

Immune Responses in Cystic Fibrosis Are They Intrinsically Defective?

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Cystic fibrosis (CF), the most common lethal single-gene disorder affecting Northern Europeans and North Americans, is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Cftr is a chloride channel and a regulator of other ion channels, and many aspects of the CF phenotype are directly related to ion channel abnormalities attributable to CFTR mutation. Lung disease is the most common limitation to the quantity and quality of life for patients with CF. One aspect that continues to be enigmatic is the observed alterations in innate and adaptive immune responses to certain pathogens. Altered responses to *Pseudomonas aeruginosa* and *Aspergillus fumigatus*, with an increase in neutrophil chemoattractants in the former case and a hyper-IgE-like state in the latter, are common in CF. Several lines of evidence suggest that the proinflammatory cytokine response to bacterial infection is exaggerated in CF. A literature search reveals that, although the abnormalities in CF immune cells have been recognized since the 1970s, few studies until recently have appreciated the role that CFTR plays in these cell types. A growing body of evidence has emerged that points to neutrophils, macrophages, and T cells as being central to the infectious and pulmonary pathology, accounting for the majority of CF mortality. Primary CFTR defects in T cells are providing new insights into the misorchestration of the CF immune system due to aberrant signaling pathways. Defective CFTR function disrupts the balance of intracellular ion concentrations, including $[Ca^{2+}]$, which is known to drive gene expression pathways. New evidence links this hypothesis to anomalies in immune activation observed across CF cell types, which could shed light on the inability of individuals with CF to effectively clear pathogens. This review focuses on the emerging role of Cftr in gene expression and other functions in cells of the innate and adaptive immune system.

Keywords: cystic fibrosis; T cells; calcium; macrophages; allergy

Cystic fibrosis (CF) is the most common fatal single-gene disorder in North Caucasians, affecting approximately 30,000 children and adults in the United States. CF is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which is an ATP-driven chloride channel in the cell membrane. CF has variably penetrant pathology in multiple organ systems, but the respiratory system accounts for the vast majority of morbidity and mortality. Patients with CF develop chronic infections by opportunistic bacteria such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Haemophilus influenzae*, and *Burkholderia cepacia* (but not the more commonly occurring *Streptococcus pneumoniae*) at a greater

incidence than the general population. Infection is accompanied by exaggerated inflammation with migration of large numbers of neutrophils to the lungs. Pulmonary infection by *P. aeruginosa* is usually the terminal complication in CF, with high mortality.

Until recently, the main focus on CF pathology has been the function of the Cftr channel in epithelial cells, with its malfunction leading to subsequent air-liquid interface dehydration in the airways, thick mucus deposition, and impaired mucociliary clearance. This phenomenon is hypothesized to allow bacterial trapping, resulting in chronic lung infections. Although this is responsible for much of the morbidity in CF, the intrinsic effects of mutant CFTR on the immune system may exacerbate the pathology by promoting allergic reactions and hindering the proper clearance of pathogens in the CF lung.

In the last decade, an increasing number of investigators have noted peculiar signaling abnormalities in Cftr-deficient cells, particularly in the NFAT (1–3) and MAPK/ERK signaling pathways (4). These pathways are responsible for the transcription of inflammatory mediators and many critical cellular processes whose disruption could cause a plethora of possible pathologies. These molecular findings resonate with observations that patients with CF have a unique profile of proinflammatory cytokines even in the absence of infection (5), together with a tendency toward asthma (6–8), dermatitis (9), and allergic reactions (10–14). Of particular concern is the high frequency of adverse drug reactions among patients with CF because antibiotic treatment is critical for their longevity.

Although most of these CFTR-related signaling abnormalities are being studied in the context of hyperinflammation of airway epithelial cells (AECs), they also play a major role in the orchestration of inflammatory responses by the immune system. These pathways have largely been described and discovered in immune cells. This has led several groups to question whether a primary CFTR-mediated defect in granulocyte, monocyte, and lymphocyte lineages may contribute to the infectious pathology in CF. Although the relative contribution of compromised immunity and lung epithelium to CF mortality must be clarified, new insights regarding this crucial question are bringing it to the forefront and may have significant implications for the development of effective treatment strategies for CF. This review focuses on the new insights and disease models that have emerged from these studies in CF immunology.

CFTR IN AIRWAY EPITHELIAL CELLS AND NEUTROPHILS

One puzzling feature of CF is excessive inflammation in the lungs. Large numbers of neutrophils are present in the lungs of adults and children with CF even in the absence of pathogens (5, 15), where they cause proteolytic degradation of elastin and destruction of lung architecture (16). Is the excessive neutrophil activity due to abnormal AEC secretion of neutrophil chemoattractants,

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or is it due to an intrinsic neutrophil defect? It appears that it may be both, as we review in this section.

The etiology of CF lung inflammation in the field has been a topic of debate. Some studies have found no difference in cytokine secretion between CF and non-CF cells and have questioned whether CF lung inflammation is caused by an intrinsic CFTR defect (17–19). These studies have been primarily conducted in epithelial cells, which may have left critical interactions with immune cells unanalyzed. In fact, the negative results drawn by studies in cultured epithelial cells could be interpreted to suggest that an intrinsic CFTR defect in immune cells, not AECs, may be the source of CF lung inflammation. *In vitro* studies are conducted in an artificial environment, and immune cells are notorious for behaving differently *in vitro* than *in vivo*. Therefore, conclusions from *in vitro* immunological studies must be analyzed with caution.

Interpretation of lung inflammation from *in vivo* studies can be difficult because popular *Pseudomonas* delivery methods involve inoculation of the lungs with *Pseudomonas*-laden alginate beads, and these can elicit inflammation by themselves even in wild-type controls or create chronic infection that even wild-type mice cannot clear (20–22). Newer, optimized models have made such studies more feasible and show that an intrinsic CFTR defect is responsible for excessive lung inflammation and for the inability to clear *P. aeruginosa* in mice (23, 24). It is not clear whether these conclusions would hold true in humans because these models use a nonmucoid strain of *P. aeruginosa*, whereas clinical isolates from patients with CF are typically biofilm-forming strains. Moreover, expression of CFTR and other Cl^- channels may differ between mice and humans, which can further confound the translatability of such studies.

AECs play a significant role in the infectious pathology of CF and represent an interface between pathogens and the immune system. Thus, the intrinsic changes in Cftr-deficient AECs are important not only in how they affect interaction with pathogens but also in how they affect communication with immune cells. Several groups have described up-regulation of signaling pathways associated with proinflammatory cytokine transcription in CF epithelial cells (25–30). In particular, Cftr-deficient AECs that have been exposed to *P. aeruginosa* demonstrate increased activation of the proinflammatory transcription factor nuclear factor- κB (NF- κB), which subsequently drives the increased expression of IL-8 (1, 2). IL-8 is a potent neutrophil chemoattractant, and its overexpression by AECs could partly explain the pulmonary hyperinflammation seen in CF. CF AECs secrete increased quantities of IL-8 in response to other pathogens as well, such as human rhinovirus (31). There are other broad differences in Cftr-deficient AECs that seem to exacerbate neutrophil-mediated inflammation. Cftr-deficient AECs also produce less glutathione (32, 33) but have higher levels of COX2 and prostaglandin E2 (34, 35). Glutathione is critical for neutralizing the damaging reactive oxygen species produced by oxidative processes in response to infection, whereas prostaglandins produced by COX2 are responsible for mediating a broad spectrum of inflammatory processes. Coupled with excessive numbers of neutrophils, these conditions are a recipe for havoc in the CF lung (Figure 1).

