

## Lung injury in cystic fibrosis

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The major cause of death in cystic fibrosis is respiratory failure associated with pulmonary hypertension and cor pulmonale. This follows a variable period of chronic pulmonary infection and progressive lung injury associated with a considerable morbidity. Lung injury is caused mainly by a continuous and exuberant host inflammatory response to chronic endobronchial bacterial infection. The initiation of infection and the regulation of the inflammatory response are poorly defined and greater understanding of both might indicate ways of protecting the lungs and prolonging survival.

### Pathology of the lung in cystic fibrosis

Histological abnormalities of the lung are detectable within the first few days of life.<sup>1</sup> Before any infective episodes submucosal gland hypertrophy, duct obstruction, and mucus cell hyperplasia occur. These abnormalities are probably related to the underlying genetic defect and encourage colonisation of the respiratory tract with microorganisms. Repeated pulmonary infection causes bronchiolitis, mucus impaction, cyst formation, and ultimately bronchiectasis, which has been observed in children as young as 2 months.<sup>2</sup> This leads to a vicious circle of endobronchial and endobronchiolar infection and inflammation with impaired ciliary function and reduced clearance of mucus.<sup>3-5</sup> Further bronchial and bronchiolar damage occurs, leading to obstruction, bronchial compression, and secondary alveolar injury with atelectasis.<sup>5,6</sup>

### Role of infection

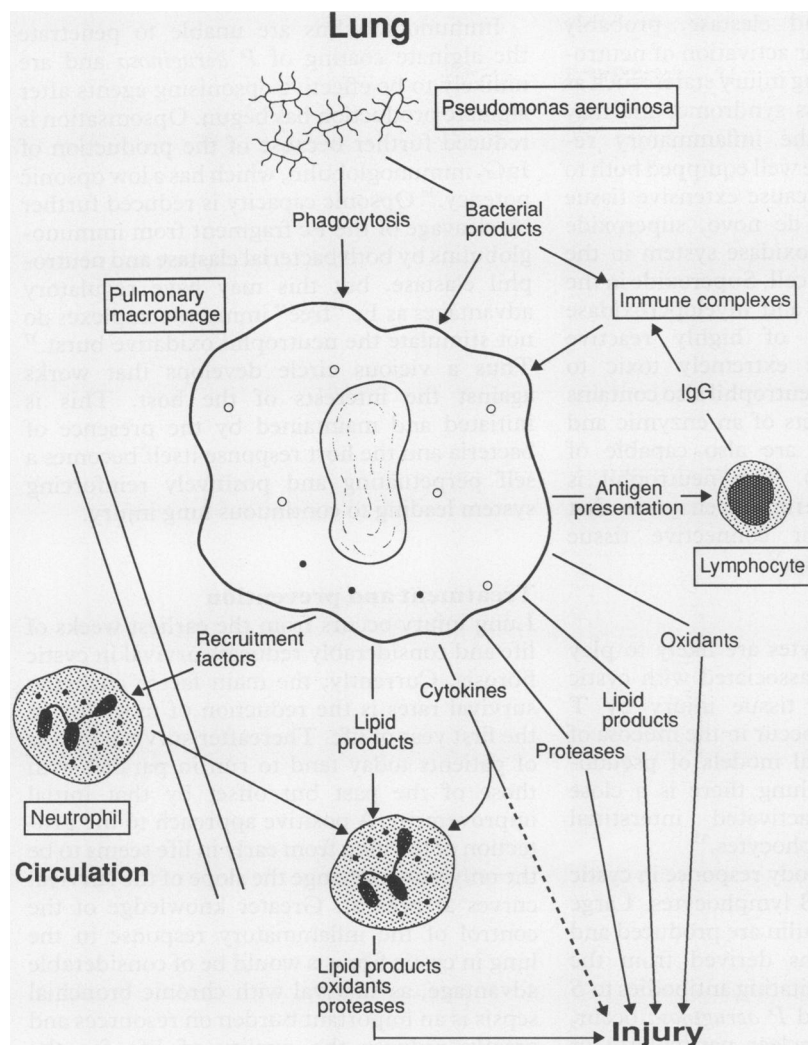
Viral infections may compromise airway defences and encourage secondary bacterial infection of the lower airways, but this remains unproved.<sup>7</sup> The main bacteria infecting the lungs in cystic fibrosis are *Staphylococcus aureus*, *Haemophilus influenzae*, and *Pseudomonas aeruginosa*. The relation between the presence of an organism in the sputum and lung injury is not clear. This has led to the use of the terms "colonisation" to indicate a non-injurious infected state and "infection" to indicate injury associated with infection. The use of the term "colonisation" without proof of the absence of lung injury has led clinicians to underestimate the potential for early lung injury in cystic fibrosis.

