Immunological aspects on cystic fibrosis lung disease

Carlsson, Malin

2007

Immunological aspects on cystic fibrosis lung disease

Malin Carlsson

Dept of Laboratory Medicine
Faculty of Medicine
Lund University

Immunological aspects on cystic fibrosis lung disease

Malin Carlsson

Departments of Laboratory Medicine and

Clinical Sciences in Lund

LUND
UNIVERSITY
Faculty of Medicine

2007

Cover: *Pseudomonas aeruginosa* grown on agar plates. The greenest strain is an environmental isolate that produce the pigment pyocyanin. The others are cultured from CF patients, showing the adaption of the bacteria during colonization.

ISBN 978-91-85559-06-0

Layout: Thomas Hellmark

Printed at E-husets tryckeri,

Lunds tekniska högskola, Sweden

Original papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals (I-IV).

I. *Pseudomonas*-induced lung damage in cystic fibrosis correlates to bactericidal-permeability increasing protein (BPI)-autoantibodies. Malin Carlsson, Leif Eriksson, Inger Erwander, Jörgen Wieslander, Mårten Segelmark. Clinical and Experimental Rheumatology, 21(Suppl 32):S95-S100;2003.

II. Autoantibody responses to BPI predict disease severity and outcome in cystic fibrosis. Malin Carlsson, Leif Eriksson, Tania Pressler, Ragnhild Kornfält, Lena Mared, Peter Meyer, Allan Wiik, Jörgen Wieslander, Mårten Segelmark. Journal of Cystic Fibrosis, doi:10.1016/j.jcf. 2006.10.005

III. Deficiency of the mannan-binding lectin pathway of complement and poor outcome in cystic fibrosis: bacterial colonization may be decisive for a relationship. Malin Carlsson, Anders G. Sjöholm, Leif Eriksson, Steffen Thiel, Jens C. Jensenius, Mårten Segelmark, Lennart Truedsson. Clinical and Experimental Immunology, 139:306-313;2004.

IV. *Pseudomonas aeruginosa* in cystic fibrosis: Pyocyanin negative strains are associated with BPI-ANCA and progressive lung disease. Malin Carlsson, Ann-Cathrine Petersson, Catarina Andersson, Leif Eriksson, Mårten Segelmark, Thomas Hellmark. *Manuscript.*

Grants

This work was supported by grants from the Faculty of Medicine, Lund University, The Swedish Medical Research Council, Riksförbundet för cystisk fibros and Bengt Andreassons stiftelse.

Contents

Abbreviations

ANCA	anti-neutrophil cytoplasmic antibody
BPI	bactericidal/permeability increasing protein
C	complement protein
CF	cystic fibrosis
CFTR	cystic fibrosis transmembrane conductance regulator
CR	complement receptor
ELISA	enzyme-linked immunosorbent assay
FEV1	forced expiratory volume in 1 second
Ig	immunoglobulin
IIF	indirect immunofluorescence
IL	interleukin
LPS	lipopolysaccharide
Map	MBL-associated protein
MASP	MBL-associated serine protease
MBL	mannan binding lectin
MPO	myeloperoxidase
NFκB	nuclear factor κB
OD	optical density
PMN	polymorphonuclear granulocyte
PR3	proteinase 3
QS	quorum sensing
SLE	systemic lupus erythematosus
TLR	toll like receptor

Introduction

The immune system consists of cells and soluble molecules with the ability to distinguish self from non-self in order to protect the host from invading microorganisms. It can adapt its specificity and thus direct its action against the invader, and then remember this invader, creating a more powerful defence at the next attack. Immunological defence brings about inflammation, which is necessary in order to clear the pathogen. Adequate resolution of inflammation is an active process, and perpetuated inflammation is harmful to the host. In autoimmune diseases, the effector molecules of the immune system are directed against host tissues, creating a perpetuated inflammatory process.

In cystic fibrosis (CF), invading pathogens colonize the airways. The immune system fails to clear the infection and in some patients the inflammation causes extensive tissue damage. CF is not an autoimmune disease, but for unknown reasons the CF patients with the most severe lung damage have developed autoantibodies. Their role in this destructive inflammation is unclear. This thesis deals with the role of the immune system in CF lung disease.

Cystic fibrosis, mechanisms of disease

Historical overview

CF is the most common lethal recessively inherited disease in Caucasians and has an incidence of 1 in 2500-5600 livebirths.[1-3] The disease was first described in 1936 by Fanconi as 'zystische Pancreasfibromatose und Bronchiektasien' and then in 1938 by Anderson as 'cystic fibrosis of the pancreas', referring to the destrucion of exocrine pancreas.[4,5] In 1950, Dickey again reported a correlation between cystic fibrosis of the pancreas and pulmonary disease and in 1953, Di Sant'Agnese demonstrated elevated sodium and chloride concentrations in sweat from CF patients, indicating an abnormal electrolyte transport.[6,7] The molecular background of CF was described in 1987 when the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) was described.[8] Life expectancy in CF has improved substantially in recent years due to centralized medical care in CF units, antibiotic therapy, more efficient nutrition, substitution of pancreatic enzymes, and daily chest physiotherapy. Some decades ago, few CF patients survived into adulthood, whereas today, the adult CF population is larger than the pediatric.[2] Predicted life expectancy in chidren born in the 1990s is over 40 years.[9]

Molecular genetic backgound of CF

CF is manifested in individuals with mutations in the gene encoding the CFTR on both allelles. CFTR is a cAMP (cyclic adenosine monophosphate) regulated ion channel that is expressed mainly on the apical surface of airway ciliated cells and on gastrointestinal epithelial cells.[10] The gene encoding the CFTR is located on chromosome 7.[8] At present 1525 mutations have been described.[11] Five classes of

CFTR mutations are defined according to the mechanism of protein synthesis disruption; I. CFTR is not synthesised. II. Defect in processing resulting in no CFTR expression on the cell surface. III. Defective regulation of CFTR by cAMP. IV. Defective conduction by CFTR. V. Reduced amount of functional CFTR on cell surface.[12] The most common mutation worldwide is the class II mutation, ΔF508. It accounts for about 70% of the alleles in CF patients.[13] The phenotypical implications of these differences in expression concerns mainly the pancreatic function. Class I, II and III mutations are associated with pancreas insufficiency, whereas most patients with mutations belonging to group IV and V have preserved pancreatic function. The correlation between CFTR genotype and lung function is far less distinct even though class IV and V often have mild disease presentation. The majority of patients have class I or II mutations, and within these groups, there are wide phenotypic variations.[14,15] As every CF patient has two mutated alleles, the milder of these two determines the functional expression of CFTR. When CFTR genotypes are studied, the consensus is to divide the genotypes into four groups: A, homozygosity for ΔF508; B, combination of two mutations from classes I, II or III; C, one or two class IV or V mutations; and D, one or two unknown alleles.[16]

Pathogenesis of CF lung disease

The pulmonary disease is the major cause of mortality and morbidity in CF.[17] In the airways, lack of CFTR creates conditions that are favourable for chronic bacterial colonization.[18] *Pseudomonas aeruginosa* is the most significant pathogen in CF. Many mechanisms are sugggested to explain the link between mutated CFTR and susceptibility to chronic infection.

Consequences of loss of CFTR

On the epithelial cells of the airways, the apical surface is covered by cilia, that move in a liqid layer called the periciliary liquid (PCL), transporting mucus and inhaled bacteria in a proximal direction. In the PCL important salt-sensitive antimicrobial peptides are abundant.[18] Mutated CFTR alters the salt and water composition of the PCL. Two main hypotheses have been proposed to explain the effect of mutated CFTR on PCL. The low volume hypothesis is presently the most recognized and suggests that abnormal sodium absorption leads to a thin but isotonic PCL. The ciliary transport will be impaired as the space for movement is limited, resulting in the entrapment of bacteria (figure 1).[19,20] The second hypothesis is the salt depletion hypothesis which suggests that the salt concentration in the airway surface liquid is elevated, inhibiting the salt-sensitive antimicrobial peptides.[21]

CFTR is normally expressed also in the submucosal glands, which beside mucus secrete a variety of products important for host defence, such as secretory immunoglobulin A (IgA) and protease inhibitors. Loss of CFTR changes the composition of the secretions once again impairing defence and mucociliary clearance.[19,22] Loss of CFTR also facilitates bacterial colonization by creating conditions that alter the glycosylation of epithelial components. The number of the

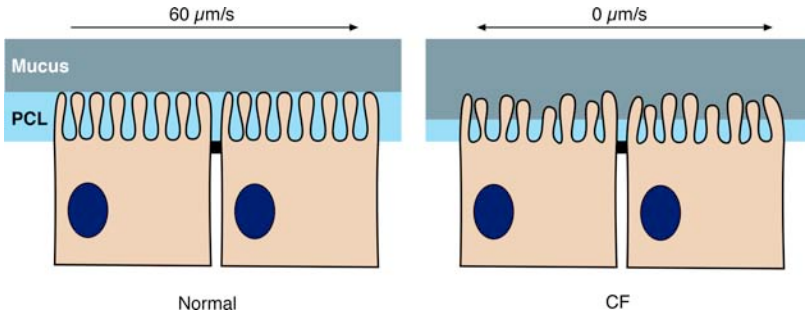

Figure 1. In the healthy (normal) airway, the cilia of the epithelial cells beat synchronically in the periciliar liquid layer (PCL), which moves the overlying mucus layer in a proximal direction. Particles and bacteria trapped in the mucus can thus be exhaled. In CF, the PCL is thin and the beating of the cilia is impeded by the mucus, resulting in the entrapment of bacteria.

cell surface glycolipid asialoGM1, containing a sequence that binds *P. aeruginosa* flagella, is increased in CF airway epithelium.[23,24] It has also been suggested that CFTR itself functions as a receptor for clearance of *P. aeruginosa* [25].

