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# A new model of cystic fibrosis pathology: Lack of transport of glutathione and its thiocyanate conjugates

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**Summary** Many of the symptoms of cystic fibrosis are not explained by the current disease mechanisms. Therefore, the authors conducted an extensive literature review and present a new model of cystic fibrosis pathology, which is the culmination of this research. Understanding that the cystic fibrosis transmembrane conductance regulator (CFTR) is responsible for glutathione (GSH) transport, the authors hypothesize that mutations of the CFTR, which create abnormal GSH transport, will lead to aberrations of GSH levels in both the intracellular as well as the extracellular milieu. These alterations in normal cellular GSH levels affect the redox state of the cell, thereby affecting the intracellular stress protein, metallothionein. The authors describe how this disruption of the redox state caused by excess cellular GSH, will naturally prevent the delivery of zinc as a cofactor for various enzymatic processes, and how these disruptions in normal redox may cause alterations in both humoral and cell-mediated immunity. Moreover, the symptom of thick sticky mucus in these patients might be explained through the understanding that oversulfation of mucus is a direct result of elevated cellular GSH and cysteine. The issues of hyperinflammation, altered pH and the imbalance of fatty acids that are typical in cystic fibrosis are addressed—all of which may also be linked to disruptions in GSH homeostasis. Additionally, this new model of cystic fibrosis pathology, clarifies the relationship between the CFTR and the multi-drug resistance proteins, and the lack of cell-mediated immunity by predicting that the substrate of these proteins is a glutathione adduct of thiocyanate. Finally, a new therapeutic strategy by using isothiocyanates to rectify the GSH imbalance and restore the immune system is suggested for the treatment of cystic fibrosis patients.


## Introduction

Cystic fibrosis (CF) is an autosomal recessive disorder characterized by repeated and destructive lower respiratory infections, resulting in the gradual destruction of the lung tissue in patients, and ending in an early death. The disease affects approximately 30,000 children and adults in the United States. The etiology of the disease has been traced to the mutation of the cystic fibrosis transmembrane conductance regulator (CFTR) protein which has long been thought to govern chloride

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ion conductance in epithelial cells. And, while loss of chloride conductance is the ''gold standard'' in terms of diagnostic parameters, loss of this function does not fully explain the diverse pathologies seen in this disease.

Since the discovery of the CFTR and its respective gene in 1989, there has been an enormous drive to further understand the disease and in fact, try to correct the imperfection via gene therapy. Because CFTR has been the center of attention in CF research, it has become much clearer what its function is as a membrane protein, and there is a better understanding of its similarities to other ATP-binding cassette (ABC) membrane proteins [1]. It is now known that the CFTR, just as the other ABC proteins, is permeable to glutathione (GSH) and that the lining of the CF lung which has a defective CFTR is abnormally deficient in this anti-oxidant protein [2]. There is now a better understanding of the disease, but the cause of the various pathologies associated with CF still remains elusive.

In order to shed further light on the various disease mechanisms in CF, it is important to consider the role of the CFTR, its relationship to GSH, and the intracellular and extracellular resulting effects due to transport deficiency. Further, there has been a great deal of CF related papers published which discuss the activity of enzymes and various DNA factors, that are either inactive or overactive, all of which depend on zinc as a cofactor. Owing to zinc's ability to act as a cofactor in various enzymatic reactions, and because the proper activity of zinc is dependent upon the redox state of the cell, our interest has been the effects of altered zinc homeostasis due to the abnormalities of GSH homeostasis.

Moreover, the immune system in CF is compromised, particularly in the respiratory tract, which remains a major cause of mortality. The immune system in CF seems to be skewed toward the humoral immune response, while cell-mediated immunity is lacking [3,4]. The obvious result is hyperinflammation, which is destructive to the lung, and creates an inability to combat invading bacteria, such as *Pseudomonas aeruginosa*, which colonizes the lungs of these patients at a very young age. Certain immune factors such as the lactoperoxidase system and neutrophil activity are faulty in CF. And, anti-inflammation therapy remains central in most treatment protocols.