Cftr appears to maintain homeostatic expression of up to 843 genes, according to one study in cultured human AECs (36), and 702 to 943 genes in a mouse study (37). When grouped, the majority of these genes were found to be involved in phagocytosis, complement activation, apoptosis, DNA replication, T-cell selection, B-cell activation, and chemotaxis. By what mechanism does CFTR, a chloride channel, affect all of these pathways?

Several groups have proposed that Cftr has physiological roles besides chloride conductance. Some researchers have shown that

Cftr interacts with β -adrenergic receptors (38), phosphodiesterases (39), and other ion channels (40, 41). The significance of these interactions is open to speculation, although it is likely that additional signaling pathways are affected.

Balghi and colleagues (42) recently proposed a new model in AECs that may explain many if not all of these alterations. They showed nearly 2-fold elevation in the expression of ORAI1, which is a Ca^{2+} release-activated Ca^{2+} channel in the cell membrane, in Cftr-deficient AECs. They also measured a 2-fold increase in the calcium I_{CRAC} current in these cells, a 2-fold increase in total intracellular Ca^{2+} , and a 2-fold increase in the secretion of IL-8. The I_{CRAC} current was normalized by increasing CFTR levels at the cell surface. Their data demonstrate that store-operated calcium entry is excessive in *Cftr*^{-/-} cells; this can lead to inappropriate cytokine expression by Ca^{2+} -sensitive gene expression pathways.

Although many important lessons have been learned from studies of CFTR in AECs, data are emerging that a primary CFTR defect in cells of the innate immune system contributes significantly to CF lung pathology. When healthy mice are reconstituted with *Cftr*^{-/-} neutrophils, they develop severe lung inflammation and injury upon LPS challenge compared with mice receiving wild-type neutrophils (43); this inflammation is driven by increased NF- κB transcription, which seems to be increased in multiple Cftr-deficient cell types. Because neutrophils are the first line of defense against *P. aeruginosa* infection, proper neutrophil function is critical in patients with CF and warrants further investigation.

One question that has been posed is whether CFTR mutation disrupts phagolysosomal function in neutrophils. Morris and colleagues (44) showed that CF neutrophils from patients with CF have a reduced phagocytotic capacity, although the authors could not detect CFTR by Western blot; indeed, some still question whether CFTR is expressed in neutrophils at all. It is not clear if the phagocytic defect is intrinsic to neutrophils, but this group suggests the possibility that mutant CFTR may be expressed during neutrophil maturation in the bone marrow and causes an irreversible functional defect. This notion is supported by the observation that a human leukemic cell line up-regulates CFTR when induced to differentiate into neutrophils (45). Others have also reported a defect in CF neutrophil respiratory burst, attributed to disrupted chloride transport to the phagolysosome (45–48). Studies in zebrafish have confirmed this finding as well (49).

CFTR plays a role in early neutrophil development, as is suggested by the fact that other cells of the granulocyte lineage behave peculiarly in CF. A recent study demonstrated that subpopulations of mast cells are present at an altered ratio in the lungs of patients with CF (50). These CF mast cells secrete increased levels of IL-6, a proinflammatory cytokine that stimulates neutrophil production in the bone marrow and inhibits the activity of regulatory T cells. Little is known about the activity of CFTR in hematopoietic stem cells or its effect on differentiation. To expand on this theme, CF platelets have been shown to be more readily activated than non-CF platelets, and this can contribute to the recruitment of neutrophils to the lung and other inflamed tissue (51, 52). This activation is not directly due to the lack of platelet CFTR but rather is due to CF plasma factors as well as perhaps intrinsic changes in gene expression in the hematopoietic stem cell lineage. Nevertheless, this is further evidence for a systemic hyperinflammatory state not limited to the lung.

Putting these anomalies in perspective, it has been found that CFTR mutation affects the expression of approximately 89 genes in neutrophils alone (53); these genes include signal transduction molecules, interleukin receptors, chemokines, and all three

colony-stimulating factors (G-, M-, and GM-CSF). Recently, miRNAs have joined the growing list of disturbed pathways in CF, where 5-fold higher levels of miR-155 in CF AECs and neutrophils result in up-regulation of the PI3K/Akt signaling pathway (54); alterations in this broad signaling pathway would be difficult to predict but would be expected to have a significant impact on cell behavior.

Other interesting changes in neutrophil physiology have been documented. One group observed that toll-like receptor 4 (TLR4) is up-regulated in CF neutrophils, whereas TLR2 is down-regulated (55); this could compromise the effectiveness of the innate immune response in clearing pathogens and result in dysregulation of inflammatory pathways. Similar findings have been observed in the CF monocyte lineage.

Another group recently found that the expression of Annexin A1, an endogenous antiinflammatory protein, is controlled by a CFTR-dependent pathway and that addition of recombinant Annexin A1 to CF mice corrects the exaggerated neutrophil migration to the pancreas (56). It would be interesting to see if Annexin A1 can also correct the abnormal leukocyte migration to the CF lung and whether the corrected neutrophils are able to adequately respond to pathogens.

The nature of the intrinsic defect in CF neutrophils is complex and remains to be fully elucidated. The practical measure for the competence of CF neutrophils lies not in the variation in cytokines but rather in their ability to clear infection. Despite the ensuing pulmonary inflammation with associated mortality, CF mice are able to clear acute *P. aeruginosa* infection in approximately the same time as wild-type mice (20, 24, 57), but they are unable to clear chronic infection. This compels us to separately recognize two aspects of the lung pathology: the excessive inflammatory response and the inability to clear chronic infection. In both cases, it may be that mutant CFTR impairs the ability of the adaptive immune system to clear chronic bacterial infection and to halt the excessive neutrophil inflammation.

In summary, CFTR appears to affect multiple gene expression pathways in AECs and neutrophils, and the effects from both cell types complement one another to produce excessively destructive inflammation in the CF lung. We have learned that increased intracellular Ca^{2+} levels can alter the expression of many genes in affected CF cells, with possible impact on the ability to clear pathogens and on the severity of the inflammatory response. CFTR-associated perturbations in intracellular Ca^{2+} may have direct effects on a variety of immune cells.

CFTR IN MONOCYTES AND MACROPHAGES

Alveolar macrophages mature from circulating blood monocytes and play a major role in the direct clearance of pathogens in the lung as well as the presentation of antigens to helper T cells. Some investigators have hypothesized that their function is compromised in CF because patients are unable to clear chronic infection with *P. aeruginosa* and have worse outcome in *P. aeruginosa* sepsis compared with patients without CF.

