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Lymphocyte dysregulation plays an important role in pathogenesis of cystic fibrosis

Abstract

Cystic fibrosis (CF) is the most common genetic disorder in the Caucasian population. CF is usually associated with lung disease and patients suffer from persistent infections with strong pulmonary inflammation. It has been shown that fluid and electrolyte transport across epithelial membranes is affected in CF. However, the single gene that is affected is widely expressed in many tissues, including immune cells such as T-lymphocytes. The purpose of this thesis is to analyse available literature on the influence of the genetic defect in CF on the adaptive immune system. The idea that the immune system is involved is not new, but recently new insights supporting this hypothesis have been published. These indicate that the CF immune system is already in a proinflammatory state and prone to a T_H2 response before contact with microorganisms.

Introduction

Cystic fibrosis is a multifactorial disease caused by a defect in the cystic fibrosis transmembrane conductance regulator (CFTR) (Riordan et al., 1989). It is the most common lethal autosomal recessive inherited disease in the Caucasian population with 1 in 3500 newborns being affected (Davis et al., 1996). Although over 1500 disease-associated mutations have been identified, most cases of CF are caused by a deletion of a single codon. This codon encodes for a phenylalanine at position 508 in the protein (CFTR-Fdel508). This deletion is found in 70% of all CF patients and 90% of all patients carry at least one allele (http://www.genet.sickkids.on.ca/cftr). The loss of the amino acid does reduce channelling function, but more importantly CFTR-Fdel508 misfolds and is reatined within the ER by strict quality control mechanisms. Wildtype CFTR is prone to misfolding and only 33% of all polypeptides make it to the plasmamembrane (Ward and Kopito, 1994). CFTR-Fdel508 misfolds almost completely which results in identification and subsequent degradation by the endoplasmic reticulum quality control system (Du et al., 2005). Folding can somewhat be repaired if cells are grown at lower temperature: CFTR-Fdel508 increasingly reach the plasmamembrane and has functional activity (Denning et al., 1992). The other mutations also affect folding and result in loss of production, conductivity or channel opening.

CFTR is present in many different tissues, but not all tissues affected by CFTR deficiency have a clear phenotype. CF is mostly associated with disease in the lungs and this causes almost all morbidity and mortality. Another clinical feature due to this genetic defect is pancreatic insufficiency caused by obstructed ducts and autolysis (Davies et al., 2007). Furthermore, lymphocytes were shown to express CFTR, but its function in these cells is not known. It has been shown that chloride permeability plays a

role in dividing lymphocytes of both normal and CF origin (Bubien et al., 1990; Krauss et al., 1992). The most common hypothesis for symptoms in the lungs is that it causes volume reduction and thickening of the mucus which prevents efficient clearance of bacteria, resulting in persistent infections. These infections are hard to clear even with the use of antibiotics. Not only lung epithelium is affected by this defect, but almost all excreting epithelia. However, the persistent infections can not only be explained by a defect in the mucosal clearance by the lung epithelium (Berger, 2002). It appears that more than the mucus is affected, for example the liquid extracted from lungs of CF patients via bronchoalveolar lavage (BAL) contains evidence for an overstimulated immune system (Cantin, 1995). Another indication is that the immune response that is observed is mostly T_H2 in CF patients (Hartl et al., 2006). The inflammatory response in CF patients to bacteria is on average ten times stronger than in unaffected individuals (Muhlebach et al., 1999). The purpose of this thesis is to determine whether, by studying available literature, this dysregulation of the immune system can be attributed to the persistent infections or due to a vital function of the CFTR gene in lymphocytes.

Nowadays in most countries, newborns are genetically screened to identify CF as early as possible. The disease is generally further diagnosed by measuring sweat contents for high concentrations of chloride. Symptoms only occur when both genes are affected. However not everyone with mutations in both genes shows symptoms, this is due to the large amount of different mutations which have been identified (Davies et al., 2007). Males with two mild mutations may not show any symptoms except absence of the vas deferens, which causes infertility. Females affected with two mild mutations might not show any symptoms at all. There is an unexplained difference between life expectancy of males and females, where males generally live longer with CF (Coakley et al., 2008; Rosenfeld et al., 1997). Homo- and heterozygosis is important not only for development of CF disease, but have been associated with other disorders, like allergic bronchopulmonary aspergillosis.

