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Animal models of cystic fibrosis

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Abstract

Animal models of cystic fibrosis, in particular several different mutant mouse strains obtained by homologous recombination, have contributed considerably to our understanding of CF pathology. In this review, we describe and compare the main phenotypic features of these models. Recent and possible future developments in this field are discussed.

Keywords: Cystic fibrosis transmembrane conductance regulator, Genetics; Lung diseases, Microbiology; Pathology; Disease models, Animal; Intestines, Pathology; Mice, Genetics

1. Introduction

As described elsewhere in this issue [Bals, 2004; Willems, 2004;Ulrich, 2004; Davidson, 2004], epithelial cells in culture allow us to perform many important studies in the field of CF. Yet, despite progress recently made, these models lack the specific differentiation pattern, and structural complexity of intact organs. Therefore, animal models are still essential in the study CF pathogenesis, and in the evaluation of novel therapeutic strategies.

A comprehensive review of all available literature on this subject, more than 2500 monographs at the latest count, is beyond the scope of this paper. To give the reader access to this field, we will highlight several available animal models that have been used frequently in CF research, and point out relevant forthcoming developments. For further information on handling, acquiring and using the models, we refer to the website of the European Working Group CFTR Expression [81].

2. Mouse models of CF

The cloning of the CF gene in 1989 [1] boosted CF research. One of the novel approaches that emerged from this breakthrough was the generation of mouse strains with a mutation of the mouse *Cftr* gene. This could be done with a technique pioneered by Thomas and Capecchi [2], which uses homologous recombination in mouse embryonic stem cells to target a mutation to a specific site in the mouse genome. The resultant mutant embryonic stem cells are injected into blastocysts, to produce first chimeric mice, and subsequently a mutant strain through germ-line transmission of the mutant allele. Several groups have independently produced CF mutant mouse strains in this way. A list of currently available CF mouse models made by homologous recombination is shown in a table which is available in hyperlinked form at the above-mentioned website [81].

3. Knockout and residual function mouse models of CF

The initial mouse models of CF were created with mutations that resulted in a complete loss of function, using

Abbreviations: AML, amiloride; *Bc*, *Burkholderia cepacia*; BMT, bumetanide; Ca-ion, Ca^{2+}-ionophore ionomycin; DIDS, 4,4′ -diisothiocyanatostilbene-2,2′ -disulfonic acid; ENaC, epithelial sodium channel; FABP, fatty-acid-binding protein; FSK, forskolin; *Hi*, *Haemophilus influenzae*; iNOS, intracellular nitric oxide syntethase; KO, knockout; LPS, lipopolysaccharide; MCC, mucociliary clearance; *Pa*, *Pseudomonas aeruginosa*; PCL, periciliary liquid layer; RSV, respiratory syncytial virus; *Sa*, *Staphylococcus aureus*.

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a ''replacement strategy'' to produce an interruption of the *Cftr* gene. Examples of these are the mutants generated by the North Carolina (*Cftr*^{tm1Unc}) [3], Cambridge (*Cftr*^{tm1Cam}) [4], Toronto (*Cftr*^{tm1Hsc}) [5] and Baylor (*Cftr*^{tm3Bay}) [6] groups. These do not produce detectable amounts of *Cftr* mRNA and are considered complete loss of function (''knockout'', KO) mutations. In addition, models using an ''insertional strategy'' were described by the Edinburgh (*Cftr*^{tm1Hgu}) [7] and Baylor (*Cftr*^{tm1Bay}) [8] groups that produce low levels of normal mouse *Cftr* mRNA, despite interruption of the *Cftr* gene. These have been described as ''residual function'' models. In each model, mutant animals could be determined on the basis of their bioelectric phenotype, with these analyses revealing classical characteristics of CF in humans (reviewed in Refs. [9,10]). However, differences in bioelectric, and other phenotypes were apparent between the models, some of which could be attributed to their specific mutation. Thus, while these earlier mouse models have been fundamental to subsequent research in this field, it could not be assumed that the laboratory mutations would accurately replicate the effects of specific clinical mutations, in which dysfunctional or non-functional CFTR protein is produced (such as F508del and G551D).

4. Mouse models with specific CF mutations

To address this concern, another brand of recombinant CF mouse models was generated with specific clinically relevant mutations in *Cftr*. By far, the most common CF mutation in humans is F508del. This single amino-acid deletion results in defective intracellular trafficking and high turnover of the mutant protein. Importantly, it has been shown that the mutant protein can be functional when it reaches the plasma membrane. This opens the possibility to treat CF using a compound that inhibits degradation or improves trafficking of F508del CFTR. To facilitate these studies, three groups independently created F508del *Cftr* mouse models. Two of these, *Cftr*^{tm2Cam} [11] and *Cftr*^{tm1Kth} [12], were made using an exon 10 replacement strategy that left a selective marker gene inserted in one of the introns. This marker gene has an effect on transcription activity of the mutant allele, but does not affect the protein product. The third model, *Cftr*^{tm1Eur}, was made with a ''hit and run'' procedure that resulted in a mutant exon only. Therefore transcription activity of the mutant allele is identical to the normal allele [13,14]. It has been shown that the F508del mutation, in the context of the mouse *Cftr* sequence, results in a similar, temperature-dependent processing defect as the human version [14]. Recent experiments with intestinal mucosa from homozygous *Cftr*^{tm1Eur} mice in maintenance culture [15] showed that incubation at reduced temperature for up to 15 h resulted in wild-type (wt) levels of CFTR-mediated transepithelial chloride (Cl^-) secretion.

In addition to these mice, models carrying the human CF mutations G480C (*Cftr*^{tm2Hgu}) and G551D (*Cftr*^{tm1G551D}) have also been generated, using ''hit and run'' and ''replacement'' strategies, respectively [16,17]. These also aim to model relevant human mutations of particular interest. Similar to F508del CFTR, G480C CFTR has been demonstrated to be defective in its intracellular processing, and to show similar channel characteristics to wt protein when allowed to traffic in *Xenopus* oocytes. In contrast, G551D CFTR becomes appropriately localized, but is dysfunctional as an ion channel. These models also demonstrated characteristic bioelectric profiles (reviewed in Ref. [9]), and certain mutation-specific phenotypes. Finally, a transgenic KO model expressing a human CFTR with the G542X mutation under the control of the intestinal fatty-acid-binding protein (*FABP*) gene promoter has been generated and used to study the effect of aminoglycosides on suppression of this CFTR premature stop mutation [18].