Indeed, CF macrophages are deficient in their ability to destroy pathogens. Originally this was suggested to be due to defective opsonization and phagocytosis (58–60). Although this may be a contributing factor, the percentage of surviving bacteria is increased in CF macrophages even after successful phagocytosis (61). The implication is that a pathogen-killing defect is also present, although it is not immediately clear whether this is due to an intrinsic macrophage defect or the aberrant signaling of other cells.

Some groups have proposed that CFTR mutation disrupts phagosome acidification in macrophages, thus compromising the lysis of pathogens (62, 63). They showed that the capacity

for phagocytosis and lysogenic fusion is preserved in the CF macrophage. However, in 2007 Haggie and Verkman provided pharmacological evidence showing that Cftr does not participate in acidification of the phagosome and attributed the observation of the former group to a conceivable artifact of the pH-sensitive dye that was used in the experiment (64). Together, these studies seem to suggest that the pathogen-killing machinery in CF macrophages is grossly intact. Therefore, mutant CFTR must be compromising pathogen-killing via another mechanism, perhaps by inhibiting macrophage activation. Evidence suggests that this speculation is worth investigating because, although the numbers of macrophages, neutrophils, and CD3^+ T cells in CF lungs are elevated (16), the number of activated macrophages and neutrophils is reduced (65). One possibility is that mutant CFTR causes the accumulation of calcium levels sufficient to affect gene expression within the macrophage. Recently, Shenoy and colleagues showed that intracellular Ca^{2+} levels do increase in wild-type monocytes treated with inhibitors of CFTR and Ca^{2+} ATPase (66), which warrants investigation into how macrophage gene expression may be affected.

We learned from studies in AECs and neutrophils that Cftr affects a broad range of cell processes. As in other CF cell types, there appear to be aberrations in the gene expression pathways of CF monocytes, which could compromise their ability to clear pathogens. Zaman and colleagues demonstrated that the MAPK/Erk pathway is hypersensitive to stimulation by LPS in homozygote and even in heterozygote CF monocytes (4). This would have significant consequences for immune function because the MAPK/Erk pathway is involved in critical cell functions (e.g., cell division, apoptosis, and cytokine synthesis). Moreover, they found that CF monocytes produce 100-fold less IL-8 in response to LPS stimulation compared with controls, which implies decreased neutrophil recruitment to sites of infection by gram-negative bacteria. This could be significant during sepsis, where recruitment of neutrophils to the infectious source is critical to stop the spread of the pathogen. Bruscia and colleagues showed that LPS hypersensitivity in CFTR-deficient monocytes is due to ineffective turnover of TLR4, which is caused by delayed transfer to the lysosomal compartment from the early endosome (67). This is further supported by evidence of increased expression of TLR4 in monocytes of children with CF even in the absence of infection (68). The consequence of this in patients with CF appears to be an exaggerated inflammatory response upon encountering LPS but reduced overall effectiveness in clearing the pathogen. It is also worth questioning whether the up-regulation of TLR4 may allow more adjuvant-mediated hypersensitivity reactions to occur against antigens that would otherwise be tolerated in healthy individuals.

Another group found that peripheral blood monocytes from patients with CF are unresponsive to LPS despite TLR activation (69). The authors attribute this observation to the 2-fold lower levels of TREM1 in circulating CF monocytes, which is a downstream signaling protein important in leukocyte activation. The signaling events downstream of TREM1 are not well characterized, but Gibot and colleagues recently showed that TREM1 enhances survival during septic shock in mice (70). Furthermore, the increased activity of macrophage inhibitory factor in patients with CF may also contribute to an ineffective response against pathogens (71). In future studies, these findings may aid in forming hypotheses for why patients with CF have poor clinical outcome in *P. aeruginosa* pulmonary infection and sepsis.

Although the anomalies in CF monocytes and macrophages seem to be due at least in part to an intrinsic CFTR defect, their function may be further compromised by disrupted processes

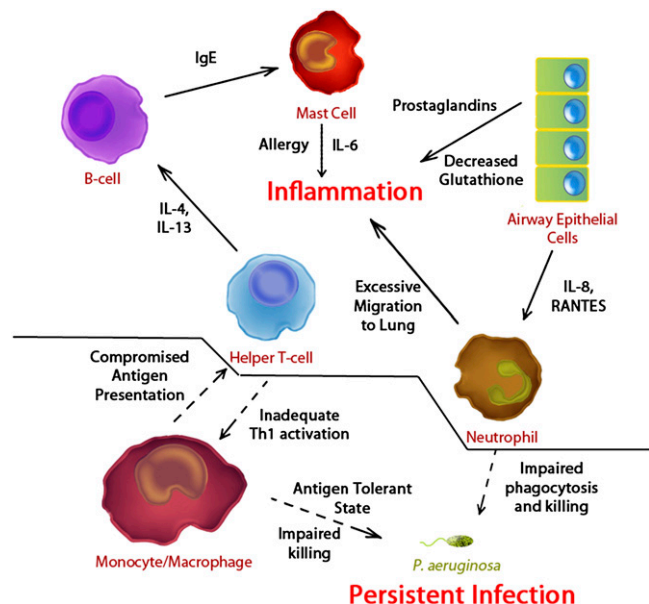


Figure 1. Snapshot of the cystic fibrosis (CF) immune system and processes affected by mutant cystic fibrosis transmembrane conductance regulator (CFTR). This diagram shows the complex interaction between various cell types, all of which lack CFTR and may have an independent phenotype. Epithelial cells secrete IL-8 and other chemokines that attract neutrophils to the lung, potentiating inflammation especially in the setting of bacterial infection. The intrinsic effect of CFTR deficiency in neutrophils and macrophages appears to be an inability to effectively kill bacteria. This is further exacerbated by inadequate Th1 activation of macrophages by CFTR-deficient helper T cells, which seem to favor a proallergic Th2-type response upon activation. This leads to increased IL-4 and IL-13 levels, which stimulate IgE antibody synthesis by B cells. IgE on mast cells potentiates allergy and inflammation, and CFTR-deficient mast cells overproduce the proinflammatory cytokine IL-6. This excessive inflammatory response is particularly damaging in the CF lung because CFTR-deficient epithelial cells produce decreased levels of glutathione and increased amounts of proinflammatory prostaglandins.

elsewhere in the CF immune system. For instance, a lack of macrophage activation by helper T cells may allow chronic *P. aeruginosa* infection to persist (Figure 1). The adaptive immune system likely plays an important role in CF macrophage pathology, although relative contributions of an intrinsic defect in macrophages and other cells have been difficult to dissect. Nevertheless, CFTR is expressed in T cells, and aberrations in these cells may have anywhere from subtle to profound effects on immune function systemically.

CFTR IN LYMPHOCYTES

One aspect that continues to be enigmatic in CF is the observed alteration in adaptive immune responses to certain pathogens. Patients with CF have a tendency toward allergic reactions, asthma, dermatitis, and hyperinflammatory immune responses. Although lymphocytes have never been directly established as the cause of these phenomena, this pattern makes one wonder whether adaptive immune responses may be involved. In another review, an investigator pointed out that stimulation of wild-type B cells with a cAMP analog results in a rapid transient increase in λ light chain secretion, but this effect is absent in CF B cells (72). A failure of regulated secretion could cause a reduced response to antigen presentation and an inability to clear *P. aeruginosa* infection, which leads to loss of lung function.

