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Pseudomonas aeruginosa Infections in Patients with Cystic Fibrosis

New Immunomodulatory Strategies

Andre Buret

Division of Molecular and Cell Biology, Faculty of Medicine, University of Newcastle, Newcastle, New South Wales, Australia

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Summary

Pseudomonas aeruginosa is the most common cause of morbidity and mortality in patients with cystic fibrosis. The pathogenesis of the fatal pulmonary infections it causes is multifactorial. A number of immunomodulatory strategies are currently being developed to improve the welfare of cystic fibrosis patients. The overall aims of these therapies are to: (a) attenuate the pathogenic effects of the bacterium's immunoevasive arsenal and the host inflammatory response; (b) boost pulmonary immunity; (c) modify the host's mucosal abnormalities.

The major and most promising strategies involve: (a) protease inhibitor therapy with α-antitrypsin, secretory leukoprotease inhibitor, or ICI 200800, a synthetic chloromethylketone antagonist; (b) anti-inflammatory therapy, using ibuprofen rather than prednisone; (c) immunomodulation of the activities of leukotriene B_4, interleukins-1 and -8 or tumour necrosis factor-α (only experimental data are available); (d) hyperimmune immunoglobulin transfer; (e) vaccination with an anti-idiotype antibody mimicking the *P. aeruginosa* mucoid exopolysaccharide, or oral immunisation with mucoid *P. aeruginosa* antigens (only experimental data are available); (f) treatment with aerosolised recombinant human deoxyribonuclease-I (DNase) and other mucolytic agents.

To date, only protease inhibitors, ibuprofen and DNase are available (or will be available in the very near future) to the physician caring for patients with cystic fibrosis. Until curative treatment becomes feasible, clinical trials investigating alternative immunotherapies must receive a high priority on the list of strategies that need to be developed in order to improve the prognosis of patients with cystic fibrosis.

The discovery in 1989 of the cystic fibrosis transmembrane regulator (CFTR) gene has raised new hopes for an eventual cure of cystic fibrosis, the most common lethal genetic disease among Caucasians.[1-4] Although virtually every organ in the body may be affected by this inherited condition, it is usually the acute or chronic exacerbation of the airway disease associated with it that is fatal to cystic fibrosis patients. In most of these cases, the aetiological agent is *Pseudomonas aeruginosa*. Therefore, until a cure for cystic fibrosis is discovered, the clinical management of affected patients must involve the control of endobronchial infections with *P. aeruginosa*.

A better understanding of the mechanisms causing pulmonary disease in cystic fibrosis is needed to resolve the controversies that still surround treatment strategies.[5-10] An increasing number of reports demonstrate that *P. aeruginosa* products can modulate host immune mechanisms. Because of the obvious role of inflammation in pathogenesis,[11,12] the development of immunomodulatory protocols to treat *P. aeruginosa* infections in cystic fibrosis is very complex. A synthesis of this knowledge is now warranted to gain a better insight into potential immunotherapy for the cystic fibrosis patient with *P. aeruginosa* pneumonia. Therefore, the aims of this review are to:

- critically discuss key developments in clinical and experimental immunomodulatory strategies against *P. aeruginosa* pneumonia in cystic fibrosis
- highlight the controversies surrounding some of these immunotherapeutic concepts
- suggest directions for future research.

The article focuses on the seminal and most recent advances in this crucial area of cystic fibrosis research in an attempt to encourage further clinical studies that may improve the prognosis of affected patients. More specifically, it discusses the promise and disadvantages of protease inhibitors, anti-inflammatory agents, cytokine therapy and immunisation in the medical management of patients with cystic fibrosis.

1. Pathogenesis of *P. aeruginosa* Infection in Cystic Fibrosis

Air pollutants and viral infections, and most importantly bacterial pathogens, all contribute to pulmonary inflammation in cystic fibrosis patients. Over the past 2 decades, a gradual decrease in colonisation with *Klebsiella ozaenae* and *Escherichia coli* and an increase in infection with *P. cepacia* have been observed in the lungs of patients with cystic fibrosis.[13,14] At present, the bacterial species most commonly recovered from respiratory secretions of patients with cystic fibrosis are *Staphylococcus aureus*, *Haemophilus influenzae*

and *P. aeruginosa*, a Gram-negative pathogen that alone accounts for more than 70% of the infections.[13-16] While *S. aureus* and *H. influenzae* can to a large extent be controlled with antibiotic therapy, a number of factors contribute to the establishment and persistence of *P. aeruginosa*.[10,13,17] Indeed, pharmacotherapeutic elimination of this organism from the airways has proven elusive and continues to be the topic of numerous investigations.[6,13,18]

The respiratory tract of patients with cystic fibrosis remains a highly susceptible target for chronic colonisation by *P. aeruginosa*. The infecting organisms gradually change from a non-mucoid to a mucoid phenotype and cause the progressive pulmonary disease that is primarily responsible for the morbidity and mortality in these patients. Clearly, until curative therapy becomes feasible, the development of therapeutic methods allowing control and/or prevention of the establishment of *P. aeruginosa* in the lungs of cystic fibrosis patients remains a goal of foremost priority. In an attempt to build a rational basis for such developments, much current research is dedicated to the pathogenic mechanisms leading to pulmonary damage during the infection.