The inflammatory response

Polymorphonuclear granulocytes (PMNs) are white blood cells essential for innate immune defence. They are recruited to the site of infection by chemotactic factors such as interleukin-8 (IL-8), which is a chemokine released from epithelial cells and macrophages as a response to bacterial stimuli. IL-8 is also crucial for activation and migration of PMNs once recruited to the site of inflammation.[26,27,28] Elevated levels of IL-8 in the airways of CF patients are reported from several groups.[29-32]

P. aeruginosa can induce IL-8 release from epithelial cells through a number of mechanisms. Activation of toll-like receptors (TLR) 2, 4 and 5 and asiylated glycolipids (for example asialoGM1) by bacterial surface structures converges downstream in the activation of the transcription factor NFκB which regulates the production of IL-8.[23,33,34] Induction of IL-8 production by substances released from bacteria is also described.[35,36]

The PMNs are essential in the first line host defence against pulmonary pathogens. They fight the pathogens by phagocytosis and through the release of degrading substances. Subsequently the PMNs will go into apoptosis. Resolution of inflammation after the elimination of the pathogens depends on adequate apoptosis followed by phagocytosis of the PMNs by macrophages. Maintaining balance between pro- and anti-apoptotic signals is essential. Premature apoptosis of the PMNs would decrease their antibacterial effect and delayed apoptosis would lead to increasing number of apoptotic PMNs in the airways, overwhelming the macrophage capacity and leading to secondary PMN necrosis. Accumulation of necrotic PMNs in the airways causes tissue by perpetuating the inflammation.[37,38] *P. aeruginosa* is

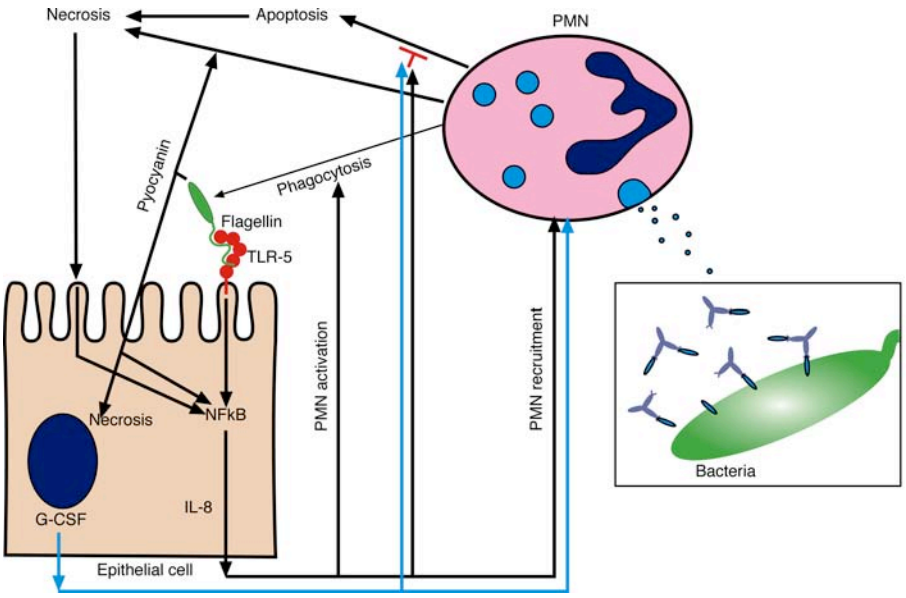

Figure 2. Bacteria can induce an inflammatory response through different mechanisms. *Pseudomonas aeruginosa* can bind via its flagellum to toll-like receptor 5 (TLR-5), which triggers the release of interleukin 8 (IL-8) through the activation of nuclear factor κB (NFκB). Pyocyanin produced by *P. aeruginosa* can also induce the release of IL-8, as well as granulocyte colony-stimulation factor (G-CSF). IL-8 is crucial for recruitment and activation of the PMNs. G-CSF also contibutes to the recruitment of PMNs, and delays PMN apoptosis. Once activated, the PMNs release antimicrobial substances and phagocytose the dying bacteria. Elimination of the PMNs that have fulfilled their task occurs through apoptosis, followed by macrophage phagocytosis of the apoptotic PMNs. Overwhelming of the macrophage capacity leads to secondary necrosis of the PMNs, which perpetuates the inflammatory response.

known to disturb this delicate balance between pro-and anti-apoptotic mechanisms, for example by the release of pyocyanin, which will be discussed further below, and by the induction of granulocyte colony-stimulation factor (G-CSF) from epithelial cells.[39-41] Increased number of PMNs in the airways of CF patients is reported by many groups and is believed to be crucial for the tissue damage in CF lung disease (figure 2).[29,30]

Pre-infectious inflammation in CF?

It has been proposed that inflammation in the CF airways is a constitutional phenomenon, independent of bacterial stimuli. Evidence supporting this theory was found when Hubeau *et al*. detected increased number of macrophages in the airways

of CF fetuses compared to non-CF fetuses.[42] Overproduction of inflammatory mediators in CF previous to bacterial infection has been suggested by several investigators.[30,43-45] Undetectable or eradicated infections could not, however, be excluded in these studies, which is underscored by the report demonstrating that antibodies against *P aeruginosa* can be detected in serum up to two years before the microorganism can be cultured in sputum. Thus a negative culture does not exclude colonization.[46] Evidence that bacterial colonization preceeds inflammation was found by Armstrong et al. when bronchoalveolar lavage fluid from infants with CF who were detected by newborn screening was analysed. Inflammatory cytokines were measured, and no signs of inflammation were seen in the abcence of infection. The results of other investigators, reviewed by Machen in 2006, also imply that the inflammatory response in CF is initiated by bacteria.[32,47]

It has also been suggested that the inflammatory response in CF is elevated relative to the bacterial stimulus, which has been shown both in patients and *in vitro*.[30,35] A plausible explanation of the hyprerinflammatory conditions in CF might be that colonization that is not detectable by conventional sputum culture induces the inflammatory response and that the signalling is enhanced secondary to loss of CFTR.[22,47] At this time point, most data indicate that the inflammation in CF is caused by bacteria colonizing the airways and that factors related to the loss of CFTR may exaggerate the inflammatory response.

Bacterial colonization in CF

Historically, the dominant bacterial pathogens isolated from the airways of CF patients have been *Staphylococcus aureus*, non-capsulate *Haemophilus influenzae* and *P. aeruginosa*. *S. aureus* and *H. influenzae* are frequenly found in pediatric patients before the onset of *P. aeruginosa* colonization. Their role in the development of CF lung disease is not clear, but the presence of *S. aureus* is believed to predispose to colonization with *P. aeruginosa*. Methicillin-resistant *S. aureus* (MRSA) is also an emerging problem in CF patients worldwide.[48] Moreover, the aggressive antibiotic treatment regimens in CF patients during recent decades has caused the emergence of a new flora of Gram-negative bacteria. *Burkholderia cepacia* is a major problem in many clinics, and it is associated with fatal necrotizing pneumonia and bacteremia. Another Gram-negative species not associated with such poor prognosis is *Stenotrophomonas maltophilia*.[49] Non-tuberculosis mycobacteria and fungi are other significant airway pathogens in CF.[50,51]

P. aeruginosa

P. aeuginosa is a Gram-negative motile aerobic bacillus. It can be isolated from many natural environments, such as soil, marshes and plants, and also from food, cut flowers and sinks. *P. aeruginosa* is an opportunistic human pathogen that causes infections in immuno-compromised individuals, burn victims and CF patients.[52]

Definition of chronic colonization

Intermittent colonization is almost invariably followed by chronic colonization, which is a poor prognostic sign in most patients. The definition of chronic colonization that is most widely used is three successive positive sputum cultures, as defined by a European consensus.[53] However, expectorate may not be representative of the lower airways, but rather of the throat. Serological methods for detecting *P. aeruginosa* in CF become more and more widely used, and a rise in the level of antibodies against *P. aeruginosa* indicates colonization even if culture is negative. [46,54] Some CF centres have chosen to define chronic colonization according to antibody levels.[55]

Alginates

After the establishment of chronic colonization, the initial isolates of *P. aeruginosa* resemble environmental isolates in that they have a non-mucoid phenotype and are sensitive to antibiotic treatment. Later the phenotype changes into mucoid and the strains become resistant to antibiotics.[56] The mucoid phenotype is caused by the production of an exopolysaccharide called alginate. Alginate promotes the persistence of *P. aeruginosa* in the airways, as it reduces phagocytosis and PMN chemotaxis and also inhibits complement activation.[57-60]

Biofilm and quorum-sensing

Another hallmark of *P. aeruginosa* growth in the CF airways is biofilm formation. Biofilms are surface-attached communities of bacteria embedded in an extracellular matrix. Both mucoid and non-mucoid strains of *P. aeruginosa* can form biofilms.[61,62] The biofilm mode of growth contributes to the inability of host immune defence to clear the infection, as biofilm bacteria are by nature resistant to antibiotics.[52,63].

Quorum-sensing (QS) is the term used to describe the cell-to-cell communication that makes a bacterial community work as a primitive multicellular organism. In a bacterial population, certain small-molecule autoinducers are released. When a threshold concentration correlated to bacterial density is reached, a cascade reaction that alters gene expressions population-wide is initiated. *P. aeruginosa* use QS signals to coordinate biofilm formation[64,65] even though biofilm without QS occurs. [66] QS also controls among other things the expression of many virulence factors that in order to be efficient require co-ordinated production from the whole bacterial population.[67]

Pili and flagella

Pili are hair-like proteins extending from the surface of many bacterial species including *P. aeruginosa*. They are important for bacterial adhesion to epithelial cells and for initiation of biofilm formation through moving by twitching motility. The pili are composed of subunits called pilin.[68] Induction of inflammatory response by *P. aeruginosa* pilin is described.[69]

P. aeruginosa expresses one single polar flagellum that also contributes to motility. Flagella are potent inducers of inflammatory response from epithelial cells. Direct binding of flagella to TLR5 causes release of IL-8 and binding to asialoGM1 initiates a TLR2-dependent IL-8 release, by mechanisms described above. Expression of flagella is an early event in CF lung disease, as the flagellar genes are expressed only in the environmental, planktonically growing bacteria during initial colonization. In isolates from chronically colonized patients, the flagellar genes are down-regulated.[23,70]

Lipopolysaccharides

Lipopolysaccharides (LPS) are important virulence factors in Gram-negative bacteria. Binding of LPS to TLR4 initiates inflammatory signaling. *P. aeruginosa* LPS is however much less immunogenic than LPS from *Escherichia coli* or *Salmonella*.[27]

Pyocyanin

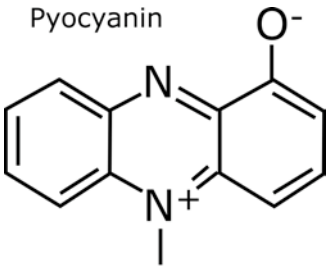

Figure 3. Structure of pyocyanin.