*S aureus* is associated with infection in the first few years of life, but there remains controversy about the importance of such infection as an initiator of lung injury. Deterioration of lung function with chronic *S aureus* infection, before colonisation by *P aeruginosa*, indicates the organism's capacity to initiate lung injury,<sup>8</sup> as does radiographic evidence of lung destruction associated with high titres of IgG antibody to staphylococcal teichoic acid.<sup>9</sup> The lack of consensus on the importance of *S aureus* has led to a diversity of treatment regimens, ranging from no treatment<sup>10</sup> to intermittent intensive courses aimed at eradication<sup>11</sup> and continuous long term prophylaxis.<sup>10,12</sup> Chronic *S aureus* infection usually precedes *P aeruginosa* infection, but how such infection or its treatment relates to the development of chronic infection with *P aeruginosa* is not clear.<sup>13</sup>

Non-capsulate strains of *H influenzae* are frequently isolated from the respiratory tract of children with cystic fibrosis, though whether they act as pathogens is unknown.<sup>14,15</sup> In adults non-capsulate strains are an important pathogen in the respiratory tract and in children with otitis media and community acquired pneumonia.<sup>16,17</sup> *H influenzae* has increased mucosal adherence during viral infection and produces various virulence factors, which lyse IgA<sub>1</sub>, cause ciliary dyskinesia, release histamine, and stimulate mucus secretion.<sup>18-20</sup> Patients with cystic fibrosis had a higher isolation rate for *H influenzae*, with a further increase at the time of a symptomatic respiratory deterioration, than asthmatic patients.<sup>21</sup> In addition, *Haemophilus parainfluenzae* was isolated, though it may not be a pathogen in cystic fibrosis.<sup>21</sup> In adults the role of *H influenzae* infection is probably underestimated as this organism may coexist with *P aeruginosa* infection in a spheroplast form and not be isolated without the use of selective media.<sup>22</sup> In patients with cystic fibrosis infected with *P aeruginosa* 80% were coinfecting with *H influenzae* when sputum was specifically cultured for the latter organism.<sup>23</sup> Most interest has focused on the role of chronic infection with *P aeruginosa*, which is associated with chronic lung injury and a reduced survival.<sup>24,25</sup> Much of the knowledge of the host response in cystic fibrosis and its relation to lung injury comes from the study of interaction between the host and *P aeruginosa*.

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Interactions between *Pseudomonas aeruginosa* and the host humoral and cellular responses in the lung, indicating the coordinating role of the pulmonary macrophage in various pathways of injury.

### The host response to pulmonary infection

In cystic fibrosis there is no convincing evidence to suggest a defect in host defences, though there are reports of individual cases of abnormality. Infection at sites other than the lung are no more common in patients with cystic fibrosis than in individuals without cystic fibrosis and systemic spread of pulmonary infection is rare, despite the enormous bacterial load and often debilitated state of the patient with cystic fibrosis. The inflammatory response in cystic fibrosis is not fully understood but includes both cellular and humoral aspects.