These mouse models with specific CF mutations provide a clinically relevant in vivo system to enable the pre-clinical testing of compounds that emerge from large scale screening programs and mutation-specific therapeutic approaches.

5. Genetic background affects the phenotype of CF mouse models

Because the most widely used mouse embryonic stem cell line is derived from 129/Ola mice, and the recipient blastocysts are preferably from C57/bl6 or FVB mice, all recombinant mouse models initially were of mixed genetic background. This is not a major problem with many experiments, though it may add to experimental variation. However, experiments aimed at the identification of ''modifier genes'', microarray experiments [Galvin, 2004 this issue] and proteome analysis [Edelman, 2004 this issue] will depend heavily on the availability of isogenic ('backcrossed') strains. One advantage of mouse models is that such a comprehensive genetic analysis is possible by a combination of classical backcross experiments and state-of-the-art micro-array analysis. Indeed, several groups have generated inbred CF mutant lines in different genetic background and observed interesting differences between resultant strains. By backcrossing experiments, supported by genetic analysis this pointed to several genetic *loci* that modify the CF phenotype in mice [19].

In contrast to CF KO mice, F508del mice show low but distinct residual CFTR activity in several epithelial tissues, dependent on the expression level of the mutant *Cftr* allele. Interestingly, this is also observed in a sub-population of F508del CF patients [20]. Presumably, the balance between inactivation and proper processing of the mutant protein depends on the genetic repertoire of the individual. Identification of genes that are associated with residual ΔF508CFTR activity could lead to new therapeutic approaches.

6. Phenotypic properties of CF mice

Mouse models of CF have provided researchers with the opportunity to start to dissect the pathogenesis of CF, and to design and test novel therapies, by targeting specific phenotypes downstream of *Cftr* mutation. Although the phenotypes of the different mouse models of CF bear most of the same hallmarks, important differences have been observed, relating to the specific mutation, genetic background, and environmental factors. As a result, direct comparisons between the different models must be made with attention to these modifying influences.

6.1. Intestinal disease

Intestinal disease is the most prominent feature of CF mutant mice, with symptoms comparable to CF in humans. Homozygous KO mice are born at Mendelian ratios, but pups often die with signs of intestinal obstruction both before and after weaning. Mouse models of CF demonstrate a wide range of severity in intestinal phenotypes, from negligible effects on survival in *Cftr*^{tm1Eur} and *Cftr*^{tm2Hgu} mice, to severe intestinal obstruction and 95% mortality in the *Cftr*^{tm1Unc}, *Cftr*^{tm1Cam} and *Cftr*^{tm2Cam} mice, consistent with the multi-organ deficiency of an epithelial Cl^- channel involved in the regulation of iso-osmotic water transport (reviewed in Ref. [9]). Interestingly, the *Cftr*^{tm1G551D} mice display less severe intestinal disease and consequent mortality than the KO models, in keeping with the effects of this mutation in humans, in whom a reduced incidence of meconium ileus is observed [17,21]. Intestinal histology shows dilated crypts filled with mucous material and goblet cell hyperplasia, of varying severity. Electrophysiological analysis, e.g. Ussing chamber experiments [De Jonge, 2004], confirms the absence of a CFTR dependent Cl^- current after stimulation with cyclicAMP agonists, with the exception of the *Cftr*^{tm2Hgu} mice. CF mice also generally have reduced body weight of varying severity. This is probably related to reduced lipid resorption, in particular fatty acid uptake, in CF mouse intestine [22]. The molecular mechanism of CF intestinal disease, in particular the exact relationship between CFTR deficiency and lipid malabsorption, remains to be established. We conclude that CF mice provide an interesting and most likely valid model in this field.

However, this powerful phenotypic manifestation of murine *Cftr* mutation presents an obvious difficulty in the analysis of additional phenotypes. Whereas good survival of the *Cftr*^{tm1Eur}, *Cftr*^{tm2Hgu} and *Cftr*^{tm1Hgu} allowed longer-term studies, the high mortality of other models required alternative solutions to be found. Death around the time of weaning appears to result from the consumption of solid food, and the use of a liquid diet has been shown to prolong the lifespan of *Cftr*^{tm1Unc} mice [23]. However, observations on mice weaned using these diets suggest care must be taken that subsequent phenotypic observations are not simply a result of malnutrition [24]. An alternative approach utilised the expression of human CFTR cDNA in the intestinal tract of *Cftr*^{tm1Unc} mice, under the control of the rat intestinal *FABP* gene promoter. These *Cftr*^{tm1Unc}-TgN$^{(FABPCFTR)}$ mice demonstrated decreased lethality from the intestinal defect in the mutant mice and a longer survival, despite inappropriate cell-specific expression [25], and has enabled longer-term studies of mice bearing this mutation.

6.2. Pancreatic disease

Pancreatic insufficiency is a is a major problem in many CF patients, but it has not been convincingly demonstrated in most mouse models of CF. In one study, *Cftr*^{tm1Unc} mice weaned on a liquid diet demonstrated significant differences in pancreatic growth and specific enzyme activities [24]. However, controls showed similar, albeit less severe, abnormalities on this diet in comparison with solid diet fed mice. A further study in *Cftr*^{tm1Unc} mice, also using a liquid elemental diet, reported luminal dilatation and the accumulation of zymogen granules at the apical pole of the ductal epithelial cells [26]. This phenotype has since been used, and corrected, in a study of the role of dietary fatty acids in CF [27] The less severe nature of pancreatic disease in mouse model of CF appears to be the result of lower levels of expression of *Cftr* in the murine pancreas and the presence of an alternative fluid secretory pathway, which is activated by increases in intracellular calcium [28]. This indicates that other ion channels might be capable of compensating for the loss of CFTR and suggests novel therapeutic approaches in humans, to identify and utilize such pathways.