Although our understanding of this cascade of events is still incomplete, findings from these investigations indicate that the pathogenesis of *P. aeruginosa* pneumonia in cystic fibrosis is multifactorial (fig. 1). The first disease-promoting elements may reside in host factors such as the increased number of receptors for the bacteria on the surface of epithelial cells,[19] and the unusual viscosity of pulmonary mucus. The mucus is abnormally dehydrated in cystic fibrosis because of defective chloride secretion and increased sodium absorption through the epithelia.[20] Pulmonary epithelial cells in cystic fibrosis contain less fully-sialylated superficial glycolipids, and the acidification of their intracellular organelles is defective as a consequence of the abnormal CFTR function.[21] These changes are associated with increased numbers of epithelial receptors for *P. aeruginosa* such as asialoganglioside G_{M1}.[22] This

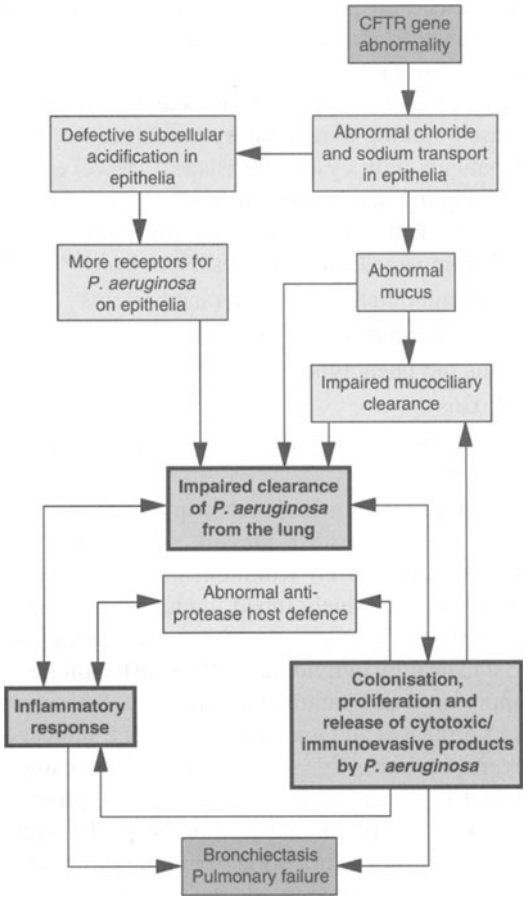

Fig. 1. Schematic diagram illustrating the pathogenic mechanisms of *Pseudomonas aeruginosa* infections in the airways of patients with cystic fibrosis. The cystic fibrosis transmembrane regulator (CFTR) gene abnormality causes mucosal abnormalities in the lung, and these in turn are responsible for the impaired clearance of bacterial pathogens. *P. aeruginosa* is resistant to antibiotic treatment, and colonisation with this pathogen initiates an inflammatory cycle involving bacterial products and host inflammatory components that is responsible for the severe tissue damage and fatal outcome of chronic infections.

substance is a ligand for pilin, a major bacterial adhesin. In addition, the phosphatidylglycerol content of pulmonary mucus in cystic fibrosis is significantly reduced, an alteration that has been implicated in the impaired mucociliary clearance in the lung.[23] All these factors contribute to the delayed elimination of infectious bacteria and the

subsequent self-perpetuating cycle of infection and inflammation that eventually leads to pulmonary failure (fig. 1).

P. aeruginosa does not appear to exhibit increased binding to respiratory mucins in the lungs of patients with cystic fibrosis.[24] However, further help in the colonisation process is provided by the organisms themselves, via mechanisms that further impair clearance processes such as:[19,25-33]

- pili and other outer membrane proteins
- biofilm mode of growth
- mucoid phenotype
- toxins
- proteases, especially alkaline protease and elastase
- pathogenic pigments such as pyocyanin.

This complex host-parasite relationship provides an optimal environment in cystic fibrosis patients for the finely tuned immunoevasive arsenal of the pathogen, an environment that may not exist in healthy individuals. Although the immunosuppressive and pathogenic effects of *P. aeruginosa's* proteases and toxins have been the subject of several reviews,[11,12,14,17] severe tissue damage to the lung results from both the bacterial products and the host inflammatory response to the pathogen. The constant formation of immune complexes with persistent antigens, and the ongoing release of proteolytic enzymes such as neutrophil elastase, have been implicated in the pulmonary lesions associated with the infection.[34-39] The potential to target some of these features for the prevention and treatment of *P. aeruginosa* pneumonia in cystic fibrosis provides a fertile topic for future clinical investigation.

2. Protease Inhibitor Therapy

2.1 Rationale

The use of antibiotics does not suffice in controlling the devastating effects of chronic *P. aeruginosa* infections in cystic fibrosis, and other means of blunting the virulence of the pathogen are being sought. Two proteolytic enzymes released by *P. aeruginosa*, alkaline protease and most importantly elastase, damage the pulmonary tissue and are potent modulators of the host immune responses. In addition to inducing mucosal proteolysis, they have been shown to:[11,12,28]

- cleave IgG and IgA
- inactivate interleukin (IL)-2, interferon-γ (IFNγ) and tumour necrosis factor-α (TNFα)
- degrade complement components C1 to C9, fibronectin, α-antitrypsin and the natural killer effector-target cell receptor.

Although many of these effects have yet to be demonstrated *in vivo* in cystic fibrosis, these bacterial proteases have become logical targets for the development of immunomodulatory therapies. Moreover, neutrophil elastase, a major proteolytic enzyme released by host neutrophils in response to a bacterial infection, shares many of its effects with bacterial elastase. Currently, there is wide agreement that neutrophil proteases play a primary pathogenic role in *P. aeruginosa* pneumonia.[11,38]

Serine protease inhibitors such as α-antitrypsin or α_2-macroglobulin are secreted into the bronchoalveolar space, and the secretory leukoprotease inhibitor is produced by mucosal epithelial cells. However, in cystic fibrosis these host inhibitors are clearly overwhelmed by the massive quantities of elastase produced in the inflamed lung. Bacterial and neutrophil elastase can proteolytically degrade and inactivate endobronchial α-antitrypsin, implying that both increased elastase release and inactivation of α-antitrypsin contribute to the elevated levels of active elastase in the colonised lungs of cystic fibrosis patients.[36,37,40,41]

In an attempt to prevent injury and to maintain normal immunity in the respiratory mucosa, treatments with elastase inhibitors have been tested in clinical trials.

