PEOPLE WITH CYSTIC FIBROSIS (CF) are surviving in larger numbers into adulthood and even middle age. Over time, acquisition of antibiotic-resistant gram-positive and gram-negative bacteria in the airways leads to progressive inflammation and cycles of pulmonary exacerbations that drive obstructive lung disease and decline in lung function. CF patients have an exuberant immune system aided by ever more powerful antibiotics that prevent sepsis and death by limiting spread beyond the airways. Yet at the same time, despite aggressive intervention to improve mucociliary clearance and fight infection from infancy, the airways become chronically colonized with specific bacteria that are difficult to eradicate. Periodically, bacteria become more invasive causing pulmonary exacerbations that lead to progressive airways damage and irreversible structural changes. The paradox between a strong immune reaction and failure to eradicate infection supports the hypothesis that there is a primary defect in innate immunity in CF.

**Innate Immune Defect in CF**

Bajmoczi and colleagues have advanced the hypothesis that *Pseudomonas aeruginosa* (PA), a common destructive pathogen that inhabits 85% of CF lungs, must be internalized by airway epithelial cells, thereby initiating innate immunity, clearance of pathogen, and subsequent cellular apoptosis. Pier et al. have described a defect in PA internalization in CF cells that is attributed to the absence of a functional CFTR at the apical plasma membrane (5) since internalization rate directly correlates with the amount of CFTR (4). This defect is not uniformly manifest in all epithelial tissues. For example, in the cornea, disruption of internalization of PA by perturbing membrane cholesterol reduces bacterial levels and is protective against PA keratitis (18). Others (16) hypothesize that PA internalized through lipid raft-mediated endocytosis is protected from degradation and can survive to cause pneumonia. These contradictory observations suggest that there may be additional factors present in CF airways disease that modify the impact of failure to internalize PA (6).

The article published by Bajmoczi et al. (1) describes an internalization mechanism in a CF bronchial epithelial cell line (IB3–1) engineered to overexpress wild-type (WT) cystic fibrosis transmembrane conductance regulator (CFTR)-green fluorescent protein (GFP). They demonstrate that a functional CFTR together with caveolin-1 in a lipid raft is required to internalize PA. PA has been shown to invade nasal and bronchial epithelial cells through lipid rafts, which are regions of the membrane that are detergent insoluble, cholesterol and sphingolipid rich, and migrate in density gradients as low-density membrane fractions. When present in lipid rafts, caveolin proteins are a component that is necessary for the generation of morphological caveolae; caveolin-1 is found on most cell types, including type 1 pneumocytes and bladder epithelial cells, both defending areas of the body known to become infected with PA. The caveolin family, made up of three 21- to 24-kDa proteins, is involved in signal transduction, endocytosis, and lipid raft formation. Drugs that disrupt membrane cholesterol inhibit lipid raft and caveolae-mediated endocytosis.

**In Vitro Models of Initiating Events in CF Inflammation**

Is the IB3–1 cell line a reasonable model for CF in vivo? The endogenous CFTR protein is derived from the dF508 allele. Levels of dF508 CFTR are so low that immunofluorescence or immunoblotting often fail to detect the dF508 protein. Another CFTR allele, W1282X, produces a mutant mRNA transcript that is unstable thus preventing accumulation of truncated protein. The authors made stable clones of IB3–1 that overexpress GFP-tagged CFTR. Despite the GFP moiety, the tagged protein. The authors made stable clones of IB3–1 that overexpress GFP-tagged CFTR. Despite the GFP moiety, the tagged CFTR efficiently traffics to the cell membrane and conducts chloride. But IB3–1 does not form tight junctions or polarized monolayer cultures, even at an air:liquid interface. Restoration of WT-CFTR to IB3–1 does not restore polarization to the clones; however, remarkably, such restoration reverts IB3–1 to a non-CF phenotype with respect to cAMP-regulated chloride transport, secretion of IL-8, and half-life of mature CFTR (2, 3, 11). The authors incubated their clones with G418, an aminoglycoside that can induce read-through of stop codon mutations. The G418 was necessary to select for expression of the GFP-CFTR cDNA. To control for read-through of W1282X, the authors exposed untransfected clones to G418. Only approximately one-fourth of cells acquired the WT phenotype and PA internalization, suggesting that not all cells achieved high enough levels of normal CFTR. Removal of G418 3–4 days before experiments performed with PA and the GFP-CFTR-transfected clones prevented measurement of aminoglycoside-induced read-through of endogenous W1282X CFTR.

**Interactions Between Bacteria and CFTR**

Endogenous WT-CFTR is expressed at very low levels on airway luminal membranes in vivo (15). Normally, cough and mucociliary clearance protect cells from PA. However, under unusual circumstances low numbers of PA may gain access to...
luminal surface where they are ingested through caveolae and trigger a complex chain of events leading to rapid release of IL-1, nuclear factor-κB nuclear translocation, cytokine gene transcription and release, inflammation, and apoptosis (10). In addition to PA, other bacteria can colonize and infect CF airways. Many become multiply resistant to more than one class of antibiotic. Among these CF pathogens are _Stenotrophomonas maltophilia_, _Acinetobacter_, and _Staphylococcus aureus_. It is not known whether CFTR mediates interaction of these organisms with epithelial cell membranes. As discussed by Bajmoczi et al. (1a) not all cell types have CFTR-mediated clearance of PA. In some cells the CFTR is apical but the caveosomes and lipid rafts are basolateral. This may explain one reason for the resistance of some organs, such as the bladder and the cornea, to PA infection in CF patients.

Despite effective low level clearance of PA by airway epithelial cells, other conditions can lead to chronic colonization with PA. Immunosuppression is a risk factor, as well as is implantation of a foreign body, such as a tracheostomy tube. Acquisition of the mucoid phenotype through mutation and environmental pressure leads to complex colony formation and production of a slime-like biofilm. Thus PA does not exert all its damage at the airway epithelial surface membrane. These social networks of PA form highly structured communities that communicate through quorum sensing of small secreted molecules and coordinate gene expression (9). These PA are relatively invulnerable to antibiotics, and additional therapeutic strategies such as chronic exposure to azithromycin provides a means to control the organism.

**CFTR-Mediated Internalization of PA Occurs Through Lipid Rafts**

In the study of Bajmoczi et al. (1a), two of the GFP-CFTR transduced clones did not revert to WT phenotypes, one due to insufficient CFTR and the other due to a defect in lateral diffusability of GFP-CFTR within the lipid raft. There are at least 150 known protein components of lipid rafts, and the authors have shown that function with respect to internalization of PA also involves components of the cytoskeleton. Polymorphisms or variations in the function of one or more of these components might explain the resistance of some CF patients to PA colonization. Perhaps one or more of the genes coding for components of this mechanism (7, 14, 17) may behave as a modifier gene in CF.

There are several novel CFTR-specific pharmacological therapies in clinical trial, raising the hopes of a more direct treatment strategy in the near future. If the dF508 CFTR can be corrected with respect to mislocalization and thus induced to reside at the cell surface, will PA once more be internalized and innate immunity improve? DF508 and the milder conductance regulator from a novel adeno-associated virus promoter. J Biol Chem 268: 3781–3790, 1993.


REFERENCES