Lung infections · 3

Series editor: S L Hill

Pseudomonas aeruginosa and other related species

Robert Wilson, Ruth B Dowling

Pseudomonas aeruginosa was first obtained in pure culture by Gessard in 1882 from cutaneous wounds which had a blue green discolouration[1] and is the major human pathogen from a large genus of strictly aerobic Gram-negative rods which are widely distributed in nature.[2] The majority of *P aeruginosa* strains produce at least two pigments, a fluorescent yellow pigment and a blue pigment called pyocyanin, which together give the characteristic colour noted above when the bacterium is grown on agar.[3] *P aeruginosa* is motile by means of a single flagellum and thrives in moist environments; it is extremely versatile biochemically and can grow in many habitats including soil, surface waters, plants and various foods such as vegetables eaten by man.[2 4] In hospitals *P aeruginosa* can be found in sinks, respirators, humidifiers, etc, and is occasionally found on the hands of medical personnel.[2]

P aeruginosa is an opportunistic pathogen which only causes disease in patients with impaired host defences. The patient's defences may be generally weakened by debility or cancer, or there may be specific humeral or cellular defects. Neutropenic patients are especially susceptible to pseudomonas infection and to subsequent septicaemia. Alternatively, the body's defences may be specifically breached as in corneal ulceration or skin burns, or artificially overcome as with assisted ventilation or by an indwelling urinary catheter.[4] Patients with bronchiectasis are particularly prone to chronic infection, and delayed mucociliary clearance may be responsible.[5 6] The use of broad spectrum antibiotics may kill commensal flora or more antibiotic-sensitive pathogenic species causing infection, and promote colonisation by the intrinsically resistant pseudomonas.[7] *P aeruginosa* is particularly associated with progressive and ultimately fatal chronic respiratory infection in cystic fibrosis. Clues about the biological basis of this host-bacterial interaction which occurs almost inevitably are just being discovered. In this review we will only cover chronic airway infections, although some of the information is relevant to acute pneumonia and septicaemia which are most commonly seen in immunocompromised patients.

Two other pseudomonas species which cause disease in humans will be mentioned briefly. *P (Burkholderia) cepacia* is a distant relation of *P aeruginosa* and was first described as a cause of soft rot in onions. It is ubiquitous in the environment and is frequently found in association with soil, water and plants. Like *P aeruginosa*, it is virtually non-pathogenic in healthy people, but it can cause disease in those with reduced host defences, and it has been recognised as an important pathogen in cystic fibrosis.[8] *P cepacia* may be isolated alone or together with *P aeruginosa*. This may lead to problems in isolating *P cepacia* because *P aeruginosa* rapidly outgrows it on agar unless selective media are used.[8] *P pseudomallei* is widely distributed in the soil and water of rice paddy fields and causes melioidosis, which is a major cause of death from community acquired septicaemia in Thailand and is endemic throughout south east Asia and northern Australia.[9]

Epidemiology

Although the initial isolation of *P aeruginosa* from sputum may be intermittent in cystic fibrosis and other forms of bronchiectasis, once chronic infection is established it is rarely possible to eradicate it even with intensive antibiotic therapy.[5 6 10 11] A number of longitudinal bacteriological studies of cystic fibrosis patients have shown that most of them harbour the same *P aeruginosa* clone for many years.[12–14] Once a particular clone has colonised the lung DNA fingerprinting may reveal shifts in the macrorestriction fragment patterns, indicating subclonal variation, which may result from sequence alterations in restriction recognition sites, genomic rearrangements, and incorporation of extrachromosomal DNA—for example, from bacteriophages.[15] Available evidence suggests that acquisition of *P aeruginosa* is commonly from the environment, but that patient to patient spread can occur particularly if contact density is high such as can occur at cystic fibrosis centres and recreation camps.[15–17]

P cepacia can cause respiratory tract infection in cystic fibrosis,[8 18] although it is much less common in non-cystic fibrosis bronchiectasis. Strains are usually very antibiotic resistant and have in some studies been associated with rapid clinical deterioration,[1 8 19] although this is not always the case.[19 20] Anxiety has also been increased by reports of cross-infection between patients[21–23] although not all studies have found evidence of this.[24] Nevertheless, some centres

Host Defence Unit, Imperial College of Science, Technology and Medicine, National Heart and Lung Institute, London SW3 6LR, UK
R Wilson
R B Dowling

Correspondence to:
Dr R Wilson.

Table 1 Virulence factors of Pseudomonas aeruginosa

Virulence factor	*Biological action*
Mucoid exopolysaccharide (alginate)	Adherence to epithelium; barrier to phagocytes and antibiotics; inhibits antibody and complement binding
Protease enzymes	Tissue damage; epithelial cell tight junction separation; degrade fibronectin; cleave antibodies creating non-functional blocking antibodies; inactivate α_1-antiproteinase, complement components and cytokines; cleave C3b receptors from neutrophils; stimulate mucus secretion
Exotoxin A	Cytotoxic by inhibiting protein synthesis; toxic to macrophages; T cell mitogen; inhibits granulocyte and macrophage progenitor cell proliferation
Lipopolysaccharide	Dominant antigenic determinant on cell surface; loss of sugar unit side chains during chronic infection creates "rough" LPS and serum sensitivity; less potent endotoxin properties than other Gram-negative species
Pigments eg. pyocyanin, 1-hydroxyphenazine, pyoverdin	Inhibit ciliary beat; siderophores; toxic to other bacterial species and human cells; enhance oxidative metabolism of neutrophils; inhibit lymphocyte proliferation
Phospholipase C	Haemolysis; tissue damage; destroy surfactant
Rhamnolipid	Haemolysis; inhibit ciliary beat; stimulate mucus secretion, affect ion transport across epithelium
Pili	Adherence to epithelium
Lipase	Tissue damage
Histamine	Impair epithelial integrity
Exoenzyme S	Adherence to epithelium; cytotoxic
Leukocidin	Cytotoxic to neutrophils and lymphocytes

Compiled from references 2, 3, 10, 11, 25, 26, 31–35, 41, 42, 49, 64, 68, 78, 81, 82.

have segregated patients carrying *P cepacia*. The benefits of such a policy need to be clearly defined because of the psychosocial implications of segregation and further epidemiological data are urgently needed.[8 20]