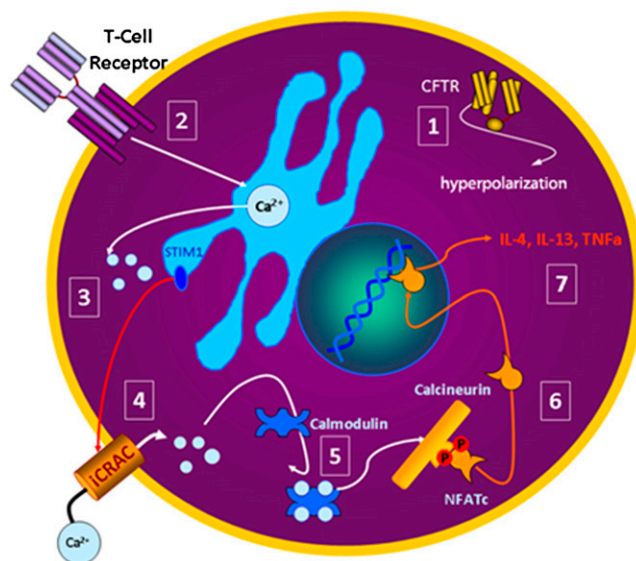


Figure 2. Proposed model showing how the lack of functional CFTR and resultant hyperpolarization of the helper T-cell membrane leads to increased Ca^{2+} entry upon activation of the T-cell receptor. This results in the opening of calcium-gated Ca^{2+} channels and Ca^{2+} -mediated activation of the NFAT transcriptional pathway. The final result is increased expression of proinflammatory cytokines, characteristic of a Th2-type immune response.

The unresponsiveness of CF lymphocytes against *P. aeruginosa* has been known since the late 1970s (73–79), and recent insights are beginning to shed some light on these observations. Central to the CF T-cell phenotype appears to be a predilection to mount a type 2 helper T-cell (Th2) response (80), which is proallergic and appropriate for fighting parasites, but not pathogens such as *P. aeruginosa*. Indeed, clinical data show that approximately 10% of patients with CF develop allergic bronchopulmonary aspergillosis upon exposure to *A. fumigatus* (13, 81), with increased levels of IL-4, IL-13, and IgE in what appears to be a hyperinflammatory, Th2-dominated immune response (82). In CF mice, increased levels of IgE are observed compared with controls after exposure to *A. fumigatus* (83, 84), accompanied by a significant shift to a predominantly IL-4 and IL-13 cytokine profile and a greater sensitivity of CF B cells to IL-4 stimulation (85). Our group confirmed that this is due to the lack of functional Cftr in T cells by recapitulating the exaggerated IgE levels in T cell-specific CFTR knockout mice and in immunodeficient mice that were transplanted with CF splenocytes (3). These findings establish a role of Cftr-deficient lymphocytes in causing allergic inflammation in CF, but more importantly they reveal the capacity of CF T cells to misorchestrate the immune response to pathogens. This may severely compromise the ability of individuals with CF to efficiently clear *P. aeruginosa* and could allow infection by *B. cepacia* and other unusual pathogens.

There is evidence that T-cell and macrophage unresponsiveness to *P. aeruginosa* may be the result of Th2 skewing in CF. Moss and colleagues showed that helper T cells from patients with CF produce lower levels of IFN- γ , a Th1 cytokine, and higher levels of IL-10, a Th2 cytokine (86). IL-10 expression in CF has been a subject of debate, but it has been confirmed to be up-regulated by Casaulta and colleagues (87). This is interesting because the ability of IL-10 to down-regulate IFN- γ production and decrease costimulatory molecules on macrophages can hinder antigen presentation and a proper immune response to *P. aeruginosa*, *A. fumigatus*, and other pathogenic

species. Further studies are needed to establish if antigen presentation by macrophages is indeed compromised.

A Th1 immune response may be critical for successfully clearing *P. aeruginosa* infection. Moser and colleagues showed that when CFTR^{-/-} mice are repeatedly infected with *P. aeruginosa*, recovery from infection is accompanied by a shift to a Th1 response and increased levels of IL-12 but no change in IgG levels (88). The surviving mice are resistant to reinfection and have improved survival. CF mice colonized with *P. aeruginosa* have a higher antibody titer against *P. aeruginosa* antigens than do colonized wild-type mice but still develop chronic infection (89). This suggests that a Th1-type immune response is protective against *P. aeruginosa* infection independent of antibody production and compels us to examine the mechanism of a CFTR-mediated defect in T cells, which seems to favor inappropriate Th2 differentiation.

One emerging explanation for the phenomenon of Th2 skewing is the hypothesis that mutant Cftr causes increased Ca²⁺ flux across the T-cell membrane, thereby perturbing Ca²⁺-sensitive gene expression pathways. Ca²⁺ influx is known to be critical for T-cell activation and is tightly regulated by a number of ion channels, including Cftr (42). Disruption of this delicate balance could lead to abnormal expression of many genes in T cells, including those responsible for Th1/Th2 differentiation. Our group found increased intracellular Ca²⁺ entry in helper T cells of CF mice upon T-cell receptor stimulation, followed by increased translocation of the transcription factor NFAT into the nucleus (Figure 2) (3). NFAT is known to drive the expression of Th2 cytokines, including IL-4, IL-13, and IL-6 (90–92), which lead to IgE synthesis and inflammation.

Different isoforms of NFAT promote different cytokine expression profiles and determine helper T-cell-type differentiation. In particular, the balance between NFATc1 and NFATc2 appears important in determining T-cell differentiation (93). Unchecked NFATc1 activity promotes Th2 differentiation and production of IL-4, IL-5, and IL-6 in helper T cells and extremely high levels of IgE in B cells. IL-4 and IgE are overexpressed in individuals with CF, with an associated skewing toward Th2 differentiation. Few studies have been conducted exploring the regulation of cytokine expression by NFAT isoforms in Cftr-deficient T cells or their potential dependence on Cftr function.

Although the downstream effects of Cftr dysfunction are important, it is events at the cell membrane that drive the Cftr-mediated gene expression aberrations in T cells and warrant further attention. In 2001, Fanger and colleagues (94) showed that Ca²⁺ entry in Jurkat T cells resulted in activation of K_{Ca} channels, creating an efflux of K⁺ ions, which prevents cell membrane depolarization and allows continued Ca²⁺ entry; these Ca²⁺ levels are sufficiently high to cause the signaling necessary for transcriptional activation. If so, then defective Cftr function and subsequent intracellular retention of Cl⁻ ions would be expected to further hyperpolarize the T-cell membrane and augment Ca²⁺-activated signaling. Because it is possible that defective Cftr function may further hyperpolarize the T-cell membrane, ostensibly by intracellular retention of negatively charged chloride ions, Ca²⁺-activated signaling may be augmented in CF T cells (Figure 2). The Orail/Stim1 complex is also present in the T-cell membrane. Studies are needed to determine whether it is similarly affected by mutant Cftr and leads to abnormal gene expression.