CFTR function

The CFTR is an anion channel that facilitates chloride and other anion transfer across the apical membrane of epithelium cells. It is part of a large family of transporters called the ATP-binding cassette (ABC) transporters. ABC transporters were first found in prokaryotes and later also in eukaryotes, where they perform many different functions (Gros et al., 1986; Higgins et al., 1982). They are named for the characteristic nucleotide binding domains of which each family member has two homologs. Most of these function by hydrolysing ATP molecules at both sites. However, CFTR belongs to a sub-population where hydrolysis only occurs at one site (Aleksandrov et al., 2002). General information on the different domains in CFTR and their function has been elucidated. However the structure of CFTR has not yet been determined as it is difficult to create crystals. Only low resolution images with electron microscopy have been made.

CFTR is the only known member of the ABC transporter family that functions as an ion channel that can be opened or closed by cellular stimuli (Riordan, 2008). CFTR has a unique R domain that restrains channel gating unless phosphorylated by protein kinase A (PKA). This allows for control of

the flow of ions by changing the concentration of PKA (Chappe et al., 2005). A high concentration of PKA will cause a continual uptake of chloride. This is favourable in the sweat glands, resulting in maximal salt recovery. In other epithelia it is favourable to have inactive CFTR until stimulated, for example in the pancreas or the lungs.

Besides its function as an ion channel, CFTR has been associated with a function as a regulator of other channels or transporters, hence its name. An important downstream target of CFTR is the epithelial sodium channel (ENaC), which is responsible for sodium absorption in lung epithelia (Knowles et al., 1983; Reddy et al., 1999). This lack of regulation is suggested to cause mucus thickening and reduces mucosal clearance in the lung, leading to an increased susceptibility of bacterial infections, although new data in the CF pig appears conflicting (Chen et al., 2010).

Mouse model in CF research

Mouse models are a popular and very widely used model in medical research. Soon after identification of CFTR as the causative gene of CF it was knocked out in mice. (Colledge et al., 1995; Snouwaert et al., 1992). These mice did show a deficiency in Cl^- secretion. The first mice models that were generated died due to severe gastrointestinal CF-like phenotype that could be prevented with dietary adjustments. Nowadays hybrid strains contain a transgene expressing CFTR from a promoter specific for gut epithelium to prevent this phenotype (Zhou et al., 1994). Unlike humans, CFTR-deficient mice did not develop lung disease. The mouse models were made using homologous recombination, which causes the exon 10 containing the first nucleotide binding domain of the gene to be deleted altogether. Newer models were developed mimicking human disease further, by creating the F508 codon deletion. Models with mutations allows for partial expression, which closer resembles conditions in humans.

Another model that has been developed and can be used in CF research is the βENaC overexpressing mouse. This mouse-model has normal CFTR function, but over expresses the Na^+ channel ENaC and thereby causes an increased Na^+ uptake. This results in a phenotype in the lungs that is similar to that in CF patients (Mall et al., 2004; Zhou et al., 2011).

It was shown that mice lacking CFTR did have dysfunction at chloride secretion in the nasal epithelium but in the lower airways secrete normal chloride levels (Grubb et al., 1994b). This can be explained due to the presence of another chloride channel in mice, called Ca^{2+}-activated Cl^- channel (CaCC). Functioning of CaCC is thought to cause normal Na^+ uptake and normal mucus production, preventing characteristic CF-like symptoms in the lungs in these mice. CaCC has recently been located to the TMEM16A gene, which creates new opportunities to elucidate its function and prospects of new and possibly more accurate CF-model in mice (Clarke et al., 1994; Grubb et al., 1994a).

The mouse model has been proven to be a useful model with distinct disadvantages due to inherent differences between lung epithelium of human and mice. New models are being developed now, among which are the sheep, monkey, ferret and pig (Rogers et al., 2008a; Rogers et al., 2008b; Stoltz

et al., 2010; Sun et al., 2010). Hopefully these new models will provide us with an accurate model for CF lung disease.