Bacterial pathogenesis

P aeruginosa does not cause infection in the absence of impaired host defences, yet a wide array of potential virulence factors have been described which may contribute to its pathogenicity in the compromised patient. A review of the literature is summarised in table 1. The failure of the bacterium to infect the healthy lung—or even the mildly compromised defences of, say, a patient with chronic bronchitis—means that no single virulence factor is by itself that potent, but that the whole array should be viewed as contributing to the "pathogenic personality" of the bacterium. Once colonisation of the airways is established, *P aeruginosa* is rarely eliminated despite an exuberant host inflammatory response.[6 25]

The mucoid form of *P aeruginosa* produces large amounts of an extracellular polysaccharide called alginate, and this form accounts for up to 90% of isolates from patients with cystic fibrosis.[2 26] Typically, the first time that *P aeruginosa* is isolated it is non-mucoid but after a variable period, often one or two years, it becomes mucoid. Although patients infected by mucoid strains tend to have worse lung function and nutritional state,[26] it is not clear that a shift to the mucoid phenotype is responsible. The mucoid character is chromosomally encoded and is probably selected for by the in vivo environment including sublethal concentrations of antibiotics.[2] The mucoid phenotype is also seen in other chronic infections such as non-cystic fibrosis bronchiectasis and the urinary tract.[2]

The attachment of bacteria to mucosal surfaces is considered an important event in the pathogenesis of most infectious diseases. In a histological study of the lungs of patients with cystic fibrosis infected by *P aeruginosa*, most bacteria associated with secretions were intraluminal, while adherence to the epithelial surface occurred when there was cell damage or exposure of underlying connective tissue.[27] The importance of epithelial damage (fig 1) in facilitating *P aeruginosa* adherence has been noted in numerous studies, and the bacterium does not seem to adhere to normal epithelium.[28] Cell damage might remove defence mechanisms such as ciliary beating which would otherwise protect the epithelium,[3] and also expose new receptors for bacterial adhesins on damaged cells, on newly exposed surfaces, and on cells that grow to repair the damage.[28–30] Pili have been identified as an important adhesin of *P aeruginosa*[31 32] but do not account for all the adhesive properties and other adhesins such as a protein linked with flagellar biosynthesis,[33] exoenzyme S,[34] and alginate[35] have been identified.

P aeruginosa has a high affinity for human tracheobronchial mucus in vitro, mucus of organ cultures (fig 1) and in the airways.[27 28 36] Bacterial adherence to mucus probably involves both specific and non-specific interactions.[37–40] *P aeruginosa* proteases and rhamnolipid also stimulate mucus production.[41 42] In organ cultures *P aeruginosa* grows as continuous sheets over the mucus surface[28] and it has been shown that growth in such biofilms is resistant to opsonophagocytic killing by neutrophils.[43 44] *P aeruginosa* adherence to mucus, and its lack of adherence to normal epithelium, may explain why it does not infect the normal airway which has efficient mucociliary defences. However, mucociliary clearance is slow in patients with cystic fibrosis[45] and other forms of bronchiectasis,[46] allowing *P aeruginosa* to colonise mucus which is poorly cleared, giving the bacterium time to produce toxins that establish the infection.

There is a special association between cystic fibrosis and *P aeruginosa*, and infection can occur in patients with cystic fibrosis and bronchiectasis before there is significant damage to the lung.[47 48] The recent discovery of cystic fibrosis transmembrane conductance regulator (CFTR) has begun to lead to an understanding of why this might be.[49] Cystic fibrosis epithelial cells in primary culture bind approximately

Figure 1 Tropism of Pseudomonas aeruginosa for mucus and damaged epithelial cells in a human respiratory tissue organ culture (magnification ×3000). P aeruginosa does not adhere to the normal epithelial cells.

twice the number of *P aeruginosa* that bind to normal cells,[50] and subsequent work has suggested that this is due to alteration in the number of receptors for *P aeruginosa* adhesins on the cell surface[51] which in turn is influenced by CFTR.[52] The type of defect in CFTR correlates both with the age at colonisation[53] and the extent of binding of *P aeruginosa* to the epithelial cells of patients with cystic fibrosis.[54] *P aeruginosa* binds to the glycolipids asialoganglioside 1 (aGM_1) and aGM_2, but not to the sialylated homologues,[55] although some strains may bind to sialylated residues by non-pilus adhesins.[49 56] Glycosylation and sulphation of superficial glycoconjugates may be altered in cystic fibrosis, perhaps as a consequence of abnormal CFTR function.[57] Thus, *P aeruginosa* may bind with increased affinity to cystic fibrosis cells and also their secretions[58] because of altered glycosylation. Very recently CFTR has also been implicated in the uptake of *P aeruginosa* by cultured human airway epithelial cells.[61] Cells expressing the ΔF508 allele of CFTR were defective in the uptake of bacteria. The clinical relevance of this in vitro observation is as yet unclear, but it is hypothesised that ingestion of bacteria by airway epithelial cells followed by cellular desquamation may protect the lung from infection. An exciting recent observation is that airway epithelial cells produce an antibacterial peptide, human β-defensin-1, which kills *P aeruginosa* and other bacterial species. The peptide is inactive in the abnormally high NaCl concentrations that may be found in the airway surface fluid of the lung in patients with cystic fibrosis, which may explain their susceptibility to bacterial infection.[59 60]

Lung damage by inflammatory processes

In cystic fibrosis and most other forms of bronchiectasis there is an exuberant inflammatory response to chronic bacterial infection of the airways.[6 25] Large numbers of activated neutrophils are attracted into the airway lumen by host—for example, C5a, LTB4, IL-8—and bacterial chemotaxins.[62] There is a strong antibody response in serum, saliva, and pulmonary secretions to many pseudomonas antigens[63 64] and cystic fibrosis patients with chronic *P aeruginosa* infection have high levels of circulating immune complexes[65] which are also found in sputum.[66] There is a strong correlation between severity of lung disease and the titre of anti-pseudomonas antibodies.[67] This inflammatory response prevents systemic spread of infection but fails to eradicate it from the airways.[6 10 11 25]

Chronic inflammatory processes cause damage, both to the epithelium[68] and to the structural proteins of the lung,[69] which is probably more serious than the damage caused by the bacterium itself. This concept is supported by the observations that cystic fibrosis patients with hypogammaglobulinaemia have significantly less severe lung disease than do patients with normal or elevated levels of immunoglobulins,[70] and that immunosuppressive agents can benefit patients with cystic fibrosis.[71]