The repertoire of *P. aeruginosa* virulence factors include a number of substances that are released from the bacteria. One of the best studied exoproducts is the redox-active, blue pigment pyocyanin (figure 3). Together with the green pigment pyoverdin, it creates a bright green color that is characteristic for *P. aeruginosa*, and is used for species identification in microbiological laboratories. Non-pigmented strains of *P. aeruginosa* occur mainly in CF patients.[71] Despite the fact that CF isolates often do not exhibit a pyocyanin-producing phenotype, pyocyanin is believed to play an important role in *P. aeruginosa* pathogenicity in CF lung disease.[72]

Many mechanisms for how pyocyanin mediates inflammation and tissue damage have been described. In a mouse model, pyocyanin-producing wild type *P. aeruginosa* strains caused acute lobar pneumonia, while the non-pyocyanin producing mutant strains caused a less aggressive bronchopneumonia-like disease, with areas of acute inflammation spread throughout the lungs.[73] *In vitro* experiments have demonstrated that pyocyanin inactivated epithelial ATPase, resulting in reduced expression of CFTR.[74] It also induced increased expression of IL-8 and intracellular adhesion molecule 1 (ICAM-1) from epithelial cells.[36,75] Induction of neutrophil apoptosis by pyocyanin has also been reported.[39,41]

Modifier genes, prognostic markers and markers of disease activity

Clinical outcome in CF is highly variable between individuals. A minority of the patients carry mild CFTR mutations that are associated with favourable outcome but

for most patients, outcome seems to be determined by other factors.[16,76,77] Chronic colonization with *P. aeruginosa* is associated with poor prognosis. Little is however known about individual susceptibility (which persons become colonized and at what age), as well as individual development of tissue damage (which persons will develop serious lung damage following colonization). Great efforts have been made to find factors that will predict outcome. Studies on mono- and dizygotic twins have not indicated any clear-cut role for non-CFTR genetic factors (modifier genes) as determinants of pulmonary outcome.[78] Two recent studies have compared the features of the patients with the lowest and the highest lung functions. In one of the studies, 808 patients homozygous for ΔF508 were investigated. The patients were genotyped concerning ten candidate modifier genes and the presence of mutations was compared between the patients with the highest and the lowest quartiles of lung functions. Only the mutation of one gene, that of transforming growth factor β (TGFβ), was significantly overrepresented in the group with severe disease.[79] The other study investigated the gene expression in nasal epithelium using oligonucleotide microarray analysis. Differences between patients with preserved lung function and severe lung damage were reported in the expression of genes involved in airway defence and mitochondrial oxidoreductase activity, perhaps identifying new candidate modifier genes.[80]

One of the most extensively investigated candidate modifier gene is the gene encoding mannan-binding lectin (MBL), which is a protein involved in complement activation. The complement system and its role in CF will be discussed further below. More candidate modifier genes are presented in table 1. Many of the genes involve immune defence and factors important for inflammatory response. It should be noted that the table gives a brief overview of suggested modifier genes in CF, and that the presented studies are not quite comparable, as inclusion criteria, as well as definitions of end-points vary.

Exacerbations in CF lung disease

Acute exacerbations in CF often trigger long-term deterioration and are recognized by clinical signs such as dyspnea, fatigue and increased coughing.[81] Signs of acute phase reaction, such as fever and rise in CRP and white blood cell count, are discrete. Total serum IgG is of some clinical use in determining overall inflammatory activity (L Eriksson, Lund, personal communication), and elevated IgG concentration and elevation of certain IgG subclasses are associated with lowered lung function.[82-84] Elevation of CRP is sometimes seen in connection with an exacerbation.[85] The rise is however discrete in comparison with an acute pulmonary infection and its potential to predict an exacerbation is limited. There is evidence that the *P. aeruginosa* strain involved in an exacerbation in most cases has been carried for a long time, and that exacerbations are rarely caused by the acquisition of a new strain.[86] In the daily care of CF patients, physicians have few parameters besides their clinical judgement to determine disease activity and to predict exacerbations.

Table 1. Suggested modifier genes in cystic fibrosis

Gene product	Author	Year	Ref.	*n*	Association to FEV1	Association to PA
MBL	Garred et al.	1999	175	*146*	+	(+)
	Gabolde et al.	1999	177	*164*	+	(+)
	Yarden et al.	2004	178	*179*	(+)	0
	Davies et al.	2004	179	*558*	+	0
	Carlsson et al.	2005	156	*112*	0	-
	Drumm et al.	2005	79	*808*	0	n.i.
	Muhlebach et al.	2006	180	*149*	(+)	n.i.
	Olesen et al.	2006	181	*109*	-	n.i.
	Buranawuti et al.	2007	202	*254*	+	n.i.
TGFβ	Arkwright et al.	2003	203	*68*	+	n.i.
	Drumm et al.	2005	79	*808*	+	n.i.
	Brazova et al.	2006	204	*118*	0	0
	Buranawuti et al.	2007	202	*254*	0	n.i.
TNFα	Hull and Thomson	1998	205	*53*	+	0
	Arkwright et al.	2003	203	*68*	0	n.i.
	Yarden et al.	2005	206	*180*	+	+
	Drumm et al.	2005	79	*808*	0	n.i.
	Schmitt-Grohe et al.	2006	207	*53*	0	0
	Buranawuti et al.	2007	202	*254*	+	n.i.
α1AT	Doring et al.	1994	208	*215*	0	+
	Mahadeva et al.	1998	209	*79*	0 (-?)	n.i.
	Frangolias et al.	2003	210	*714*	0	n.i.
	Drumm et al.	2005	79	*808*	0	n.i.
NOS-1	Grasemann et al.	2002	211	*70*	n.i.	+
	Texereau et al.	2004	212	*59*	+	n.i.
β-AR	Buscher et al.	2002	213	*126*	-	0
	Drumm et al.	2005	79	*808*	0	n.i.

FEV1, Forced expiratory volume in 1 second, measure of lung function; PA, *Pseudomonas aeruginosa*; MBL, Mannan binding lectin; TGFβ, Transforming growth factor β; TNFα, Tumor necrosis factor α; α1 AT, α1 antitrypsin; NOS-1, Nitric oxide synthetase 1; β-AR, β adrenergic receptor; +, correlation between mutated gene and reduced lung function or colonization with *P. aeruginosa;* (+), not significant correlation; –, better lung function or less *P. aeruginsa* colonization correlated to mutated gene; n.i, not investigated.

Extrapulmonary manifestation of CF

Gastrointestinal manifestations develop secondary to highly viscous mucus obstructing various ducts. Insufficiency of the exocrine pancreas is found in 90% of the patients,[87] and enzyme supplementation is often necessary to avoid malabsorption. Moreover, the prevalence of CF related diabetes mellitus following pancreas destruction has increased with improved life expectancy. Liver injury is found in 25 % of CF patients and is responsible for 2-3 % of the mortality. The injury arises from bile duct plugging followed by bile-acid-related toxicity.[88] In males, infertility is common, due to obstrucion of the genital tract.

Vasculitis is an unusual complication in CF, occuring in approximately 3 % of the patients and present as a purpuric rash on the legs, sometimes accompanied by fever and arthralgia. It occurs mainly in patients with severe pulmonary disease and is usually associated with poor prognosis. Histological examination of the rash is consistent with leukocytoclastic vasculitis.[89-91]

The adaptive immune system and autoantibodies

Every individual is born with an innate ability to fight invading pathogens by means of a repertoire of white blood cells, such as the PMNs, and molecules, such as complement proteins, TLRs and cytokines. The immune system also has the ability to adapt and direct its action according to the antigens encountered by each individual. The most important cells of the adaptive immune system are the lymphocytes. Their initial maturation takes place in the central lymphoid organs, which are the thymus (T-lymphocytes, T-cells) and the bone marrow (B-lymphocytes, B-cells). After migrating to peripheral lymphoid organs such as lymph nodes, the lymphocytes undergo additional maturation and are ready to exert their action. Adaptive immunity begins when T- and B-cells recognize a non-self antigen and go through a mutual activation.[92]

The antibody response

Antibodies are the most important effector molecules of the adaptive immune system. They are produced by B-cells in response to antigens recognized as non-self. Each antibody consists of two heavy chains and two light chains, forming a Y-shaped molecule. Both the light and the heavy chains have variable domains together forming the variable, antigen-specific regions of the antibody, which are part of the $F(ab')_2$ fragment. The Fc region of the antibody is made up of two constant domains of the two heavy chains. Specific binding by the variable part of an antibody to its antigen leads to elimination of the pathogen, for example by complement activation or enhancement of phagocytosis. During an antibody response to an invading pathogen, different isotypes of antibodies are formed. The first antibodies to be produced are IgM, which often are of low affinity. They are formed as pentamers and are due to their large size mainly found in the bloodstream. IgM molecules are powerful activators of complement. During the course of the immune response, the B-cells change into expressing IgG, IgA or IgE with the same variable region found during the IgM response. This isotype switching is induced by cytokines released from T-cells. Due to somatic mutations in the variable region, these antibodies often have higher affinity than the preceding IgM antibodies. IgG, IgA and IgE all exist as monomers, which allows them to diffuse into extravascular sites. IgG are the most important antibodies in the blood and extracellular fluid where they opsonize pathogens for phagocytosis and activate complement. IgA exist both as monomers and dimers and act mainly as neutralizing antibodies on epithelial surfaces, most importantly in the intestinal and respiratory tract. IgA is the isotype

that is produced in the largest amounts. The main function of IgE is activation of mast cells, inducing local inflammation in the skin or on mucosal surfaces.[92]

Natural antibodies

Natural antibodies occur in the circulation without previous specific immunization. These IgM antibodies are highly cross-reactive and bind both self and microbial antigens with low affinity. Binding of natural antibodies to invading pathogens provides protection by complement activation until an adaptive immune response has developed. Natural antibodies are also important in the scavenging of metabolic waste from inflammatory processes and apoptosis by their ability to react with certain self-antigens. It has also been proposed that natural antibodies prevent autoimmune reactions by blocking the reaction between self-reactive antibodies and their antigen.[92,93]

Tolerance and loss of tolerance

Tolerance is the ability of the immune system to prevent immunological activation against self-antigens. Several mechanisms are involved in maintaining tolerance and preventing self-reactive lymphocytes from attacking host tissues, and thus errors in these mechanisms can lead to the development of autoimmune diseases.