6.3. Lung disease

The most significant impact of CF in affected individuals is progressive pulmonary disease, which causes 95% of the morbidity and mortality. This lung disease is not evident at birth, but develops over repeated acute exacerbations of pulmonary infections with a characteristic spectrum of pathogens (including respiratory syncytial virus (RSV), *Staphylococcus aureus* (Sa), *Haemophilus influenzae* (Hi), *Pseudomonas aeruginosa* (Pa) and *Burkholderia cepacia* (Bc)), chronic microbial colonisation, tissue damaging inflammation, and irreversible deterioration of lung function [29–31]. Chronic pulmonary infection with *Pa* is the primary concern, with the characteristic transition of this organism to mucoid phenotype clearly correlated with poor prognosis and clinical decline [32]. However, it remains unclear whether CFTR dysfunction results directly in an increased predisposition to infection with this organism, or a broader susceptibility resulting in repeated infection with organisms such as *Sa* and inappropriate inflammatory responses generating a lung environment that favours subsequent infection with *Pa*. This distinction is of clear importance in designing novel therapeutic approaches, but remains difficult to resolve in clinical studies. The develop-

ment of mouse models of CF has provided in vivo systems to assist in dissecting the pathogenesis of CF lung disease and a number of lung models have been described. However, despite various unequivocal bioelectric phenotypes, developing an appropriate patho-physiological model of infectious and inflammatory lung disease in CF mice has proved challenging.

6.4. Bioelectric phenotype of murine CF airways

The hyperactivity of the amiloride (AML)-sensitive epithelial sodium (Na^+) channel, ENaC, a prominent feature of CF in human nasal and bronchial epithelium and exploited as a prime diagnostic criterion [33,34] is faithfully reproduced in nasal epithelium from CF mice, irrespective of the genetic background or specific mutation (Fig. 1A) [10,35,36]. In this tissue, CFTR immunoreactivity is confined mainly to the *microvilli* of the olfactory supporting cells (brush cells; [37]) where it is co-localized with ENaC [38], presumably facilitating their functional interaction. The importance of ENaC as a major determinant of Na^+ and fluid absorption in the airways and a regulator of the periciliary liquid layer (PCL) height and mucociliary clearance (MCC) has been illustrated recently in transgenic mouse models in which overexpression of the β-subunit of ENaC results in a significant reduction in PCL height and MCC, mucus retention, goblet cell metaplasia and severe mucus plugging, causing asphyxia and death [39].

Interestingly, the defect in cAMP-activated Cl^- secretion, a hallmark of CF in human airway epithelium, could be demonstrated at the level of nasal [10,35,36] and tracheal [40] epithelium in some CF mouse models but not in others (Fig. 1B). The forskolin (FSK)-activated Cl^- current that we observed in our homozygous F508del *Cftr*^{tm1Eu} mice (FVB/129 and FVB congenics) and in the *Cftr*^{tm1Cam}-null mice (C57Bl/6/129 background) appeared to be 4,4′-diisothiocyanatostilbene-2,2’-disulfonic acid (DIDS)-insensitive and could not be reproduced by calcium-linked agonists (e.g., Ca^{2+}-ionophore; thapsigargin; ATP; UTP; Fig. 1B and data not shown), arguing against a role of cAMP-provoked (dependent?) Ca^{2+} signaling in the Cl^- secretory response. These and other data (not shown) suggest that some, but not all strains of CF mice, dependent on their genetic background and environment, are able to express a “pseudo-CFTR” Cl^- channel in their airways, which is cAMP-sensitive and differs from a Ca^{2+}-regulated Cl^- conductance. The elucidation of the molecular identity of this non-CFTR channel and a more detailed knowledge of its mechanism of activation and induction may suggest novel approaches to antagonize the Cl^- secretory defect in CF airways and to reduce surface liquid volume depletion.

6.5. Pathophysiological changes in murine CF airways

Initial characterisation of the different CF mice demonstrated no gross lung disease, suggesting that CF pulmonary pathology might not be effectively modelled without exposure to pathogens. Although this may be largely true, a variety of interesting observations have subsequently been made in the apparent absence of infection. These include excessive inflammation observed by histology in *Cftr*^{tm1Hgu} mice [41], abnormal MCC in the *Cftr*^{tm1Hgu} and *Cftr*^{tm1Unc} mice [41,42], an increase in goblet cells, with decreased volume of airway surface liquid in the nasal epithelium of *Cftr*^{tm1Unc} mice [43], a more distal extension of submucosal glands in *Cftr*^{tm1Hgu} and *Cftr*^{tm1G551D} mice [44], hypersensitivity of bone marrow-derived macrophages from *Cftr*^{tm1G551D} mice to bacterial lipopolysaccharide (LPS) [45], and abnormalities in intracellular nitric oxide syntethase (iNOS) expression in *Cftr*^{tm1Unc} and *Cftr*^{tm1Kth} mice [46,47].

These observations suggest that more subtle pulmonary phenotypes do indeed occur secondary to *Cftr* mutation, which might represent the earliest stages of CF lung disease, and raise the issue of time scale over which one might expect the development of severe CF pathology to develop in a mouse model, or indeed whether it is realistic to expect the same endpoints in a different species. In addition to these studies, pulmonary abnormalities have been described in *Cftr*^{tm1Unc} mice bred to congenicity on the C57Bl/6 strain [48], suggesting complex interplay between mutation and strain type (and thus independently segregating modifier genes), in addition to the more obvious environmental influences, such as infectious agents. The range of mutations generated in CF mice, and the potential to exploit murine strain differences to study genetic modifiers, should provide powerful opportunities to study these early changes, in addition to studies of overt infectious models. Indeed, genetic modifier *loci* have been identified for submucosal gland distribution [49] and mononuclear cell interstitial infiltrate and fibrosis [19] in CF mice.

