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Symptoms in a 'leaky' mouse

Since the gene for cystic fibrosis (CF), a common and fatal genetic disease, was cloned in 1989, there has been no shortage of interest or time invested in trying to create convincing mouse models to mimic the disease. Four such models, using the technique of gene targeting in embryonic stem cells, have been reported so far[1–4]. The research groups concerned, located in North Carolina (UNC), Edinburgh, Cambridge and Houston, have used various strategies to disrupt and potentially to inactivate the murine homologue of the CF gene (*cftr*). Three of these groups engineered mutations (see Table) that created null alleles with no residual expression of *Cftr*. The results were mice with extremely severe homozygous phenotypes, with most (>80%) of the animals dying at or soon after birth from complete intestinal obstruction.

By contrast, the mice created in 1992 by Julia Dorin, David Porteous and colleagues at the MRC Human Genetics Unit in Edinburgh[2] were only mildly affected. They had created a 'leaky' insertional mutation in exon 10 ($cftr^{m1HGU}$), so that low levels of residual *Cftr* expression remained as a result of alternative splicing of the gene. Although the mice showed electrophysiological abnormalities reminiscent of CF, the incidence of intestinal blockage — and neonatal death — was reduced to 5–10%, similar to the incidence of neonatal intestinal blockage (meconium ileus) in CF patients. None of the mice reported so far can be said to serve as an ideal model of the most common forms of CF — indeed, work is in progress to produce the most common single amino-acid CFTR substitutions and deletions in the mouse gene, which may reflect more closely the human disease. But the improved survival of the Edinburgh mice offers important advantages in studying the phenotype of affected mice, even though that potential has taken more than two years to realize (see below).

The value of the CF mouse models is difficult to overstate, particularly in studying a number of aspects of *CFTR* gene replacement. Liposome trials in humans would not have been possible without first demonstrating their success in gene-targeted mice, for example[5,6]. And in a successful attempt to circumvent the cause of the short life-span of the first mouse model, Jeffrey Whitsett's team in Cincinnati was recently able to show that a human *CFTR* transgene, expressed specifically in the small intestine, could rescue the severe gastrointestinal phenotype of the UNC mice as judged by cAMP-stimulated chloride secretion and much improved survival[7].

The need for a 'milder' model for CF is not just to provide a more realistic setting to test gene therapy approaches, however. A formidable problem in the disease remains the inability to eradicate some of the opportunistic bacterial lung

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Microbe management. Top: The custom-built apparatus used by the Edinburgh group to administer bacterial aerosols to mice. Bottom: The results of plating lung homogenates of wildtype (left) and *cftr*-deficient (right) mice following exposure to *S. aureus*. (Courtesy of D. Porteous and J. Govan.)

Table Mouse models for cystic fibrosis

Origin	Mutation	Intestinal phenotype	Survival	Reference
North Carolina	exon 10 replacement	severe	Poor	2
Edinburgh	exon 10 insertion	mild	Good	3
Cambridge	exon 10 replacement	severe	Poor	4
Baylor	exon 3 duplication	severe	Poor	5

infections, which are the most common source of morbidity in CF patients. Although the transgenic mice may now offer the opportunity to study this aspect of lung pathology in CF, the Edinburgh mouse offers this advantage naturally because of the presence of small amounts of the wild-type *cftr* message. The Edinburgh group has now managed to examine bacterial lung infections in CF mice. Writing on page 351 of this issue, Davidson *et al.* show for the first time that their mutant mice are susceptible to at least two characteristic CF pathogens — *Staphylococcus aureus* and *Burkholderia cepacia*[8].

The Edinburgh group first noticed that its $cftr^{m1HGU}/cftr^{m1HGU}$ mice were more susceptible to bacterial infection when housed in standard conditions. So the group infected both $cftr^{m1HGU}/cftr^{m1HGU}$ mice and their normal counterparts with isolates of the two bacterial strains (cultured from the sputum of CF patients) by administering the bacteria with a nebulizer over 1–2 months. The $cftr^{m1HGU}/cftr^{m1HGU}$ mice showed increased incidence of bronchiolitis, mucus retention and inflammation in response to *S. aureus*, and pneumonia following repeated administration of *B. cepacia*. Although some of the normal mice also showed adverse effects from the treatment, the Edinburgh group points out that the changes in the $cftr^{m1HGU}/cftr^{m1HGU}$ mice were significantly more widespread.

A crucial challenge now is to study the infection of $cftr^{m1HGU}/cftr^{m1HGU}$ mice with the most notorious and damaging CF pathogen, *Pseudomonas aeruginosa*. Although not as early a colonizer of lungs of CF patients as *S. aureus*, for example, *P. aeruginosa* eventually infects more than 90% of patients, making it virtually diagnostic for the disease. However, the study and indeed the potential interpretation of *P. aeruginosa* infection is complicated by the existence of several subtypes of the organism, and its slow transformation from a non-mucoid to a mucoid form during human infection. The results of these studies will be awaited with considerable interest. If these findings are confirmed with other organisms and mouse models, they may offer the best hope yet to find a way to combat the devastating infections associated with CF. Despite considerable improvements in intravenous antibiotic treatment for the disease in recent years (increasing the average life expectancy of patients to 29 years) and its ability to keep the growth of *P. aeruginosa* under control, it is not possible using current therapies to eradicate the organism.

Management of CF has been aided by the use of Genentech's newly approved recombinant DNase drug, Pulmozyme® to reduce the thickness of the sputum in patients' lungs. The drug is gaining popularity in the United States, and is increasingly being used in various European countries. But despite evidence that a daily regimen of the drug improves lung function, its effect on longevity remains unclear, and it obviously does not tackle the root of the problem in CF. And while there is understandable excitement about the use of gene therapy, whether using adenoviruses[9,10], liposomes[11] or other methods, there remain doubts that gene therapy alone will be sufficient to ward off all symptoms of the disease[12].

The CF mice doubtless have much more to teach us about the human disease. As more is learned about the circumstances and mechanisms surrounding bacterial airway infections in *cftr*-deficient mice, the prospect of being able to combat the deadly infections in humans may be edging a little closer.

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

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