#### PULMONARY MACROPHAGES

There are subpopulations of macrophages in the lung, comprising the alveolar macrophage, the interstitial macrophage, and the intravascular pulmonary macrophage.<sup>26</sup> The role of these subtypes in the pathogenesis of lung injury is not clear but pulmonary macrophages, with their range of functions, seem likely to be fundamental in the initiation and maintenance of the host response to infection (figure). The alveolar macrophage is probably the first line of

defence met by invading bacteria. Phagocytosis of bacteria will activate the cell, leading to a range of signals that affect the function of other inflammatory cells.<sup>27</sup> Such factors include surface expression or secretion (or both) of various cytokines, neutrophil chemotactic factors, HLA class I and II antigens, and the presentation of processed foreign antigen. By this means the acute inflammatory-phagocytic response, mediated by neutrophils and monocytes, and the inflammatory-immune response, mediated by lymphocytes, are activated and maintained. All these functions are essentially protective, and with its stimulatory effects on fibroblast proliferation and the secretion of connective tissue the pulmonary macrophage links the normally self limiting inflammatory response with the first phases of healing. In cystic fibrosis, however, this process is exaggerated by the continual presence of bacteria and their antigenically active byproducts. The host response then perpetuates the vicious cycle with more tissue injury leading to more infection and further inflammation.<sup>3</sup> The alveolar macrophage has a limited capacity for bacterial clearance and generally is more effective against Gram negative than Gram positive organisms. Rat alveolar macrophages exposed in vitro to formalin killed *P. aeruginosa* release neutrophil chemotactic factor in a dose dependent manner, whereas in vivo instillation of the same bacteria lead to a pulmonary neutrophilia 24 hours later. Thus the alveolar macrophage has the key function, at least in the earliest phases of infection, of coordinating the inflammatory response, but later it is likely that the interstitial macrophage maintains the host mucosal inflammatory response and leads to cell mediated tissue injury via T lymphocytes. Intravascular pulmonary macrophages provide a link between the lung and the systemic response to infection. This is probably by the release of cytokines, chemotactic factors, and a facilitation of the endothelial aspect of cell transit from the vascular compartment. The intravascular pulmonary macrophage in particular is an efficient particle removing cell,<sup>28</sup> and may be an important regulator of circulating immune complexes arising from the lung in cystic fibrosis. The presence of circulating cytokines, such as tumour necrosis factor and various interleukins, augment and maintain the inflammatory response.<sup>29</sup> Peripheral metabolic effects of tumour necrosis factor alpha may also specifically mediate the development of the cachexia associated with chronic sepsis in cystic fibrosis.

#### POLYMORPHONUCLEAR NEUTROPHILS

Neutrophils are the predominant cells of the alveolar space in patients with cystic fibrosis and chronic pseudomonas infection. These represent a substantial systemic circulatory component of lung inflammation in that cells such as the neutrophil and monocyte are largely recruited from this compartment by various chemoattractant stimuli. This is shown by increased neutrophil transit into the lung and also the presence of neutrophil granule products in the circulation.<sup>29 30</sup> Granule products,

such as lactoferrin and elastase, probably derive from intravascular activation of neutrophils, as seen in other lung injury states, such as adult respiratory distress syndrome, and may be used to monitor the inflammatory response.<sup>31</sup> Neutrophils are well equipped both to destroy bacteria and to cause extensive tissue injury. They produce, de novo, superoxide anion via the NADPH oxidase system in the plasma membrane of the cell. Superoxide in the presence of halide ions and myeloperoxidase will produce a range of highly reactive metabolites, which are extremely toxic to surrounding cells. The neutrophil also contains many preformed products of an enzymic and cationic nature, which are also capable of extensive injury (table). The neutrophil is remarkably potent in terms of enzymes that break down the major connective tissue proteins found in the lung.<sup>32</sup>