Recent insights in T-cell electrophysiology are also giving new perspective to local Cftr functions in the T-cell membrane. According to Cahalan and Chandy (40), some ion channels (Orail, STIM1, Kv1.3, and KCa3.1) cluster at the antigen presentation site after contact with an antigen-presenting cell

(APC). They hypothesize that this clustering could be important for stabilizing the interaction between the T cell and the APC and in generating large extracellular K⁺ concentrations sufficient to depolarize the APC and the T cell. This may be important for efficient antigen presentation because MHC class II molecule expression on dendritic cells has been shown to double within 1 minute of K⁺-induced depolarization (95). The downstream effects of this may be speculated; regardless, these mechanisms may be affected by the lack of functional Cftr at the cell membrane.

CONCLUSIONS

CF is a systemic disease where multiple organ systems are affected with much overlapping pathology. Isolating the pathologic mechanisms has been difficult in part because *Cftr* has profound effects on gene expression. This is of particular concern in helper T cells because signaling abnormalities in these cells could manifest themselves clinically as vague comorbidities (e.g., allergy, dermatitis) alongside more prominent CF symptoms. These can be easily overlooked in the context of life-threatening lung infections or attributed to malnutrition. In fact, mutant Cftr in helper T cells could account for much of the mortality of patients with CF due to the less effective performance of the immune system as a whole (Figure 1). Efforts to develop therapeutic strategies for CF should encompass immune cells as well as other cell types. In particular, Ca²⁺ channels may be attractive drug targets in light of recent data on the dysregulation of store-operated calcium entry and perturbation of Ca²⁺-sensitive gene expression pathways in CF.

One major question that remains for the field is the degree to which immune dysfunction accounts for CF mortality, as compared with CFTR-related defects in AECs. This is critical to establish to best focus resources on an effectively targeted treatment strategy. Because lung-directed treatments short of transplantation have little appreciable effect in the long-term survival and quality of life of patients, an alternative approach aimed at correcting the CFTR defect in immune cells may turn out to be of clinical value.

The recent years have been pivotal in our understanding of CF because a cause-and-effect relationship has been established between ion transport and gene expression in CF immune cells. As the details of this mechanism and their greater impact on immune function continue to be elucidated, we can only hope that these findings will advance clinical efforts to treat this disease and improve the duration and quality of life for patients.

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References

1. Venkatakrishnan A, Stecenko AA, King G, Blackwell TR, Brigham KL, Christman JW, Blackwell TS. Exaggerated activation of nuclear factor-κB and altered IκB-β processing in cystic fibrosis bronchial epithelial cells. *Am J Respir Cell Mol Biol* 2000;23:396–403.
2. Rottner M, Kunzelmann C, Mergey M, Freyssinet JM, Martinez MC. Exaggerated apoptosis and NF-κappaB activation in pancreatic and tracheal cystic fibrosis cells. *FASEB J* 2007;21:2939–2948.
3. Mueller C, Braag SA, Keeler A, Hodges C, Drumm M, Flotte TR. Lack of cystic fibrosis transmembrane conductance regulator in CD3+ lymphocytes leads to aberrant cytokine secretion and hyperinflammatory adaptive immune responses. *Am J Respir Cell Mol Biol* 2011;44:922–929.
4. Zaman MM, Gelrud A, Junaidi O, Regan MM, Warny M, Shea JC, Kelly C, O'Sullivan BP, Freedman SD. Interleukin 8 secretion from monocytes of subjects heterozygous for the deltaF508 cystic fibrosis transmembrane conductance regulator gene mutation is altered. *Clin Diagn Lab Immunol* 2004;11:819–824.

