Preclinical Modeling for Therapeutic Development in Cystic Fibrosis

Patients with cystic fibrosis (CF) suffer from the consequences of deficient CFTR (CF transmembrane conductance regulator gene) activity (1). CFTR controls epithelial chloride and sodium transport, with deficient function altering tissue milieu hydration, cellular membrane potential, and cellular functions, resulting in a multiorgan disease (2). The consequences of this pathophysiology results in increased mucous viscosity, mucus plugging, inflammation, and infection that ultimately destroys the lungs (3, 4). Unfortunately, to date, there are no therapeutic panaceas for CF. Even with small molecule CFTR correctors and potentiators, the established pulmonary damage still requires supplemental avenues of therapeutic intervention (5). The pursuit of new treatments requires the right model of choice for determining potency, efficacy, and adverse effects (6). The modeling system requires that the tissue pathophysiology be consistent both with the target of treatment and with the complexity of enhanced mucus viscosity/plugging, inflammation, and infection (7).

The primary challenge to developing new therapeutics for CF is the lack of a readily accessible, cost-effective model system that allows for the simultaneous evaluation of mucociliary clearance abnormalities, infection susceptibilities, and inflammation (8). The *Cftr*-deficient mouse model does not have mucus/mucociliary clearance manifestations but does provide important insight into immune mechanisms associated with CF-deficient management of infection and inflammation. The βENaC (β epithelial sodium channel) mouse model has altered sodium ion transport, resulting in enhanced mucous viscosity and sustained inflammation, but does not have many of the other anomalies associated with deficient *Cftr* (9). In the case of the βENaC mouse model, additional therapeutic testing translated to a *Cftr*-deficient scenario assures an evaluation of other potential complications because of deficient *Cftr*. The cost of breeding mice, the short reproductive cycle, and the production of large litters streamlines numbers for efficiently powered studies with a lower cost in husbandry than the larger animal counterparts (10, 11). However, mice are not humans, and they do not recapitulate all aspects of CF pathophysiology.

The CF research community has continued to create *in vivo* models that more effectively reproduce many of the pathophysiological consequences of deficient CFTR function. The CF pig and ferret have been highly interrogated and are effective at recapitulating many of the components of CF (9, 11–13). Both the CF pig and ferret have similar pulmonary anatomy to humans, and a pathophysiology that more closely mimics that of CF in humans, including inefficient mucociliary clearance, excessive inflammation, and susceptibility to infection (13, 14). The CF rabbit and rat are newer models, less well established for translatability to the human disease, but the developers are steadily making progress (11, 15, 16). Each of these models has contributed their own major clinical advancement to the understanding of CF, but not without caveats of accessibility, financing, breeding issues, survivability, and translatability because of associated deficiencies in disease pathophysiology resulting from deficient CFTR. In addition, in some instances, extension into understanding the pathophysiology of disease is hindered because of the lack of biological agents specific for the species.

In this issue of the *Journal*, Kim and colleagues (pp. 313–324) focus on demonstrating that losartan, an angiotensin blocker, has therapeutic benefits for CF (17). The preclinical models used by this group include both an *in vitro* setting using primary CF patient F508del airway epithelial cells in an air–liquid interface and an *in vivo* functional CFTR-deficient sheep model. The *in vivo* model uses a CFTR inhibitor (CFTRinh172) in combination with TGF-β (transforming growth factor β) and hNE (human neutrophil elastase) to generate reproducible mucociliary dysfunction and inflammation. In later experiments, the addition of exogenous TGF-β was discontinued because the hNE itself in combination with deficient CFTR function induced endogenous TGF-β, implicating the cytokine in the pathophysiology. The combination of the CFTRinh172 and hNE resulted in an *in vivo* model with prolonged tracheal mucus velocity, mucus dehydration, and increased endogenous TGF-β without the complexities of deficient CFTR genetics. In interrogating the effect of losartan, the investigators also demonstrated that the CFTR deficient/hNE inflammatory sheep model had inefficient $Ca^{+}2$-activated and voltage-dependent K^{+} channel (BK) function, which is critical for mucociliary clearance similar to patients with CF. The losartan rescued the BK channel function in the absence of functional CFTR, further emphasizing losartan's clinical potential. The improvement in the BK channel function was also associated with improved mucus transport and hydration and decreased inflammation. The authors summarize that losartan is a good therapeutic candidate for CF because of the ability to improve mucociliary clearance, decrease mucous plugging, and indirectly decrease inflammation.

Ex vivo models of human primary airway epithelial cells do not completely replicate the *in situ* abnormalities of deficient CFTR, but they do provide important information regarding how losartan potentially affects epithelial cells' function in lieu of directly administering losartan to people with CF. The sheep model is not a genetically CFTR-deficient model, but it does provide a model for monitoring changes in mucus clearance abnormalities. The models used in Kim's studies aide in understanding abnormal mucociliary clearance and inflammation in the context of CFTR dysfunction, without the complexities of superimposed infection or other tissue abnormalities, which cause fragility in many other CF animal models. Furthermore, losartan is not an antiinflammatory drug aimed at altering immune cell function; therefore, the ultimate requirement of testing in the context of infection is not as concerning when compared with evaluating direct immune regulators. That being said, it will be exciting to see whether the

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improved mucociliary clearance and mucous viscosity induced by losartan will enhance infection resolution.

The question remains: is blocking CFTR function the perfect setting for the preclinical development of losartan? If not, what model really reproduces a reasonable, cost-effective means by which to explore specific therapies in the context of consistent and reproducible complexities of CF lung disease? Kim and colleagues' manuscript introduces the potential of losartan as a CF therapeutic and also highlights innovations in model development used for open-minded investigations and for the systematic development of novel therapeutics for CF. Innovation here is the key. ■

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Improving Pulmonary Immunity to Bacterial Pathogens through *Streptococcus pneumoniae* Colonization of the Nasopharynx

Streptococcus pneumoniae is a common cause of bacterial pneumonia, especially in the elderly and patients with significant comorbidities, and is also frequently associated with exacerbations of chronic obstructive pulmonary disease (1, 2). Existing *S. pneumoniae* vaccines have partial strain coverage, may lack efficacy in high-risk groups, and generally seem to have poorer efficacy against pulmonary infection than against systemic infection (3, 4). Hence, alternative strategies to conventional vaccines may be required to prevent the persistent high morbidity and mortality caused by *S. pneumoniae* lung infections.

Mitsi and colleagues present data obtained using the experimental human pneumococcal colonization (EHPC) model that suggest one such alternative strategy for preventing pneumonia caused by multiple bacterial pathogens, including *S. pneumoniae*. Repeated episodes of *S. pneumoniae* colonization throughout life induce and repeatedly boost protective antibody to both capsular and multiple protein antigens, as well as poorly defined cellular immunity (5–8). In a study presented in this issue of the *Journal*, Mitsi and colleagues (pp. 335–347) used the EHPC model to investigate the effects of *S. pneumoniae* colonization on alveolar macrophage (AM) function in healthy volunteers and identified a novel mechanism by which successful colonization improves lung immunity to multiple bacterial pathogens (9). The phagocytic capacity of *S. pneumoniae* AMs (recovered by BAL) improved from 69% in uncolonized EHPC subjects to 80.4% in EHPC subjects who were successfully colonized. This was a convincing change that was strengthened by a significant correlation to the density of *S. pneumoniae* colonization of the nasopharynx. Matched pre- and

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