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Revisiting the mouse lung model for CF

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After the identification of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene in the late 1980s,[1] an animal model of cystic fibrosis (CF) was keenly sought to facilitate the development of gene and other therapies. However, none of the several mouse models produced were found to develop the lung disease characteristic of CF spontaneously,[2] although it was soon discovered that lung disease could be induced by exposure to high levels of bacteria.[3] In a recent issue of the *Proceedings of the National Academy of Sciences USA*, Jim Hu *et al*[4] present an intriguing study that not only further validates the efficacy of their *K18* promoter driven *CFTR* construct but also upholds the bacterially challenged CF mouse lung as a viable model system for assaying *CFTR* transfer.

Although the common recessive condition CF affects many organs, it is the associated lung disease that accounts for 95% of morbidity. Lack of functional CFTR protein results in thickening of secretions from exocrine glands. In the lung, the resulting sticky mucus fails to be effectively removed by mucociliary clearance, thus providing a growth medium for microorganisms such as bacteria. Pathogenic effects of colonizing bacteria combine with the enhanced chronic inflammatory response in CF (manifested most strikingly in a pronounced neutrophilia) to produce ultimately fatal lung damage. These considerations emphasize the value of a good model of CF lung disease.

The Hu group has developed the *K18* promoter system to restrict expression of *CFTR* after gene transfer to certain epithelial cell types in the belief that suppressing exogenous expression in this way would minimize immune stimulation. For this approach to work therapeutically, the pattern of expression of the *K18* gene should encompass those cells in which *CFTR* expression is necessary to prevent CF lung disease. *K18* expression is certainly observed in all the right places at the tissue level, but is unlikely to mirror exactly the marked heterogeneity of *CFTR* expression seen at the cellular level in airway epithelia, an observation whose biological significance remains unclear.[5] Nevertheless, there is no doubt that the *K18* promoter is a superior alternative to nonspecific viral promoters such as P_{CMV}. The authors also make a virtue of the fact that *K18* is more strongly expressed than *CFTR*. However, it is by no means certain that strong expression of *CFTR* in a small proportion of transduced cells is functionally equivalent to weak expression in a high proportion of cells, as seen in normal airways. Indeed, there is evidence that overexpression may hinder CFTR processing.[6]

The present study[4] goes some way to addressing the question, being the first demonstration that *CFTR* under control of a tissue-specific promoter can positively affect a CF-related bacterial phenotype in the mouse lung. The pertinent result is that nasal administration of a helper-dependent adenoviral K18CFTR vector (K18CFTR-HD-AD) to CF knockout mice protects against subsequent pulmonary infection by a clinical strain of *Burkholderia cepacia* complex (Bcc), a particularly virulent pathogen of the human CF lung. In brief, as well as presenting immunohistochemical and RNA evidence of recombinant CFTR expression, the authors show that only those knockout mice pretreated with K18CFTR vector are able to clear Bcc to basal levels indistinguishable from normal mouse controls. The model of Bcc infection was refined in earlier work,[7] in which wild-type mice repeatedly instilled with Bcc cleared the bacteria within 9 days, whereas CF knockout mice retained Bcc and succumbed to severe bronchopneumonia, exhibiting many of the histological signs of human CF lung disease including neutrophilia. The Bcc model is more compelling than the agar bead model of *Pseudomonas aeruginosa*,[8] as it requires no immobilizing agents.

How does this study advance the cause of clinical CF gene therapy? Firstly, it shows that the lungs of CF mice *can* be used to evaluate *CFTR* gene transfer using the functional and clinically relevant readout of protection from Bcc infection. It should therefore encourage the CF gene therapy field to reconsider positively the usefulness of the bacterially exposed mouse lung as a test bed for *CFTR* gene transfer. Secondly, it shows that K18CFTR-HD-AD offers improved longevity of expression (albeit tested only up to 4 weeks) over earlier generation Ad vectors. However, we feel it is a weakness of the work that the authors chose to deliver *CFTR* to bacteria-free mice *before* introducing Bcc: the human CF lung is likely to have been chronically infected with bacteria (although mostly not Bcc) by the time gene therapy can be contemplated. While modelling the complexities of CF-associated chronic bacterial infection in the mouse would be challenging, it would have been of considerable interest to know how the K18CFTR-HD-AD vector performed in a mouse lung already infected with Bcc or other bacteria. This would have generated valuable data on the ability of the vector to transduce airway epithelia in the context of exuberant inflammatory response to bacterial infection. We already know that normal mice airways infected with the *P. aeruginosa* bead system are 50% less efficiently transduced (using earlier generation adenovirus expressing a reporter gene) than uninfected mice.[8]

What accounts for the longevity of expression of the K18CFTR-HD-AD vector? We know that HD-Ads are able *per se* to promote expression for longer periods than earlier generation Ads. It is also clear that strong viral promoters are prone to cytokine-mediated suppression. However, the authors have not demonstrated that the K18CFTR cassette contributes to longevity of expression: they would have to show that K18CFTR-HD-AD provided longer-term expression than an HD-Ad harbouring a *CFTR* cassette driven by a strong *constitutive* cellular promoter. As things stand, longevity of expression may be solely a

consequence of the HD-Ad. It is important to establish the contribution of K18CFTR to longevity since the use of Ad as a vector for human lung use is fraught with problems, not all biological. For example, the authors pretreat the lungs with EGTA to disrupt tight junctions thus allowing the vector to reach basolaterally located Ad receptors. Such a procedure is highly unlikely to be approved in CF patients. Also, given the difficulties of repeatedly administering Ad to the airways,[9] other (especially nonviral) vectors of K18CFTR should be considered and tested. So what do we see as the next significant milestone? As well as determining how well the cassette controls pre-existing infection, we suggest that there is also an absolute need to develop a vector capable of repeat delivery through CF mucus. ■

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