In the last couple of decades, the average life span of a person with CF has increased remarkably due to new treatment options and a better understanding of nutrition. However, treatments for CF, as with most diseases, are geared toward easing symptoms. Since CF is a systemic disease, involving multiple organ pathophysiology, stopping its progression has been difficult. Thus, because the authors believe that the aberrations in GSH homeostasis are crucial in terms of the pathophysiology of the disease, they performed an extensive literature review, and in this paper they propose a new model of disease pathology, and present a new treatment, which might prove to be ameliorative to both the symptoms of CF as well as its lethal effects on the lungs.

## Discussion

### Alterations in GSH homeostasis and GSH—thiocyanate adduct transport

Recent research clearly shows that glutathione (GSH) is either directly or indirectly effluxed through the CFTR of epithelial cells [5,6]. And this research states that the lack of functioning CFTR protein in the cells lining the airways is most likely the cause of the deficit of GSH in epithelial lining fluid that is seen in cystic fibrosis. However, logically, a deficit of GSH in the extracellular space caused by a lack of functioning CFTR protein, requires the assumption that either there is a surplus of GSH in the intracellular space of CF cells, and/or that other GSH transport mechanisms are operating in these cells. If either or both of these statements are true, it could explain the pleiotropic pathology seen in this disease.

Moreover, the multi-drug resistance proteins, which have been shown to be functionally redundant to the CFTR protein, transport glutathione adducts of chemotoxins [7,8]. Since the CFTR has also been shown to be permeable to thiocyanates, which are a part of the lactoperoxidase anti-bacterial defense system, this fact is of particular interest [9,8]. Given these facts, it is not only likely that the CFTR transports adducts of glutathione, but that GSH—thiocyanate is transported by the CFTR, as well as by the multi-drug resistance proteins. Furthermore, the lack of transport of this adduct by the non-functional CFTR could very well be implicated in causing much of the pathology seen in this disease. That is, a deficit of extracellular thiocyanate would at least partly explain the lack of cell-mediated immunity due to a faulty lactoperoxidase system in CF [10—12]. This lack of GSH transport and its conjugate, logically, may also cause a sustained surplus of intracellular GSH. Following this line of reasoning, this increase in intracellular GSH, and the resulting abnormal redox state, could also explain the concomitant in-

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creased humoral immunity seen in CF, caused by an intracellular and extracellular zinc deficiency (as a result of the disrupted redox state), and a deficit of extracellular GSH.

It is well documented that extracellular levels of GSH, particularly in the lung, are below normal in CF patients. Hanrahan and Linsdell provided an explanation for this deficiency by showing that the CFTR is permeable to GSH [5]. Further studies have shown that GSH is indeed effluxed through the CFTR and into the extracellular space, not only in epithelial tissue of the lung, but the CFTR has also been located in blood vessel endothelia, and in various organs as well [13–15]. Finally, although it has been suggested that restoration of lung ELF glutathione levels may ameliorate some of the pathology seen in this disorder, no studies have directly addressed the likelihood of supraphysiological intracellular GSH levels and the pathology associated with CF.

## Intracellular GSH levels and apoptosis

In support of this paradigm, which would naturally follow from a model in which the essential function of the CFTR protein is a GSH/GSH adduct transport—while GSH levels in CF patients' ELF have been shown to be lower—the levels of GSH in the intracellular space of these patients' epithelial cells may actually be higher than those having normal CFTR function. The most convincing evidence of this phenomenon was discovered by Jungas et al. In this study the authors compared CFTR deficient and normal CFTR expressing epithelial cells and studied their ability to undergo apoptosis triggered by hydrogen peroxide ($H_2O_2$) treatment. It was discovered that sensitivity to apoptosis could be correlated to GSH levels. That is, BCL2-associated X protein (BAX) activation, which leads to apoptosis and is a direct effect of cytosolic GSH depletion, was slower in CFTR deficient cells than in normal cells. Further, they found that mutant cells not expressing CFTR were depleted of GSH slower and underwent apoptosis at a slower rate. Thus, elevated levels of GSH may protect the cells from undergoing normal cell death. Accordingly, at least one study supports our model of elevated cellular GSH by showing decreased apoptotic rates in infected CFTR mutant epithelial cell lines in CF patients [16]. Moreover, because there is a defect in apoptosis in these cells, bacterial clearance is affected, and thus the defect in apoptosis may contribute to the pathogenesis of the disease [17]. While a reduced intracellular state is normal in epithelial cells, during the process of apoptosis, depletion of GSH is required and it has been repeatedly demonstrated that CF epithelial cells appear to have a defect in this particular step in the process of normal cell death [18].