2.2 Clinical Trials with Protease Inhibitors

When infected cystic fibrosis patients were given purified human plasma α-antitrypsin 1.5 to 3.0 mg/kg every 12 hours for 1 week by aerosol, their epithelial lining fluid α-antitrypsin levels

were increased compared with baseline values.[42] This in turn:

- suppressed active endobronchial elastase levels
- enhanced the anti–neutrophil elastase capacity
- reversed the ability of respiratory secretions to interfere with neutrophil killing of *P. aeruginosa*.

Intravenous α-antitrypsin had no effect.

Another trial[43] suggested that recombinant secretory leukoprotease inhibitor 100mg every 12 hours in aerosol also increased the levels of the inhibitor in epithelial lining fluids and suppressed elastase activity. In addition, this treatment caused a marked decrease in gene expression of the pro-inflammatory cytokine IL-8 by bronchial epithelial cells, and dramatically reduced the concentration of IL-8 recovered from respiratory secretions.

These strategies may therefore enhance host defence while breaking the cycle of inflammation in the lung, and show promise for the clinical management of cystic fibrosis patients whose lungs are colonised with *P. aeruginosa*. Also underway are other clinical trials of synthetic drugs such as the chloromethylketone antagonist ICI 200 800, a competitive inhibitor of neutrophil elastase.

3. Anti-Inflammatory Therapy

3.1 Rationale

A growing number of studies have established that pulmonary inflammation plays a critical role in the pathogenesis of respiratory failure in cystic fibrosis patients.[11,12,34,39,44] From this inventory, it appears that 2 major host factors are involved in the deleterious effects on the mucosa:

- Neutrophils chronically accumulate on an airway epithelial surface from which *P. aeruginosa* cannot be cleared. These leucocytes then release large amounts of neutrophil elastase, the nonspecific proteolytic enzyme that targets the bacteria but concurrently damages the endobronchial epithelium and interferes with host immunity.
- The constant formation of immune complexes is responsible for serious tissue damage in the lungs of cystic fibrosis patients.[34,39,44] It has been established that there is a direct correlation between serum IgG levels and severity of pulmonary disease, and mortality is greatest in patients with hypergammaglobulinaemia.

The serum level of IgG_2 is elevated in chronically colonised cystic fibrosis patients, and IgG_2-containing immune complexes impair phagocytosis by pulmonary macrophages.[44] Lung function tests, such as FEV_1, forced vital capacity (FVC) and forced expiratory flow between 25 and 75% of vital capacity (FEF), have been used to show that high serum levels of IgG_3, and to a lesser extent IgG_2, anti–*P. aeruginosa* antibodies correlate with abnormal pulmonary function in colonised cystic fibrosis patients.[45,46] Poor clinical condition and high serum IgG_3 titres also correlated with increased neutrophil chemotactic indices.

Taken together, these observations constitute the basis for developing anti-inflammatory therapy that will blunt the detrimental effects of pulmonary inflammation in cystic fibrosis patients chronically colonised with *P. aeruginosa*.

3.2 Corticosteroids

Corticosteroids have been proposed for anti-inflammatory therapy in cystic fibrosis. An initial clinical trial indicated that prednisone 2mg/kg orally every other day may be efficacious in controlling inflammation and improving lung function in patients with cystic fibrosis.[47] However, further investigation showed that long term usage and/or higher dosages induced severe adverse effects, such as reduced bone density, cataracts and diabetes.[48-50]

In order to evaluate further the risk-benefit ratio of corticosteroid therapy, a current multicentre long term study has assessed the effects of oral prednisone 1mg/kg/day in 6- to 14-year-old cystic fibrosis patients with mild to moderate disease and colonised with *P. aeruginosa*. The as yet unpublished findings suggest that such treatment was preferable to the regimen outlined above,[47] as it significantly improved airflow and prevented decline in lung function. The only statistically

significant adverse effect over a 4-year period was a higher incidence of growth suppression in the prednisone group.

3.3 Nonsteroidal Anti-Inflammatory Drugs

The increased understanding of the pharmacokinetics of nonsteroidal anti-inflammatory drugs (NSAIDs)[51] and the adverse effects observed with long term and high dosage use of corticosteroids in cystic fibrosis patients have focused clinical research on the use of NSAIDs in these patients. Ibuprofen is a potent inhibitor of the cyclo-oxygenase and 5-lipoxygenase pathways in neutrophils,[52] and significantly reduces the production of the neutrophil chemoattractant leukotriene (LT) B_4.