5. Muhlebach MS, Stewart PW, Leigh MW, Noah TL. Quantitation of inflammatory responses to bacteria in young cystic fibrosis and control patients. *Am J Respir Crit Care Med* 1999;160:186–191.
6. Morgan WJ, Butler SM, Johnson CA, Colin AA, FitzSimmons SC, Geller DE, Konstan MW, Light MJ, Rabin HR, Regelman WE, et al. Epidemiologic study of cystic fibrosis: design and implementation of a prospective, multicenter, observational study of patients with cystic fibrosis in the U.S. and Canada. *Pediatr Pulmonol* 1999;28:231–241.
7. Konstan MW, Butler SM, Schidlow DV, Morgan WJ, Julius JR, Johnson CA. Patterns of medical practice in cystic fibrosis: part I. Evaluation and monitoring of health status of patients: investigators and coordinators of the epidemiologic study of cystic fibrosis. *Pediatr Pulmonol* 1999;28:242–247.
8. Balfour-Lynn IM. Asthma in cystic fibrosis. *J R Soc Med* 2003;96:30–34.
9. Bernstein ML, McCusker MM, Grant-Kels JM. Cutaneous manifestations of cystic fibrosis. *Pediatr Dermatol* 2008;25:150–157.
10. Chavez A, Mian A, Scurlock AM, Blackall D, Com G. Antibiotic hypersensitivity in CF: drug-induced life-threatening hemolytic anemia in a pediatric patient. *J Cyst Fibros* 2010;9:433–438.
11. Troncone R, Santamaria F, Ercolini P, Raia V, Panza G, de Ritis G. Increased serum antibody levels to dietary antigens in cystic fibrosis. *Acta Paediatr* 1994;83:440–441.
12. Parmar JS, Nasser S. Antibiotic allergy in cystic fibrosis. *Thorax* 2005;60:517–520.
13. Agarwal R, Khan A, Aggarwal AN, Gupta D. Link between CFTR mutations and abpa: a systematic review and meta-analysis. *Mycoses* (In press)
14. Burrows JA, Nissen LM, Kirkpatrick CM, Bell SC. Beta-lactam allergy in adults with cystic fibrosis. *J Cyst Fibros* 2007;6:297–303.
15. Tabary O, Corvol H, Boncoeur E, Chadelat K, Fitting C, Cavaillon JM, Clement A, Jacquot J. Adherence of airway neutrophils and inflammatory response are increased in CF airway epithelial cell-neutrophil interactions. *Am J Physiol Lung Cell Mol Physiol* 2006;290:L588–L596.
16. Ulrich M, Worlitzsch D, Viglio S, Siegmann N, Iadarola P, Shute JK, Geiser M, Pier GB, Friedel G, Barr ML, et al. Alveolar inflammation in cystic fibrosis. *J Cyst Fibros* 2010;9:217–227.
17. Becker MN, Sauer MS, Muhlebach MS, Hirsch AJ, Wu Q, Verghese MW, Randall SH. Cytokine secretion by cystic fibrosis airway epithelial cells. *Am J Respir Crit Care Med* 2004;169:645–653.
18. Pizurki L, Morris MA, Chanson M, Solomon M, Pavirani A, Bouchardy I, Suter S. Cystic fibrosis transmembrane conductance regulator does not affect neutrophil migration across cystic fibrosis airway epithelial monolayers. *Am J Pathol* 2000;156:1407–1416.
19. Black HR, Yankaskas JR, Johnson LG, Noah TL. Interleukin-8 production by cystic fibrosis nasal epithelial cells after tumor necrosis factor- α and respiratory syncytial virus stimulation. *Am J Respir Cell Mol Biol* 1998;19:210–215.
20. van Heeckeren AM, Schluchter MD. Murine models of chronic *Pseudomonas aeruginosa* lung infection. *Lab Anim* 2002;36:291–312.
21. Gosselin D, Stevenson MM, Cowley EA, Griesenbach U, Eidelman DH, Boule M, Tam MF, Kent G, Skamene E, Tsui LC, et al. Impaired ability of cfr knockout mice to control lung infection with *Pseudomonas aeruginosa*. *Am J Respir Crit Care Med* 1998;157:1253–1262.
22. McMorran BJ, Palmer JS, Lunn DP, Oceandy D, Costelloe EO, Thomas GR, Hume DA, Wainwright BJ. 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:L740–L747.
23. van Heeckeren AM, Schluchter MD, Drumm ML, Davis PB. Role of cfr genotype in the response to chronic *Pseudomonas aeruginosa* lung infection in mice. *Am J Physiol Lung Cell Mol Physiol* 2004;287:L944–L952.
24. van Heeckeren AM, Schluchter MD, Xue W, Davis PB. Response to acute lung infection with mucoid *Pseudomonas aeruginosa* in cystic fibrosis mice. *Am J Respir Crit Care Med* 2006;173:288–296.
25. Schwiebert LM, Estell K, Probst SM. Chemokine expression in CF epithelia: implications for the role of cfr in rantes expression. *Am J Physiol* 1999;276:C700–C710.
26. Estell K, Braunstein G, Tucker T, Varga K, Collawn JF, Schwiebert LM. Plasma membrane cfr regulates rantes expression via its c-terminal PDZ-interacting motif. *Mol Cell Biol* 2003;23:594–606.
27. Hallows KR, Fitch AC, Richardson CA, Reynolds PR, Clancy JP, Dagher PC, Witters LA, Kolls JK, Pilewski JM. Up-regulation of amp-activated kinase by dysfunctional cystic fibrosis transmembrane conductance regulator in cystic fibrosis airway epithelial cells mitigates excessive inflammation. *J Biol Chem* 2006;281:4231–4241.
28. Hallows KR, Kobinger GP, Wilson JM, Witters LA, Foscett JK. Physiological modulation of cfr activity by AMP-activated protein kinase in polarized t84 cells. *Am J Physiol Cell Physiol* 2003;284:C1297–C1308.
29. Hallows KR, McCane JE, Kemp BE, Witters LA, Foscett JK. Regulation of channel gating by AMP-activated protein kinase modulates cystic fibrosis transmembrane conductance regulator activity in lung submucosal cells. *J Biol Chem* 2003;278:998–1004.
30. Hallows KR, Raghuram V, Kemp BE, Witters LA, Foscett JK. Inhibition of cystic fibrosis transmembrane conductance regulator by novel interaction with the metabolic sensor amp-activated protein kinase. *J Clin Invest* 2000;105:1711–1721.
31. Sutanto EN, Kicic A, Foo CJ, Stevens PT, Mullane D, Knight DA, Stick SM. Innate inflammatory responses of pediatric cystic fibrosis airway epithelial cells: effects of nonviral and viral stimulation. *Am J Respir Cell Mol Biol* 2011;44:761–767.
32. Gao L, Kim KJ, Yankaskas JR, Forman HJ. Abnormal glutathione transport in cystic fibrosis airway epithelia. *Am J Physiol* 1999;277:L113–L118.
33. Gao L, Broughman JR, Iwamoto T, Tomich JM, Venglarik CJ, Forman HJ. Synthetic chloride channel restores glutathione secretion in cystic fibrosis airway epithelia. *Am J Physiol Lung Cell Mol Physiol* 2001;281:L24–L30.
34. Chen J, Jiang XH, Chen H, Guo JH, Tsang LL, Yu MK, Xu WM, Chan HC. Cfr negatively regulates cyclooxygenase-2-PGE(2) positive feedback loop in inflammation. *J Cell Physiol* 2011;227:2759–2766.
35. Freedman SD, Weinstein D, Blanco PG, Martinez-Clark P, Urman S, Zaman M, Morrow JD, Alvarez JG. Characterization of LPS-induced lung inflammation in cfr $^{-/-}$ mice and the effect of docosahexaenoic acid. *J Appl Physiol* 2002;92:2169–2176.
36. Virella-Lowell I, Herlihy JD, Liu B, Lopez C, Cruz P, Muller C, Baker HV, Flotte TR. Effects of cfr, interleukin-10, and *Pseudomonas aeruginosa* on gene expression profiles in a CF bronchial epithelial cell line. *Mol Ther* 2004;10:562–573.
37. Haston CK, Cory S, Lafontaine L, Dorion G, Hallett MT. Strain-dependent pulmonary gene expression profiles of a cystic fibrosis mouse model. *Physiol Genomics* 2006;25:336–345.
38. Naren AP, Cobb B, Li C, Roy K, Nelson D, Heda GD, Liao J, Kirk KL, Sorscher EJ, Hanrahan J, et al. A macromolecular complex of beta 2 adrenergic receptor, cfr, and ezrin/radixin/moesin-binding phosphoprotein 50 is regulated by pka. *Proc Natl Acad Sci USA* 2003;100:342–346.
39. Penmatsa H, Zhang W, Yarlagadda S, Li C, Conoley VG, Yue J, Bahouth SW, Buddington RK, Zhang G, Nelson DJ, et al. Compartmentalized cyclic adenosine 3',5'-monophosphate at the plasma membrane clusters PDE3A and cystic fibrosis transmembrane conductance regulator into microdomains. *Mol Biol Cell* 2010;21:1097–1110.
40. Cahalan MD, Chandy KG. The functional network of ion channels in T lymphocytes. *Immunol Rev* 2009;231:59–87.
41. Stutts MJ, Rossier BC, Boucher RC. Cystic fibrosis transmembrane conductance regulator inverts protein kinase a-mediated regulation of epithelial sodium channel single channel kinetics. *J Biol Chem* 1997;272:14037–14040.
42. Balghi H, Robert R, Rappaz B, Zhang X, Wohlhuter-Haddad A, Evangelidis A, Luo Y, Goepp J, Ferraro P, Romeo P, et al. Enhanced Ca $^{2+}$ entry due to orail plasma membrane insertion increases IL-8 secretion by cystic fibrosis airways. *FASEB J* 2011;25:4274–4291.
43. Su X, Looney MR, Su HE, Lee JW, Song Y, Matthay MA. Role of cfr expressed by neutrophils in modulating acute lung inflammation and injury in mice. *Inflamm Res* 2011;60:619–632.
44. Morris MR, Doull IJ, Dewitt S, Hallett MB. Reduced iC3b-mediated phagocytotic capacity of pulmonary neutrophils in cystic fibrosis. *Clin Exp Immunol* 2005;142:68–75.
45. Painter RG, Valentine VG, Lanson NA Jr, Leidal K, Zhang Q, Lombard G, Thompson C, Viswanathan A, Nauseef WM, Wang G. Cfr expression in human neutrophils and the phagolysosomal chlorination defect in cystic fibrosis. *Biochemistry* 2006;45:10260–10269.
46. Painter RG, Bonvillian RW, Valentine VG, Lombard GA, LaPlace SG, Nauseef WM, Wang G. The role of chloride anion and cfr in killing of *Pseudomonas aeruginosa* by normal and CF neutrophils. *J Leukoc Biol* 2008;83:1345–1353.

