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EDITORIAL

Cystic fibrosis transmembrane conductance regulator protein: What is its role in cystic fibrosis?

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Cystic fibrosis (CF) is a disease involving many organs including sweat glands, pancreas, gastrointestinal tract and airways. Disordered airway function is the main cause of illness and death in the increasing numbers of CF sufferers who survive infancy. At one time it was thought that a circulating CF factor or factors might cause the airway malfunction, but studies of normal lungs transplanted into CF patients have called this into question. Airways in normal lungs transplanted into CF patients have a normal epithelial potential difference and show only the same rate of infection with *Pseudomonas aeruginosa* and *Staphylococcus aureus* as do lungs transplanted into non-CF recipients [1]. Thus, the airway lesions in CF seem to depend in large part on the local expression of a disordered gene, probably within the mucosa.

It is now just over three years since the gene locus responsible for CF was found and its gene cloned and sequenced [2, 3, 4]. The gene product is a membrane-spanning protein, cystic fibrosis transmembrane conductance regulator (CFTR), which acts as a chloride channel regulated *via* the cyclic AMP (cAMP) pathway [5]. In cells from CF patients, stimulation of cAMP release fails to open the channel. However, introduction of DNA coding for the correct protein into these cells restores their ability to conduct chloride *via* this channel [6]. This and other observations led to the hypothesis that lack of functional CFTR protein in the apical membrane of the epithelium leads to the abnormalities seen in CF lungs [7]. Surface epithelium reabsorbs sodium ions from the liquid in the airway lumen and can secrete chloride into it. In man Na^+ reabsorption is the chief ion movement. Water moves along the osmotic gradient created by ion movements. In patients with CF, chloride movement into the lumen cannot be stimulated by cAMP and sodium reabsorption is greater than normal, though the reason for this is unknown. It has been suggested that these abnormalities would dehydrate mucus, giving it abnormal physical properties, and reduce the layer of periciliary fluid, both of which would slow mucociliary clearance. This in turn might explain why CF airways are liable to infection. This hypothesis is seductive but cannot explain the entire pathophysiology of the CF airway. For example, patients with 'immotile cilia syndrome' have a more profound slowing of airway mucus transport than that seen in CF but, typically, they suffer only mild airway infections [8]. Also, the hypothesis predicts that preventing sodium reabsorption from the airway with the drug amiloride should prevent the withdrawal of water from the airway lumen and so mitigate the disease. In a trial of amiloride on CF patients with previously stable lung disease, KNOWLES *et al.* [9] first treated airway infections vigorously with antibiotics and then withdrew conventional treatments. The patients were split into two groups, one treated with amiloride aerosol and the other receiving placebo. Over the next 6 months, FVC declined in both groups but more slowly in the amiloride-treated group. However, a subsequent 6 month cross-over trial of amiloride aerosol *versus* placebo given in addition to conventional therapy showed no benefit [10]. Thus, partial correction of the abnormal ion movement seems, at the moment, only to provide a marginal benefit in CF.

Could absence of functional CFTR protein at other sites in the airway also be important in the genesis of the CF syndrome? In this issue of the journal, JACQUOT *et al.* publish their finding that airway submucosal gland cells express CFTR protein, some of it in the basal and apical membranes of the secretory cells, some in the membranes of the secretory granules and some in the cell cytoplasm [11]. This independently confirms the recent report of ENGELHARDT *et al.* who describe submucosal gland as the main site of CFTR in the airway [12]. Thus, CF patients lack functional CFTR in submucosal gland cells, and this finding suggests alternative explanations for the lung manifestations of the disease, some of which are reviewed below:

1) The role of CFTR as a chloride channel in providing the water for submucosal gland secretions. In airways with submucosal glands, the main bulk of secretion appears to come from these glands. Could chloride secretion *via* the CFTR protein account for the production of water by the submucosal glands? Under basal conditions human submucosal glands in culture do not secrete Cl^- but activation of either the Ca^{++} or cAMP second messenger pathway stimulates Cl^- secretion in non-CF glands [13]. In CF glands, however, stimulation of either pathway fails to produce Cl^- secretion [14]. Agents acting through these

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second messenger pathways may also have weaker effects on mucin secretion in CF than in normal bronchi [15]. In contrast, CORRALES *et al.* [16] measured the rate at which they could collect fluid from ducts of individual submucosal glands in feline trachea *in vitro* and tested how this responded to stimulation with phenylephrine. They found that blocking chloride secretion did not alter the flow of mucus in the ducts in response to phenylephrine. This appears to contradict the idea that the CFTR protein is important in providing Cl^- and water for submucosal gland secretion. They proposed instead that the secretory granules contain osmotically active particles which, on their release, take up water and cause the granules to swell, an idea supported by VERDUGO [17].

A recent study has shown that the CFTR protein introduced into Xenopus oocytes transports water as well as anions in response to cAMP. It is not yet certain that it plays the same role in submucosal glands in the human airway. Thus CFTR protein in human submucosal gland may play a part in conducting Cl^- and water into mucus and failure of this mechanism may be important in CF.