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Disrupted ion transport in CF disease

The mucus of the lungs plays a major role in defence against bacteria and depends on an intricate balance between different ions to keep it functioning. There are clear indications that Na^+ uptake plays an important role in CF. The increased Na^+ absorption has been described 30 years ago, before the responsible gene was found and has been a hallmark in CF ever since (Boucher et al., 1986; Knowles et al., 1983). This has been suggested as one of the possible mechanisms by which the disease develops. Due to an increased sodium uptake the airway surface liquid (ASL) becomes less liquid and thicker reducing its ability to function. The increased sodium uptake has been attributed to a more active ENaC. Experiments *in vitro* have shown that Na^+ absorption is increased in epithelial cells due to a higher activity of ENaC (Mall et al., 1998). This is thought to be due to the lack of inhibitory signals from the absent CFTR. This chapter is focused on innate immunity, because it shows that innate immunity is thought to be a key factor, but cannot explain all symptoms of CF lung disease.

As described, mice overexpressing ENaC show symptoms in the lungs characteristic of CF (Mall et al., 2004). These include thickening of the mucus, ASL depletion and chronic inflammation. This was further supported in a new mouse model. Here the regulator of ENaC was knocked out and showed CF symptoms as well (Kimura et al., 2011). Contradicting these results are human patients suffering from Liddle's syndrome, where ENaC is overexpressed due to a mutation, which prevents ubiquitylation and therefore degradation of the channel. These patients suffer from kidney dysfunction, but no lung disease has been reported.
The hyperabsorption of Na^+ has been observed a long time ago, but the effect of Na^+ absorption *in vivo* remains heavily debated. Recently a new hypothesis was postulated. Herein was stated that instead of Na^+ absorption, deficiency in HCO_3^- secretion by CFTR could be the cause of thickening of the mucus (Quinton, 2008). This would cause an acidified ASL, which could cause the reduction of mucosal clearance and the subsequent infections.

More recently glands from CF patients were tested for Na^+ conductance as well as Cl^- conductance. These results showed that there was no increased Na^+ conductance and that ENaC might not be active in CF glands. This indicates towards defective anion secretion as the cause of reduced mucosal clearance (Joo et al., 2006). Similar results were found in excised as well as cultured CFTR deficient cells. This work has later been supported by other results in the newly-developed pig models for CF (Joo et al., 2010). Recently, epithelium of CF patients was examined for conductance as well, and showed that in contrast to previous results there was no increased Na^+ absorption in lung epithelium. Cl^- conductance was reduced and could not be stimulated with an increase of cAMP. (Itani et al., 2011). This indicates that Na^+ absorption might not play such a big role in CF disease as previously thought.

Blockers for EnaC are known and have been postulated as possible therapies. Ameloride was the first candidate to be tested in preventing CF symptoms. Trials with this substance were unsuccessful due

to rapid clearance in the lungs (Pons et al., 2000). Next generation of sodium channel blockers, like Benzamil, have been tested as well. Again rapid clearance prevented a positive result. Although the lack of effect is attributed to the rapid clearance, it could also indicate that Na^+ absorption does not have a large effect as previously thought.

In summary, the viscous mucus that builds up in CF lungs is clearly a causative factor for CF disease, and relies on disturbed innate mechanisms relying on ion transport in epithelia. The exact underlying mechanism causing viscous mucus in humans remains heavily debated.

Cytokine expression and the dysregulation of the immune system

Persistent infections are common in CF patients and eventually all patients become infected. There is a specific subset of bacteria that infect CF patients, most notably are the *Staphylococcus aureus* and *Pseudomonas aeruginosa* (figure 1). These infections are the major cause of morbidity and mortality in CF. Patients are treated with antibiotics, but are unable to clear the bacteria. This is an indication that the immune system is compromised and cannot clear infections even when bacteria are weakened by antibiotics. This is supported by analysis of infections in CF patients after lung transplantation. Lung transplantation is performed as a last resort if the lungs are failing. If the reduced clearance by mucus would be the only cause of persistent infections, lung transplantation should cure the patient since the transplanted lungs contain normal secretion systems. Unfortunately, patients that have undergone lung transplantation do develop recurrent infections with the same bacteria (Ramirez et al., 1992). In patients with Liddle's disease a transplantation of the affected organ cures the patient.