The first description of abnormal pulmonary responses to infection came from outbred, mixed background *Cftr*^{tm1Hgu} mice exposed to bacteria by aerosolisation. These mice displayed defective airway clearance of *Sa* and *Bc*, and developed more severe, pathogen-dependent lung pathology in response to repeated exposure to these pathogens [50]. Another study has also described a similar phenotype of decreased clearance, with increased pulmonary pathology, in response to repeated exposure to *Bc* in *Cftr*^{tm1Unc} mice [51]. The observation of more severe pathology in response to a CF clinical isolate of the most frequent early CF lung pathogen *Sa* has more recently been replicated in *Cftr*^{tm1Hgu} mice and *Cftr*^{tm1Hgu}/*Cftr*^{tm1Unc} compound heterozygote mice bred to congenicity on the C57Bl/6 strain, confirming this enhanced susceptibility [52]. The failure of *Cftr*^{Hgu}/*Cftr*^{tm1Unc} compound heterozygote mice to develop a more severe phenotype than the “residual function” *Cftr*^{tm1Hgu} homozygote mice indicates that a decrease in residual wt *Cftr* mRNA from approximately 10% to 5% of normal levels does not exacerbate this lung phenotype. This suggests that the lung may be very sensitive to loss of

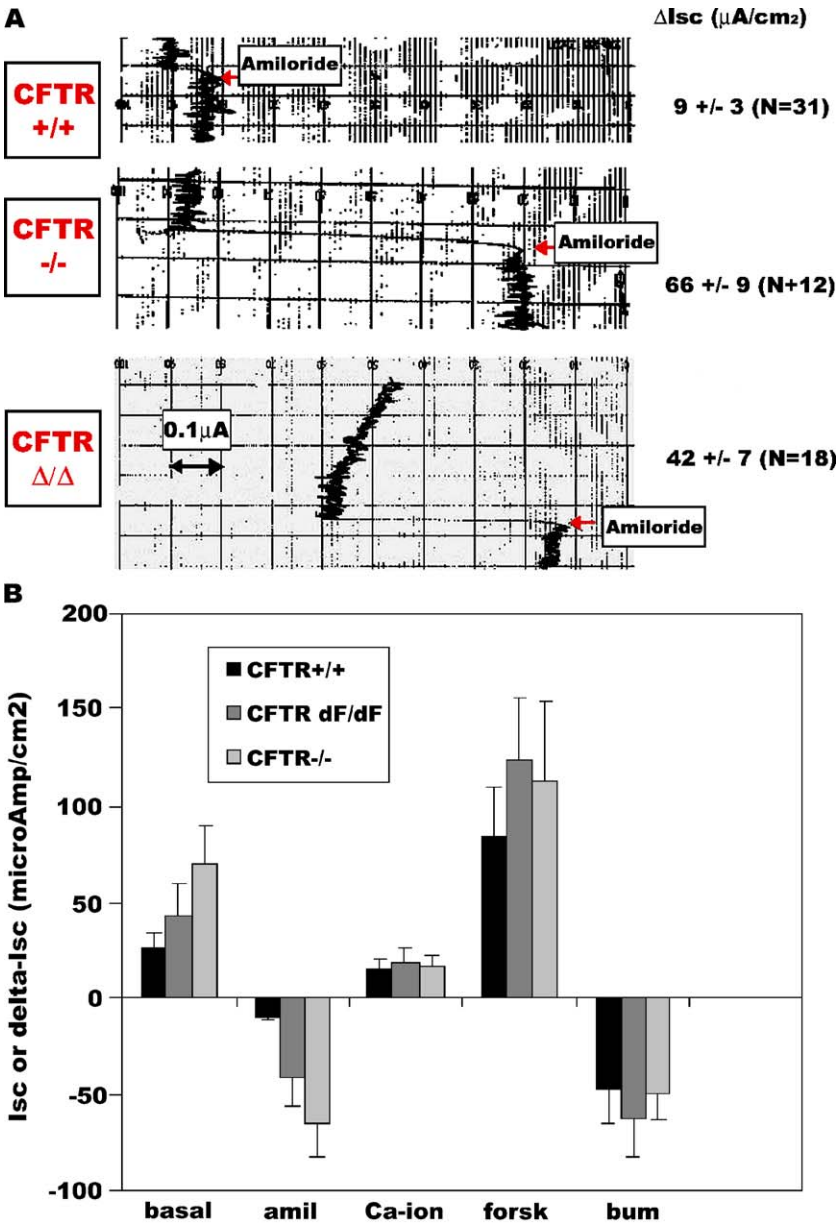

Fig. 1. (A) Na^+ hyperabsorption in CF mice. Recorded traces of freshly excised nasal epithelium from CFTR-wt (CFTR+/+: FVB/129), mutant (CFTR F508del/F508del; FVB/129 littermates), and null mice (CFTR −/−; C57Bl/6/129). Under short-circuit current conditions, the absorptive AML-sensitive Na^+ current is enhanced by 4.7- and 7.3-fold in CFTR mutant and null mice, respectively. Short-circuit current responses to AML were similar in CFTR null mice in the FVB background (ΔIsc: 72 ± 16; $n = 5$). AML, 10 μM, apical side only. (B) Bioelectrics of Na^+ absorption and Cl^- secretion in nasal epithelium of CF mice. Short-circuit current (Isc) responses were monitored following serial addition of AML (10 μM, apical; measures AML-sensitive Na^+ absorption), the Ca^{2+}-ionophore ionomycin (Ca-ion, 5 μM, apical (A) + basolateral (B); measures Ca^{2+}-activated Cl^- secretion), the cAMP agonist FSK (10 μM; A + B; measures cAMP-activated Cl^- secretion), and bumetanide (BMT 100 μM, B; inhibits Cl^- secretion by blocking $NaKCl_2$ cotransport at the basolateral membrane). Data are means ± S.E.M., $n = 8–12$. For specification of mouse genetic backgrounds, see (A). The large rise in Isc in response to FSK in the CFTR null mice (not significantly different from wt mice; seen also in the absence of Ca-ion), in contrast to the modest response to Ca-ion, suggests that the FSK-activated Cl^- secretion is not due to cAMP-triggered mobilization of intracellular Ca^{2+} or cAMP-promoted Ca^{2+} influx.

functional CFTR, and that a critical level may exist for normal lung responses to infection, below which further decreases will have no additional impact. This is consistent with observations in CF individuals, the vast majority of who succumb to severe lung infections despite very different defects in CFTR transcription, translation or function.