Activated neutrophils do not differentiate between bacteria and bystander lung tissue. They spill proteinase enzymes[68] and oxygen radicals[72] which, because of the number of neutrophils present, overwhelm the ability of the lung defences to neutralise them. The epithelial damage that ensues, together with stimulation of mucus production by proteinase enzymes,[73] promotes continued bacterial infection and more inflammation. *P aeruginosa* produces a low molecular weight factor which stimulates the production of the powerful neutrophil chemoattractant IL-8 from epithelial cells.[74] Neutrophil elastase in secretions may itself attract more neutrophils into the airway lumen by inducing IL-8 production from epithelial cells[75] and impairs opsonophagocytosis by cleavage of complement receptors from neutrophils and complement components from bacteria.[76 77] Thus, a self-perpetuating "vicious circle" of events is generated.[6]

High levels of granulocyte elastase have been found in the sputum of patients with cystic fibrosis and bronchiectasis in several studies.[78] Older patients with cystic fibrosis, those colonised by *P aeruginosa*, and those with advanced disease have higher levels than younger patients, those not colonised by *P aeruginosa*, and patients in good clinical condition.[77] However, younger patients with cystic fibrosis with good lung function still have raised elastase levels in secretions and signs of ongoing infection and inflammation.[79] DNA released by degenerating white cells makes secretions more viscous[79 80] and difficult to clear. *P aeruginosa* toxins may enhance the damage caused by inflammation—for example, by inactivating α_1 antiproteinase[81] or enhancing neutrophil oxidative metabolism.[82] The relative

Table 2 Antibiotics used against Pseudomonas aeruginosa

Category	*Examples*	*Comment*
Carboxypenicillins	Ticarcillin	Greater antipseudomonas activity and less sodium load than carbenicillin
	Temocillin	6α-methoxy substitution gives long half life and more resistance against β-lactamase enzymes
Ureido and piperazine penicillins	Azlocillin	Acyl derivative of urea as side chain
	Piperacillin	Piperazine side chain; not used in cystic fibrosis because of adverse reactions
Cephalosporin	Ceftazidime	Third generation
Aminoglycosides	Gentamicin, tobramycin, amikacin	Toxicity of aminoglycosides is based on accumulation, major side effects are on ear and kidney. Measure serum trough and peak levels at third dose, and regularly afterwards
Quinolone	Ciprofloxacin	Only oral antipseudomonal antibiotic
Monobactam	Aztreonam	Narrow spectrum of action; Gram-positive superinfection may be a problem if used alone
Carbapenem	Meropenem, imipenem	Members of a new class of β-lactam called the thienamycins; imipenem has to be combined with inhibitor cilastatin to block renal metabolism, but meropenem is stable to the renal enzyme
Beta lactamase inhibitor	Tazocin	Piperacillin and tazobactam
Polymyxin	Colomycin	Usually given by inhalation because of possible side effects when given parenterally

Compiled from references 2 and 7.

importance of different bacterial mechanisms which either inactivate or enhance the inflammatory response may change depending on the stage of the infectious process. The former might be more important early in the infectious process when the host defences are relatively intact, while the latter might predominate as airway damage increases and chronic bacterial infection is established.

Whilst the major inflammatory cell in the airway lumen of patients with chronic bacterial infection is the neutrophil, mononuclear cells predominate in the bronchial wall.[83] Many of these are T cells with the suppressor phenotype and may represent a secondary response to continued bacterial infection. However, mononuclear cells probably play an important part in the orchestration of the "vicious circle". High levels of a number of cytokines have been measured in the sputum of patients with cystic fibrosis and bronchiectasis.[84 85] The levels are much higher than those found in serum, which suggests local production.

Antibiotic management of pseudomonas lung infections

P aeruginosa is inherently resistant to many antibiotics at concentrations that can be achieved in vivo[2 7] and, with the notable exception of ciprofloxacin, those to which it is sensitive need to be given intravenously. *P cepacia* is even more resistant.[8 19] Bacteria are located intraluminally in association with mucus, or in contact with the epithelium, particularly if the epithelial surface is damaged.[27 29] To reach the site of infection the antibiotic must therefore penetrate into the bronchial epithelium and secretions. Antibiotic penetration into the mucosa in patients with cystic fibrosis and bronchiectasis may be reduced by thickening and scarring of the bronchial wall.[5 7 27] The secretions themselves may provide a barrier to antimicrobial penetration, as may alginate of mucoid strains,[2] and the concentration of antibiotic at the site of infection may therefore be quite different from the concentration in the serum.[86 87] Antibiotics vary in their ability to penetrate into bronchial mucosa and secretions and, in general, beta lactams, cephalosporins and aminoglycosides penetrate less well than quinolones.[87] Mutations produce strains which are resistant to antipseudomonal antibiotics by mechanisms which include hyperproduction of chromosomal β-lactamase, altered DNA gyrase, and membrane changes reducing drug accumulation.[88]

There appears to be no obvious choice of a particular antibiotic or combination of antibiotics, judging by the large number of reported trials which, unfortunately, often differ markedly in their design, thus making simple comparison difficult.[2] Table 2 lists those antibiotics that have proved to be clinically useful in the treatment of *P aeruginosa*. A semisynthetic penicillin or third-generation cephalosporin is usually used in combination with an aminoglycoside antibiotic. The logic of this combination is to obtain additive benefit from antibiotics that have a different mechanism of action, together with the aim of avoiding development of resistance.[7] The pharmacokinetics of cephalosporins and particularly aminoglycosides may be altered in cystic fibrosis. Increased extracellular volume associated with malnutrition, and elevated renal clearance of these drugs in patients with cystic fibrosis, means that higher doses may be needed to obtain adequate serum levels than would be the case in non-cystic fibrosis patients.[2]

A number of studies have shown that intravenous antibiotic treatment against *P aeruginosa* lowers the proteinase concentration in secretions, maintains or improves lung function, and improves survival.[89–93] For these reasons it has been suggested that courses of intravenous antibiotics should be given at regular intervals in a planned manner, irrespective of exacerbations, in order to reduce lung inflammation presumably by reducing the bacterial burden.[5 92] Long term oral ciprofloxacin has also been used in patients with bronchiectasis who suffer frequent exacerbations. It improved symptoms and lung function, decreased the number of exacerbations, and reduced hospital admissions.[94] In a number of patients *P aeruginosa* was eradicated by this long course of antibiotic, but in others resistance developed.