#### LYMPHOCYTES

Both T and B lymphocytes are likely to play part in the lung injury associated with cystic fibrosis. Cell mediated tissue injury by T lymphocytes is likely to occur in the mucosa of these patients. In animal models of pseudo-monal infection of the lung there is a close association between activated interstitial macrophages and T lymphocytes.<sup>33</sup>

The pronounced antibody response in cystic fibrosis is mediated by B lymphocytes. Large amounts of immunoglobulin are produced and directed against antigens derived from the infecting bacteria. Precipitating antibodies to *S aureus*, *H influenzae*, and *P aeruginosa* occur, but their role is largely unclear, particularly for *H influenzae* and *S aureus*. In *P aeruginosa* infection there is a massive antibody response to bacterial exoproducts, but later antibodies to cell wall lipopolysaccharide predominate, with the formation of immune complexes both within the lung and in the circulation. Early on such antibodies may be protective, especially if they are opsonically active<sup>34</sup>; whereas in the later phases of this disorder high levels of immune complexes form and spill over into the circulation from the lung and indicate the terminal phase of pulmonary inflammation.<sup>35</sup> Immune complexes can maintain the inflammatory response by direct stimulation of various inflammatory cells.

#### *Injurious neutrophil products*

##### *Oxidants*

Superoxide ion  
Hydrogen peroxide  
Hydroxyl radical  
Hypochlorous acid  
Singlet oxygen

##### *Specific granule products*

B<sub>12</sub> binding proteins  
Lactoferrin  
Lysozyme

##### *Azurophil granule products*

β glucuronidase  
Collagenase  
Elastase  
Lysozyme  
Myeloperoxidase

Immunoglobulins are unable to penetrate the alginate coating of *P aeruginosa* and are unlikely to be effective opsonising agents after alginate production has begun. Opsonisation is reduced further because of the production of IgG<sub>2</sub> immunoglobulin, which has a low opsonic potency.<sup>36</sup> Opsonic capacity is reduced further by cleavage of the Fc fragment from immunoglobulins by both bacterial elastase and neutrophil elastase, but this may have regulatory advantages as Fc "free" immune complexes do not stimulate the neutrophil oxidative burst.<sup>37</sup> Thus a vicious circle develops that works against the interests of the host. This is initiated and maintained by the presence of bacteria and the host response itself becomes a self perpetuating and positively reinforcing system leading to continuous lung injury.

#### Treatment and prevention

Lung injury occurs from the earliest weeks of life and considerably reduces survival in cystic fibrosis. Currently, the main factor changing survival rates is the reduction of mortality in the first year of life. Thereafter survival curves of patients today tend to run in parallel with those of the past but offset by that initial improvement. A positive approach to the protection of the lung from early in life seems to be the only way to change the slope of the survival curves at present. Greater knowledge of the control of the inflammatory response in the lung in cystic fibrosis would be of considerable advantage, as survival with chronic bronchial sepsis is an important burden on resources and greatly reduces the quality of life for the survivors. A more subtle approach to controlling specific inflammatory mechanisms at an early stage may halt injury, or at least reduce the rate of injury, leading to a better survival and a higher quality of life. In cystic fibrosis corticosteroid treatment preserves lung function, though the mechanisms are unknown.<sup>38</sup> Various other potential treatments are being studied. Non-steroidal anti-inflammatory agents acting by cyclo-oxygenase inhibition reduce lung injury in experimental pseudomonas pneumonia in animals.<sup>39</sup> The future may yield specific antielastase compounds, antioxidants, antagonists for receptors for specific cytokines, monoclonal antibodies to specific cytokines, and agents like pentoxifylline, which has a wide range of anti-inflammatory effects and protects against lethal bacterial infection in animals.<sup>40</sup>

Even with knowledge of the cystic fibrosis gene and of the cystic fibrosis transmembrane regulator protein, many patients for several decades are likely to be at risk of a shortened lifespan because of chronic pulmonary infection. For these patients more research needs to be aimed at defining the processes underlying lung injury.

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