In studies of animals chronically or acutely infected with *P. aeruginosa*, ibufopren at large dosages (35 mg/kg orally every 12 hours) significantly impaired 2 critical pathogenic features of the colonised lung from cystic fibrosis patients, neutrophilic infiltration and development of the inflammatory cascade.[53,54] Indeed, neutrophils from treated animals produced significantly lower levels of LTB_4 *in vitro*, an observation which is consistent with the pathogenic role of this eicosanoid in inflamed lungs.[53,55]

Pharmacokinetic studies have been carried out in children with cystic fibrosis to assess the tolerability of this agent. Peak plasma concentrations of ibuprofen in excess of 50 μg/ml could be achieved in children with cystic fibrosis without any sign of toxicity.[56] Animal studies had shown that concentrations >35 μg/ml were necessary to inhibit the specific inflammatory functions of neutrophils.[53]

These observations indicate that ibuprofen, and possibly other NSAIDs, may be of significant clinical benefit to cystic fibrosis patients with chronic *P. aeruginosa* infections and pulmonary inflammation. The results of large multicentre clinical trials are expected with great anticipation. However, the well known adverse effects of NSAIDs in the gastrointestinal tract[57,59] are likely to stimulate further research into the mechanisms of action of these compounds and lead to the development of novel alternatives.

4. Cytokine and Eicosanoid Therapy

With the increasing interest in using cytokines and/or anticytokines to control parasitic and other microbial infections,[60,61] a number of studies are currently investigating the benefits and risks of either controlling or augmenting the activity of these agents in the setting of the lung infected with *P. aeruginosa*. As pro-inflammatory cytokines such as IL-1, IL-8 and TNFα and the eicosanoid LTB_4 drive the pathogenic inflammation in the chronically colonised lungs of cystic fibrosis patients, attempts are being made to modulate the gene expression and/or production of these agents *in situ*. Indeed, there is strong clinical and experimental evidence to suggest that the inflammatory disease in acute pulmonary infection with *P. aeruginosa* is partly mediated by persistently high levels of endobronchial LTB_4, IL-1 and TNFα.[55,62-68]

4.1 Interleukin-1 and Tumour Necrosis Factor-α

IL-1 and TNFα have been implicated in other inflammatory diseases such as rheumatoid arthritis and colitis, and various methods are currently being developed to inhibit their production and/or activity *in vivo* in an attempt to control their deleterious effects.[61,69] Experimental strategies for controlling IL-1 bioactivity include the administration of corticosteroids, NSAIDs, transforming growth factor-β, IL-4, IL-10, anti–IL-1 antibodies, soluble IL-1 receptors, anti-receptor antibodies or IL-1 receptor antagonist.[61,70,71]

A recent study has found that exposure of ovalbumin-sensitised guinea-pigs to an aerosol of IL-1 receptor antagonist (50μg/30 min) prevented airway dysfunction and reduced the generation of TNFα in the antigen-challenged lung.[72] In another report, it was shown that intrapulmonary deposition of IL-4 or IL-10 protected against immune complex–induced lung injury in rats.[73] The dosage-dependent protective effects of each cytokine were associated with a significant reduction

of TNFα production in the bronchoalveolar space. Similarly, blockade of IL-1 receptors with recombinant IL-1 receptor antagonist 5mg/kg reduces the inflammatory responses in experimental immune complex colitis in rabbits.[74] Separate investigations have demonstrated that IL-4 and IL-10 increase the synthesis of IL-1 receptor antagonist and concurrently decrease that of IL-1.[61,70,71] Finally, experiments using a neutropenic rat model have shown that an intravenously administered combination of monoclonal antibodies against *P. aeruginosa* lipopolysaccharide and TNFα provided significantly greater protection than when either monoclonal antibody was given alone. Such immunotherapy may be of potential benefit in the clinical management of Gram-negative bacterial sepsis.[75]

Similar approaches would be worth investigating in the context of *P. aeruginosa*–infected airways. However, immunotherapy directed at IL-1 or TNFα has been very little explored in this setting, although a clinical trial currently underway evaluates the benefits of pentoxifylline, an inhibitor of TNFα transcription, in patients with cystic fibrosis.

When designing novel protocols for cytokine therapy of *Pseudomonas* infection in cystic fibrosis, it should be remembered that small physiological concentrations of IL-1 and TNFα are necessary for the continuation of host defence mechanisms. For example, mice given IL-1 intramuscularly or intraperitoneally are protected from a subsequent lethal systemic infection with *P. aeruginosa*, and rats intratracheally inoculated with recombinant TNFα significantly enhanced their clearance of *P. aeruginosa* from the lungs. These data further support the importance of pro-inflammatory cytokines in protection against this micro-organism.[62,76-78] As other authors have suggested,[61] these observations indicate that a complete blocking of IL-1 or TNFα is not warranted, but that a significant reduction of the persistent high cytokine levels at the chronically inflamed mucosal site may prove beneficial to the host.

4.2 Neutrophil Chemoattractants

IL-8 and LTB_4 are the most potent chemoattractants for neutrophils, and, as mentioned above, both appear to play a critical pathogenic role in cystic fibrosis patients infected with *P. aeruginosa*.[38,55,67,79] Moreover, leukotrienes augment IL-1 production by human monocytes.[80] Therefore, the rationale is clearly there for controlling the chronic and massive production of these compounds in the inflamed airways.

There is recent clinical evidence for the *in vivo* suppression of IL-8 levels in the cystic fibrosis respiratory tract by treatment with aerosolised recombinant secretory leukoprotease inhibitor.[43] An initial clinical trial in cystic fibrosis patients has shown that eicosapentaenoic acid 2.7g in fish oil capsules daily for 6 weeks modulated LTB_4 metabolism, an effect that was associated with a significant decrease in sputum production and improvement in clinical score and spirometry.[55] Aerosol and oral administration of a prodrug of 2,6-disubstituted 4-(2-arylethenyl)phenol successfully and specifically inhibited antigen-induced synthesis of leukotrienes in the lungs of monkeys,[81] perhaps pointing towards another means of using leukotriene antagonists and biosynthesis inhibitors in the treatment of inflammatory diseases. The potential clinical benefits in cystic fibrosis of such immunotherapeutic strategies, used alone or in combination with other procedures, deserve to be investigated further.