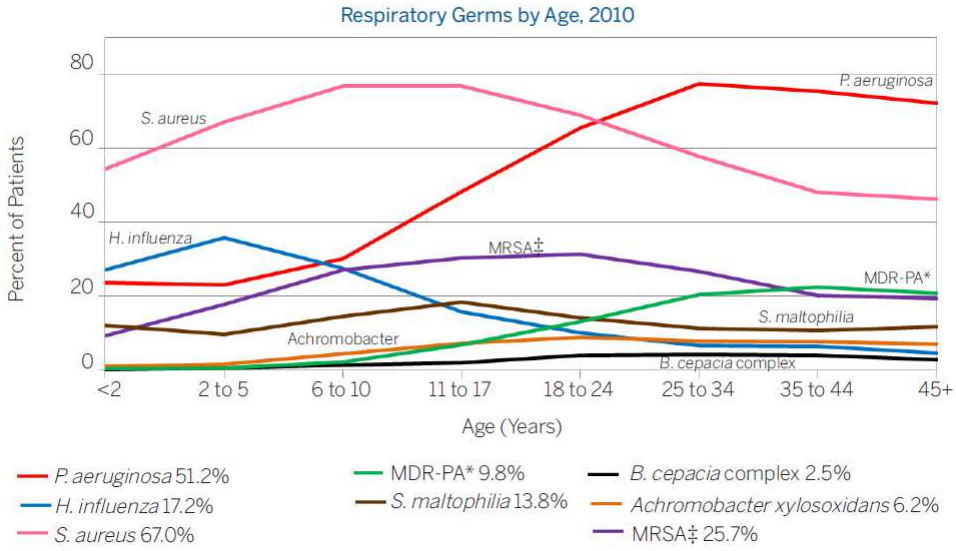

Figure 1, *Distribution of pathogens in CF patients.*
This graph shows the distribution of pathogens that infect CF patients. *S. aureus* and *P. aeruginosa* are the two bacteria causing most infections. It also shows that *P. aeruginosa* infections generally develop later in life (Cystic Fibrosis Foundation, 2010).

Defects in the innate immune system have been identified in CF. Neutrophils were shown to release significantly more oxidants (Witko-Sarsat et al., 1996) and a reduced ability to chlorinate proteins from *P. aeruginosa*, which potentially results in a reduced phagocytotic efficacy of this pathogen, was recently described (Painter et al., 2006). Macrophages have been shown to be affected in their ability to kill bacteria (Di et al., 2006). Recently, adoptive transfer experiments with macrophages have proven that CFTR in the immune cell compartment plays an important role in the aberrant pro-inflammatory cytokine responses in mice (Bruscia et al., 2009).

CFTR is more than a chloride channel shown by its influence on the Na^+ channels. Besides that, it has been shown that it regulates chemokine expression directly. This was shown in the case of RANTES. This stands for regulated upon activation, normal T-cell expressed, and presumably secreted. It functions as a chemotactic cytokine, which plays a role in recruiting leukocytes to the site of infection.

The protein was first thought to be expressed by T-cells only, but has been shown to be expressed by many different cell types including endothelial and epithelial cells.
An aberrant RANTES expression was observed in a CF cell line when compared to the wildtype in response to *P. aeruginosa* (Kube et al., 2001). They also showed that these cell lines have higher IL-6 and IL-8 secretion after stimulation with *P. aeruginosa* (Kube et al., 2001). Subsequent research using deletions of specific domains of CFTR showed that the expression of RANTES was independent from the ability to conduct chloride across the membrane. This indicates that the impaired regulation of proinflammatory factors by CFTR is not dependent on an ion gradient, but that there is another mechanism regulating this aberrant expression. The PDZ-interacting motif on the C-terminus was found to regulate RANTES expression (Estell et al., 2003). This shows that expression patterns of epithelial cells are affected by deficiency of CFTR. Furthermore, it shows that CFTR regulates RANTES expression directly, and that similar mechanisms in other cells such as immune cells may be responsible for enhanced production of pro-inflammatory cytokines, independent of infection.