## Similarities of function and expression of multi-drug resistant and CFTR proteins

Perhaps the most compelling evidence of the role that glutathione efflux, or lack of this function, as the case may be, plays in terms of the pathology of this disease comes from the field of multi-drug resistance protein research. As previously stated, the CFTR and the multi-drug resistance (MDR)/multi-drug resistance associated (MRP) proteins are functionally redundant. Substrates of the multi-drug resistance proteins have been shown to block CFTR conductance of chloride anions, and synthetic-chloride-channel-forming peptides correspondingly restore glutathione secretion in cystic fibrosis airway cells [19,20]. As well, the CFTR protein has been shown to mediate, in a direct manner, nucleotide-regulated glutathione flux [21]. Finally, not only do the CFTR and the MRP share certain characteristics of protein structure, but it has been shown that the CFTR and the MRP share a complimentary pattern of expression; i.e., when the expression of the CFTR is increased, the expression of MRP is decreased, and vice versa [22,23]. Moreover, transfectants over expressing both proteins are known to modify membrane electrical potential (delta psi) and thus affect the accumulation of chemotherapeutic drugs [24,25]. More importantly, these drugs are pumped out of the cell in the form of GSH adducts. Since GSH is negatively charged, the depletion of GSH from the intracellular compartment is just as likely to affect delta psi, as well as the pH, in cells expressing functional CFTR, as it is to affect the efflux of $Cl^-$ anions. Indeed, overexpression of either MDR or CFTR proteins not only confers resistance to chemotherapeutic drugs, but also results in changes in both membrane psi and intracellular pH [25].

## Glutathione, hydrogen peroxide, and Th-2 immune response

In this model of CF pathology, an increase in intracellular levels of GSH in epithelial cells would result in a deficiency of intracellular $H_2O_2$, via the $H_2O_2$/GSH cycle (Fig. 1). However, because $H_2O_2$ is electrically neutral and thus passes easily through the cell membrane, this model also explains reports of increases in extracellular hydroperoxides and their byproducts in CF tissues [26]. Without the CFTR protein to transport intracellular

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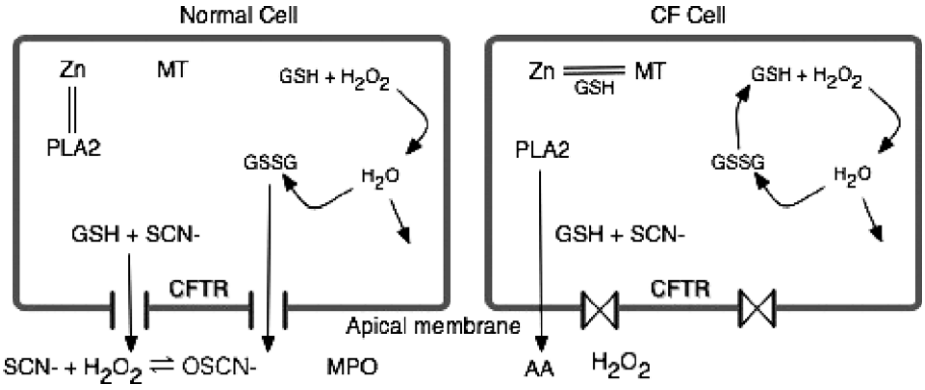