The first checkpoints of tolerance are the thymus and the bone marrow, where self-reactive clones are deleted. Lack of this central clonal deletion is probably inconsistent with life. Clonal deletion of self-reactive clones also occurs in peripheral lymphoid tissues. Another peripheral mechanism by which autoimmunity is prevented is clonal anergy. Naïve T-cells need co-stimulatory signals from antigen-presenting cells, such as dendritic cells, for maturation and clonal expansion. Absence of these signals leads to the inactivation of self-reactive clones by clonal anergy. Regulatory T-cells are a subset of T-cells that are important for regulating the maturation and activation of lymphocytes. They are believed to play a role in preventing autoimmunity by counteracting the development of self-reactive clones. Autoimmunity can thus arise from dysfunctional regulation of lymphocyte development. It can also arise from errors in antigen presentation. Under certain circumstances such as during extensive cell death or traumatic injury, the immune system can misinterpret a self-antigen as non-self and thus evoke an immune response.[92,93]

Molecular mimicry

Infectious diseases can trigger autoimmune disease through a mechanism known as molecular mimicry. The autoimmune disease is caused by autoantibodies or self-reactive T-cells directed against an epitope common for microorganism and host. Removal of the microorganism by antibiotic treatment leads in most cases to cessation of the immune response. The tissue damage caused by it can however be irreversible.[94]

A widely known example of molecular mimicry is rheumatic fever, where infection with *Streptococcus pyogenes* induces an immune response and specific T-cells infiltrate the heart valves leading to valvular lesions and heart failure. In this case, the M-protein of the bacteria shares a homologous sequence with a valvular glycoprotein.[95]

Guillain-Barré syndrome is a neurological disease characterized by acute progressive limb weakness and areflexia. Antibodies against *Campylobacter jejuni* carbohydrates that also recognize ganglioside carbohydrates are believed to be the causative agent. Epidemiolgical studies have shown that 1/1000 patient with *Campylobacter*-enteritis develop Guillain-Barré syndrome.[96]

Helicobacter pylori codes for α-carbonic anhydrase, which shares homology with human carbonic anhydrase II. Autoantibodies against carbonic anhydrase II are associated with autoimmune pancreatitis, and an epidemiological connection between infection with *H. pylori* and this disease has been demonstrated.[94]

Another interesting example of molecular mimicry is that antibodies against the intestinal pathogen *Klebsiella* are significantly elevated in patients with ankylosing spondylitis. The pathogen shares homology with human leukocyte antigen (HLA) B27 and spinal collagens. Moreover, a starch free diet, resulting in eradication of *Klebsiella* from the digestive tract has been reported to lead to clinical improvement of the patients.[97]

Antineutrophil cytoplasmic autoantibodies

The ANCA antigens

Antineutrophil cytoplasmic autoantibodies (ANCA) is the common name of autoantibodies directed against granula proteins of PMNs. The ANCA antigens are found mainly in the azurophilic granules (also known as primary granules) and their antimicrobial properties are important for the elimination of microorganisms by PMNs. Leukocyte elastase, cathepsin G, proteinase 3 (PR3) and azurocidin are examples of serine proteases found in these granules. Other antimicrobial proteins are bactericidal/permeability increasing protein (BPI), which will be described further below, and the enzyme myeloperoxidase (MPO).

Enzyme linked immuno-sorbent assay (ELISA, see Methods for further description) using purified protein as coating antigen is the most widely used method to detect and decide the specificity of ANCA. ANCA can also be detected by indirect immunofluorescence (IIF) using ethanol-fixed normal donor granulocytes. PR3-ANCA then gives a cytoplasmic pattern known as c-ANCA whereas MPO-, elastase-, cathepsin- and azurozidin-ANCA give a perinuclear pattern, known as p-ANCA.[98,99] BPI-ANCA shows no clear-cut IIF pattern, but can yield either a c- or a p- ANCA pattern. Serum samples positive for BPI-ANCA when tested with ELISA have also frequently for unknown reasons shown to be negative when tested with IIF (figure 4).[100,101]

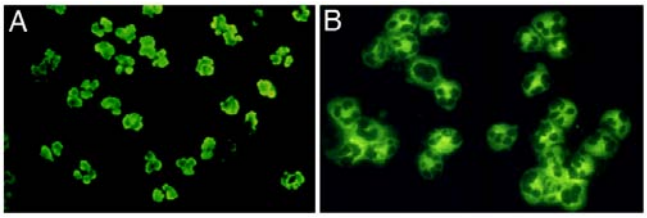

Figure 4. ANCA detected by indirect immunofluorescence on human polymorphonuclear granulocytes. A, p-ANCA pattern; B, c-ANCA pattern.

Clinical implications of ANCA

ANCA are most widely known as antibodies associated with types of small vessel systemic vasculitis, such as Wegener's granulomatosis and microscopic polyangitis. The first report to clarify this association was published in 1985 by van der Woude, even though antibodies that stained leukocytes in patients had been reported earlier[102-104]. ANCA-associated vasculitis is characterized by inflammation and necrosis of blood vessels and often also granuloma formation. Organs involved are mainly the kidneys, respiratory tract and skin. MPO and PR3 are the most important antigens in connection with ANCA-associated small vessel vasculitidies. In these diseases, ANCA occurs in more than 80 % of untreated patients. The diseases typically run in flares with intervening phases of remission. A rise in the ANCA level accompanies the flares in most cases, and the ANCA level falls following successful treatment and during remission.[99]

ANCA is also frequently found in inflammatory bowel diseases. In one study 60% of the patients with ulcerative colitis were ANCA positive by IIF and 28% of the Crohn patients. About half of these IIF positive patients were positive for BPI-ANCA in serum when tested by ELISA.[105] Other researchers have found similar figures for prevalence. No relation to disease severity or activity was, however, reported.[106-109]

Pathogenesis of vascular inflammation by ANCA

The reason why ANCA develops is not clear. There is however emerging evidence that ANCA, once formed, plays a role in disease development of ANCA-associated vasculitides. The most widely accepted theory suggests that some external stimuli induce the release of pro-inflammatory cytokines that will activate the PMNs, resulting in translocation of the ANCA antigens to the cell surface, as well as up-regulation of adhesion molecules. The binding between ANCA and ANCA antigens on the PMNs stimulates PMN adhesion and migration over the endothelial lining, resulting in the release of degrading and reactive substances in the tissue. *In vivo* evidence for the pathogenic role of ANCA was given in a mouse model, where anti-MPO-positive splenocytes were transferred to *Rag2*$^{-/-}$ mice that lacked functioning T- and B-cells. This resulted in circulating MPO-ANCA accompanied by the development of systemic vasculitis engaging the kidneys and other organs.[110,111]

BPI

BPI is a 55 kD boomerang-shaped cationic protein that share 45 % sequence homology with LPS-binding protein (LBP).[112,113] While LBP binds to LPS in order to support bacteria-induced inflammation, BPI has the opposite effect thereby being a potent inhibitor of damaging bacterial effects. The amino-terminal domain of the molecule binds with high affinity to LPS, which besides preventing LPS from inducing an inflammatory response, results in an increased permeability of the outer bacterial membrane, leading to bacterial growth inhibition. Bacterial killing is induced by the penetration of the inner bacterial membrane with the help of complement activation.[114,115] The ability of BPI as well as an amino-terminal fragment of BPI to kill *P. aeruginosa* and other Gram-negative bacteria *in vitro* was reported already in 1992.[116] BPI also enhances bacterial phagocytosis by mediating opsonization with its carboxy-terminal domain (figure 5).[117]

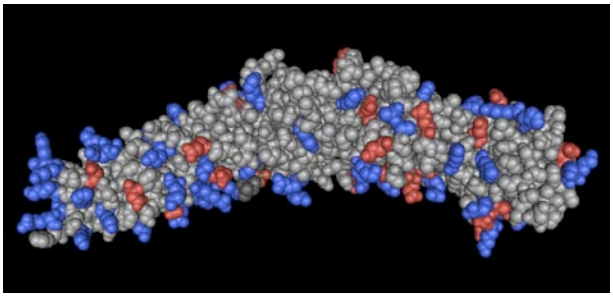

Figure 5. Structure of the 55 kD bactericidal/permeability increasing protein. Negatively charged domains are shown in red and positively in blue.

A murine orthologue to human BPI was recently demonstrated in testis and epididymis cells, indicating that BPI is an important antimicrobial protein in many vertebral species throughout evolution.[118] The main source of BPI in humans is PMNs.[119] Expression of BPI in mucosal epithelium of the gastrointestinal and reproductive tracts as well as in fibroblasts has also been reported.[120-124]

Recently, BPI was demonstrated at both protein and mRNA levels in cells extracted from pulmonary fluid of CF patients. The majority of the cells were PMNs, but BPI was also found in another cell type that had the appearance of epithelial cells when examined by light microscopy. BPI was also found in cell-free sputum supernatants. Moreover, it was demonstrated that BPI was released from PMN granula after stimulation with *P aeruginosa* in a dose-dependent way and in strong correlation with the release of IL-8.[125]

BPI-ANCA

In 1991, Charles *et al.* described antibodies against a 57 kD cationic protein (CAP-57).[126] This protein was identical with BPI, which was recognized as an ANCA antigen in 1995.[127,128] In patients with vasculitis whose sera were tested with IIF and found positive for c-ANCA or p-ANCA, but negative when tested with ELISA against PR3 and MPO, the target antigen in many cases turned out to be BPI. Other patients who were BPI-ANCA positive had a c-ANCA or more rarely a p-

ANCA pattern on IIF. The prevalences of BPI-ANCA in patients with vasculitis were reported to be less than 10%.[100,101,107,128] In a case report, BPI-ANCA was demonstrated in a patient with vasculitis secondary to bronchiectasis and infection with *P. aeruginosa*. In this patient, successful antibiotic treatment was accompanied with a fall in the BPI-ANCA level.[129] Another case report describes a patient with bronchiectasis and *P. aeruginosa* colonization with no evidence of vasculitis and with elevated levels of both BPI- and MPO-ANCA. In this patient, eradication of the *P. aeruginosa* infection by lung resection lead to normalization of the ANCA levels.[130]

The connection between BPI-ANCA and chronic pulmonary disease and subsequent infection with Gram-negative bacteria has been illustrated in many reports. Diffuse pan-bronchiolitis (DPB) is an idiopathic inflammatory disease, almost solely abundant in Japan. It is characterized by progressive obstructive airway disease and bacterial colonization, often with *P. aeruginosa*, that leads to bronchiectasis and respiratory failure. Two studies have been conducted on BPI-ANCA in DPB patients and the prevalences of BPI-ANCA were reported to be 85 % (n=16) and 93 % (n=25) respectively. Moreover, the BPI-ANCA levels were reported to be correlated to disease activity.[131,132]

Table 2. BPI-ANCA in cystic fibrosis

Author	Year	Ref.	*n*	median age (range)	Prevalence of BPI-ANCA (%)		Association to	
					IgG	IgA	FEV1	PA
Eftimiou et al.	1991	133	*30*	(5-27)	63*		n.i.	+
Zhao et al.	1996	134	*66*	23 (13-37)	91	83	+	n.i.
Sediva et al.	1998	144	*71*	(0.5-18)	77	29	0	0
Mahadeva et al.	1999	139	*148*	24.0 (3-50)	70	55	+/0**	+
Aebi et al.	2000	135	*71*	20 (13-27)	39	37	+	+
Schultz et al.	2000	141	*27*	(1-27)	48	11	0	0
Cooper et al.	2000	100	*14*		79	43	n.i.	n.i.
Sediva et al.	2003	143	*28*	(1-19)	57	n.i.	n.i.	n.i.
Carlsson et al.	2003	136	*46*	25 (18-45)	72	59	+	+
Dorlöchter et al.	2004	138	*29*	17 (6-40)	71	33	+	n.i.
Schultz et al.	2004	142	*23*	13 (5-32)	65	n.i.	n.i.	n.i.
Rotschild et al.	2005	140	*18*	15 (5-36)	67	n.i.	n.i.	n.i.
Carlsson et al.	2006	137	*366*	20 (0.5-55)	62	30	+	+

FEV1, Forced expiratory volume in 1 second, measure of lung function; PA, *Pseudomonas aeruginosa*; *, sera only investigated with indirect immunofluorescensce; **; Correlation between FEV and IgG-BPI-ANCA only; +, correlation BPI-ANCA and reduced lung function or colonization with *P. aeruginosa*; –, better lung function or less *P. aeruginsa* colonization correlated to BPI-ANCA; n.i, not investigated.