4.3 Colony-Stimulating Factors

Finally, granulocyte and granulocyte-macrophage colony-stimulating factors (G-CSF and GM-CSF) are proving of great value in treating infections in a variety of clinical and experimental settings.[82,83] Subcutaneous injections of G-CSF in mice infected intramuscularly with *P. aeruginosa* significantly increased survival and suppressed bacterial growth.[84] The potential of this agent in cystic fibrosis patients with pulmonary *P. aeruginosa* infections has not yet been assessed.

4.4 Overview

Taken together, these observations illustrate the potential clinical benefits of modulating the action of cytokines and eicosanoids in the lungs of patients with cystic fibrosis. Such treatments may control inflammatory damage in the chronically colonised lung and/or optimise host defence mechanisms against *P. aeruginosa*. However, the majority of these approaches towards novel therapy remain theoretical or experimental.

The importance of preserving host immune defences in cystic fibrosis patients must be emphasised, particularly in young and newly identified carriers of *P. aeruginosa*. Thus, procedures that will interfere with phagocytic defence mechanisms in the lung, such as therapies directed against IL-1, TNF and/or IL-8, may only prove indicated in chronically colonised patients. In these individuals the immune response has clearly become detrimental to the host, and pathogenic inflammation must be controlled to prevent further pulmonary damage. This issue will be discussed in more detail when analysing the advantages and disadvantages of boosting immune function in the cystic fibrosis lung.

5. Immunisation

The adverse effects of anti-inflammatory therapy have stimulated research into the development of alternative immunomodulating strategies. In an attempt to boost the effector mechanisms of host immunity, immunisation procedures have been tested for the treatment and/or prevention of *P. aeruginosa* pneumonia in cystic fibrosis patients. These procedures include:

- passive immunisation, i.e. transfer of immunoglobulins to the patient
- active immunisation, i.e. vaccination.

5.1 Immunoglobulin Transfer

Animal experiments have demonstrated that intravenous administration of IgG against *P. aeruginosa* combined with antibiotics enhanced bacterial killing and survival of lethally infected hosts.[85,86] Functional IgG and IgA are deficient in lungs colonised with *P. aeruginosa*, partly because of proteolytic inactivation.[11,12,87-90] These observations provided the rational basis for assessing the therapeutic value of immunoglobulin transfer to cystic fibrosis patients.

Intravenous administration of immunoglobulin and antibiotics combined with chest physiotherapy in cystic fibrosis patients with an acute exacerbation of *P. aeruginosa* infection significantly increased FVC, FEV_1 and serum IgG levels.[91] However, quantitative sputum cultures for *P. aeruginosa* did not correlate with the experimental group or improved clinical condition.

Another study determined the effects of intravenously administered hyperimmune globulin (enriched >5-fold for anti–*P. aeruginosa* lipopolysaccharide) on chronic *Pseudomonas* infection in cystic fibrosis patients in combination with conventional therapy such as intravenous antibiotics, chest physiotherapy, bronchodilators and nutritional supplementation.[92] In treated patients, there was an increase in the serum levels of anti–*P. aeruginosa* lipopolysaccharide IgG antibodies, combined with a decline in sputum *P. aeruginosa* density. These changes were associated with improved pulmonary function as assessed by FVC and FEV_1.

Together, these findings suggest that passive administration of hyperimmune globulin is a safe and beneficial adjunctive therapy that may be indicated in moderately ill patients during a pulmonary exacerbation caused by antibiotic-resistant *P. aeruginosa*. However, the underlying mechanisms of these effects must be elucidated. The results from the extensive clinical trials of immunoglobulin therapy presently underway will help determine whether this strategy can be applied routinely to patients with cystic fibrosis.

5.2 Vaccination

Development of a vaccine against pulmonary infection with *P. aeruginosa* is the subject of several studies. However, all attempts to prevent the establishment of the bacteria in the lungs of cystic

fibrosis patients have either failed or not been fully assessed,[93-96] even though immunisation with *P. aeruginosa* lipopolysaccharide can protect against infections associated with burn wounds.[97-99] Experimental vaccines have been based on *P. aeruginosa* lipopolysaccharide, purified outer membrane proteins, and surface epitopes such as protein conjugates, mucoid exopolysaccharides (MEP) and monoclonal anti-idiotype antibodies. Two particular experimental approaches have yielded promising findings: systemic immunisation with an anti-idiotype mimicking MEP, and intestinal immunisation with whole *P. aeruginosa* antigens.

5.2.1 Anti-Idiotype Vaccines

An anti-idiotypic antibody has been generated in mice that is directed to an opsonic monoclonal antibody specific for the *P. aeruginosa* MEP.[100,101] Experiments *in vitro* showed that this antibody fixed complement to mucoid *P. aeruginosa* isolates and successfully opsonised the bacteria for phagocytosis by human neutrophils.

The advantage of an anti-idiotype vaccine resides in its production of specific antibodies to MEP without the concurrent activation of negative feedback mechanisms that are apparently initiated by standard immunisation protocols. However, it remains to be shown whether such a vaccine can elicit protective immunity in humans. Moreover, in light of the potent immunoevasive activities displayed by *P. aeruginosa*, in particular its proteolytic effects on immunoglobulin molecules, a vaccine not solely based on the production of specific antibodies would seem more suitable for use in cystic fibrosis. As suggested previously,[11] the optimal *Pseudomonas* vaccine should be able to:

- overcome at least part of the immunoevasive activities of the bacteria
- enhance cellular mechanisms of immunity that are specific to the bacteria
- prevent the onset of the first infection with *P. aeruginosa*
- increase bacterial clearance in patients with ongoing infections.