**Figure 1** Contrast between a normal and a CF cell. Inside a normal cell, zinc is often bound to metallothionein (MT). Intracellular $H_2O_2$ can strip off the zinc to make it available to enzymatic processes. In a CF cell, excess GSH oxidizes a higher percentage of the $H_2O_2$, which results in zinc remaining bound to MT and unavailable to enzymatic processes, and the increased intracellular GSH acts to bind zinc to MT. In a normal cell, SCN conjugates with GSH and the conjugates are transported out of the cell through the CFTR. In a CF cell, the conjugates are not transported out of the cell. In a normal cell, zinc can occupy an inhibitory site of phospholipase A2, which prevents the release of arachadonic acid (AA). In a CF cell, because free zinc is not available, PLA2 promotes the release of AA from the cell membrane. In the normal cell, myeloperoxidase catalyzes the conversion of thiocyanate ($SCN^-$) by $H_2O_2$ to hypothiocyanate (OSCN). In the CF cell, no such process takes place, because the CFTR protein is not available to transport the $SCN^-$/GSH conjugate to the extracellular space.

GSH to the extracellular space and neutralize the additional $H_2O_2$, humoral (Th-2) immune mediators are increased, resulting in the increase in oxidant stress seen in this disease [27]. In conclusion, without a functional CFTR to move the surplus GSH to the extracellular space, epithelial cell apoptosis would be affected, neutrophils would be deficient in GSH, leaving much of the cell-mediated immune defenses lost in CF cells; while a lack of extracellular GSH provides a high $H_2O_2$ oxidant burden, for an increase in Th-2 immune responses.

## Relationship between GSH and mucus viscosity

One of the most obvious effects of the sustained supraphysiological levels of GSH in this disease is displayed in the products of the mucus secreting cells of these patients. It has long been known that CF mucus is oversulfated, and that this property is a result of an intracellular mucus processing defect [28,29]. Since the biosynthesis of the carbohydrate chains forming mucins is a stepwise process involving many sulfotransferases, an increase in the intracellular sulfur content, stemming from supraphysiological levels of GSH—which serve as a reservoir for cysteine—could very well account for the increased sulfation, and the concomitant increase in viscoelasticity vis à vis increased molecular bonds, seen in CF mucus. Indeed, the fact that the clearance of certain compounds, such as oral contraceptives containing estrogen, and acetaminophen, both subject to clearance via the detoxification process of sulfation, is increased in CF patients, gives support to this explanation [30,31].

## Metallothionien and the redox couple—the mechanism for the disruption of metal homeostasis

Moreover, central to the pathology associated with this Th-2 immune response is the relationship of the glutathione redox couple, GSSG/GSH, to metallothionein (MT) or more specifically, the effect of these compounds on zinc homeostasis. Metallothionein, a 61 kDa protein thought to be largely responsible for copper and zinc homeostasis, contains 20 cysteine residues together capable of binding either 12 copper ions, seven zinc ions, or 20 nitric oxide ions. More importantly, MT has been shown to be both an acceptor, in its apo-protein state, as well as a donor of zinc [32–35]. Although it is only recently that both the mechanics and the particular enzymes involved in these exchanges are coming to light, it is well known that GSH acts as the bonding agent for these metals to the protein, and perhaps more importantly, that the major cellular disulfide GSSG, acts to release zinc from MT [36–39].

Of particular importance in regard to CF, is the fact that the cysteine-sulfur ligands of MT are redox sensitive [38]. In a reducing cellular environ-

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ment, MT will tightly bind metals, and in an oxidative environment, it will release them [40]. Accordingly, metals can be chelated from the intracellular environment in the presence of MT, or they can be chelated directly from enzymes, under reducing conditions. Finally, the controlled binding and releasing of metal ions by MT is important to the normal functioning of certain cellular proteins and enzymes, because the metal ions often act as cofactors governing the metabolic functions of these proteins [41]. Specifically, in the CF cell, a disruption of the normal redox state happens because of the high intracellular concentrations of GSH and MT tightly binds any transient metal ions available in the cytosol, as well as in the mitochondria; this sequestration leading to the disruption of the normal delivery of these metals to their respective enzymes and metabolic processes [42]. According to this model of CF pathology, enzymes that are dependent on zinc delivery to inhibitory sites are abnormally active; enzymes that are dependent on zinc delivery to active sites are abnormally inactive; the expression of proteins governed by transactivating factors requiring zinc are downregulated, and the metabolic processes requiring zinc are either non-existent or abnormal. Most importantly, these patients exhibit all of the generalized negative effects associated with zinc deficient states in terms of intracellular zinc-activated and zinc-inhibited enzymes; and since MT is a zinc-induced protein, these patients also lack the ability to have this state corrected by zinc repletion therapy [43].