A number of studies indicate that antibiotics may not just benefit patients by killing bacteria. Clinical improvement in cystic fibrosis following antibiotics is often associated with only a small decrease in the viable count of pseudomonas in the sputum or no change at all.[95] The benefit of antibiotic treatment might also be explained partly by reduction in exoproduct production which occurs with subinhibitory concentrations of antibiotic in vitro[96] and from strains isolated from patients after intravenous antibiotic treatment,[97] or perhaps by killing subpopulations of bacteria adherent to the mucosa or infecting the parenchyma.[27] It should be remembered, however, that patients admitted to hospital for intravenous antibiotics also receive supportive care such as physio-

therapy and intravenous rehydration.[98] It will be interesting to compare the results from home intravenous antibiotic programmes when the supportive care may be less good than that obtained in hospital.[99 100]

Because the concentration of antibiotic at the site of airway infection is important, the idea of delivering high concentrations of antibiotic directly onto the mucosa by inhalation is appealing.[101] A number of regimens of nebulised antibiotics, including β-lactams, aminoglycosides and colomycin, either singly or in combination, have been shown to improve symptoms and lung function and reduce hospital admissions of cystic fibrosis patients colonised by *P aeruginosa*.[102–106] They are best used in a prophylactic manner to delay relapse, and are less effective during acute exacerbations, probably because they are deposited centrally due to blockage of small airways by secretions and bronchospasm.[107] They should be used after physiotherapy and bronchodilator treatment and prescribed with a suitable air compressor and nebuliser to allow effective dispersal through the bronchial tree. A one-way valve system should be used with an outlet so that exhaled antibiotics can be discharged via a window, preventing exposure of family or other patients to the antibiotics.

Continuous erythromycin is commonly used in Japan to treat patients with diffuse panbronchiolitis and other forms of chronic bronchial sepsis involving *P aeruginosa*.[108] Some recent observations might explain the unexpected benefits that have been reported and justify further clinical studies. Erythromycin reduces exotoxin production by *P aeruginosa* at concentrations which do not affect bacterial growth,[109] and suppresses biofilm formation.[110] Erythromycin also has anti-inflammatory actions such as inhibition of neutrophil chemotaxis[111] and generation of reactive oxygen species,[112] and is also an inhibitor of mucus secretion in vitro.[113]

An important issue which would influence management but remains undecided is the role of *P aeruginosa* in disease progression in non-cystic fibrosis bronchiectasis. *P aeruginosa* is associated with worse lung function[114] and worse quality of life,[115] but it is not clear whether chronic *P aeruginosa* infection causes an accelerated decline in lung function or whether it is simply a marker of those patients whose lung function is already declining rapidly.

Other forms of management

With the successful development of DNA vectors, somatic gene therapy for patients with cystic fibrosis has come closer to reality. However, *P aeruginosa* lung infections will continue to be a major problem in cystic fibrosis for many years to come. There has been a relative failure of antibiotics to eradicate *P aeruginosa* or to halt the increased morbidity and mortality following infection.[5 6 11 25] It seems unlikely that any new antibiotic will change this outcome, so preventative strategies and adjunct therapies are very important.

One study has suggested that aggressive antibiotic therapy on first isolation of *P aeruginosa* may prevent chronic infection.[116] There has been much research into development of an effective pseudomonas vaccine, but most results to date have been disappointing and, indeed, in some circumstances have led to clinical deterioration, presumably by enhancing inflammation.[64] Recent research has focused on inducing opsonic antibodies which are not readily formed during natural infections.[117] There have been some promising clinical results in small trials, and vaccination prior to *P aeruginosa* colonisation seems to be a logical approach.[118]

A major problem in *P aeruginosa* bronchial infections is poor clearance of mucus which harbours bacteria and their products, as well as host inflammatory factors. Thus poor clearance perpetuates and enhances the inflammatory response which causes lung damage. Nebulised amiloride may enhance mucus clearance in cystic fibrosis by blocking excess sodium absorption.[119] Recombinant human DNase reduces viscosity of cystic fibrosis sputum with some clinical benefit[120] but results have not been as good in non-cystic fibrosis bronchiectasis,[121] perhaps because the DNA content of the sputum is less.

A number of approaches are being investigated that seek to control the exuberant inflammatory response to *P aeruginosa* infection. These include oral corticosteroids[71] which may be successful but have unacceptable side effects at the dosage required,[122] non-steroidal anti-inflammatory agents[123] and elastase inhibitors given by inhalation.[124 125]

1 Forkner CE. *Pseudomonas aeruginosa* infections. In: Wright IS, ed. *Modern medical monographs No. 22.* New York and London: Grune and Stratton, 1960: 1–5.
2 Pitt TL. Biology of *Pseudomonas aeruginosa* in relation to pulmonary infection in cystic fibrosis. *J R Soc Med* 1986;**79**:13–18.
3 Wilson R, Pitt T, Taylor G, *et al*. Pyocyanin and 1-hydroxyphenazine produced by *Pseudomonas aeruginosa* inhibit the beating of human respiratory cilia in vitro. *J Clin Invest* 1987;**79**:221–9.
4 Neu HC. The role of *Pseudomonas aeruginosa* in infections. *J Antimicrob Chemother* 1983;**11**:1–13.
5 Rayner CFJ, Cole PJ, Wilson R. The management of chronic bronchial sepsis due to bronchiectasis. *Clin Pulm Med* 1994;**1**:348–55.
6 Wilson R, Cole P. Respiratory tract infections. In: Barnes PJ, ed. *Royal Brompton Review Series Volume 2: Respiratory medicine—recent advances.* London: Butterworths, 1993: 95–122.
7 Wilson R, Tsang KWT. Antibiotics and the lung. In: Page CP, Metzger WJ, eds. *Drugs and the lung.* New York: Raven Press, 1994: 347–81.
8 Stableforth DE, Smith DL. *Pseudomonas cepacia* in cystic fibrosis. *Thorax* 1994;**49**:629–30.
9 Leelarasamee A, Bovornkitti S. Melioidosis: review and update. *Rev Infect Dis* 1989;**11**:413–25.
10 Govan JRW, Harris GS. *Pseudomonas aeruginosa* and cystic fibrosis: unusual bacterial adaptation and pathogenesis. *Microbiol Sci* 1986;**3**:302–8.
11 Fick RB Jr. Pathogenesis of the *Pseudomonas* lung lesion in cystic fibrosis. *Chest* 1989;**96**:158–64.
12 Ogle JW, Janda JM, Woods DE, *et al*. Characterisation and use of a DNA probe as an epidemiological marker for *Pseudomonas aeruginosa*. *J Infect Dis* 1987;**155**:119–26.
13 Speert DP, Campbell ME, Farmer SW, *et al*. Use of a pilin gene probe to study molecular epidemiology of *Pseudomonas aeruginosa*. *J Clin Microbiol* 1989;**27**:2589–93.
14 Loutit JS, Tompkins LS. Restriction enzyme and southern hybridization analyses of *Pseudomonas aeruginosa* strains from patients with cystic fibrosis. *J Clin Microbiol* 1991;**29**: 2897–900.
15 Romling U, Fiedler B, Bobhammer J, *et al*. Epidemiology of chronic *Pseudomonas aeruginosa* infections in cystic fibrosis. *J Infect Dis* 1994;**170**:1616–21.
16 Tummler B, Koopmann UTA, Grothues D, *et al*. Nosocomial acquisition of *Pseudomonas aeruginosa* by cystic fibrosis patients. *J Clin Microbiol* 1991;**29**:1265–76.
17 Cheng K, Smyth RL, Govan JR, *et al*. Spread of beta-lactam resistant *Pseudomonas aeruginosa* in a cystic fibrosis clinic. *Lancet* 1996;**348**:639–42.