Research involving measurement of cytokines and other indicators of immune activation, like neutrophil elastase, in the lungs showed extensive hyperactivation. It started in the mid 1990s when BAL in combination with bronchoscopy was introduced. This created opportunities to measure the lungs of both healthy and very young CF patients. Results showed that the onset of inflammation was earlier than previously thought. Previously it was assumed that the lungs were colonized with harmless infections, and afterwards developed into serious infections. However the new data showed that there were continuous infections (Cantin, 1995). Further research using BAL has shown that infected CF patients have higher cytokine concentrations than non-infected patients. Compared to normal healthy controls CF patients have much higher concentrations of IL-8 (Dakin et al., 2002). Not all investigations showed the same expression patterns, as contrasting data has been published. (Hubeau et al., 2004). Elevated pro-inflammatory cytokine levels were also found in the BAL in the absence of detectable pathogens, further pointing towards a pro-inflammatory phenotype of CF cells that is intrinsically regulated by CFTR (Khan et al., 1995). Together, the data points out that CF patients display an aberrant inflammatory cytokine response in the absence and presence of pathogens.

It remained unclear whether dysregulation was present in naive cells or whether it was triggered by the reaction to infecting bacteria. The BAL results where cytokines were measured of patients with or without infections did not show any conclusive results of the cause underlying this dysregulation. In 2000, experiments were conducted where mice were transplanted with naïve or mature human fetal airway grafts from non-CF and CF patients (Tirouvanziam et al., 2000). These were transplanted into SCID mice to prevent graft rejection. Mice that were transplanted with CF lungs do not develop infections on their own. The CF lungs did produce significantly more IL-8. If the mice are challenged with *P. aeruginosa* there is a large difference between CF and non-CF. Non-CF lungs did not show any disruptions of the epithelium within six hours, while CF lungs showed exfoliation after three hours and intense shedding after six hours (Tirouvanziam et al., 2000). This indicates that innate

mechanisms in the CF lung contribute to pathogen-associated damage, independent of the immune system.

Despite the discrepancies, most papers point towards a dysregulation of cytokine expression that is generally skewed towards a T_H2 response (See box 1) (Hartl et al., 2006). This was shown again by BAL analysis of healthy controls, non-infected CF patients and infected patients. Lymphocytes were quantified and measured using flow cytometry for the presence of either $CXCR3^+$ or $CCR4^+$. $CXCR3^+$ is a marker for T_H1 and $CCR4^+$ for T_H2. Furthermore concentrations of chemokines for both T_H1 and T_H2 were quantified. This showed that there is a prevalent T_H2 response, characterised by expression of IL-4, IL-5 and IL-13, in CF patients infected with *P. aeruginosa* (Hartl et al., 2006). The T_H2 based response in not beneficial to CF patients since this is associated with a reduced clearance of *P. aeruginosa* (Moser et al., 2002; Zuercher et al., 2006). This was demonstrated by re-infecting wildtype BALB/c mice. During the second infection there was a shift towards a T_H1 dominant response, which resulted in improved clearance. Although this was not tested in CFTR deficient mice, it does show that the induced T_H2 response found in CF patients may reduce clearance of *P. aeruginosa*. These results show that due to CFTR deficiency there is a dysregulation resulting in a T_H2 response to common infections.

Box 1, Subsets of T lymphocytes

T lymphocytes are generally distinguished between cytotoxic $CD8^+$ lymphocytes and helper cells characterised by $CD4^+$. Within the group of $CD4^+$ cells there are different subsets. Best described are the T helper 1 and T helper 2 (T_H1 & T_H2) sets (Mosmann and Coffman, 1989). Others like T_H17 have been described (Harrington et al., 2005; Park et al., 2005). Distinction between the sets is mostly based on cytokine secretion. The mechanism by which $CD4^+$ cells differentiate into the different subsets is not yet understood. The different subsets have different activity shaping the adaptive immune response: T_H1 leads to a more cell-mediated immune response whereas T_H2 stimulates a humoral response. The induction of specific T_H subsets has an influence on the outcome of certain diseases and has been described for some to negatively influence outcome when the less beneficial subset is induced.

This was further supported by findings in humans that showed that $CD4^+$ lymphocytes derived from CF patients have lower IL-10 secretion after polyclonal activation (Moss et al., 1996). Freshly isolated CF peripheral blood mononuclear cells as well as $CD4^+$ T-cells were subsequently shown to have reduced interferon-gamma secretion after various stimuli (Moss et al., 2000). This suggests that lymphocytes are dysregulated by CFTR deficiency in humans.