Despite these promising observations, the most problematic phenotype to establish in mouse models of CF has been perhaps the most critical: pulmonary infection with *Pa*. Initial studies using the *Cftr*^{tm1Unc}, *Cftr*^{tm1Hgu} and *Cftr*^{tm1Kth} mice found no abnormalities in response to this pathogen [53–55], significantly contrasting with CF lung disease in humans. More promising recent studies have demonstrated a defect in the epithelial cell ingestion of *Pa*, with greater bacterial lung burden after 4.5 h in *Cftr*^{tm1Kth} mice [56], and *Pa* oropharyngeal colonisation, with some evidence of pulmonary spread and mucoid transformation in *Cftr*^{tm1Unc}-TgN$^{(FABPCFTR)}$ mice following oral infection [57]. In addition, following infection with *Pa,* defective airway epithelial cell apoptosis, required for pulmonary clearance of this organism [58], has been demonstrated in *Cftr*^{tm1Kth} and *Cftr*^{tm1G551D} mice [59]. These observations suggest that careful optimisation may yet enable the development of a model of pulmonary infection with motile *Pa*, subsequent colonisation, bacterial phenotypic transformation, and fibrotic lung damage, characteristic of human CF disease [60]. However, to date, clear evidence of persistent infection and gross differences in response to *Pa* have only been achieved using the agar bead model to mimic chronic colonisation, both in *Cftr*^{tm1Unc} mice [61,62] and *Cftr*^{tm1G551D}/*Cftr*^{tm1G551D} mice [63]. These studies use agar to prevent normal clearance of the bacteria, revealing phenotype differences in survival and cytokine response, but less pronounced or no differences with regard to bacterial proliferation and lung pathology. Although use of this technique may prove to be effective in the study of the host response to established infection, by superimposing bacterial retention on an otherwise unaffected lung, it seems less likely to provide informative with regard to the initiation and development of early stage lung disease. Indeed, if predisposition to *Pa* infection in CF is secondary to previous cycles of infection with other organisms and subsequent inflammatory damage, or even the consequent antibacterial chemotherapy received, difficulty in modelling this infection in mouse models of CF, reared in sterile conditions, may not be surprising. It may be that pulmonary exposure to *Pa* following, or in conjunction with, repeated exposure to *Sa* could prove to be more effective in triggering mouse lung disease.

Thus in conclusion, despite some tantalising similarities between CF lung disease in humans and mouse models of CF, significant differences are evident. The development of an ideal mouse model of CF lung disease, to enable the dissection of pathogenesis, or testing of novel therapeutics, is yet to be achieved. Nevertheless, mouse models of CF clearly demonstrate a range of abnormal pulmonary phenotypes as result of the *Cftr* mutation. It is possible that species differences such as basic lung physiology and airway epithelial cellular composition [64], alternative Cl^- channels [65] and less widespread submucosal glands in the murine airways [44], host cationic peptides with different antibacterial profiles [66,67], or altered specificity or expression of innate pattern recognition receptors [68,69], might ultimately prevent the generation of such a model. However, further careful development of phenotypes already described should at least offer the opportunity to study relevant elements of the disease pathogenesis secondary to *Cftr* dysfunction, even if classical endstage human disease might not be modelled. Indeed, although the suitability as endpoints for therapeutic testing remains controversial, some of the models described above have already been used as in vivo systems to test novel therapeutic strategies [70,71].

6.6. Hepatic disease

Gallbladder epithelium in mouse and human expresses CFTR, and is involved in iso-osmotic water transport. cAMP-stimulated Cl^- transport and fluid secretion is absent in gallbladder of CF KO mice, whereas resorption is normal [72]. Though direct measurements have not been performed, it is tempting to hypothesise that a similar condition occurs in the intrahepatic bile duct epithelium in human CF patients. This could help to explain bile duct obstruction and cirrhosis observed in many CF patients [73,74].

6.7. Vas deferens dysfunction

Human CF males generally suffer from infertility caused by bilateral absence the *vas deferens,* which contains another ductal epithelium that requires CFTR for proper function. CF mutant male mice also show severely reduced fertility. Abnormalities of *vas deferens*, mucoid obstruction and luminal collapse, were indeed observed in both *Cftr*^{tm1Eur} and *Cftr*^{tm1Cam} homozygous males [75], but not the complete absence of the structure, which is characteristic of human CF males, even in subjects with only mild CFTR dysfunction [76]. Here too, we observe that CFTR dysfunction in mice leads to a phenotype comparable to, but not identical with the human disease, presumably because other gene products strongly affect its expression.

7. Other animal models

CF mutant mouse models clearly have limitations, especially as a model for CF lung disease. Therefore, other animals are being studied in the CF field, in particular sheep, pig and ferret. One important advantage of these models is that the lung function, size and architecture of these animals more closely resemble the human situation. In particular, the role of submucosal glands, which are only found in the proximal trachea in mice, can be studied in these models [77,78]. So far, only mice have been successfully used in modelling by homologous recombination and transgenesis. Despite intensive efforts, CF sheep [79] or ferrets [80] have not been created but may become a valuable contribution to the field in the future.

References

[1] Riordan JR, Rommens JM, Kerem B, et al. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science 1989;245(4922):1066–73.

[2] Ledermann B. Embryonic stem cells and gene targeting. Exp Physiol 2000;85(6):603–13.

[3] Snouwaert JN, Brigman KK, Latour AM, et al. An animal model for cystic fibrosis made by gene targeting. Science 1992;257(5073):1083–8.