## The role of zinc in CF pathophysiology

Central to the topic of CF pathology in terms of the inflammation seen in this disease is the arachidonic acid (AA)-releasing enzyme, phospholipase A2 (PLA2), which contains a zinc inhibitory site [44]. In CF, it has been shown that AA is more rapidly processed out of the cellular membrane, which leads to an imbalance in the fatty acids, docosahexaenoic acid (DHA) and AA, and contributes via the processing of AA by the cyclooxygenase and lipoxygenase systems, to an increase in the pro-inflammatory mediators, prostaglandin E2 and leukotriennes [45–47]. Intrinsic to the initiation of this particular inflammatory cascade is the binding by the S100A8/S100A9 complex to AA. The complex of S100A8 and S100A9, collectively known as the cystic fibrosis antigens, is present in the serum of CF patients and, at lower levels, in the serum of CF heterozygotes [48]. In a recent study by Kerkhoff et al., zinc binding to S100A8/S100A9, acts to reverse the AA binding capacity of the complex [49]. In our CF model, MT would sequester zinc, preventing it from being delivered properly to the cystic fibrosis antigens, or the inhibitory site of PLA2. Therefore, the lack of this zinc binding would act to promote the steps and the events leading to inflammation, which is unquestionably one of the hallmarks of CF pathology.

Zinc also affects enzymes that are not known to have a zinc inhibitory site. Thus, a deficiency can act to increase the activity of these enzymes. Accordingly, CF patients have increased energy expenditure that can be explained by differences in the activity of two mitochondrial enzymes: Complex I (NADH dehydrogenase) and Complex III (cytochrome *c* reductase) [50–52]. Not surprisingly, zinc inhibits all four mitochondrial complexes, but it inhibits complexes I and III with high affinity. Since MT has been shown to be imported into the mitochondrial intermembrane space, it is likely that it is the delivery vehicle for zinc to these complexes. It is also likely that the increased activities of these enzymes can be explained by the increase in intracellular GSH, and concomitant increase in the sequestration of zinc by MT, resulting from the inability of the CF cell to transport GSH adducts [53].

In addition to its ability to deliver metal ions to zinc inhibitory sites on enzymes and proteins, MT also functions to deliver metal ions to zinc-activated enzymes and proteins. And, once again, in a disrupted redox environment, such as that postulated within the CF cell, this transfer is not likely to take place. A key example, in this instance, is the lack of zinc saturation of thymulin in CF cells. Thymulin, a hormone which modulates cell-mediated immunity, requires zinc to be biologically active [54]. While total zinc-saturable thymulin fraction are near normal in CF patients, it has been shown that the active protein is extremely reduced in these patients, and there is a concomitant high level of inactive thymulin present in their plasma [55,56]. These factors clearly point to a lack of saturation of this protein by zinc ions.

Interestingly, alpha 2-macroglobulin (alpha 2M), which has a higher affinity for zinc than thymulin, and which has been shown to be increased in CF tissues, has been suggested as a factor in the lower levels of zinc-saturated thymulin in these patients [55]. Alpha 2M, a plasma protease inhibitor second to only alpha 1-anti-tripsin in its inhibitory action of proteolytic enzymes, was once considered to be a central factor in CF pathology [57,58]. It has been noted that not only is there a decreased formation of alpha 2M-protease complexes, but that both the breakdown and the uptake of these com-

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plexes is decreased in CF sera [59,60]. Since the discovery of the CFTR protein in 1989, however, interest in alpha 2M as a factor in CF pathology has waned. Nevertheless, because alpha 2M has also been shown to play a part in zinc delivery, conceivably substituting for some of the zinc delivery functions of MT, it is of particular interest in this model of CF pathology [61]. Indeed, some of the alterations seen in the functional and structural attributes of this protein in CF sera can be explained by its compensatory role in zinc transport since increased zinc binding of the protein has been shown to cause changes in both its structure as well as decreases in its trypsin-binding activity [62].

