruginosa* infection, unfortunately, cannot be eradicated. Treatment "on demand" for acute exacerbations of pulmonary symptoms is used in many centres, but the response to treatment may be disappointing as some acute exacerbations may be precipitated by virus infections.^{3 34 35} Chronic *P aeruginosa* infection can be treated intensively with antibiotics, so that near normal lung function may be maintained for many years. The principles of this "maintenance treatment" or "chronic suppressive treatment" are based on the observation that lung function improves during antibiotic treatment and that this effect is still detectable one to two months after completion of treatment.^{19 36} The principle is therefore to restore lung function repeatedly by regular two week courses of intensive intravenous treatment every three months in the cystic fibrosis centre. Treatment is intensified in patients with unstable clinical conditions by adding daily inhalations of colomycin between the courses of intravenous antibiotics and, sometimes, by administering oral ciprofloxacin in addition during these intervals. These principles of maintenance treatment have been used since 1976 in the Danish Cystic Fibrosis Centre, and some patients have now received more than 40 two week courses of anti-pseudomonas treatment.³⁷ The antibiotics used and their dosages are given in table 2. Many antibiotics have altered pharmacokinetics in patients with cystic fibrosis and the dose of agents such as tobramycin exceeds that recommended for other patients.^{19 36 38 39} Individualised dosing regimens are recommended, based on serum concentrations, as better results are produced by such a strategy.³⁸ If admission to hospital for treatment is not possible treatment with inhaled aminoglycosides and β lactam antibiotics or oral ciprofloxacin (or all of these) is an alternative.⁴⁰⁻⁴²

The results of treatment are encouraging because survival has improved substantially. More than 90% of patients survive 10 years of chronic pseudomonas infection whereas

Table 2 Antibiotics commonly used to treat chronic *Pseudomonas aeruginosa* infection in cystic fibrosis

Drug	Route	Dosage mg/kg/24 h
Tobramycin in combination with Piperacillin or Cefsulodin or Ceftazidime or Aztreonam or Thienamycin and Colistin	Intravenous	10-20 (-30)* 300 150-200 150-200 150-200
	Inhalation	50-75 Total dose: 2-4 million units (1 mg = 30 000 units)
and/or Ciprofloxacin	Oral	25-50

Probenecid is given orally to all patients receiving beta-lactam antibiotics eliminated by tubular excretion to achieve higher concentrations for prolonged periods of time.

*The dosage is adjusted to give a trough concentration in serum of 1-2 μ g/ml.

previously only 54% survived five years.^{19 37} It is, however, an expensive mode of treatment with some side effects. Many patients become allergic to some of the β lactam antibiotics, and *P aeruginosa* often develops resistance to some antibiotics.³⁷ Few side effects of tobramycin treatment have been recorded despite the repeated high dose regimen over many years.³⁷ The psychological impact of the very intensive treatment and the separation of the various groups of patients with cystic fibrosis have caused some concern, but the experience of the Danish Cystic Fibrosis Centre is that most patients accept such treatment.

In the light of our understanding of the immunopathology of chronic *P aeruginosa* infection the use of anti-inflammatory drugs in patients with cystic fibrosis seems rational; but as most patients are children, and some also have diabetes mellitus or pulmonary hypertension, the risk of side effects must be considered. A controlled trial in patients with or without *P aeruginosa* infection showed significant benefits of alternate day (2 mg/kg body weight) prednisone treatment for four years without serious side effects.⁴³ Further studies are in progress and will, we hope, further improve the treatment of patients with cystic fibrosis and chronic *P aeruginosa* lung infection.

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