[4] Ratcliff R, Evans MJ, Cuthbert AW, et al. Production of a severe cystic fibrosis mutation in mice by gene targeting. Nat Genet 1993;4(1):35–41.

[5] Rozmahel R, Wilschanski M, Matin A, et al. Modulation of disease severity in cystic fibrosis transmembrane conductance regulator deficient mice by a secondary genetic factor. Nat Genet 1996;12(3):280–7.

[6] Hasty P, O'Neal WK, Liu KQ, et al. Severe phenotype in mice with termination mutation in exon 2 of cystic fibrosis gene. Somat Cell Mol Genet 1995;21(3):177–87.

[7] Dorin JR, Dickinson P, Emslie E, et al. Successful targeting of the mouse cystic fibrosis transmembrane conductance regulator gene in embryonal stem cells. Transgenic Res 1992;1(2):101–5.

[8] O'Neal WK, Hasty P , McCray PB Jr., et al. A severe phenotype in mice with a duplication of exon 3 in the cystic fibrosis locus. Hum Mol Genet 1993;2(10):1561–9.

[9] Davidson DJ, Dorin JR. The CF mouse: an important tool for studying cystic fibrosis. Exp Rev Mol Med 2001; 12 March, http://www-ermm.cbcu.com.ac.uk/01002551h.htm

[10] Grubb BR, Boucher RC. Pathophysiology of gene-targeted mouse models for cystic fibrosis. Physiol Rev 1999;79(Suppl. 1):S193–214.

[11] Colledge WH, Abella BS, Southern KW, et al. Generation and characterization of a delta F508 cystic fibrosis mouse model. Nat Genet 1995;10(4):445–52.

[12] Zeiher BG, Eichwald E, Zabner J, et al. A mouse model for the delta F508 allele of cystic fibrosis. J Clin Invest 1995;96(4):2051–64.

[13] van Doorninck JH, French PJ, Verbeek E, et al. A mouse model for the cystic fibrosis delta F508 mutation. EMBO J 1995;14(18):4403–11.

[14] French PJ, van Doorninck JH, Peters RH, et al. A delta F508 mutation in mouse cystic fibrosis transmembrane conductance regulator results in a temperature-sensitive processing defect in vivo. J Clin Invest 1996;98(6):1304–12.

[15] Wilke M, Bot A, Jorna H, Rensen S, De Jonge H. Correction of the dF508-CFTR processing defect by low temperature incubation of mouse intestine. Pediatr Pulmonol 2003;98(Suppl. 25):34.

[16] Dickinson P, Smith SN, Webb S, et al. The severe G480C cystic fibrosis mutation, when replicated in the mouse, demonstrates mistrafficking, normal survival and organ-specific bioelectrics. Hum Mol Genet 2002;11(3):243–51.

[17] Delaney SJ, Alton EW, Smith SN, et al. Cystic fibrosis mice carrying the missense mutation G551D replicate human genotype–phenotype correlations. EMBO J 1996;15(5):955–63.

[18] Du M, Jones JR, Lanier J, et al. Aminoglycoside suppression of a premature stop mutation in a Cftr−/− mouse carrying a human CFTR–G542X transgene. J Mol Med 2002;80(9):595–604.

[19] Haston CK, McKerlie C, Newbigging S, Corey M, Rozmahel R, Tsui LC. Detection of modifier loci influencing the lung phenotype of cystic fibrosis knockout mice. Mamm Genome 2002;13(11):605–13.

[20] Bronsveld I, Mekus F, Bijman J, et al. Chloride conductance and genetic background modulate the cystic fibrosis phenotype of Delta F508 homozygous twins and siblings. J Clin Invest 2001;108(11):1705–15.

[21] Hamosh A, King TM, Rosenstein BJ, et al. Cystic fibrosis patients bearing both the common missense mutation Gly–Asp at codon 551 and the delta F508 mutation are clinically indistinguishable from delta F508 homozygotes, except for decreased risk of meconium ileus. Am J Hum Genet 1992;51(2):245–50.

[22] Bijvelds M, Verkade H, Havinga R, Sinaasappel M, De Jonge H. Both defective lipolysis and impaired fatty acid uptake contribute to fat malabsorption in cystic fibrosis mice. Pediatr Pulmonol Suppl 2003;25:340.

[23] Kent G, Oliver M, Foskett JK, et al. Phenotypic abnormalities in long-term surviving cystic fibrosis mice. Pediatr Res 1996;40(2):233–41.

[24] Ip WF, Bronsveld I, Kent G, Corey M, Durie PR. Exocrine pancreatic alterations in long-lived surviving cystic fibrosis mice. Pediatr Res 1996;40(2):242–9.

[25] Zhou L, Dey CR, Wert SE, DuVall MD, Frizzell RA, Whitsett JA. Correction of lethal intestinal defect in a mouse model of cystic fibrosis by human CFTR. Science 1994;266(5191):1705–8.

[26] De Lisle RC. Increased expression of sulfated gp300 and acinar tissue pathology in pancreas of CFTR(−/−) mice. Am J Physiol 1995;268(4 Pt 1):G717–23.

[27] Freedman SD, Katz MH, Parker EM, Laposata M, Urman MY, Alvarez JG. A membrane lipid imbalance plays a role in the phenotypic expression of cystic fibrosis in cftr(−/−) mice. Proc Natl Acad Sci U S A 1999;96(24):13995–4000.

[28] Gray MA, Winpenny JP, Verdon B, McAlroy H, Argent BE. Chloride channels and cystic fibrosis of the pancreas. Biosci Rep 1995;15(6):531–41.

[29] Mearns M. Cystic fibrosis: the first 50 years. In: Dodge JA, Brock DJH, Widdicombe JH, editors. Cystic fibrosis current topics15. Chichester: J. Wiley & Sons, 1992. pp. 217–50.

[30] Oppenheimer EH, Esterley JR. Pathology of cystic fibrosis: review of the literature and comparison with 146 autopsied cases. In: Rosenberg HS, Bolande RP, editors. Perspectives in paediatric pathology15. New York: Yearbook Medical, 1976. pp. 241–78.