A novel vaccine protocol has recently been developed with the aims of: (a) enhancing the effectiveness of antigen-presenting cells, and (b) directing the host's immune system to an appropriate T helper response.[102,103] In this approach, biochemical coupling of an antigen with a strongly immunogenic cytokine such as GM-CSF or IFNγ creates a potent immunogen that induces idiotype-specific antibodies. Before this exciting new technology can be tested in the context of *P. aeruginosa*–infected cystic fibrosis patients, more needs to be known about the immunology of this host/parasite interaction, particularly in the areas of T helper lymphocyte subsets and effector mechanisms of immunity.

5.2.2 Mucosal Vaccines

The concept of a common mucosal immune system has been used for the development of vaccines effective in the lung and at other mucosal sites.[104] In this concept, gut-associated lymphoid tissue is the induction site and the appropriate effector components, the antibody and/or cellular immune response, reside at the pulmonary surface. This approach has provided the basis for the development of another promising immunotherapeutic strategy for the prevention and/or treatment of *P. aeruginosa* infections in cystic fibrosis.

Animals intestinally immunised against mucoid *P. aeruginosa* were able to achieve significant protective immunity against subsequent bacterial challenge in the lung.[105] Immunological studies with this model[62,63] demonstrated that:

- pulmonary protection was achieved regardless of the immunoevasive activities of the microorganism
- the immune response was mediated by T cells that primed alveolar macrophages for enhanced recruitment and activity
- T cells and activated macrophages then stimulated bronchoalveolar neutrophils for accelerated infiltration and activation.

Hence a dramatic increase in phagocytic capacity of both alveolar macrophages and bronchoalveolar neutrophils was the main final effector mechanism of protective immunity in this model

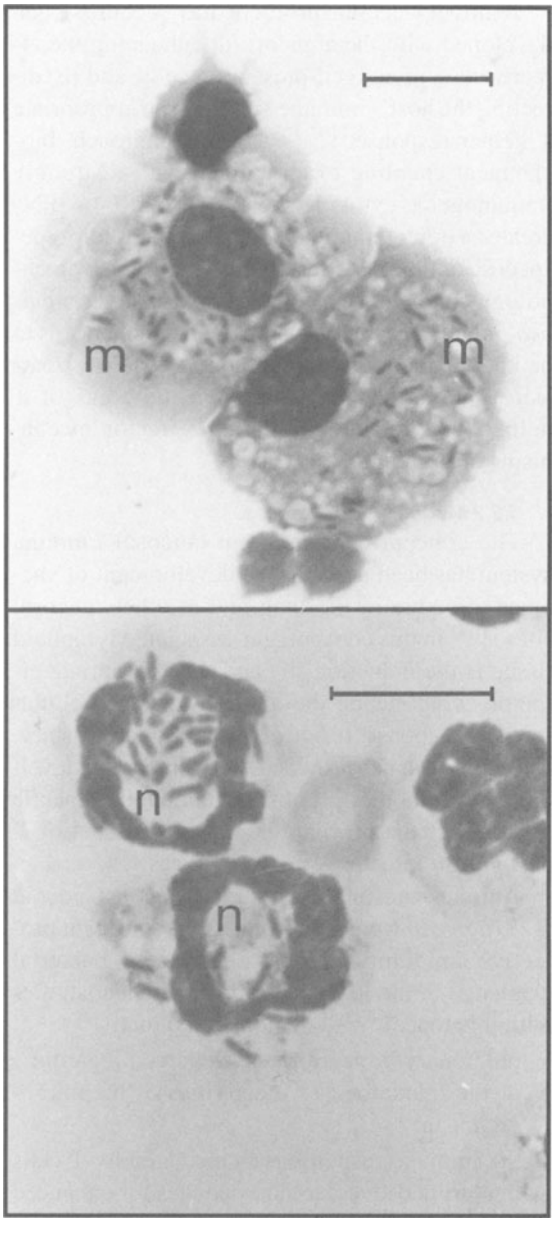

Fig. 2. Light micrographs of cytospin preparations from the bronchoalveolar lavages of rats intestinally immunised with mucoid *Pseudomonas aeruginosa* and subsequently challenged with live bacteria for 30 min (above) or 4 hours (below). High numbers of *P. aeruginosa* organisms can be seen within alveolar macrophages (m) and bronchoalveolar neutrophils (n). In this model, the phagocytic capacity of both cell types was significantly enhanced via intestinal immunisation, a response that was associated with pulmonary protection from subsequent infection.[62,63] Scale bars = 10μm.

(fig. 2). Intestinal immunisation also primed the lung for accelerated production of TNFα, but it controlled the massive release of LTB_4, IL-1α and TNFα seen in non-immunised hosts infected with *P. aeruginosa*. Further investigations using this experimental system will help characterise the pivotal mediators of protective immunity in the lung and assist in the development of novel strategies relevant to oral vaccination against *P. aeruginosa* colonisation in the lungs of cystic fibrosis patients.

Despite the optimism fuelled by such novel achievements, it seems unlikely however that a commercial *Pseudomonas* vaccine will be available for cystic fibrosis patients in the immediate future. Clearly, both passive and active immunisation strategies deserve a high priority on the list of immunotherapies that must be tested and analysed in the clinical setting.