18 Isles A, Maclusky I, Corey M, *et al. Pseudomonas cepacia* infection in cystic fibrosis: an emerging problem. *J Pediatr* 1984;**104**:206–10.
19 Taylor RF, Gaya H, Hodson ME. *Pseudomonas cepacia*: pulmonary infection in patients with cystic fibrosis. *Respir Med* 1993;**87**:187–92.
20 Gladman G, Connor PJ, Williams RF, *et al*. Controlled study of *Pseudomonas cepacia* and *Pseudomonas maltophilia* in cystic fibrosis. *Arch Dis Child* 1992;**67**:192–5.
21 John M, Ecclestone E, Hunter E, *et al*. Epidemiology of *Pseudomonas cepacia* colonisation among patients with cystic fibrosis. *Pediatr Pulmonol* 1994;**18**:108–13.
22 Lipuma JJ, Dasen SE, Nielsen DW, *et al*. Person to person transmission of *Pseudomonas cepacia* between patients with cystic fibrosis. *Lancet* 1990;**336**:1094–6.
23 Tablan OC, Chorba TL, Schidlow DV, *et al. Pseudomonas cepacia* colonisation in patients with cystic fibrosis: risk factors and clinical outcome. *J Pediatr* 1985;**107**:382–7.
24 Steinbach S, Sun L, Jiang RZ, *et al*. Transmissibility of *Pseudomonas cepacia* infection in clinic patients and lung transplant recipients with cystic fibrosis. *N Engl J Med* 1994;**331**:981–7.
25 Pier GB. Pulmonary disease associated with *Pseudomonas aeruginosa* in cystic fibrosis : current status of the host bacterium interaction. *J Infect Dis* 1985;**151**:575–80.
26 Pedersen SS, Hoiby N, Espersen F, *et al*. Role of alginate in infection with mucoid *Pseudomonas aeruginosa* in cystic fibrosis. *Thorax* 1992;**47**:6–13.
27 Baltimore RS, Christie CD, Smith GJ. Immunohistopathological localisation of *Pseudomonas aeruginosa* in lungs from patients with cystic fibrosis. Implications for the pathogenesis of progressive lung deterioration. *Am Rev Respir Dis* 1989;**140**:1650–61.
28 Plotkowski MC, Chevillard M, Pierrot D, *et al*. Differential adhesion of *Pseudomonas aeruginosa* to human respiratory epithelial cells in primary culture. *J Clin Invest* 1991;**87**: 2018–28.
29 Tsang KWT, Rutman A, Tanaka E, *et al*. Interaction of *Pseudomonas aeruginosa* with human respiratory mucosa in vitro. *Eur Respir J* 1994;**7**:1746–53.
30 de Bentzmann S, Plotkowski C, Puchelle E. Receptors in the *Pseudomonas aeruginosa* adherence to injured and repairing epithelium. *Am J Respir Crit Care Med* 1996;**154**: S155–62.
31 Woods DE, Straus DC, Johanson WG Jr, *et al*. Role of pili in adherence of *Pseudomonas aeruginosa* to mammalian buccal epithelial cells. *Infect Immun* 1980;**29**:1146–51.
32 Ramphal R, Sadoff JC, Pyle M, *et al*. Role of pili in the adherence of *Pseudomonas aeruginosa* to injured tracheal epithelium. *Infect Immun* 1984;**44**:38–40.
33 Simpson DA, Ramphal R, Lory S. Characterization of *Pseudomonas aeruginosa* fli O, a gene involved in flagellar biosynthesis and adherence. *Infect Immun* 1995;**63**:2950–7.
34 Baker NR, Minor V, Deal C, *et al. Pseudomonas aeruginosa* exoenzyme S is an adhesin. *Infect Immun* 1991;**59**:2859–63.
35 Ramphal R, Pier GB. Role of *Pseudomonas aeruginosa* mucoid exopolysaccharide in adherence to tracheal cells. *Infect Immun* 1985;**47**:1–4.
36 Vishwanath S, Ramphal R. Adherence of *Pseudomonas aeruginosa* to human tracheobronchial mucus. *Infect Immun* 1984;**45**:197–202.
37 Vishwanath S, Ramphal R. Tracheobronchial mucin receptor for *Pseudomonas aeruginosa*: predominance of amino sugars in binding sites. *Infect Immun* 1985;**48**:331–5.
38 Ramphal R, Carnoy C, Fievre S, *et al. Pseudomonas aeruginosa* recognises carbohydrate chains containing type 1 (Galβ1–3GlcNAc) or type 2 (Galβ1–4GlcNAc) disaccharide units. *Infect Immun* 1991;**59**:700–4.
39 Reddy MS. Human tracheobronchial mucin: purification and binding to *Pseudomonas aeruginosa*. *Infect Immun* 1992; **60**:1530–5.
40 Sajjan U, Reisman J, Doig P, *et al*. Binding of non-mucoid *Pseudomonas aeruginosa* to normal human intestinal mucin and respiratory mucin from patients with cystic fibrosis. *J Clin Invest* 1992;**89**:657–65.
41 Somerville M, Richardson PS, Rutman A, *et al*. Stimulation of secretion into human and feline airways by *Pseudomonas aeruginosa* proteases. *J Appl Physiol* 1991;**70**: 2259–67.
42 Somerville M, Taylor GW, Watson D, *et al*. Release of mucus glycoconjugates by *Pseudomonas aeruginosa* rhamnolipid into feline trachea in vivo and human bronchus in vitro. *Am J Respir Cell Mol Biol* 1992;**6**:116–22.
43 Jensen ET, Kharazmi A, Lam K, *et al*. Human polymorphonuclear leucocyte response to *Pseudomonas aeruginosa* grown in biofilms. *Infect Immun* 1990;**58**:2383–5.
44 Buret A, Ward KH, Olson ME, *et al*. An in vivo model to study the pathobiology of infectious biofilms on biomaterial surfaces. *J Biomed Mater Res* 1991;**25**:865–74.
45 Yeates DB, Sturgess JM, Kahn SR, *et al*. Mucociliary transport in trachea of patients with cystic fibrosis. *Arch Dis Child* 1976;**51**:28–33.
46 Currie DC, Pavia D, Agrew JE, *et al*. Impaired tracheobronchial clearance in bronchiectasis. *Thorax* 1987;**42**: 126–30.
47 Abman SH, Ogle JW, Harbeck RJ, *et al*. Early bacteriologic, immunologic, and clinical courses of young infants with cystic fibrosis identified by neonatal screening. *J Pediatr* 1991;**119**:211–7.
48 Armstrong DS, Grimwood K, Carzino R, *et al*. Lower respiratory tract infection and inflammation in infants with newly diagnosed cystic fibrosis. *BMJ* 1995;**310**:1571–2.
49 Prince A. Adhesins and receptors of *Pseudomonas aeruginosa* associated with infection of the respiratory tract. *Microb Pathogen* 1992;**13**:251–60.