[31] Govan JR, Nelson JW. Microbiology of lung infection in cystic fibrosis. Br Med Bull 1992;48(4):912–30.

[32] Parad RB, Gerard CJ, Zurakowski D, Nichols DP, Pier GB. Pulmonary outcome in cystic fibrosis is influenced primarily by mucoid *Pseudomonas aeruginosa* infection and immune status and only modestly by genotype. Infect Immun 1999;67(9):4744–50.

[33] Wilschanski M, Yahav Y, Yaacov Y, et al. Gentamicin-induced correction of CFTR function in patients with cystic fibrosis and CFTR stop mutations. N Engl J Med 2003;349(15):1433–41.

[34] Boyle MP, Diener-West M, Milgram L, et al. A multicenter study of the effect of solution temperature on nasal potential difference measurements. Chest 2003;124(2):482–9.

[35] Grubb BR, Vick RN, Boucher RC. Hyperabsorption of Na+ and raised Ca(2+)-mediated Cl− secretion in nasal epithelia of CF mice. Am J Physiol 1994;266(5 Pt 1):C1478–83.

[36] MacVinish LJ, Goddard C, Colledge WH, Higgins CF, Evans MJ, Cuthbert AW. Normalization of ion transport in murine cystic fibrosis nasal epithelium using gene transfer. Am J Physiol 1997;273(2 Pt 1):C734–40.

[37] Bot A, Jorna H, Willemsen R, De Jonge H. Detection of CFTR protein in human and mouse tissues by immunocytochemistry. J Cystic Fibros 2004;273:C734 [current issue, B1.4.].

[38] Menco BP, Birrell GB, Fuller CM, Ezeh PI, Keeton DA, Benos DJ. Ultrastructural localization of amiloride-sensitive sodium channels and Na+,K(+)-ATPase in the rat's olfactory epithelial surface. Chem Senses 1998;23(2):137–49.

[39] Mall M. Overexpression of ENaC in mouse aiways: a novel animal model for chronic bronchitis and cystic fibrosis lung disease. Pediatr Pulmonol 2003;121(Suppl. 25):137 [S7.3].

[40] Goddard CA, Ratcliff R, Anderson JR, et al. A second dose of a CFTR cDNA–liposome complex is as effective as the first dose in restoring cAMP-dependent chloride secretion to null CF mice trachea. Gene Ther 1997;4(11):1231–6.

[41] Zahm JM, Gaillard D, Dupuit F, et al. Early alterations in airway mucociliary clearance and inflammation of the lamina propria in CF mice. Am J Physiol 1997;272(3 Pt 1):C853–9.