BPI-ANCA in CF

ANCA in CF patients was first described by Eftimiou in 1991, who investigated the sera of 30 CF patients with IIF and found a c-ANCA pattern in 19 of the patients. Moreover, a strong association between bacterial colonization was reported, as well as decreasing ANCA titres 2-4 weeks after antibiotic treatment.[133] The subsequent report on ANCA in CF was published in 1996 where 66 patient sera were

investigated with ELISA for specific antibodies against BPI. In this study, 91 % were found to be positive for IgG-BPI-ANCA, and 83 % for IgA-BPI-ANCA.[134] Since then seven additional cross-sectional investigations have been published concerning BPI-ANCA in CF (not including three publications from our group), table 2. Prevalences reported for IgG-BPI-ANCA were 39-91 % and for IgA-BPI-ANCA 11-83 %. In all cohorts studied, IgG-BPI-ANCA was more prevalent than IgA-BPI-ANCA although it should be noted that the reference intervals were not uniformly defined. Three other investigators have reported a correlation between BPI-ANCA of both isotypes with bacterial colonization, principally with *P. aeruginosa*, as well as with lung damage. In the study where the lowest prevalence of BPI-ANCA was reported, no association with lung damage and *P. aeruginosa* colonization was found. In this study, however, recombinant BPI was used as coating antigen, while all other investigators used BPI purified from buffy coat. No data on the longitudinal development of BPI-ANCA in relation to the disease course and onset of colonization had been published.[100,135-144]

Pathogenetic role of BPI-ANCA?

The association between BPI-ANCA and bacterial colonization and severe disease presentation in CF lead to the hypothesis that BPI-ANCA could contribute to bacteria induced tissue damage by blocking the antimicrobial properties of BPI. In 1999, Mahadeva *et al.* showed that affinity purified BPI-ANCA recognizing the carboxy-terminal domain of the protein from the sera of 13 patients with CF, vasculitis or inflammatory bowel disease could inhibit the effect of BPI. The antibodies were incubated with leukocytes, BPI and bacteria (*E. coli*). BPI induced as expected phagocytosis, and this phagocytosis was significantly inhibited by BPI-ANCA.[139] The same effect of BPI-ANCA was shown when heat-inactivated sera from BPI-ANCA positive CF patients inhibited PMN-mediated killing of *P. aeruginosa* in vitro.[143]

The antibacterial effects of the amino-terminal domain of BPI have also been shown to be blocked by BPI-ANCA, despite the fact that most of the BPI-ANCA found in patients' sera is directed against the carboxy-terminal.[139,141] Bacterial growth is inhibited by the amino-terminal domain of BPI, as described above. When *E. coli* was incubated with BPI and sera from BPI-ANCA positive patients, the inhibitory effect of BPI on bacterial growth was reversed. BPI-ANCA positive sera from both CF patients and patients with inflammatory bowel disease were able to inhibit BPI.[142 145] BPI-ANCA was also shown to inhibit LPS-induced elastase release from PMNs.[132]

Most BPI-ANCA found in patients' sera is directed against the carboxy-terminal domain of the protein, even though antibodies against the amino-terminal exist. No correlation between the specificity of the antibodies and the clinical presentation of the associated disease has been shown. Nor have any differences in the biological properties of the antibodies depending on their specificity been shown *in vitro.*[139,142,145]

The complement system with special regard to the mannan-binding lectin pathway

Activation of complement

The complement system consists of more than 30 plasma and membrane bound proteins that work together, bridging innate and adaptive immunity, in order to eliminate invading pathogens. Several complement deficiencies are associated with increased susceptibility mainly to bacterial infections.[146] Activation of complement may occur when pathogens, or pathogen-bound antibodies are recognized by a starting molecule of one of the three complement pathways: the classical, the alternative or the MBL pathway. Activation initiates a cascade of cleavage and complex-forming reactions on the pathogen surface, ultimately leading to the formation of a membrane attack complex that may bring about bacteriolysis. Some of the complement cleavage products are important active components functioning as chemo-attractants or opsonins.

The classical pathway is activated mainly when specific IgM or IgG antibodies are bound to an antigen, even though antibody-independent activation may occur. C1 (C1q in complex with $(C1r)_2$ and $(C1s)_2$ molecules) will then bind to the antibody complex and start the reaction sequence. The alternative pathway is activated by the formation of complexes between C3b and factor B. These complexes are formed spontaneously in the circulation and on cell surfaces, but they are degraded by a number of molecules present on host cells, such as factor H and decay-activating factor. It is only on "complex protective surfaces", for example on pathogens where down regulation of the complex formation is decreased, that activation of the alternative pathway is triggered. Indirect antibody-dependent activation of the alternative pathway may also occur.[92]

MBL is the recognition molecule of the third activation pathway, which is activated by innate mechanisms. Activation of complement by MBL was described as late as in 1992.[147] In the circulation, MBL exists only in complex with the MBL-associated serine proteases, MASP-1, MASP-2, MASP-3 and a 19 kD MBL-associated protein (Map 19). The complexes can contain different combinations of MASPs or Map 19, but fully functional MBL/MASPs complexes contain mainly MASP-2 (figure 6).[148] The MBL/MASPs complexes bind to certain saccharide residues such as mannose on pathogen surfaces.

All three activation pathways converge in the formation of C3 convertases. The MBL/MASPs complex, in analogy with the C1q/C1r/C1s complex cleaves C4 and C2, leading to the formation of the C3 convertase C4b2a. The C3 convertase of the alternative pathway, C3bBb, is composed of C3b and the large factor B-fragment Bb. The C3 convertases cleave more C3 into C3a, which is an inflammatory mediator, and C3b that may bind the C3 convertase and thereby create a C5 convertase. The C5 convertase activates the terminal components of the complement system, starting with the cleavage of C5 into C5b and the anaphylatoxin, C5a. C5b

triggers the assembly of the membrane attack complex consisting of one molecule each of C5b, C6, C7 and C8, and up to 16 molecules of C9 in a polymerized form. The membrane attack complex forms a channel that may disrupt the bacterial membrane.[92,149]

Facilitating the uptake and destruction of pathogens and apoptotic cells by phagocytes is one of the most important functions of the complement system. Defects in phagocytosis are associated with a number of infectious and autoimmune conditions. Complements receptors 1 and 3 (CR1 and CR3) are present on phagocytic cells such as macrophages. CR1 binds to C3b and both receptors bind to an inactive form of C3b called iC3b. This interaction activates the macrophages. C5a, and also to a lesser extent C3a and C4a, act as inflammatory mediators that induce vascular changes at the site of inflammation resulting in the recruitment of antibodies, complement and inflammatory cells. The complement system also reinforces the humoral immune response. Complement receptor 2 (CR2) is located on B-cells and acts like a co-receptor to the antigen-specific immunoglobulin receptor. Binding of the C3b degradation product C3dg to CR2 augments the antigen signal, leading to a more powerful antibody response.[92]

MBL structure and genetics

MBL belongs to a group of immune defence proteins called collectins (collagen-like molecules with a lectin domain). Other proteins that belong to this group are the lung surfactant proteins SP-A and SP-D, ficolins 1-3 and C1q. MBL is an oligomer composed of triple helix 25 kD polypeptide chains. Each polypeptide chain contains four domains: the amino-terminal cysteine-rich domain, the collagen-like domain, the coiled neck domain, and the carboxy-terminal globular carbohydrate-recognition domain (figure 6). Most of the circulating MBL consists of trimers and tetramers in complex with MASPs, even though both higher and lower oligomeric forms exist. [150,151] MBL binds to surface structures of a wide range of pathogens, both Gram-positive and Gram-negative. Among pathogens relevant for CF, *S. aureus* shows the strongest binding. *B. cepacia* also binds strongly to MBL, while *P. aeruginosa* and *H. influenzae* only show a weak binding.[152,153]

MBL is encoded by the *MBL-2* gene located on chromosome 10, (*MBL-1* is a pseudogene that does not encode any protein). The *MBL-2* gene contains four protein encoding exons. In the gene, a number of polymorphisms associated with low levels of functional circulating MBL are present. In the region of exon 1 encoding the collagen region, single base substitutions in codons 54, 57 and 52 are referred to as variants B, C and D respectively. Variant A indicates the wild type. The B, C and D variants (commonly referred to as 0 (zero)-variants) are associated with low MBL levels.[154] Polymorphisms in the promoter region also influence the serum level of MBL, the most important being the H/L-variant, the X/Y-variant and the P/Q-variant located at nucleotide positions -550, -221 and +4 respectively (figure 6). The three loci are closely linked. The most common haplotypes are LXP, LYP, LYQ and HYP, where the HYP haplotype is associated with high serum level of MBL,

