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Cystic fibrosis: ironing out the problem of infection?

David W. Reid,[1] Gregory J. Anderson,[2] and Iain L. Lamont[3]

[1]*Menzies Research Institute, Hobart, Tasmania, and* [2]*Iron Metabolism Laboratory, Queensland Institute of Medical Research, Brisbane, Queensland, Australia; and* [3]*Department of Biochemistry, University of Otago, Dunedin, New Zealand*

THE STUDY BY MOREAU-MARQUIS et al. (3) in this issue of *AJP-Lung* advances our understanding of lung disease in cystic fibrosis (CF). Their findings are important because they may largely explain the peculiar propensity to early *Pseudomonas aeruginosa* infection that characterizes this most common of lethal genetic diseases. Moreau-Marquis et al. (3) demonstrate that immortalized human CF airway epithelial cells cocultured with *P. aeruginosa* promote the formation of antibiotic-resistant biofilms. Their series of experiments demonstrate that this is due to abnormal accumulation and loss of iron by CF epithelial cells and that biofilm formation can be prevented by either iron chelation therapy or by increasing normal cell membrane cystic fibrosis transmembrane regulator (CFTR) expression using the novel compound Corr4a. The observations of Moreau-Marquis et al. (3) confirm what has been clinically suspected in CF with respect to iron promotion of infection with *P. aeruginosa*. Previous studies have demonstrated that the airways of CF patients contain increased amounts of total iron and the iron-binding protein ferritin as well as a relationship between airway iron content and quantitative *P. aeruginosa* load assessed by colony-forming units per milliliter of sputum (5). This study also found an increase in iron in sputum in CF patients who had not previously been infected with *P. aeruginosa*, suggesting that elevated airway iron content may predate *P. aeruginosa* acquisition. Further evidence to support a problem with iron trafficking comes from the CF G551D mouse model. The teeth of these mice exhibit abnormal iron transport in pigment ameloblasts compared with wild-type mice, resulting in unusually white dentition (7). The study by Moreau-Marquis et al. (3) lends biological rationale to these earlier observations and brings two key questions into focus. First, what is the underlying link between abnormal or absent CFTR and cell iron homeostasis? Second, how does this impact on the CF host and *P. aeruginosa* interaction?

AIRWAY EPITHELIAL CELL IRON HOMEOSTASIS

Very little is known about lung cell iron homeostasis, even in health. The study by Moreau-Marquis et al. (3) offers no mechanistic explanation for their findings. Like all cells, airway epithelial cells and other cell types in the lung require iron for their normal metabolic functions, but they also play a role in regulating iron levels in the extracellular fluid. This latter function is important, as the lung is exposed to substantial amounts of environmental iron on a daily basis. Iron is a redox active metal and can catalyze reactions leading to the formation of toxic oxygen radicals. By removing potentially damaging iron from the surface of the airways, epithelial cells and macrophages play an important role in iron detoxification. As the current study shows, iron is also a virulence factor for bacteria, so iron removal also helps to limit bacterial growth.

Airway epithelial cells can acquire iron in several ways. Most iron in the circulation is bound to transferrin, and this iron can be utilized through receptor-mediated endocytosis by lung epithelial cells. Much of the iron in the airway extracellular fluid is not bound to transferrin, but epithelial cells can still utilize it through divalent metal transporter-1 (DMT1). Since DMT1 uses ferrous (Fe^{2+}) iron as a substrate, the oxidized iron that is most likely to be found in the airways must be reduced before it can be used. Although there are several candidates for this iron reductase, none have been well-characterized. The transferrin homolog lactoferrin is also found in lung fluids and can be taken up by airway epithelial cells after interaction with a specific receptor. Once within the cell, iron can be sequestered in a nontoxic form within the storage protein ferritin. All cells can synthesize ferritin to store iron, but macrophages are particularly well-adapted for this role. Although there are several potential pathways for iron entering the cell, iron efflux largely reflects the activity of a single protein, the membrane iron exporter ferroportin (FPN). FPN is expressed by both epithelial cells and macrophages in the lung. Iron release in the form of ferritin has been described for some cells, and this may also be operating in the airways as ferritin is readily detected in the extracellular fluids of the lung. The iron complement of a cell under any given set of conditions will therefore reflect a balance between iron influx and efflux. In their study, Moreau-Marquis and colleagues (3) demonstrated that airway epithelial cells expressing ΔF508-CFTR released more iron into the extracellular medium than cells expressing the wild-type protein. However, they also found a net increase in the iron content of airway epithelial cells, suggesting that iron uptake is predominating with ΔF508-CFTR expression. To explain this apparent paradox will require a more detailed investigation of the kinetics of iron transport by these cells as well as data on the expression of the various iron transport proteins. In addition, it would be valuable to determine precisely in what form the iron is released. These are fruitful areas for future investigations.