6. Immune Response in the Lung of Cystic Fibrosis Patients: Friend or Foe?

Discussion of the potential clinical benefits of anti-inflammatory therapy and immunisation may appear paradoxical, as one advocates controlling the host response and the other promotes its augmentation. Studies of uninfected and colonised cystic fibrosis patients, as well as recent experimental findings using immune and nonimmune animals infected with *P. aeruginosa*, may shed more light on this apparent contradiction and help in the assignment of appropriate clinical indications for these opposing immunotherapeutic strategies.

The problem stems from the broad range of clinical presentation associated with *P. aeruginosa* infections in the lungs of patients with cystic fibrosis. There is no doubt that pulmonary inflammation is a major contributor to pathogenesis in the end-stage of lung disease in cystic fibrosis (fig. 1). However, pathogenesis is initiated by the permissive environment in the lungs of cystic fibrosis patients that allows the first establishment of *P. aeruginosa* in the airways. These observations emphasise that the choice of immunotherapeutic

strategies in cystic fibrosis will be dictated by the clinical status of each individual patient.

6.1 Prevention of Infection

Prevention of the crucial first infection with *P. aeruginosa* seems to be of paramount importance. This justifies a significant therapeutic boosting of immunity, either via immunisation or other cytokine-enhancing therapy, in an attempt to attenuate the initial pathogenic colonisation by bacteria. Experiments using rats intestinally vaccinated against *P. aeruginosa* have suggested that significantly increased macrophage and neutrophil activity constitutes a central effector mechanism of pulmonary immunity, and that acutely elevated levels of TNFα (but not of IL-1) in the bronchoalveolar space mediate such a response.[62,63] These characteristics are therefore possible clinical targets for the development of effective preventive immunotherapy against *P. aeruginosa*.

6.2 Place of Anti-Inflammatory Therapy

In contrast, in patients whose pulmonary defence mechanisms have long been overwhelmed by chronic infections the host response is the major driving force towards lung damage. Anti-inflammatory therapy may prove of great clinical benefit in such patients. However, future studies are needed to establish whether boosting immunity under such circumstances may result in detrimental clinical effects, as it may amplify the chain of reactions that is the cause of pathology.

In summary, rather than being paradoxical, the duality of immunomodulatory concepts in cystic fibrosis addresses different clinical situations. Within this framework of extreme strategies, various degrees of combined therapy may prove of great clinical benefit. A better understanding of the pathogenesis and effector mechanisms of immunity in *P. aeruginosa* pneumonia will help identify the appropriate clinical targets, or combinations thereof, for immunotherapy in cystic fibrosis patients.

7. Other Therapies

7.1 Deoxyribonuclease

Therapeutic alteration of the abnormal mucus viscosity in the lungs of patients with cystic fibrosis has become an important topic for pharmaceutical research. The thick and sticky respiratory secretions reduce expiratory flow rates and thus play a critical role in the pathogenesis of pulmonary disease in cystic fibrosis (fig. 1). In response to airway infection, neutrophils release large quantities of DNA which significantly increases the viscosity of mucus. Preliminary studies showed that recombinant human DNase cleaved the DNA in cystic fibrosis mucus *in vitro*.[106] The use of aerosol delivery of DNase was successful in initial clinical trials as it was associated with significantly increased DNase activity and improved FVC and FEV_1 in treated patients throughout the experimental period.[107,108] No adverse effects were observed, but measurements taken 1 week after therapy showed that pulmonary function had again declined towards baseline values.[107] Subsequent clinical studies[109] leading to licensing have now established that treatment with DNase is free from adverse effects and that improved pulmonary function could be maintained for at least 1 year if the drug is used regularly. Therefore, aerosolised DNase treatment appears to be particularly suitable for the maintenance of pulmonary function in cystic fibrosis patients with chronic purulent airway secretions.

7.2 Gelsolin

Gelsolin, a protein that severs actin filaments, has been shown to significantly reduce the viscosity of mucus from patients with cystic fibrosis.[110] In the lungs of such patients, the naturally occurring plasma gelsolin proteins may be overwhelmed by the enormous quantities of filamentous actin derived from inflammation, leading to an increase in mucus viscosity. *In vitro*, human plasma gelsolin rapidly severed noncovalent bonds between monomers of individual actin filaments, and this shortening of actin filaments was associated with a 62%

Table I. Immunotherapeutic strategies for patients with cystic fibrosis and pulmonary *Pseudomonas aeruginosa* infections

Strategy	Target	Clinically tested	References
Protease inhibitor therapy α-Antitrypsin (aerosol) Recombinant secretory leukoprotease inhibitor (aerosol) ICI 200800	Control of increased elastase levels in the infected lung	Yes	42, 43
Anti-inflammatory therapy Prednisone (oral) Ibuprofen (oral)	Control of pulmonary inflammation (neutrophilia)	Yes	47, 48, 50 53, 54, 56
Cytokine/eicosanoid therapy	Modulation of activity of inflammatory mediators such as interleukins-1 and -8, tumour necrosis factor-α and/or leukotriene B_4 in the lung	No[a]	43, 55, 61, 62, 69-71, 75, 81
Immunoglobulin transfer	Enhancement of antibody-dependent protective immunity	Yes	91, 92
Vaccination	Prevention and/or reduction of colonisation with *P. aeruginosa* in the lung	Yes (failed)[b]	93-96
DNase (aerosol)	Reduction of mucus viscosity	Yes	106-109
Gelsolin	Reduction of mucus viscosity	No	110
Distearoyl phosphatidylglycerol (aerosol)	Improvement of mucociliary clearance	No	23
Amiloride (aerosol)	Reduction of pulmonary Na^+ absorption	Yes?[c]	112, 115-117

a Recombinant secretory leukoprotease inhibitor aerosol therapy suppresses endobronchial interleukin-8 in the lung,[43] and oral eicosapentaenoic acid modulates leukotriene B_4 metabolism and improves clinical parameters in patients with cystic fibrosis.[55] A phase III clinical trial of pentoxifylline, an inhibitor of tumour necrosis factor-α transcription, is currently underway in cystic fibrosis patients.

b Other vaccination strategies such as inducing idiotype-specific antibodies[100-103] and oral immunisation[62,63,105] have shown promising experimental results, but have not yet been clinically tested in patients with cystic fibrosis.

c Controversial findings.

mean decrease in viscosity of sputum samples. Overall, the effect of gelsolin *in vitro* was kinetically and stoichiometrically more efficient than that of DNase.[110] The potential therapeutic benefits of gelsolin administration should now be assessed within the clinical setting.