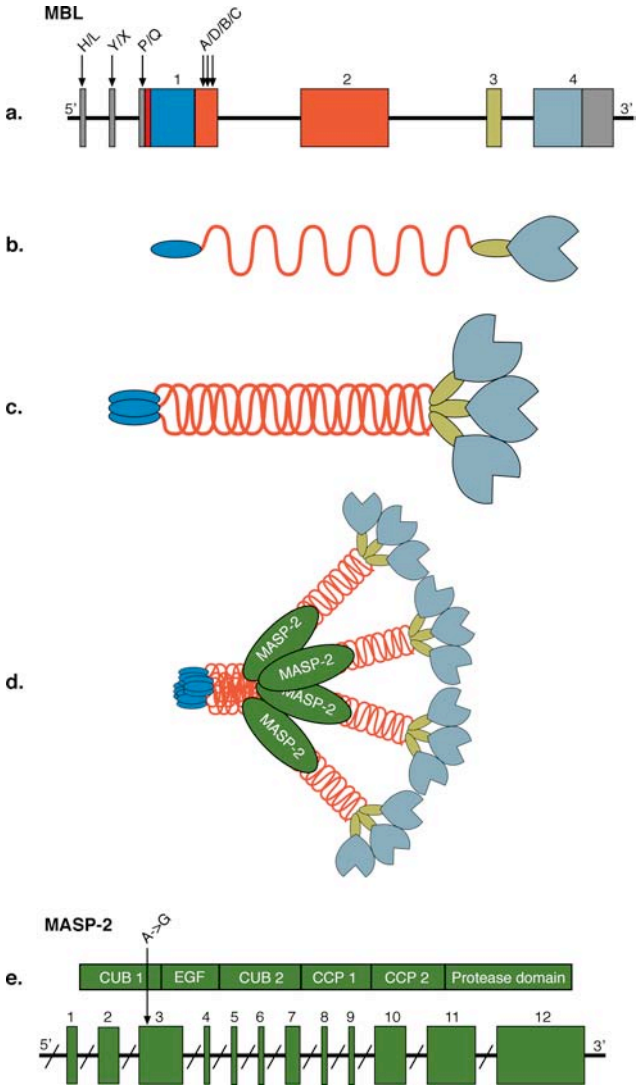

Figure 6. Schematic overview of the gene and protein structure of mannan binding lectin (MBL); a, The *MBL-2* gene comprising four exons (1-4) and show three polymorphisms in the promoter region (H/L, Y/X and P/Q); b, MBL 25 kD polypeptide chain containing four domains : The amino-terminal cysteine-rich domain, the collagen-like domain, the coiled neck domain, and the carboxy-terminal globular carbohydrate-recognition domain; c, The MBL polypeptide chains form triple-helix chains; d, The triple-helix chains form MBL trimers or tetramers in complex with MBL-associated serine proteases, *e.g.* MASP-2; e, The MASP-2 protein is comprised of 6 domains with a known polymorphism in the first domain indicated. The gene encoding MASP-2 is shown below and contains 12 exons. Arrows indicate known mutations and polymorphisms in the genes.

and LXP with low level.[155] *MBL-2* genotype frequencies concerning the structural gene, the X/Y- and the L/H loci, and the mean level of MBL in each genotype group in healthy blood donors are shown in table 3.

Table 3. *MBL-2* genotypes and serum concentrations of MBL in 200 healthy blood donors.[156]

			***MBL-2* genotype frequencies (%)**	**Mean serum level of MBL (μg/L)**
MBL-sufficient genotypes	A / A	HYA / LYA	15.5	2217
		LYA / LYA	3.5	2179
		HYA / HYA	6.5	1965
		LYA / LXA	13.5	1316
		HYA / LXA	13.5	1150
		LXA / LXA	5.5	379
	YA / 0	HYA / HYD	7.5	692
		LYA /HYD	4.0	504
		HYA / LYC	0.5	463
		HYA / LYB	10.0	297
		LYA / LYB	6.0	238
MBL-deficient genotypes	XA / 0	LXA / HYD	1.5	141
		LXA / LYB	5.0	21
		LXA / LYC	0.5	<5
	0 / 0	LYB / HYD	1.0	39
		HYD / HYD	2.0	28
		LYB / LYB	3.0	19
		LYC / HYD	1.0	11

A, normal structural gene; B, C, D, mutated structural genes (0= B, C or D alleles); Y, promoter variant associated with high MBL expression; X, promoter variant associated with low MBL expression; L, H, promoter variants with non-significant association with MBL expression.

MBL deficiency

MBL deficiency is often defined by *MBL-2* genotypes and their described association with low serum levels of functional MBL. These genotypes are homozygote 0-variants of the structural gene, or heterozygote structural gene A-variant and promoter LXP-variant.[154,155] In Caucasian populations, 10-15% are MBL-deficient by this definition. When serum level of MBL is used to define deficiency, the upper limit is usually 100 ng/ml.[150,151] In table 3, MBL genotype frequencies and the mean MBL serum levels in each genotype in Swedish healthy blood donors are presented.[156]

MBL deficiency and susceptibility to infection

The clinical consequence of MBL deficiency is not clear. Reported associations with infectious conditions have in many cases not been possible to reproduce. In individuals with normal, fully developed adaptive immunity, MBL deficiency has been reported to be overrepresented in patients with septicemia.[157,158] Increased susceptibility to certain pathogens for example *Mycoplasma* and severe acute respiratory syndrome (SARS) corona virus were also reported.[159,160] However, in a study based on a large population no increased susceptibility for infectious diseases was found.[161]

MBL deficiency in children and immunocompromised patients

In children admitted to hospital for respiratory tract infections, MBL-deficient individuals were reported to be overrepresented.[162] Another report suggested an increased risk for severe respiratory infections in MBL-deficient individuals between 6 and 17 months of age, when maternal immunoglobulins have waned, and adaptive immunity is still immature.[162,163] Also in patients immunocompromised due to chemotherapy, MBL deficiency is reported to be associated with increased risk for severe infections.[164] In children with malignant diseases and neutropenia, MBL deficiency was associated with prolonged febrile episodes.[164,165] Others report no correlation between MBL deficiency and infectious complications to chemotherapy.[166,167]

MBL deficiency in autoimmune diseases

MBL variant alleles were reported to be risk factors for developing systemic lupus erythematosus (SLE) in one British and one Chinese population[168,169], while this association was not reproducible in a Japanese population.[170] Severe disease presentation and increased susceptibility to infection in SLE patients with MBL deficiency were also reported.[171] Another investigation, however, found no such association.[172] In rheumatoid arthritis, there are conflicting data on the association between MBL deficiency and poor outcome.[173,174]

MBL deficiency in CF

As previously mentioned, Garred *et al.* have reported an association between MBL deficiency and early death from CF. In this study, 146 patients were included and lowered lung function as well as increased mortality were found in patients with 0/0 and YA/0 genotypes.[175] A case report where MBL substitution in an *MBL-2* variant homozygous patient with severe CF lung disease led to a stabilized clinical condition was also published.[176] The association between MBL deficiency and severe CF presentation was confirmed by Gabolde *et al,* who identified eleven out of 164 patients with 0/0 genotypes. These patients were matched for age and gender with eleven control patients with A/A genotypes. More serious lung damage and a tendency to higher prevalence of *P aeruginosa* colonization were seen in the 0/0-group.[177] In 179 CF patients from Belgium and the Czech Republic, non-significant tendencies towards lowered lung functions were seen in MBL-deficient patients both when A/A patients were compared with A/0 and 0/0, and when A/A and YA/0 were compared to XA/0 and 0/0-patients.[178]

An association between 0/0 genotypes and reduced lung function and frequent hospital admission was found in adults but not in children, when 558 CF patients from the UK were investigated.[179] One more study suggested a correlation only in adults, although no significant correlations were seen. Here, no *MBL-2* genotyping had been performed, but patients with MBL serum levels below 200 ng/ml were regarded as MBL deficient.[180] In a study where the prevalence of suggested CF modifier genotypes was investigated in 263 patients with severe CF phenotype and

545 patients with mild CF phenotype, *MBL-2* 0-variants were not overrepresented in the patients with severe phenotype.[79] Olesen *et al.* investigated 109 CF patients who were stratified according to *MBL-2* genotype as 'high' (YA/YA and YA/XA), 'intermediate' (YA/0 and XA/XA) and 'low' (XA/0 and 0/0). Surprisingly the 'high' group had significantly worse lung function than the other groups.[181] In summary, data on the association between MBL deficiency and CF are conflicting. There is a tendency for recent studies and studies in children to show less marked correlations than older studies and studies in adults (table 1).

Production of MBL

MBL is mainly produced by hepatocytes. Reports of rising MBL levels in patients following surgery or road accidents led to the definition of MBL as an acute phase reactant.[182,183] In patients suffering from acute poststreptococcal glomerulonephritis, the MBL levels were elevated compared to controls.[184] Other studies on patients undergoing surgery, and on patients with various severe infections have not shown any clear-cut elevation of MBL during such events.

Extrahepatic production of MBL has earlier been reported in murine intestinal epithelial cells.[185] More recently mRNA of the human *MBL-2* gene was found in intestinal epithelium, testis cells and also in a monocyte like cell line after LPS stimulation. The authors suggested that local production of MBL could play a role in innate immune defence.[186] A substantial increase in MBL levels during pregnancy, and a decrease to about 50% of baseline immediately postpartum were recently described.[187]

MASP-2

MASP-2 is necessary for adequate function of the MBL pathway. The MASP-2 gene and protein are illustrated in figure 6. An A→G mutation in exon 3 resulting in exchange of glycine for aspartic acid at position 120 (position 105 in the mature protein) is reported to be associated with low serum levels of MASP-2. The reported allele frequency for this mutation in a Danish population was 5.5 %. The first described patient homozygous for this mutation manifested severe autoimmune and infectious diseases.[188] Another homozygous individual had CF with severe disease presentation, indicating that MASP-2 or the MBL activation pathway may play a role for outcome in CF.[181]

Aims of the present studies

The aim of this thesis was to further increase our knowledge concerning the events underlying the development of lung damage in CF and to elucidate the interaction between bacteria and host immune defence. The specific aims of the present studies were:

1. To investigate the relationship between the formation of autoantibodies (BPI-ANCA), colonization with *P. aeruginosa* and the development of lung damage. (I, II)
2. To evaluate BPI-ANCA as a prognostic marker in CF. (I, II)
3. To assess the importance of the MBL pathway of complement in CF lung disease. (III)
4. To investigate CF isolates of *P. aeruginosa* in order to study biological differences between isolates from BPI-ANCA positive patients with isolates from BPI-ANCA negative patients. (IV)

Patients and methods

Patients

Patients at the CF centres of the Department of Pediatrics and at the Heart Lung Division at Lund University Hospital and patients from the CF centre at Copenhagen University Hospital were included in the presented studies. CF was confirmed by genotyping with regard to CFTR mutations in all included patients. Data on CFTR genotypes, ages, lung function and microbiological conditions are given in table 4. The Swedish patients studied in paper II are essentially the same as were studied in paper III.