Another enzyme of interest in CF pathology is Carboxypeptidase A (CBA), a zinc-containing pancreatic enzyme involved in protein degradation. In accord with the exocrine pancreatic dysfunction that is pathognomic of CF, Hansson et al. found that the activity of this enzyme is inhibited in CF leading to malabsorption of nutrients [63]. Interestingly, the inhibited forms of CF CBA and control CBA are reversed by treatment with zinc. Furthermore, a further examination of the variant protein produced by CF patients suggested that differences in catalytic activity of the enzyme were not due to defects in the enzyme itself [64].

Additionally, patients with CF typically exhibit increased rates of bone resorption and, as a result, also show elevated levels of the bone turnover biomarker, alkaline phosphatase [65]. However, although the concentration of this enzyme is increased in patients with CF, the activity of the enzyme has been shown to be decreased [66]. Since alkaline phosphatase is an enzyme that requires zinc for activation, a model which describes metallothionein in its reduced state—which would inhibit the transfer of zinc ions from MT to alkaline phosphatase—may explain the relative inactivity of the enzyme in patients with CF [67]. Indeed, there are at least two studies showing that MT is responsible for transferring ionic zinc to apo-alkaline phosphatase [36,68].

Perhaps one the most obvious indicators of decreased intracellular zinc delivery in CF is the decrease in the activity of carbonic anhydrase, which facilitates the release of carbon dioxide ($CO_2$). In fact, it has been suggested that the lack of function of this enzyme can also explain the disparity between sodium ($Na^+$) absorption and $Cl^-$ secretion in the short circuit current across airway epithelia [69]. Carbonic anhydrase requires zinc for activation and zinc-deficiency has been proven to result in a reduction of the activity of this enzyme [70]. It has been shown that the CFTR controls the movement of bicarbonate ($HCO_3^-$) across both airway and pancreatic epithelia, and has even been suggested that this transport provides more of a key to the pathology seen in this disease than the lack of $Cl^-$ transport by CFTR defective cells [71,72]. Intracellular zinc deficiency caused by a sequestration of zinc on MT, may explain the means by which the CFTR controls $HCO_3^-$ secretion across epithelial cells, in general.

Additionally, it has long been known that zinc regulates DNA expression [73,74]. Correspondingly, a number of transcription factors contain zinc finger domains which interact with their respective DNA sequences. And, although the argument that inflammatory gene expression is dysregulated in CF airway epithelial cells is controversial, a disease model which incorporates zinc sequestration by MT via GSH-facilitated bonding of the metal to the protein, could explain this phenomena.

An example of this can be seen in the transactivating factors Sp-1 and Ap-1. Both of these factors require a zinc ion in order for them to bind to the promoter on their respective DNA start site or regulatory region [75,76]. And, although these transactivating factors are redox sensitive—that is, the levels of both of these increase when the levels of cellular GSH drops—there is no indication that the levels of these transactivating factors are anything but normal in the highly reduced cellular environment that we propose in this model of CF pathophysiology. Interestingly, however, these two factors, have been shown to be required for the transcription of IL-10, an anti-inflammatory cytokine known to be downregulated in patients with CF [77–79]. Accordingly, the non-delivery of zinc to both of the zinc finger domains of Sp-1 and Ap-1, could explain the low levels of IL-10 in these patients.

It has been noted that the CFTR protein regulates the expression of a variety of immune factors. Of particular interest in this regard is the Regulated upon Activation, Normal T cell Expressed and Secreted (RANTES) chemokine, the expression of which is a key step in the activation and proliferation of T lymphocytes [80,81]. Several studies have pointed out a dysregulation in immune response upon bacterial challenge of CF cells, and particularly, the lack of expression of RANTES in these cells [82–84]. Interestingly, RANTES expression is dependent upon the RANTES factor of Late Activated T Lymphocytes-1 (R-FLAT-1), which is a zinc-finger transcription factor that regulates gene expression in T lymphocytes, and has been shown to be inhibited in response to zinc chelators [85,86].