[42] Cowley EA, Wang CG, Gosselin D, Radzioch D, Eidelman DH. Mucociliary clearance in cystic fibrosis knockout mice infected with *Pseudomonas aeruginosa*. Eur Respir J 1997;10(10):2312–8.
[43] Tarran R, Grubb BR, Parsons D, et al. The CF salt controversy: in vivo observations and therapeutic approaches. Mol Cell 2001;8(1):149–58.
[44] Borthwick DW, West JD, Keighren MA, Flockhart JH, Innes BA, Dorin JR. Murine submucosal glands are clonally derived and show a cystic fibrosis gene-dependent distribution pattern. Am J Respir Cell Mol Biol 1999;20(6):1181–9.
[45] Thomas GR, Costelloe EA, Lunn DP, et al. G551D cystic fibrosis mice exhibit abnormal regulation of inflammation in lungs and macrophages. J Immunol 2000;164(7):3870–7.
[46] Steagall WK, Elmer HL, Brady KG, Kelley TJ. Cystic fibrosis transmembrane conductance regulator-dependent regulation of epithelial inducible nitric oxide synthase expression. Am J Respir Cell Mol Biol 2000;22(1):45–50.
[47] Kelley TJ, Drumm ML. Inducible nitric oxide synthase expression is reduced in cystic fibrosis murine and human airway epithelial cells. J Clin Invest 1998;102(6):1200–7.
[48] Kent G, Iles R, Bear CE, et al. Lung disease in mice with cystic fibrosis. J Clin Invest 1997;100(12):3060–9.
[49] Innes BA, Dorin JR. Submucosal gland distribution in the mouse has a genetic determination localized on chromosome 9. Mamm Genome 2001;12(2):124–8.
[50] Davidson DJ, Dorin JR, McLachlan G, et al. Lung disease in the cystic fibrosis mouse exposed to bacterial pathogens. Nat Genet 1995;9(4):351–7.
[51] Sajjan U, Thanassoulis G, Cherapanov V, et al. Enhanced susceptibility to pulmonary infection with *Burkholderia cepacia* in Cftr(−/−) mice. Infect Immun 2001;69(8):5138–50.
[52] Davidson DJ, Webb S, Teague P, Govan JRW, Dorin JR. Lung pathology in response to repeated exposure to *Staphylococcus aureus* in congenic residual function cystic fibrosis mice does not increase when the level of Cftr is reduced. Pathobiology 2004 71(3) 152–8.
[53] Cressman VL, Hicks EM, Funkhouser WK, Backlund DC, Koller BH. The relationship of chronic mucin secretion to airway disease in normal and CFTR-deficient mice. Am J Respir Cell Mol Biol 1998;19(6):853–66.
[54] Larbig M, Steinmetz I, Reganzerowski A, Tschernig T, Bellman B, Berhard W, et al. *Pseudomonas aeruginosa* infection in cystic fibrosis: animal model of the Cftrm1HGU mouse. 22nd European Cystic Fibrosis Conference; 1998. p. 140. Berlin.
[55] McCray Jr PB, Zabner J, Jia HP, Welsh MJ, Thorne PS. Efficient killing of inhaled bacteria in DeltaF508 mice: role of airway surface liquid composition. Am J Physiol 1999;277(1 Pt 1):L183–90.
[56] Schroeder TH, Reiniger N, Meluleni G, Grout M, Coleman FT, Pier GB. Transgenic cystic fibrosis mice exhibit reduced early clearance of *Pseudomonas aeruginosa* from the respiratory tract. J Immunol 2001;166(12):7410–8.
[57] Coleman FT, Mueschenborn S, Meluleni G, et al. Hypersusceptibility of cystic fibrosis mice to chronic *Pseudomonas aeruginosa* oropharyngeal colonization and lung infection. Proc Natl Acad Sci U S A 2003;100(4):1949–54.
[58] Grassme H, Kirschnek S, Riethmueller J, et al. CD95/CD95 ligand interactions on epithelial cells in host defense to *Pseudomonas aeruginosa*. Science 2000;290(5491):527–30.
[59] Cannon CL, Kowalski MP, Stopak KS, Pier GB. *Pseudomonas aeruginosa*-induced apoptosis is defective in respiratory epithelial cells expressing mutant cystic fibrosis transmembrane conductance regulator. Am J Respir Cell Mol Biol 2003;29(2):188–97.
[60] Davidson DJ, Currie AJ, Speert DP. *Pseudomonas aeruginosa* infections in individuals with cystic fibrosis: North American perspective. In: Hauser A, Rello J, editors. Severe infections caused by *Pseudomonas aeruginosa*. Norwell: Kluwer Academic Publishing; 2003. p. 71–89.
[61] Gosselin D, Stevenson MM, Cowley EA, et al. Impaired ability of Cftr knockout mice to control lung infection with *Pseudomonas aeruginosa*. Am J Respir Crit Care Med 1998;157(4 Pt 1):1253–62.
[62] Heeckeren A, Walenga R, Konstan MW, Bonfield T, Davis PB, Ferkol T. Excessive inflammatory response of cystic fibrosis mice to bronchopulmonary infection with *Pseudomonas aeruginosa*. J Clin Invest 1997;100(11):2810–5.
[63] McMorran BJ, Palmer JS, Lunn DP, et al. G551D CF mice display an abnormal host response and have impaired clearance of *Pseudomonas* lung disease. Am J Physiol Lung Cell Mol Physiol 2001;281(3):L740–7.
[64] Pack RJ, Al-Ugaily LH, Morris G, Widdicombe JG. The distribution and structure of cells in the tracheal epithelium of the mouse. Cell Tissue Res 1980;208(1):65–84.
[65] Grubb BR, Paradiso AM, Boucher RC. Anomolies in ion transport in CF mouse tracheal epithelium. Am J Physiol 1994;267:C293–300.
[66] Morrison G, Kilanowski F, Davidson D, Dorin J. Characterization of the mouse Beta defensin 1, defb1, mutant mouse model. Infect Immun 2002;70(6):3053–60.
[67] Maxwell AI, Morrison GM, Dorin JR. Rapid sequence divergence in mammalian beta-defensins by adaptive evolution. Mol Immunol 2003;40(7):413–21.
[68] Muir A, Soong G, Sokol S, et al. Toll like receptors in normal and cystic fibrosis airway epithelial cells. Am J Respir Cell Mol Biol 2003;30(6):777–83.
[69] Hajjar AM, Ernst RK, Tsai JH, Wilson CB, Miller SI. Human Toll-like receptor 4 recognizes host-specific LPS modifications. Nat Immunol 2002;3(4):354–9.
[70] Koehler DR, Sajjan U, Chow YH, et al. Protection of Cftr knockout mice from acute lung infection by a helper-dependent adenoviral vector expressing Cftr in airway epithelia. Proc Natl Acad Sci U S A 2003;100(26):15364–9.
[71] Van Heeckeren AM, Scaria A, Schluchter MD, Ferkol TW, Wadsworth S, Davis PB. Delivery of CFTR by adenoviral vector to cystic fibrosis mouse lung in a model of chronic *Pseudomonas aeruginosa* lung infection. Am J Physiol Lung Cell Mol Physiol 2003;286(4):L717–26.
[72] Peters RH, van Doorninck JH, French PJ, et al. Cystic fibrosis transmembrane conductance regulator mediates the cyclic adenosine monophosphate-induced fluid secretion but not the inhibition of resorption in mouse gallbladder epithelium. Hepatology 1997;25(2):270–7.
[73] Castaldo G, Fuccio A, Salvatore D, et al. Liver expression in cystic fibrosis could be modulated by genetic factors different from the cystic fibrosis transmembrane regulator genotype. Am J Med Genet 2001;98(4):294–7.
[74] Kinnman N, Lindblad A, Housset C, et al. Expression of cystic fibrosis transmembrane conductance regulator in liver tissue from patients with cystic fibrosis. Hepatology 2000;32(2):334–40.
[75] Reynaert I, Van Der Schueren B, Degeest G, et al. Morphological changes in the vas deferens and expression of the cystic fibrosis transmembrane conductance regulator (CFTR) in control, deltaF508 and knock-out CFTR mice during postnatal life. Mol Reprod Dev 2000;55(2):125–35.
[76] Gilljam M, Moltyaner Y, Downey GP, et al. Airway inflammation and infection in congenital bilateral absence of the vas deferens. Am J Respir Crit Care Med 2004;169(2):174–9.
[77] Joo NS, Krouse ME, Wu JV, et al. HCO3− transport in relation to mucus secretion from submucosal glands. JOP 2001;2(Suppl. 4):280–4.
[78] Hug MJ, Bridges RJ. pH regulation and bicarbonate transport of isolated porcine submucosal glands. JOP 2001;2(Suppl. 4):274–9.
[79] Williams SH, Sahota V, Palmai-Pallag T, Tebbutt SJ, Walker J, Harris A. Evaluation of gene targeting by homologous recombination in ovine somatic cells. Mol Reprod Dev 2003;66(2):115–25.
[80] Li Z, Engelhardt JF. Progress toward generating a ferret model of cystic fibrosis by somatic cell nuclear transfer. Reprod Biol Endocrinol 2003;1(1):83.
[81] The Virtual Repository of Cystic Fibrosis European Network. F. Animal and Cellular Models/Vectors, http://central.igc.gulbenkian.pt/cftr/vr/models.html.