50 Saiman L, Cacalano G, Gruenert D, *et al*. Comparison of the adherence of *Pseudomonas aeruginosa* to respiratory epithelial cells from cystic fibrosis patients and healthy subjects. *Infect Immun* 1992;**60**:2808–14.
51 Saiman L, Prince A. *Pseudomonas aeruginosa* pili bind to asialoGM1 which is increased on the surface of cystic fibrosis epithelial cells. *J Clin Invest* 1993;**92**:1875–80.
52 Imundo L, Barasch J, Prince A, *et al*. Cystic fibrosis epithelial cells have a receptor for pathogenic bacteria on their apical surface. *Proc Natl Acad Sci* 1995;**92**:3019–23.
53 Kubesch P, Dork T, Wulbrand U, *et al*. Genetic determinants of airways colonisation with *Pseudomonas aeruginosa* in cystic fibrosis. *Lancet* 1993;**341**:189–93.
54 Zar H, Saiman L, Quittell L, *et al*. Binding of *Pseudomonas aeruginosa* to respiratory epithelial cells from patients with various mutations in the cystic fibrosis transmembrane regulator. *J Pediatr* 1995;**126**:230–3.
55 Krivan HC, Roberts DD, Ginsburg V. Many pulmonary pathogenic bacteria bind specifically to the carbohydrate sequence GalNAcβ1–4Gal found in some glycolipids. *Proc Natl Acad Sci USA* 1988;**85**:6157–61.
56 Baker N, Hansson GC, Leffler H, *et al*. Glycosphingolipid receptors for *Pseudomonas aeruginosa*. *Infect Immun* 1990; **58**:2361–6.
57 Cheng PW, Boat TF, Cranfill K, *et al*. Increased sulphation of glycoconjugates by cultured nasal epithelial cells from patients with cystic fibrosis. *J Clin Invest* 1989;**84**:68–72.
58 Carnoy C, Ramphal R, Scharfman A, *et al*. Altered carbohydrate composition of salivary mucins from patients with cystic fibrosis and the adhesion of *Pseudomonas aeruginosa*. *Am J Respir Cell Mol Biol* 1993;**9**:323–34.
59 Smith JJ, Travis SM, Greenberg EP, *et al*. Cystic fibrosis airway epithelia fail to kill bacteria because of abnormal airway surface fluid. *Cell* 1996;**85**:229–36.
60 Goldman MJ, Anderson GM, Stolzenberg ED, *et al*. Human β-defensin-1 is a salt sensitive antibiotic in lung that is inactivated in cystic fibrosis. *Cell* 1997;**88**:553–60.
61 Pier GB, Grout M, Zaidi TS, *et al*. Role of mutant CFTR in hypersusceptibility of cystic fibrosis patients to lung infections. *Science* 1996;**271**:64–7.
62 Currie DC, Peters AM, Garbett ND, *et al*. Indium-111 labelled granulocyte scanning to detect inflammation in the lungs of patients with chronic sputum expectoration. *Thorax* 1990;**45**:541–4.
63 Hoiby N. *Pseudomonas aeruginosa* infection in cystic fibrosis. Diagnostic and prognostic significance of *Pseudomonas aeruginosa* precipitins determined by means of crossed immunoelectrophoresis. A survey. *Acta Pathol Microbiol Scand* 1977;**262**:1–96.
64 Buret A, Cripps AW. The immunoevasive activities of *Pseudomonas aeruginosa*. Relevance for cystic fibrosis. *Am Rev Respir Dis* 1993;**148**:793–805.
65 Moss RB, Hsu Y, Lewiston NJ, *et al*. Association of systemic immune complexes, complement activation, and antibodies to *Pseudomonas aeruginosa* lipopolysaccharide and exotoxin A with mortality in cystic fibrosis. *Am Rev Respir Dis* 1986;**133**:648–52.
66 Kronborg G, Fomsgaard A, Shand GH, *et al*. TNF-alpha and immune complexes in sputum and serum from patients with cystic fibrosis and chronic *Pseudomonas aeruginosa* lung infection. *Immunol Infect Dis* 1992;**2**:171–7.
67 Winnie GB, Cowan RG. Respiratory tract colonisation with *Pseudomonas aeruginosa* in cystic fibrosis: correlations between anti-*Pseudomonas aeruginosa* antibody levels and pulmonary function. *Pediatr Pulmonol* 1991;**10**:92–100.
68 Amitani R, Wilson R, Rutman A, *et al*. Effects of human neutrophil elastase and *Pseudomonas aeruginosa* proteinases on human respiratory epithelium. *Am J Respir Cell Mol Biol* 1991;**4**:26–32.
69 Bruce MC, Poncz L, Klinger JD, *et al*. Biochemical and pathologic evidence for proteolytic destruction of lung connective tissue in cystic fibrosis. *Am Rev Respir Dis* 1985;**132**:529–35.
70 Matthews WJ Jr, Williams M, Oliphint B, *et al*. Hypogammaglobulinaemia in patients with cystic fibrosis. *N Engl J Med* 1980;**302**:245–9.
71 Auerbach HS, Williams M, Kirkpatrick JA, *et al*. Alternate day prednisolone reduces morbidity and improves pulmonary function in cystic fibrosis. *Lancet* 1985;ii:686–8.
72 Feldman C, Anderson R, Kanthakumar K, *et al*. Oxidant-mediated ciliary dysfunction in human respiratory epithelium. *Free Radic Biol Med* 1994;**17**:1–10.
73 Sommerhoff CP, Nadel JA, Basbaum CB, *et al*. Neutrophil elastase and cathepsin G stimulate secretion from cultured bovine airway gland serous cells. *J Clin Invest* 1990;**85**: 682–9.
74 Massion PP, Inoue H, Richman-Eisenstat J, *et al*. Novel *Pseudomonas* product stimulates interleukin-8 production in airway epithelial cells in vitro. *J Clin Invest* 1994;**93**:26–32.
75 Nakamura H, Yoshimura K, McElvaney NG, *et al*. Neutrophil elastase in respiratory epithelial lining fluid of individuals with cystic fibrosis induces interleukin-8 gene expression in a human bronchial epithelial cell line. *J Clin Invest* 1992;**89**:1478–84.
76 Berger M, Sorensen RU, Tosi MF, *et al*. Complement receptor expression on neutrophils at an inflammatory site, the pseudomonas-infected lung in cystic fibrosis. *J Clin Invest* 1989;**84**:1302–13.
77 Tosi MF, Zakem H, Berger M. Neutrophil elastase cleaves C3bi on opsonized pseudomonas as well as CR1 on