Intracellular zinc deficiency may also affect both the expression and the activity of insulin-like growth factor I (IGF-I), an anabolic hormone re-

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quired for proper linear growth, which has been linked to abnormal growth patterns seen in CF patients [87]. And, while exogenous growth hormone treatment has been of some benefit in the clinical status of these patients, some researchers have proposed that CF patients exhibit a ''resistance'' to the effects of growth hormone [88]. This ''resistance'' may, in fact, be a result of a deficiency of available intracellular zinc, which is required for the normal synthesis and activity of IGF-I. In support of this view, studies on zinc deficient rats have shown that exogenous supplementation of growth hormone has no effect on IGF-I synthesis [89].

There are also correlations between zinc levels and levels of other biological compounds in CF. For instance, plasma retinol levels have been shown to be decreased in these patients [90]. This decrease has been attributed to a function of both malabsorption and the high oxidant stress that is common during exacerbations [91,92]. However, retinol binding protein (RBP), which is necessary for the regulation of normal retinol status, has also been shown to be decreased in the serum of CF patients [93]. And, the activity of RBP, which is responsible for the transport of Vitamin A is dependent upon zinc levels [94].

Finally, there is a substantial amount of research delineating zinc levels in patients with CF. The literature is replete with case studies describing acrodermatitis enteropathic-like skin eruptions, indicative of zinc deficiency, as a presenting symptom in CF patients [95,96]. Several studies have shown that there are reduced levels of intracellular zinc in the tissues of these patients, while the levels of zinc in the hair and nails of these patients—often a repository for metallothionein-bound metals—is increased [97,98]. These findings are understandable, in a system with a prolonged reducing state that we propose in this model. Indeed, a cell that cannot properly transport surplus GSH to the outside of the cell will have higher levels of GSH, causing a sequestering of metals by MT.

## This model might explain the increased incidence of the CF mutation

Accordingly, the incidence of the CF mutation among heterozygotes is increasing, pointing to some sort of evolutionary advantage that these individuals have, especially given that the process of natural selection should have eliminated the gene from the population long ago [99]. The current consensus is that the CF mutation confers partial immunity to cholera [100,101]. However, although there may be some merit to this claim, the locations of the cholera epidemics specific to the point of mutation of the CFTR gene do not coincide with the prevalence of the most common CFTR variant in those populations [102–104]. If we consider, though, that the inborn ability of the CF cell to bind to toxic metal ions confers protection against metal toxicity, the increased incidence of the mutation since its first occurrence can be understood in the light of the increase in lead pollution 5000 years ago, when Europeans began smelting the first lead–silver alloys. The Romans continued the use of lead during the height of the Roman Empire—a period during which lead was used to line the aqueducts, in the manufacture of eating utensils, and to sweeten the wine. The CF mutation, in such circumstances, would confer an advantage because the heterozygotic cell would have a much greater ability to sequester toxic lead.

## There is a direct relationship between disease severity and the GSTM1 mutation

An interesting adjunct to this model of CF pathogenesis is the research on genetic modifiers of disease severity in CF. In particular, it has been shown that CF patients who were homozygous for the null allele of glutathione-S-transferase M1 (GSTM1) tested significantly higher on the Chrispin–Norman chest radiographic score, and significantly lower on the Shwachman, both of which indicate an increase in disease severity [105]. If the function of GSTM1 is to catalyze the conjugation of toxins to GSH, which are then transported to the extracellular by the MDR proteins and the CFTR, patients homozygous for the null allele of this enzyme would have less of an ability to form conjugates of toxins with GSH. The result would be more cellular GSH to bind zinc to MT, and more of an ability to neutralize cellular $H_2O_2$. Thus, this explanation of the increase in disease severity associated with this mutated allele for GSTM1 could be explained through this model of CF pathology.

## More evidence of an altered redox state in CF cells

Additionally, other research results favor this model of CF pathogenesis. Levels of both nitric oxide (NO) and inducible nitric oxide synthase (iNOS), the enzyme responsible for the production of NO, have been measured in CF lung airways and bronchial epithelial tissue, and shown to be below normal levels [106]. And, while intracellular levels of GSH have been linked to the expression of the iNOS enzyme, this relationship has proven to be bipha-

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sic; resulting in lower levels of mRNA iNOS expressed as a result of both sub- and supraphysiological intracellular GSH levels [107]. Accordingly, if the CFTR protein is responsible for the transport of GSH, then a defect in this protein would result in higher levels of intracellular GSH and a subsequent downregulation of the expression of iNOS in the bronchial epithelium of these patients.