47. Painter RG, Marrero L, Lombard GA, Valentine VG, Nauseef WM, Wang G. Cftr-mediated halide transport in phagosomes of human neutrophils. *J Leukoc Biol* 2010;87:933–942.
48. Bonvillain RW, Painter RG, Adams DE, Viswanathan A, Lanson NA Jr, Wang G. RNA interference against cftr affects HL60-derived neutrophil microbicidal function. *Free Radic Biol Med* 2010;49:1872–1880.
49. Phennicie RT, Sullivan MJ, Singer JT, Yoder JA, Kim CH. Specific resistance to *Pseudomonas aeruginosa* infection in zebrafish is mediated by the cystic fibrosis transmembrane conductance regulator. *Infect Immun* 2010;78:4542–4550.
50. Andersson CK, Andersson-Sjoland A, Mori M, Hallgren O, Pardo A, Eriksson L, Bjermer L, Lofdahl CG, Selman M, Westergren-Thorsson G, et al. Activated MCTC mast cells infiltrate diseased lung areas in cystic fibrosis and idiopathic pulmonary fibrosis. *Respir Res* 2011;12:139.
51. O'Sullivan BP, Linden MD, Frelinger AL III, Barnard MR, Spencer-Manzon M, Morris JE, Salem RO, Laposata M, Michelson AD. Platelet activation in cystic fibrosis. *Blood* 2005;105:4635–4641.
52. O'Sullivan BP, Michelson AD. The inflammatory role of platelets in cystic fibrosis. *Am J Respir Crit Care Med* 2006;173:483–490.
53. Adib-Conquy M, Pedron T, Petit-Bertron AF, Tabary O, Corvol H, Jacquot J, Clement A, Cavaillon JM. Neutrophils in cystic fibrosis display a distinct gene expression pattern. *Mol Med* 2008;14:36–44.
54. Bhattacharyya S, Balakathiresan NS, Halgard C, Gutti U, Armistead D, Jozwik C, Srivastava M, Pollard HB, Biswas R. Elevated mir-155 promotes inflammation in cystic fibrosis by driving hyperexpression of interleukin-8. *J Biol Chem* 2011;286:11604–11615.
55. Petit-Bertron AF, Tabary O, Corvol H, Jacquot J, Clement A, Cavaillon JM, Adib-Conquy M. Circulating and airway neutrophils in cystic fibrosis display different tlr expression and responsiveness to interleukin-10. *Cytokine* 2008;41:54–60.
56. Dalli J, Rosignoli G, Hayhoe RP, Edelman A, Perretti M. Cftr inhibition provokes an inflammatory response associated with an imbalance of the annexin a1 pathway. *Am J Pathol* 2010;177:176–186.
57. van Heeckeren AM, Tscheikuna J, Walenga RW, Konstan MW, Davis PB, Erokku B, Haxhiu MA, Ferkol TW. Effect of pseudomonas infection on weight loss, lung mechanics, and cytokines in mice. *Am J Respir Crit Care Med* 2000;161:271–279.
58. Fick RB Jr, Naegel GP, Matthay RA, Reynolds HY. Cystic fibrosis *Pseudomonas* opsonins: inhibitory nature in an *in vitro* phagocytic assay. *J Clin Invest* 1981;68:899–914.
59. Fick RB Jr, Naegel GP, Squier SU, Wood RE, Gee JB, Reynolds HY. Proteins of the cystic fibrosis respiratory tract: fragmented immunoglobulin G opsonic antibody causing defective opsonophagocytosis. *J Clin Invest* 1984;74:236–248.
60. Vishwanath S, Ramphal R, Guay CM, DesJardins D, Pier GB. Respiratory-mucin inhibition of the opsonophagocytic killing of *Pseudomonas aeruginosa*. *Infect Immun* 1988;56:2218–2222.
61. Del Porto P, Cifani N, Guarnieri S, Di Domenico EG, Mariggio MA, Spadaro F, Guglietta S, Anile M, Venuta F, Quattrucci S, et al. Dysfunctional cftr alters the bactericidal activity of human macrophages against *Pseudomonas aeruginosa*. *PLoS ONE* 2011;6:e19970.
62. Di A, Brown ME, Deriy LV, Li C, Szeto FL, Chen Y, Huang P, Tong J, Naren AP, Bindokas V, et al. Cftr regulates phagosome acidification in macrophages and alters bactericidal activity. *Nat Cell Biol* 2006;8:933–944.
63. Deriy LV, Gomez EA, Zhang G, Beacham DW, Hopson JA, Gallan AJ, Shevchenko PD, Bindokas VP, Nelson DJ. Disease-causing mutations in the cystic fibrosis transmembrane conductance regulator determine the functional responses of alveolar macrophages. *J Biol Chem* 2009;284:35926–35938.
64. Haggie PM, Verkman AS. Cystic fibrosis transmembrane conductance regulator-independent phagosomal acidification in macrophages. *J Biol Chem* 2007;282:31422–31428.
65. Sajjan U, Thanassoulis G, Cherapanov V, Lu A, Sjolin C, Steer B, Wu YJ, Rotstein OD, Kent G, McKerlie C, et al. Enhanced susceptibility to pulmonary infection with *Burkholderia cepacia* in cftr(–/–) mice. *Infect Immun* 2001;69:5138–5150.
66. Shenoy A, Kopic S, Murek M, Caputo C, Geibel JP, Egan ME. Calcium-modulated chloride pathways contribute to chloride flux in murine cystic fibrosis-affected macrophages. *Pediatr Res* 2011;70:447–452.
67. Bruscia EM, Zhang PX, Satoh A, Caputo C, Medzhitov R, Shenoy A, Egan ME, Krause DS. Abnormal trafficking and degradation of TLR4 underlie the elevated inflammatory response in cystic fibrosis. *J Immunol* 2011;186:6990–6998.
68. Sturges NC, Wikstrom ME, Winfield KR, Gard SE, Brennan S, Sly PD, Upham JW. Monocytes from children with clinically stable cystic fibrosis show enhanced expression of toll-like receptor 4. *Pediatr Pulmonol* 2010;45:883–889.
69. del Fresno C, Gomez-Pina V, Lores V, Soares-Schanoski A, Fernandez-Ruiz I, Rojo B, Alvarez-Sala R, Caballero-Garrido E, Garcia F, Veliz T, et al. Monocytes from cystic fibrosis patients are locked in an LPS tolerance state: down-regulation of trem-1 as putative underlying mechanism. *PLoS ONE* 2008;3:e2667.
70. Gibot S, Massin F, Marcou M, Taylor V, Stidwill R, Wilson P, Singer M, Bellingan G. Trem-1 promotes survival during septic shock in mice. *Eur J Immunol* 2007;37:456–466.
71. Plant BJ, Gallagher CG, Bucala R, Baugh JA, Chappell S, Morgan L, O'Connor CM, Morgan K, Donnelly SC. Cystic fibrosis, disease severity, and a macrophage migration inhibitory factor polymorphism. *Am J Respir Crit Care Med* 2005;172:1412–1415.
72. Bubien JK. Cftr may play a role in regulated secretion by lymphocytes: a new hypothesis for the pathophysiology of cystic fibrosis. *Pflugers Arch* 2001;443:S36–S39.
73. Sorensen RU, Chase PA, Stern RC, Polmar SH. Influence of cystic fibrosis plasma on lymphocyte responses to *Pseudomonas aeruginosa* *in vitro*. *Pediatr Res* 1981;15:14–18.
74. Sorensen RU, Ruuskanen O, Miller K, Stern RC. B-lymphocyte function in cystic fibrosis. *Eur J Respir Dis* 1983;64:524–533.
75. Sorensen RU, Stern RC, Chase P, Polmar SH. Defective cellular immunity to gram-negative bacteria in cystic fibrosis patients. *Infect Immun* 1979;23:398–402.
76. Sorensen RU, Stern RC, Chase PA, Polmar SH. Changes in lymphocyte reactivity to *Pseudomonas aeruginosa* in hospitalized patients with cystic fibrosis. *Am Rev Respir Dis* 1981;123:37–41.
77. Sorensen RU, Stern RC, Polmar SH. Lymphocyte responsiveness to *Pseudomonas aeruginosa* in cystic fibrosis: relationship to status of pulmonary disease in sibling pairs. *J Pediatr* 1978;93:201–205.
78. Sorensen RU, Stern RC, Polmar SH. Cellular immunity to bacteria: impairment of *in vitro* lymphocyte responses to *Pseudomonas aeruginosa* in cystic fibrosis patients. *Infect Immun* 1977;18:735–740.
79. Van Geffel R, Hubert E, Josse M. Study of the relation between the clinical pulmonary condition of children with cystic fibrosis and the lymphoblastic response to the antigen *Pseudomonas aeruginosa*. *Ann Immunol (Paris)* 1982;133D:293–303.
80. Hartl D, Griesse M, Kappler M, Zissel G, Reinhardt D, Rebhan C, Schendel DJ, Krauss-Etschmann S. Pulmonary T(h)2 response in *Pseudomonas aeruginosa*-infected patients with cystic fibrosis. *J Allergy Clin Immunol* 2006;117:204–211.
81. Laufer P, Fink JN, Bruns WT, Unger GF, Kalbfleisch JH, Greenberger PA, Patterson R. Allergic bronchopulmonary aspergillosis in cystic fibrosis. *J Allergy Clin Immunol* 1984;73:44–48.
82. Knutsen AP, Mueller KR, Levine AD, Chouhan B, Hutcheson PS, Slavin RG. Asp f I CD4+ Th2-like t-cell lines in allergic bronchopulmonary aspergillosis. *J Allergy Clin Immunol* 1994;94:215–221.
83. Muller C, Braag SA, Herlihy JD, Wasserfall CH, Chesrown SE, Nick HS, Atkinson MA, Flotte TR. Enhanced IgE allergic response to *Aspergillus fumigatus* in cftr(–/–) mice. *Lab Invest* 2006;86:130–140.
84. Knutsen AP, Hutcheson PS, Slavin RG, Kurup VP. IgE antibody to *Aspergillus fumigatus* recombinant allergens in cystic fibrosis patients with allergic bronchopulmonary aspergillosis. *Allergy* 2004;59:198–203.
85. Knutsen AP, Hutchinson PS, Albers GM, Consolino J, Smick J, Kurup VP. Increased sensitivity to IL-4 in cystic fibrosis patients with allergic bronchopulmonary aspergillosis. *Allergy* 2004;59:81–87.
86. Moss RB, Hsu YP, Olds L. Cytokine dysregulation in activated cystic fibrosis (CF) peripheral lymphocytes. *Clin Exp Immunol* 2000;120:518–525.
87. Casaulta C, Schoni MH, Weichel M, Cramer R, Jutel M, Daigle I, Akdis M, Blaser K, Akdis CA. IL-10 controls *Aspergillus fumigatus*- and *Pseudomonas aeruginosa*-specific T-cell response in cystic fibrosis. *Pediatr Res* 2003;53:313–319.
88. Moser C, Jensen PO, Kobayashi O, Hougen HP, Song Z, Rygaard J, Kharazmi A, Hb N. Improved outcome of chronic *Pseudomonas aeruginosa* lung infection is associated with induction of a Th1-dominated cytokine response. *Clin Exp Immunol* 2002;127:206–213.