2) Plasma membrane recycling. Recently it has been shown that CFTR protein is important in the recycling of plasma membranes [18]. Proteins such as horseradish peroxidase (HRP) can be used as markers to follow the process of endocytosis. In normal cells, raised levels of cAMP within the cytoplasm inhibited endocytosis, but in CF cells raised cAMP failed to influence the process. Transfection of CF cells with the gene encoding for normal CFTR protein restored the ability of cAMP to inhibit endocytosis. This study also showed that CFTR protein regulates exocytosis of macromolecules. In normal cells cAMP stimulates this process, but not in CF cells. Again, transfection of CF cells with the normal CFTR gene produced exocytosis in response to cAMP. We do not know what part CFTR protein has in the normal regulation of endo- and exocytosis, but there is evidence that Cl^- transport is important in the latter process. Nor is it yet apparent how disordered control of endo- and exocytosis could lead to the airway lesions in CF.

3) Acidity of the Golgi apparatus. Some intracellular organelles, such as the Golgi apparatus, are normally more acid than the cell cytoplasm. The process of acidification involves the transfer of a proton (H^+) which is accompanied by a Cl^- ion, perhaps transported by the CFTR protein. BARASCH *et al.* [19] have reported that, in nasal polyp tissue, the trans-Golgi and pre-lysosomes from CF tissues are less acid than the equivalent organelles from normals by 0.2–0.3 pH units. They attribute this to the failure of faulty CFTR protein to conduct sufficient Cl^- to accompany protons in CF. They argue that the relatively alkaline interior of the Golgi apparatus in CF cells may explain the observed lack of sialic acid in the oligosaccharides of CF mucins because sialyl transferase enzymes work most efficiently at an acid pH. This could be functionally important because sialic acid is one of the terminating groups of the oligosaccharide sidechains of mucins; glycosylating enzymes continue to add further sugars to a sidechain until an end group such as sialic acid is in place. This suggests a possible way in which absence of the CFTR protein could lead to over-glycosylation of mucins in CF and so be responsible for abnormal mucus. Could the modest difference in pH between normal and CF cells really make an important difference? Furthermore, two recent studies now question the role of CFTR in acidifying intracellular organelles [20, 21]. Showing that transfection of CF cells with the CFTR gene increases mucin sialylation would make this hypothesis more persuasive.

4) Oversulphation of mucins. CF mucus from both sputum and cell cultures contains an abnormally large amount of sulphate ion in the mucin macromolecules [22]. The authors proposed that this results from an abnormal biochemical process intimately connected with the CF lesion and suggested that the altered mucins resulting from this might explain the susceptibility of CF airways to colonisation by *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Increased sulphation in CF might arise from a failure of acidification of the Golgi apparatus (see 3 above) inhibiting sialylation of the oligosaccharide sidechains, thus allowing them to grow abnormally long and providing correspondingly more positions for sulphation. Another hypothesis to explain increased sulphation is that the abnormal anion channel found in CF membranes might cause large quantities of SO_4^{--} to reach sulphation sites in the Golgi apparatus. On the other hand, altered glycosylation and sulphation of mucins with an increase in mucin acidity is a feature of prolonged airway inflammation and so might not be specific to CF [23]. Again, it would be interesting to see whether mucin sulphation returned to normal in cultures of CF airway cells after transfection with the gene for normal CFTR.

5) Transport of macromolecules. Finally, it is important to consider the possibility that CFTR protein may act as an active transporter of macromolecules, not just as a Cl^- channel. This idea arose because the predicted structure of CFTR protein resembles that of a family of proteins which act as energy requiring transporters of macromolecules. This firmly includes P-glycoprotein, responsible for the drug-resistance in many solid tumours by active cellular extrusion of chemotherapeutic agents. Recently it has become clear that P-glycoprotein can also act as a chloride channel [24]. By analogy CFTR protein might also have a dual role as macromolecular transporter and ion channel.

The idea that the submucosal glands are the site of an important lesion in CF has implications for therapy. The attractions of a topical route for drugs aimed at correcting the consequences of the CF defect, or

for genes to correct the defect itself, are obvious. If the site of the lesion is the submucosal gland, instead of (or as well as) the surface epithelium, directing the therapy to its target will be more difficult. This may delay effective therapy, but should not put it beyond the scope of human ingenuity.

The way that our knowledge of CF has advanced is instructive. Defective chloride transport was initially predicted by electrophysiologists working on CF tissues. Then molecular biologists identified the faulty gene and, together with electrophysiologists, confirmed that the gene product is a chloride channel. This has not led to an immediate understanding of the disease, but has provided techniques and ideas which are spurring cell biologists, biochemists and physiologists in their attempts to explain CF. We are now approaching the time when physicians will be able to draw together insights provided by the various basic sciences in order to treat CF effectively.

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