### How to balance humoral and cell-mediated immunity in the CF system

Despite the functional redundancy of the CFTR and the multi-drug resistance proteins, and certainly taking into account the fact that they share complimentary expression patterns, there is every reason to believe that the CFTR protein has an ascendant function in terms of the transport of toxic compounds. The multi-drug resistance proteins transport a broad range of compounds, including many chemotoxic drugs. If we assume that toxicity of the substrate is a factor, as well as the ability of the transported compound to bind with glutathione (i.e., glutathione-*S*-transferase substrate), and be transported as a GSH adduct, then the most likely candidate, as both an GSH adduct and a substrate of the MRP and its functionally redundant protein, the CFTR, is thiocyanate ($SCN^-$).

Thiocyanate is an important player in the lactoperoxidase system which is responsible for clearance of bacteria from airways. In this system, thiocyanate is converted, in the presence of $H_2O_2$, by lactoperoxidase to hypothiocyanate, a biocidal compound. Perturbation of the lactoperoxidase system, either because of a lack of, or deactivation of the enzyme itself, or because of a lack of availability of substrates $H_2O_2$ or $SCN^-$ has been suggested as a possible source of the pathology seen in CF [108]. There is ample evidence to suggest not only that $SCN^-$ is a substrate of the CFTR protein, but that it is a more preferred CFTR substrate than chloride anions [8,9].

The lack of transport of thiocyanate as a GSH adduct, could explain why CF patients have reduced airway bacterial clearance. That is, the lack of thiocyanate could be why there is a defective lactoperoxidase system which causes a decrease in cell-mediated immunity. In fact, CF patients with severe pulmonary damage have been shown to have significantly lower levels of thiocyanate than those with moderate disease [109]. Interestingly, isothiocyanate, a naturally occurring isomer of thiocyanate commonly derived from plant sources, has been shown in numerous studies to induce Phase II detoxification of cancer cells in the same manner as do chemotoxic drugs [110–112].

Finally, several recent studies clearly show that the isothiocyanate compounds are exported as GSH adducts via the multi-drug resistance proteins [113–115]. In view of this fact, and the fact that the CFTR protein and the multi-drug resistance proteins share a complimentary pattern of expression, as well as 50% homology, in terms of their structure, it is extremely likely that isothiocyanates are able to induce the expression of the multi-drug resistance proteins, and in doing so, functionally substitute for the CFTR protein.

## Conclusion

In summary, the CF epithelial cell exhibits all of the characteristics inherent in a system in which epithelial intracellular GSH and the various compounds to which it is bound, particularly thiocyanate, is not transported to the extracellular space, but instead are trapped within the intracellular space. Furthermore, another result of the ensuing increase in epithelial intracellular GSH is an increased binding of zinc to metallothionein, and a concomitant decrease in zinc-dependent enzyme activation, zinc-inhibited enzyme deactivation, and zinc-dependent DNA expression, all of which serve to decrease zinc-dependent cell-mediated immune responses, and to increase zinc-inhibited humoral immune responses.

As well, GSH, acting as a reservoir for cysteine, increases both the rate of metabolism of compounds such as estrogen and acetaminophen via Phase II detoxification processes and the sulfation of mucins produced by submucosal cells. And, finally, because the glutathione adduct of thiocyanate is likely a substrate of the CFTR protein, like GSH, it too is likely to be trapped within the cellular walls, and not available as a substrate for the lactoperoxidase enzyme, thus decreasing both bacterial killing and clearance in the airways of these patients. Exogenously supplied isothiocyanates, because of their low toxicity and the fact that they may, as GSH adducts, be transported by the CFTR protein to the extracellular space, should serve to correct both the levels of intracellular GSH of CF cells, as well as restore biocidal ability to the cells, and normal bacterial clearance, which are, inarguably, the major causes of the pathology associated with this disease.

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