89. Coleman FT, Mueschenborn S, Meluleni G, Ray C, Carey VJ, Vargas SO, Cannon CL, Ausubel FM, Pier GB. Hypersusceptibility of cystic fibrosis mice to chronic *Pseudomonas aeruginosa* oropharyngeal colonization and lung infection. *Proc Natl Acad Sci USA* 2003;100:1949–1954.
90. Hodge MR, Ranger AM, Charles de la Brousse F, Hoey T, Grusby MJ, Glimcher LH. Hyperproliferation and dysregulation of IL-4 expression in NF-ATp-deficient mice. *Immunity* 1996;4:397–405.
91. Ranger AM, Hodge MR, Gravalles EM, Oukka M, Davidson L, Alt FW, de la Brousse FC, Hoey T, Grusby M, Glimcher LH. Delayed lymphoid repopulation with defects in IL-4-driven responses produced by inactivation of NF-ATc. *Immunity* 1998;8:125–134.
92. Yoshida H, Nishina H, Takimoto H, Marengere LE, Wakeham AC, Bouchard D, Kong YY, Ohteki T, Shahinian A, Bachmann M, *et al.* The transcription factor NF-ATc1 regulates lymphocyte proliferation and Th2 cytokine production. *Immunity* 1998;8:115–124.
93. Peng SL, Gerth AJ, Ranger AM, Glimcher LH. NFATc1 and NFATc2 together control both T and B cell activation and differentiation. *Immunity* 2001;14:13–20.
94. Fanger CM, Rauer H, Neben AL, Miller MJ, Wulff H, Rosa JC, Ganellin CR, Chandy KG, Cahalan MD. Calcium-activated potassium channels sustain calcium signaling in T-lymphocytes: selective blockers and manipulated channel expression levels. *J Biol Chem* 2001;276:12249–12256.
95. Vukcevic M, Spagnoli GC, Iezzi G, Zorzato F, Treves S. Ryanodine receptor activation by Ca v 1.2 is involved in dendritic cell major histocompatibility complex class II surface expression. *J Biol Chem* 2008;283:34913–34922.

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