Table 4. CFTR genotypes, age, lung function and *P. aeruginosa* colonization in the patients included in the studies.

Paper		*n*	median age (range)	CFTR genotype group (%)				FEV1% pred	PA (%)
				A	B	C	D		
I		*46*	25 (18-45)	52	24	7	17	66	59
II	Swedish	*129*	20 (2-54)	55	18	12	15	83	40
	Danish	*237*	20 (0.5-54)	73	13	6	8	80	38
III		*112*	20 (4-54)	53	20	11	16	83	42

CFTR genotype: A, homozygosity for ΔF508; B, severe/severe mutation; C, one or two missense mutations; D, one or two unknown mutations. The genotype groups are further explained in the Introduction section; FEV1%pred, Median forced expiratory volume in 1 second, expressed as proportion of the predicted value, measure of lung function; PA, *P. aeruginosa* colonization.

Paper I: Forty-six adult patients out of the 54 who were seen at the Lund CF centre at the time of the study were included. One serum sample was drawn from each patient. Lung function and microbiological findings at this time point were obtained from the patients' records. The patients were then followed prospectively as described below.

Paper II: All CF patients at the CF centres in Lund and Copenhagen were asked to participate. In Copenhagen, 237 patients were included (Danish patients). In Lund, 129 patients were included (Swedish patients). Serum samples were drawn at inclusion, and in the Swedish patients, additional samples were drawn at intervals of six to twelve months for at least twice.

Paper III: Serum samples and blood samples for DNA extraction was obtained from 112 Swedish patients. Lung function and microbiological conditions at this time point were obtained from the patients' records.

Paper IV: CF patients who had been chronically colonized with *P. aeruginosa* for at least three years were identified for the study. Negative BPI-ANCA is rare in patients with a long history of *P. aeruginosa* colonization, although six BPI-ANCA negative patients were available. These were compared with seven arbitrarily chosen BPI-ANCA positive patients.

Ethics

The Research Ethical Committees of Lund and Copenhagen Universities approved the studies, and written informed consent was obtained from each patient, or, in the case of children, their parents.

Statistics

Non-parametrical tests were used when analysing lung functions, BPI-ANCA and MBL-pathway data. To compare medians, the Mann-Whitney test was used. To analyse contingency tables, Fisher's exact test was used, if not indicated otherwise. To calculate correlations, the Spearman rank test was used. Results were considered significant when $p<0.05$.

Measurement of lung function

Spirometry measures static and dynamic lung volumes. Forced expiratory volume in one second (FEV1) is a commonly used measurement of lung function and is known to correlate with outcome in CF lung disease.[189,190] Lung function increases physiologically until the age of approximately 20, after which it declines. FEV1 is often expressed as a proportion of a predicted value according to height, age and gender (FEV1%pred). Normal values for lung function are calculated differently in children and adults, which must be taken into consideration when investigating CF patients, as the median age of these populations often is around 20 years of age.[191,192]

In the present studies, lung function was measured by spirometry for medical purposes and FEV1%pred was calculated according to Solymar in children and in adults according to Quanjer.[191,192] In the Swedish patients (I, II, III and IV), lung function was recorded in conjunction with serum sampling. In the Danish patients (II), lung function was measured on each out-patient visit, and calculated as mean values from one year of measurement prior to inclusion. Lung function was considered normal when FEV1%pred was more than 80 % and values between 50 and 80 % were considered as indicating moderate lung damage.

Microbiological diagnosis

In the Swedish patents, colonization with *P. aeruginosa* was considered chronic after three consecutive positive cultures (I, II and III). In the Danish patients (II), colonization was considered chronic when an organism had been cultured for six consecutive months, or when there was an increase in precipitating antibodies above the normal level (II).

Serum analyses with enzyme-linked immunosorbent assays (ELISAs)

BPI-ANCA (I, II and IV)

Microtitre plates were coated with purified BPI. Patient serum was added and bound antibodies of IgG and IgA class were detected using labelled goat anti-human IgG and IgA respectively. Quantification was made from a calibrator curve and results expressed as arbitrary units.[98] In paper I, no calibrator curve was available for IgA-BPI-ANCA, and therefore the results were expressed as optical density. The positive cut-off levels were defined by the absorbance values of 42 healthy blood donors +3 SD. Positive level of IgG-BPI-ANCA was defined as serum level above 20 U/L and IgA-BPI-ANCA OD 0.14 (I) or 67 U/L (II). In paper I the patients were stratified according to BPI-ANCA levels using arbitrary high-level cut-offs. BPI-ANCA has previously been analysed in a pediatric non-CF population and the median value was found to be on the same level as in adult blood donors (personal communication L Dorlöchter, Bergen, Norway).

MBL (III) and IL-8 (IV)

Both MBL and IL-8 were measured with sandwich-ELISAs. Microtitre plates were coated with monoclonal antibodies directed against the target molecule. Sample was added and detection was made using a labelled secondary antibody directed against the target molecule. In the MBL analysis, 200 healthy blood donors were used as controls.[193]

MBL pathway function (III)

Microtitre plates were coated with mannan and serum was added. Anti-C1q antibodies were added to prevent activation of the classical pathway. MBL pathway activation was quantified by measuring C5b-9_n using a labelled antibody specific for the complex.[194]

Typing of genes encoding MBL and MASP-2 (III)

DNA from peripheral blood leukocytes was extracted by a salting out method.[195]

MBL genotypes

Known polymorphisms in exon 1 of the MBL gene were analysed by allele specific polymerase chain reaction (PCR) amplification. The wild type structural allele is designated A. A mutation allele at codon 52 of the structural gene is designated D, at codon 54 B and at codon 57 C. H and L denominate promoter variants at position -550, and X and Y denominate promoter variants at position -221.[154] 200 healthy blood donors were used as controls.[156]

MASP-2 genotypes

The 258 base pair fragment of chromosome 1 that encodes the CUB1 domain of MASP-2 was amplified by PCR and sequenced by pyrosequencing as described in paper III.[156,188,196]

Cell stimulation with clinical isolates of P. aeruginosa (IV)

Culture and preparation of *P. aeruginosa* isolates

Bacteria were stored at -80°C, thawed and grown for 18 hours on agar plates. For stimulation with bacteria, a single colony from the primary agar plate was spread on a secondary agar plate and incubated for 18 hours to give a confluent growth. Bacteria were harvested, washed, suspended in PBS and serially diluted. Bacterial density was confirmed with viable count. For stimulation with substances released from the bacteria, a colony from the primary agar plate was suspended in LB-medium and incubated for 18 hours. The bacteria were then spun down and the supernatant collected after filtration.

A549 cell culture and stimulation

Prior to stimulation, the human alveolar epithelial-like lung carcinoma cell line A549 was plated onto 6 well plates and cultured overnight to approximately 80 % confluency. In order to study the interactions of bacterial cell wall structures with the epithelial cells, whole bacteria were suspended in RPMI supplemented with Gentamicin and fetal calf serum at densities of 10^9, 10^7, 10^6 and 10^5 bacteria/ml. The interactions of released substances were studied after stimulation with 20 % supernatant in the same medium as described above.

Isolation and stimulation of PMNs

PMNs were isolated using Polymorphprep™ (Axis-Shield PoC AS, Oslo, Norway) from human blood kindly provided by five healthy volunteers. Tubes containing 10^6 PMNs and 200µl bacterial supernatant was mixed with 800 µl RPMI with Gentamicin and incubated for 18-20 hours. The percentage of PMN was 95-99 %, as determined by Türk staining. Viability, checked using Tryptan Blue exclusion test was >95 %.

Cell analysis with flow cytometry

To analyse the proportions of live, apoptotic and necrotic cells after stimulation we used flow cytometry. The PMN population was selected by gating with appropriate settings for forward scatter (FSC) and sideward scatter (SSC). The apoptotic cells were measured with the FL1 fluorescence channel that was used to record the emitted fluorescence from Alexa fluor 488-labelled Annexin V and the necrotic cells with the FL3 fluorescence channel that was used for BD Via-Probe. Cells positive

for annexin V only are from now on denominated as apoptotic cells and all cells positive for BD Via-Probe as necrotic, including double positive cells (figure 7).

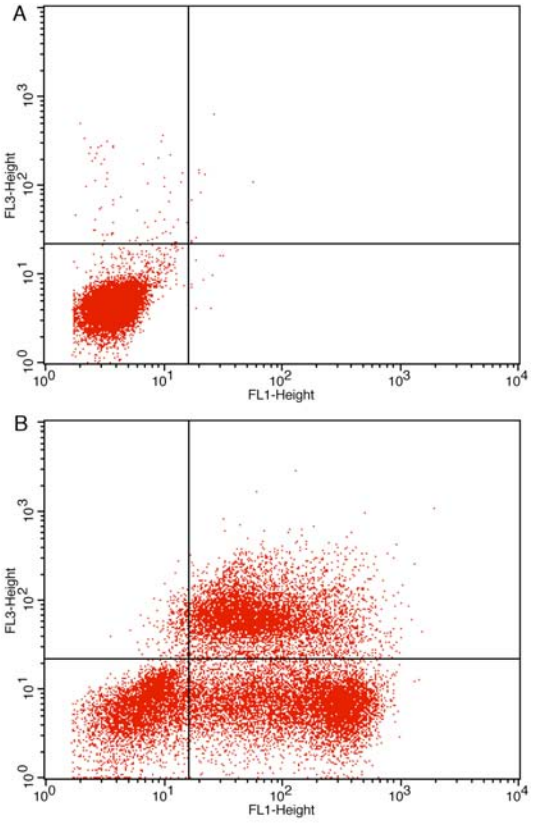

Figure 7. Scattogram from flow cytometry analysis of polymorphonuclear granulocytes stained with annexin V for detection of apoptotic cell (FL1) and BD Via-Probe for necrotic cells (FL3). A, Freshly isolated cells; B, cells incubated for 15 hours. Cells in the lower right field are apoptotic and cells in the upper right and left are necrotic.

Levels of MBL-2 mRNA in monocytes using Real-time PCR

Monocyte total RNA was purified using the RNeasy kit (Qiagen) according to the manufacturer's instructions. The RNA was reversibly transcribed into cDNA. For determination of the expression of various target genes, quantitative PCR assays were performed on an ABI PRISM 7000 Sequence Detector using Taqman chemistry. In short, fluorescence is correlated to the number of probes hybridized to their targets and cleaved by the polymerase activity. Primer/probe sets were purchased from Applied Biosystems (Stockholm, Sweden). The expression of the housekeeping gene Beta-actin was used for normalization. The level of expression was calculated on the basis of the PCR cycle number (Ct) at which the exponential growth in fluorescence from the probes passed a certain threshold value. Relative expression was determined by the difference in the Ct values for the target genes after normalization to RNA input level, using beta-actin Ct values ($\Delta\Delta$Ct).