neutrophils to create a functionally important opsonin receptor mismatch. *J Clin Invest* 1990;**86**:300–8.
78 Suter S. New perspectives in understanding and management of the respiratory disease in cystic fibrosis. *Eur J Pediatr* 1994;**153**:144–50.
79 Konstan MW, Hilliard KA, Norvell TM, *et al*. Bronchoalveolar lavage findings in cystic fibrosis patients with stable, clinically mild lung disease suggest ongoing infection and inflammation. *Am J Respir Crit Care Med* 1994;**150**:448–54.
80 Lethem MI, James SL, Marriott C, *et al*. The origin of DNA associated with mucus glycoproteins in cystic fibrosis sputum. *Eur Respir J* 1990;**3**:19–23.
81 Morihara K, Tsuzuki H, Oda K. Protease and elastase of *Pseudomonas aeruginosa* : inactivation of human plasma α_1-proteinase inhibitor. *Infect Immun* 1979;**24**:188–93.
82 Ras GJ, Anderson R, Taylor GW, *et al*. Proinflammatory interactions of pyocyanin and 1-hydroxyphenazine with human neutrophil in vitro. *J Infect Dis* 1990;**162**:178–85.
83 Lapa e Silva JR, Jones JA, Cole PJ, *et al*. The immunological component of the cellular inflammatory infiltrate in bronchiectasis. *Thorax* 1989;**44**:668–73.
84 Eller J, Lapa e Silva JR, Poulter LW, *et al*. Cells and cytokines in chronic bronchial infection. *Ann NY Acad Sci* 1994;**725**:331–45.
85 Kronborg G, Hansen MB, Svenson M, *et al*. Cytokines in sputum and serum from patients with cystic fibrosis and chronic *Pseudomonas aeruginosa* infection as markers of destructive inflammation in the lungs. *Pediatr Pulmonol* 1993;**15**:292–7.
86 Bergogne-Berezin E. Pharmacokinetics of antibiotics in respiratory secretions. In: Penington JE, ed. *Respiratory infections: diagnosis and management*. 2nd ed. New York: Raven Press, 1989: 608–31.
87 Baldwin DR, Honeybourne D, Wise R. Pulmonary disposition of antimicrobial agents: in vivo observations and clinical relevance. *Antimicrob Agents Chemother* 1992;**36**: 1176–80.
88 Chen HY, Yuan M, Ibrahim-Elmagboul IB, *et al*. National survey of susceptibility to antimicrobials amongst clinical isolates of *Pseudomonas aeruginosa*. *J Antimicrob Chemother* 1995;**35**:521–34.
89 Meyer KC, Lewandoski JR, Zimmermann JJ, *et al*. Human neutrophil elastase and elastase/ alpha$_1$-antiprotease complex in cystic fibrosis. Comparison with interstitial lung disease and evaluation of the effect of intravenously administered antibiotic therapy. *Am Rev Respir Dis* 1991;**144**:580–5.
90 Schaad UB, Wedgwood-Kruco J, Suter S, *et al*. Efficacy of inhaled amikacin as adjunct to intravenous combination therapy (ceftazidime and amikacin) in cystic fibrosis. *J Pediatr* 1987;**111**:599–605.
91 Suter S, Schaad UB, Tegner H, *et al*. Levels of free granulocyte elastase in bronchial secretions from patients with cystic fibrosis : effect of antimicrobial treatment against *Pseudomonas aeruginosa*. *J Infect Dis* 1986;**153**:902–9.
92 Szaff M, Hoiby N, Flensborg EW. Frequent antibiotic therapy improves survival of cystic fibrosis patients with chronic *Pseudomonas aeruginosa* infection. *Acta Paediatr Scand* 1983;**72**:651–7.
93 Vogelmeier C, Hubbard RC, Fells GA, *et al*. Anti-neutrophil elastase defence of the normal human respiratory epithelial surface provided by the secretory leukoprotease inhibitor. *J Clin Invest* 1991;**87**:482–8.
94 Rayner CJF, Tillotson G, Cole PJ, *et al*. Efficacy and safety of long term ciprofloxacin in the management of severe bronchiectasis. *J Antimicrob Chemother* 1994;**34**:149–56.
95 Beaudry PH, Marks MI, McDougall D, *et al*. Is anti-Pseudomonas therapy warranted in acute respiratory exacerbations in children with cystic fibrosis? *J Pediatr* 1980;**97**:144–7.
96 Grimwood K, To M, Rabin HR, *et al*. Inhibition of *Pseudomonas aeruginosa* exoenzyme expression by subinhibitory antibiotic concentrations. *Antimicrob Agents Chemother* 1989;**33**:41–7.
97 Grimwood K, To M, Semple RA, *et al*. Elevated exoenzyme expression by *Pseudomonas aeruginosa* is correlated with exacerbations of lung disease in cystic fibrosis. *Pediatr Pulmonol* 1993;**15**:135–9.
98 Gold R, Carpenter S, Heurter H, *et al*. Randomized trial of ceftazidime versus placebo in the management of acute respiratory exacerbations in patients with cystic fibrosis. *J Pediatr* 1987;**111**:907–13.
99 Kuzemko JA. Home treatment of pulmonary infections in cystic fibrosis. *Chest* 1988;**94**:162–6S.
100 Peckham D, Knox A. Intravenous antibiotic therapy in cystic fibrosis: in hospital or at home? *Respir Med* 1993;**87**: 329–30.