7.3 Distearoyl Phosphatidylglycerol

Recent studies using an *in vitro* system of distearoyl phosphatidylglycerol liposomes delivered to mucus from cystic fibrosis patients have led to another interesting therapeutic approach for improving mucociliary transport in cystic fibrosis.[23] This procedure appeared to reduce the stickiness of mucus from cystic fibrosis patients and accelerate its impaired transport on respiratory epithelia. These 2 abnormal features are partly due to the decreased levels of phosphatidylglycerol in the respiratory secretions of cystic fibrosis patients.[23] The effect was significantly more pronounced than when water was added alone, clearly implicating factors other than mucus rehydration.

Independent studies have demonstrated that liposome aerosol inhalation had no adverse effects on lung spirometry or oxygen metabolism in healthy volunteers.[111] In the light of the important role of impaired mucociliary clearance in the pathogenesis of pulmonary infections with *P. aeruginosa*, it would be worthwhile investigating whether the lubricating effect of distearoyl phosphatidylglycerol liposomes may be of significant clinical benefits to cystic fibrosis patients.

7.4 Amiloride

Significantly increased epithelial sodium absorption plays a central role in the pathogenesis of pulmonary disease in cystic fibrosis (fig. 1). Clinical trials in cystic fibrosis patients have assessed

the therapeutic benefit of nebulised amiloride, a specific sodium channel blocker. Application of amiloride to the mucosal surface of epithelia from cystic fibrosis patients reduces sodium absorption.[112] This agent also has antibacterial activity[113] and interferes with neutrophil activation.[114] Clinical investigation in cystic fibrosis patients has shown that long term amiloride inhalation further improved the enhanced mucociliary clearance observed after short term treatment with the same drug.[115] A subsequent trial found that, following a course of parenteral antibiotics, administration of nebulised amiloride to cystic fibrosis patients increased mucociliary clearance, improved sputum viscosity, and prevented the loss of FVC,[116] clearly implying a beneficial clinical effect.

In contrast with these earlier findings, a recent study showed that long term aerosol treatment of cystic fibrosis patients with amiloride does not improve FEV_1 or FVC, sputum culture and rheology, or white cell counts.[117] These observations suggest that the place of therapy with nebulised amiloride alone in patients with cystic fibrosis is not currently established. However, the potential benefits of amiloride treatment in cystic fibrosis in combination with other therapeutic strategies deserve to be evaluated further.

8. Conclusions

An ever-growing choice of new treatment modalities is becoming available to physicians managing cystic fibrosis patients, and immunotherapeutic strategies may soon become an important component of treatment (table I). More clinical trials based on novel experimental findings must be encouraged so that some of the strategies that are still in developmental phases should progress to practical utility in the treatment and/or prevention of *P. aeruginosa* infection of the lungs of patients with cystic fibrosis. In conclusion:

- Protease inhibitor therapy has shown positive clinical benefits in the treatment of cystic fibrosis patients colonised with *P. aeruginosa*. α-Antitrypsin, secretory leukoprotease inhibitor and ICI 200 800, a synthetic competitive inhibitor of neutrophil elastase, have undergone clinical trials.
- Anti-inflammatory therapy, using ibuprofen or other NSAIDs rather than corticosteroids, may be particularly beneficial in chronically infected patients as it controls the severe neutrophilic infiltration that damages the airways.
- The beneficial effects of cytokine and eicosanoid therapy need to be further assessed in clinical trials. Based on recent experimental findings, strategies involving IL-1 receptor antagonist, IL-4 or IL-10 appear to be particularly worthy of investigation.
- Transfer of hyperimmune globulin is a beneficial adjunctive therapy in moderately ill patients with cystic fibrosis who present with a *P. aeruginosa* infection.
- As yet, there is no commercially available vaccine to prevent pulmonary infections with *P. aeruginosa* in cystic fibrosis patients. To achieve protective immunisation in the lungs would represent a major breakthrough in the clinical management of these patients. Two areas seem to be particularly worthy of study: the use of an anti-idiotype vaccine, and mucosal (oral) immunisation with *P. aeruginosa* antigens.
- Therapy with DNase to decrease mucus viscosity appears to be indicated for maintenance of pulmonary function in patients with purulent airway secretions. In moderately ill patients, it may help reduce the permissivity of the pulmonary environment for bacterial colonisation, an effect which has yet to be assessed. The clinical potential of gelsolin for the reduction of mucus viscosity also remains to be tested.

In summary, novel developments in immunomodulatory therapies, although still at an early phase of development, are likely to improve the welfare of cystic fibrosis patients waiting for a cure, and thus hold new hope for the future clinical management of this devastating disease.

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Correspondence and reprints: Dr *Andre Buret*, Gastrointestinal Research Group, Faculty of Medicine, University of Calgary, 3330 Hospital Drive NW, Calgary, Alberta T2N 4N1, Canada.