It has long been described that CF mice respond with an increased T_H2 response upon stimulation with the fungus Aspergillus fumigatus, but it was not clear whether this was due to defects in the epithelium or in lymphocytes (Mueller et al., 2008; Muller et al., 2006). A recent investigation involving transfer of lymphocytes from CFTR deficient mice to mice that do not have lymphocytes showed similar results. In this experiment naïve splenocytes from $CFTR^{-/-}$ mice were transferred into $RAG^{-/-}$ mice. The mice were challenged with *Aspergillus Fumigatus* antigen. Mice which received CFTR deficient cells showed a large IgE response to the antigen compared to mice that received wildtype cells. Furthermore mice with a conditional knockout in T cell lineage showed similar results (Mueller et al., 2011). This indicates that T-cells have an aberrant phenotype which causes a hyperinflammatory response facilitating IgE production; independent from CFTR function in epithelial cells.

Increased ABPA prevalence points towards a dysfunction in immunity.

Allergic bronchopulmonary aspegillosis (ABPA) is a hypersensitivity response of the immune system aimed at a very common fungi. The fungal genus of *aspergillus* is causing most pulmonary disorders and specifically *Aspergillus fumigatus* is associated with causing ABPA. It is an opportunistic pathogen that can infect healthy individuals, but normally causes disease in severe neutropenic patients. *Aspergillus* spores can be found almost everywhere. (Slavin et al., 1988).Although this fungus is very abundant, ABPA in healthy individuals is very rare.

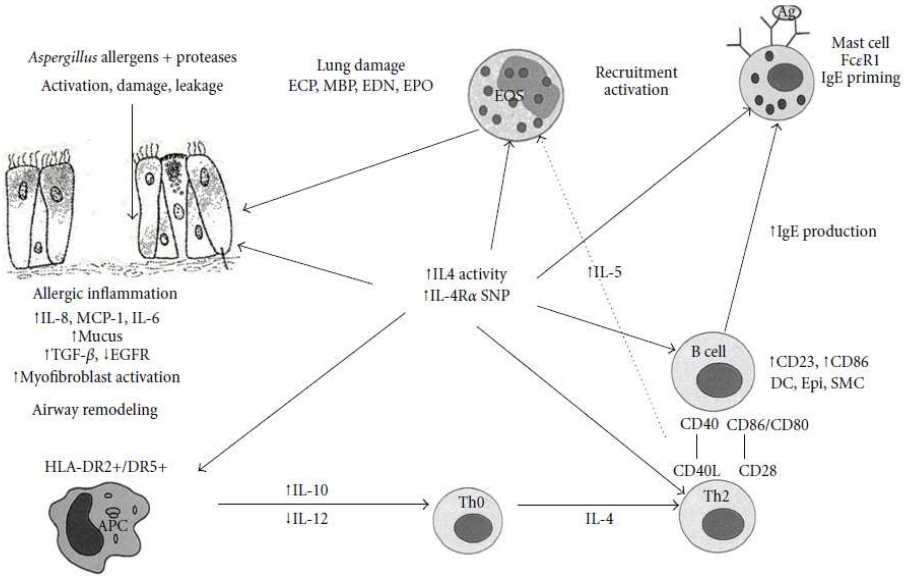

Figure 2*, Proposed immunopathogenesis of ABPA*.
Aspergillus proteins damage lung epithelium after which these are taken up and presented, which skews the T-cells towards a T_H2 response (Knutsen and Slavin, 2011).

There is a strong correlation between ABPA and asthma or CF. Especially CF patients suffer from ABPA significantly more than other people. The prevalence of ABPA among CF patients is around 10-15%, compared to only 1-2% in asthmatic patients (Greenberger and Patterson, 1988; Knutsen et al., 1994). One report suggests that ABPA patients without overt CF or asthma have at least a single CFTR mutation in 50% of the cases (Miller et al., 1996). Normally *A. fumigatus* spores are rapidly cleared from the airways by the immune system, however between 20%-40% of CF patients have spores in the lungs, indicating a reduced clearance (Haase et al., 1991; Maiz et al., 2002). ABPA is diagnosed by skin reactivity to *Aspergillus* antigens. CF patients generally have high titres for IgE and IgG to aspergillus antigens, but the IgE titer observed in ABPA patients is extremely high. There is a clear association between the occurrence of ABPA and mutations in CFTR, however, it is not clear why.