P. AERUGINOSA IRON HOMEOSTASIS

An issue that is very relevant to Moreau-Marquis et al. (3) study is why the presence of increased iron in the CF airway assists bacterial infection. Like other organisms, *P. aeruginosa* requires iron as an essential cofactor for cytochromes and other proteins involved in key metabolic pathways, and without sufficient iron it struggles to replicate, establish itself, or thrive in iron-depleted environments. In recent years, it has become clear that the amounts of iron available to *P. aeruginosa* also affect the formation of biofilms in vitro. Obtaining sufficient iron for growth is a challenge for all bacterial pathogens, and competition for available iron is fierce. For many years, it has been known that limiting the amount of iron available consti-

tutes an important mechanism by which mammals inhibit bacterial growth. Many pathogens, including *P. aeruginosa*, address this problem by secreting iron-chelating compounds (siderophores) that can scavenge iron from host proteins. The resulting iron chelates are taken up by the bacteria, and the iron is then released for incorporation into bacterial proteins. Limiting the amount of iron that is available to the bacteria inhibits biofilm formation and therefore constitutes a therapeutic intervention, although studies of such treatments in the human infection situation remain in their infancy (1, 6). When grown under conditions where low amounts of iron are available, wild-type *P. aeruginosa* are able to scavenge enough iron for in vitro biofilm formation by secreting a siderophore called pyoverdine, but mutant strains unable to make pyoverdine are not able to do so (2). Addition of pyoverdine or another iron source can restore the ability of mutant strains to make robust biofilms, indicating the importance of iron to *P. aeruginosa* survival. The data of Moreau-Marquis et al. (3) are consistent with these findings and emphasize the importance of iron to this bacterium in the infection setting in a more biologically relevant system (coculture of *P. aeruginosa* with epithelial cells) that is presumably one step closer to in vivo conditions. In particular, addition of 8 μM iron to cultures of wild-type epithelial cells enhanced biofilm formation, and addition of the iron-binding protein conalbumin inhibited biofilm formation using both wild-type and CFTR mutant epithelial cell lines. Conversely, higher iron concentrations result in decreased biofilm formation in vitro (4). These findings emphasize the critical role played by iron in biofilm development and provide strong experimental support for the emerging view that, in CF, *P. aeruginosa* adopts a biofilm-like existence (albeit, one that may be significantly different to that observed in vitro), with the elevated amounts of iron in the CF lung facilitating biofilm growth. Intriguingly, isolates of *P. aeruginosa* from patients with CF often appear unable to make pyoverdine, and they presumably obtain iron in other ways. This would be consistent with constant exposure to an iron-replete environment, as suggested by the observations of Moreau-Marquis et al. (3). It would be of interest to examine the biofilm-forming abilities of siderophore-deficient isolates in the model system developed by Moreau-Marquis and colleagues (3).

POTENTIAL TREATMENT STRATEGIES

Increased understanding of the molecular mechanisms underlying CF has rapidly expanded the number of potential treatment approaches. The majority of new therapeutic strategies target the cell membrane chloride channel or other early pathophysiological stages. Gene and stem cell therapy remains a distant hope with many barriers still to overcome. The demonstration by Moreau-Marquis et al. (3) of an inherent iron homeostatic abnormality opens the door for new therapies based on prevention of bacterial iron access. Such a strategy could be considered from the time of diagnosis. The effectiveness of the biologically occurring iron chelator conalbumin at preventing biofilm formation in Moreau-Marquis et al. (3) study using a model that much more closely mimics the in vivo situation is very encouraging. The other important message from this study is that new treatments designed to correct abnormal or absent CFTR function will need to broaden their outcome measures to include effects on CF airway epithelial iron handling as well as changes in nasal potential differences or restoration of near normal cell membrane CFTR expression. The ability of Corr4a to inhibit *P. aeruginosa* biofilm formation by increasing CFTR expression in the absence of any apparent change in the cell iron handling abnormality is difficult to explain and is clearly an area that needs further investigation but suggests that the iron homeostatic abnormality is complex and not simply due to epithelial cell surface CFTR expression. The experiments with Corr4a suggest that excess iron is necessary for biofilm formation but that this is not the whole story and that iron alone is not sufficient. Other contributory mechanisms are obviously at play.

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