101 Mukhopadhyay S, Singh M, Cater JI, *et al*. Nebulised antipseudomonal antibiotic therapy in cystic fibrosis: a meta-analysis of benefits and risks. *Thorax* 1996;**51**:364–8.
102 Hodson ME, Penketh AR, Batten JC. Aerosol carbenicillin and gentamicin treatment of *Pseudomonas aeruginosa* infection in patient with cystic fibrosis. *Lancet* 1981;ii:1137–9.
103 Stead RJ, Hodson ME, Batten JC. Inhaled cefatazidime compared with gentamicin and carbenicillin in older patients with cystic fibrosis infected with *Pseudomonas aeruginosa*. *Br J Dis Chest* 1987;**81**:272–9.
104 Carswell F, Ward C, Cook DA, *et al*. A controlled trial of nebulised aminoglycoside and oral flucloxacillin versus placebo in the out patient management of children with cystic fibrosis. *Br J Dis Chest* 1987;**81**:356–60.
105 Jensen T, Pedersen SS, Garne S, *et al*. Colistin inhalation therapy in cystic fibrosis patients with chronic *Pseudomonas aeruginosa* lung infection. *J Antimicrob Chemother* 1987;**19**: 831–8.
106 Ramsey BW, Dorkin HL, Eisenberg JD, *et al*. Efficacy of aerosolized tobramycin in patients with cystic fibrosis. *N Engl J Med* 1993;**328**:1740–6.
107 Mukhopadhyay S, Staddon GE, Eastman C, *et al*. The quantitative distribution of nebulised antibiotic in the lung in cystic fibrosis. *Respir Med* 1994;**88**:203–11.
108 Kudoh S, Uetake T, Hagiwara K, *et al*. Clinical effects of low dose long-term erythromycin chemotherapy on diffuse panbronchiolitis. *Japanese J Thorac Dis* 1987;**25**:632–42.
109 Tanaka E, Kanthakumar K, Cundell DR, *et al*. The effect of erythromycin on *Pseudomonas aeruginosa* and neutrophil mediated epithelial damage. *J Antimicrob Chemother* 1994; **33**:765–75.
110 Ichimiya T, Yamasaki T, Nasu M. In vitro effects of antimicrobial agents on *Pseudomonas aeruginosa* biofilm formation. *J Antimicrob Chemother* 1994;**34**:331–41.
111 Eyraud A, Desnotes J, Lombard JY, *et al*. Effects of erythromycin, josamycin and spiramycin on rat polymorphonuclear leukocyte chemotaxis. *Chemotherapy* 1986;**32**:379–82.
112 Anderson R. Erythromycin and roxithromycin potentiate human neutrophil locomotion in vitro by inhibition of leukoattractant-activated superoxide generation and autooxidation. *J Infect Dis* 1989;**159**:966–73.
113 Goswami SK, Kivity S, Marom Z. Erythromycin inhibits respiratory glycoconjungate secretion from human airways in vitro. *Am Rev Respir Dis* 1990;**141**:72–8.
114 Evans SA, Turner SM, Bosch BJ, *et al*. Lung function in bronchiectasis: the influence of *Pseudomonas aeruginosa*. *Eur Respir J* 1996;**9**:1601–4.
115 Wilson CB, Jones PW, O'Leary CJ, *et al*. Effect of sputum bacteriology on the quality of life of patients with bronchiectasis. *Eur Respir J* 1997;**10**:1754-60.
116 Valerius NH, Koch C, Hoiby N. Prevention of chronic *Pseudomonas aeruginosa* colonisation in cystic fibrosis by early treatment. *Lancet* 1991;**338**:725–6.
117 Schreiber JR, Pier GB, Grout M, *et al*. Induction of opsonic antibodies to *Pseudomonas aeruginosa* mucoid exopolysaccharide by an anti-idiotypic monoclonal antibody. *J Infect Dis* 1991;**164**:507–14.
118 Cryz SJ Jr, Wedgwood J, Lang AB, *et al*. Immunization of noncolonised cystic fibrosis patients against *Pseudomonas aeruginosa*. *J Infect Dis* 1994;**169**:1159–62.
119 App EM, King M, Helfesrieder R, *et al*. Acute and long term amiloride inhalation in cystic fibrosis lung disease. A rational approach to cystic fibrosis therapy. *Am Rev Respir Dis* 1990;**141**:605–12.
120 Hubbard RC, McElvaney NG, Birrer P, *et al*. A preliminary study of aerosolized recombinant human deoxyribonuclease I in the treatment of cystic fibrosis. *N Engl J Med* 1992;**326**:812–5.
121 Barker A, O'Donnell A, Mallon K, *et al*. Phase II trial of recombinant human DNase I in non-CF bronchiectasis. *Am J Respir Crit Care Med* 1995;**151**:A463.
122 Rosenstein BJ, Eigen H. Risks of alternate-day prednisolone in patients with cystic fibrosis. *Pediatrics* 1991;**87**: 245–6.
123 Konstan MW, Byard PJ, Hoppel CL, *et al*. Effect of high-dose ibuprofen in patients with cystic fibrosis. *N Engl J Med* 1995;**332**:848–54.
124 McElvaney NG, Hubbard RC, Birrer P, *et al*. Aerosol alpha-1-antitrypsin treatment for cystic fibrosis. *Lancet* 1991;**337**:392–4.
125 McElvaney NG, Nakamura H, Birrer P, *et al*. Modulation of airway inflammation in cystic fibrosis. In vivo suppression of interleukin-8 levels on the respiratory epithelial surface by aerosolization of recombinant secretory leukoprotease inhibitor. *J Clin Invest* 1992;**90**:1296–301.