Interestingly, the sensitization to A*spergillus* found in CF or asthmatic patients is higher than patients who actually suffer from ABPA. This can be explained by the observation that ABPA is related to certain HLA subtypes such as HLA-DR2 and DR5, which confer an increased risk for ABPA. On the other hand HLA-DQ2 confers protection against the disease (Chauhan et al., 1996; Chauhan et al.,

1997). There are multiple other risk factors known for ABPA, but CFTR in combination with HLA is shown to be an important one.

ABPA is characterised by a strong T_H2 response and IgE response to the allergen. Lymphocytes from ABPA and CF patients are associated with hypersensitive IL-4 receptors resulting in an overproduction of IgE antibodies directed against *aspergillus* antigen (Knutsen et al., 2004). T_H2 bias is stronger in ABPA patients suffering from CF due to increased sensitivity to IL-4.
Recently the same observations have been made in mice lacking the CFTR protein. After sensitizing these with crude protein extract from *A. fumigatus* they show similar ABPA symptoms, including T_H2-biased response and high IgE titres (Allard et al., 2006; Mueller et al., 2008; Muller et al., 2006). This further supports that the CFTR protein plays a role in the development of ABPA and is therefore associated with the adaptive immune system.

ABPA is an immunogenic disorder and the fact that it is present in so many CF patients is indicating that aberrant function of the immune system contributes to the pathogenesis of CF. However CFTR deficiency is not the only factor in ABPA and it is a very complicated disease in itself. Results obtained in ABPA research can nonetheless be very useful in providing new insights into CF.

Final remarks

Although the gene responsible for CF disease was identified more than two decades ago, the exact mechanisms underlying pathogenesis still remain unclear. The new animal models that have been developed recently should prove and sometimes have been proven to be a major asset in the field of CF (Rogers et al., 2008a).
It is clear in that there is a dysfunction in the immune system, since the lungs become infected and the bacteria can not be cleared. Innate immunity has been implicated and there has been evidence published. Mucosal clearance is part of the innate immune system, but next to that defects in neutrophils were identified (Painter et al., 2006; Witko-Sarsat et al., 1996). Macrophages have been shown to be affected both in their ability to kill the bacteria as well as in them contributing to the exaggerated inflammatory response (Bruscia et al., 2009; Di et al., 2006). So, the innate immune system is clearly affected by the loss of CFTR.

Although mice overexpressing ENaC show CF-like symptoms and therefore can be regarded a proof of concept for the onset of bacterial infections due to non-functional mucus in the lungs (Mall et al., 2004). There have recently been new insights that it might not be Na^+ hyperabsorption, but the Cl^- secretion or HCO_3^- secretion, which are facilitated by the CFTR causing the thickening of the mucus in CF patients (Chen et al., 2010; Joo et al., 2006; Quinton, 2008). Experiments performed to determine electrophysical properties have its limitations. First of all it is not possible to measure these properties *in vivo*. Secondly the cells are almost always excised from inflamed tissue, which might interfere with the results. Thickening of the mucus is an important factor, but the exact cause of the depletion is still a matter of great interest.

There is more evidence emerging about the role of adaptive immunity in CF as discussed in this thesis. Lymphocytes were already known to express CFTR, but the exact function in this type of cell is unknown. Novel insights have shown that the protein does play an important role in regulating immune responses. The balance of cytokines is clearly tipped towards a more proinflammatory state, and that the immune response is of the T_H2 type. This may play an important role in the lack of bacterial clearance as suggested from mouse experiments involving adoptive transfer with immune cells or conditional knockouts.
However, in humans most evidence is observational and the exact mechanism underlying CFTR deficiency and inflammatory dysregulation still remains unresolved. In humans, high infection rates after lung transplantation in CF recipients are possibly the best indication for lack of bacterial clearance by the CF immune system. Also, the strong genetic linkage of CFTR to the development of ABPA, a T_H2 driven allergic reaction, combined with the data from mouse studies strongly suggest that a role for CFTR in preventing T_H2 skewing in man.

CF is a multifactorial disease with a genetic defect of a protein found in many different tissues, it is therefore impossible to attribute the symptoms to a single cell-type. The innate immune system was

confirmed to play a role in CF disease. However, as outlined in this thesis the adaptive immune system is affected as well and appears partly responsible for the observed symptoms. There are clear indications showing that CFTR-deficiency in T-lymphocytes are in a T_H2 prone state that is likely to contribute to bacterial susceptibility and hypersensitivity responses to specific fungi.

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