Results and discussion

BPI-ANCA in CF lung disease

CF is not an autoimmune disease, yet autoantibodies against BPI are found in most of the patients. Moreover, previous reports have indicated that there is a close correlation between BPI-ANCA and colonization with *P. aeruginosa* and decreased lung function. The reason why CF patients develop these autoantibodies is not known. Exploring this issue is interesting, not only in order to understand CF lung disease, but to shed light on the development of autoantibodies in general. Most patients deteriorate in their lung disease after becoming colonized with *P. aeruginosa*. There are however patients who have been colonized for years with preserved lung function. Our decision was to investigate the relationship between BPI-ANCA, lung damage and *P. aeruginosa* colonization in CF patients in order to find a clue as to why BPI-ANCA is formed and what role it plays in CF lung disease. We were also interested in the clinical potential of BPI-ANCA in predicting the development of CF lung disease.

BPI-ANCA in adult patients (I)

BPI-ANCA of isotypes IgG and IgA were measured in 46 adult CF patients with a median age of 24.6 years (range 18.4 – 44.6). All patients were genotyped concerning CFTR, and 24 patients were homozygous for ΔF508. No correlation between CFTR genotype and clinical data was seen. Lung function data (FEV1% pred) and microbiological findings were recorded. *P. aeruginosa* colonization was, as expected, strongly associated with reduced lung function (table 5).

Table 5. Lung function in BPI-ANCA positive and BPI-ANCA negative patients according to colonization with *P. aeruginosa.*

	All patients		**IgG-BPI-ANCA**				**IgA-BPI-ANCA**			
			positive		**negative**		**positive**		**negative**	
	n	**FEV1% pred**	*n*	**FEV1% pred**	*n*	**FEV1% pred**	*n*	**FEV1% pred**	*n*	**FEV1% pred**
PA	*27*	**54**	*21*	**45**	*6*	**83**	*20*	**45**	*7*	**97**
not PA	*19*	**83**	*12*	**69**	*7*	**89**	*7*	**69**	*12*	**88**
All	*46*	**66**	*33*	**61**	*13*	**89**	*27*	**58**	*19*	**93**

FEV1%pred, Median forced expiratory volume in 1 second, expressed as proportion of the predicted value, measure of lung function; PA, *P. aeruginosa* colonization. Correlation between *P. aeruginosa* colonization and lung function, p=0.007, Mann-Whitney test.

Correlation with lung function within the **P. aeruginosa** *colonized group*

As shown in table 5, the BPI-ANCA negative patients did not have reduced median lung function, not even the *P. aeruginosa* colonized patients. Significantly higher levels of BPI-ANCA were found in the *P. aeruginosa* colonized patients, for both IgG- and IgA-BPI-ANCA (figure 8).

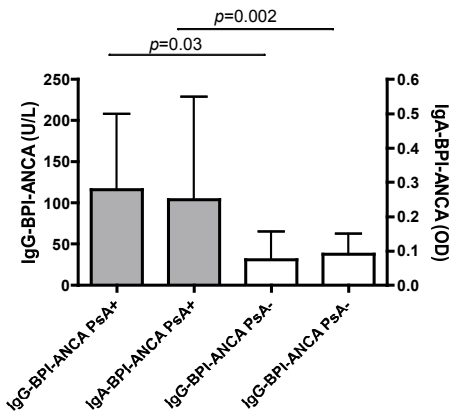

Figure 8. BPI-ANCA levels in patients colonized with *Pseudomonas aeruginosa* (PsA+) and not colonized with *P. aeruginosa* (PsA-). Medians and the 75th percentiles are shown. The Mann-Whitney test was used to compare medians.

Levels of both IgG- and IgA-BPI-ANCA were inversely correlated to lung function. The correlation was somewhat stronger for IgA-BPI-ANCA (figure 9). Significant correlation between BPI-ANCA and lung function was still seen when only the *P. aeruginosa* colonized patients were analysed, p=0.008, but in patients not colonized with *P. aeruginosa* no correlation was seen. These results led us to suspect that elevated serum levels of BPI-ANCA selected those CF patients who were likely to develope lung damage from their *P. aeruginosa* colonization. IgA-BPI-ANCA was found in fewer patients but its correlation with lung damage was slightly stronger, indicating that IgA-BPI-ANCA might have a higher specificity.

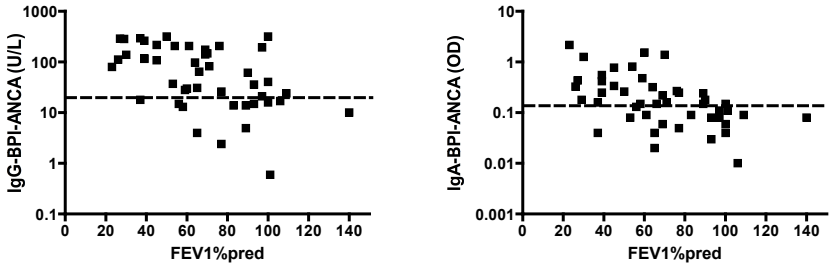

Figure 9. Both IgG-BPI-ANCA and IgA-BPI-ANCA were inversely correlated to lung function. For IgG-BPI-ANCA, r = -0.47, 95 % confidence interval (CI) -0.68 to -0.21, p=0.008. For IgA-BPI-ANCA, r = -0.57, 95 % CI -0.74 to -0.32, p=0.008, Spearman rank correlation.

Vasculitis like symptoms in CF

Secondary vasculitis and vasculitis-like symptoms such as musculoskeletal and cutaneous manifestations have occasionally been reported in CF patients.[89-91] Therefore IgG-PR3-ANCA and IgG-MPO-ANCA were measured and the patients were interviewed concerning musculoskeletal symptoms. No correlation was found, and we concluded that vasculitis-like symptoms in CF are not ANCA associated, in accordance with previous findings.[139]

IgG concentration and BPI-ANCA in relation to lung function

Hypergammaglobulinemia, and elevation of certain IgG subclasses have been reported to correlate with decreased lung function[82-84] and serum IgG concentration is of some clinical use in determining overall inflammatory activity. In this group of patients, however, no significant differences in total IgG were seen when the patients were stratified according to lung function (figure 10).

The findings from paper I implied that BPI-ANCA could prove to be a useful tool in the clinical care of CF patients and maybe even a prognostic marker. We therefore decided to follow the patients prospectively.

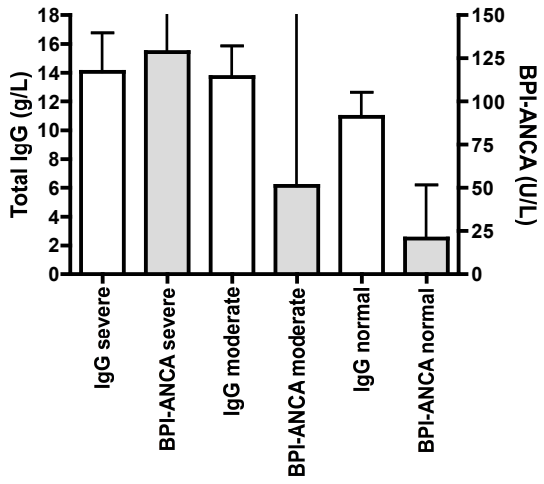

Figure 10. Serum concentrations of IgG (white bars) and IgG-BPI-ANCA (grey bars) in patients with severe lung damage (FEV% pred<50%), moderate lung damage (FEV%pred 50-80%) and normal lung function (FEV1%pred >80%). Medians and the 75th percentiles are shown.

BPI-ANCA as a predictor of severe adverse outcome (II)

Out of 46 patients included in the first study, 40 were available for follow-up. The remaining six patients had either moved (five patients) or died from a non-CF related disease. The median time elapsing from the drawing of the first BPI-ANCA sample until follow-up was made was 5.7 years. Since we knew that BPI-ANCA is strongly correlated to decreased lung function (which in itself is a poor prognostic sign) we were not surprised to find that severe adverse outcome (see II for definition) was clearly overrepresented in those who were IgA-BPI-ANCA positive

at inclusion, p=0.01. At follow-up 15/28 IgA-BPI-ANCA positive patients had severe adverse outcome, 11 had preserved lung function and three were lost to follow-up. In the IgA-BPI-ANCA negative patients, 2/18 had severe adverse outcome (3 drop-outs).

To determine the significance of IgA-BPI-ANCA beside its correlation to lung function, the patients were stratified according to FEV1%pred at inclusion. Moderate lung damage, defined as FEV1%pred between 50 and 80%, was found in 17 patients, seven IgA-BPI-ANCA negative and ten IgA-BPI-ANCA positive and within this group severe adverse outcome was overrepresented in the IgA-BPI-ANCA positive patients, p=0.05. The results are interesting, as the greatest use for a prognostic marker in CF would be in patients with moderate lung damage, in whom prognosis is most difficult to predict.

Positive IgG-BPI-ANCA was also associated with severe adverse outcome when investigating all of the 40 patients, p=0.04, although the association was not significant after stratification for lung function.

BPI-ANCA in 366 CF patients (II)

Our first study indicated that BPI-ANCA was positive only in patients whose *P. aeruginosa* colonization is associated with lung damage. The finding that a serum sample may distinguish between harmful and harmless colonization needed confirmation, and it also raised questions about the formation of BPI-ANCA. Was the BPI-ANCA response a consequence of *P. aeruginosa* colonization in certain patients, or had the BPI-ANCA formation preceded the colonization, creating conditions that facilitated tissue damage? In order to shed light on these issues, serum samples and clinical data were collected from 237 Danish and 129 Swedish CF patients, aged 0.5-54 years. In the Swedish patients, additional sera were drawn at six-month intervals at least twice. Measurement of lung function and definition of chronic colonization differed between the two cohorts. Results were, however, similar, and here the results are presented as one cohort, if not indicated otherwise. Lung transplantation had been performed in 21 patients, and due to low age no lung function data were available in 33 patients. These patients were excluded from certain calculations.

IgG-BPI-ANCA in comparison with IgA-BPI-ANCA

The levels of IgA- and IgG-BPI-ANCA showed a significant correlation (r=0.42, p<0.0001). Out of 312 patients, however, only 175 were concordant for IgG- and IgA-BPI-ANCA positivity. The double-positive (A^+G^+) and the double-negative (A^-G^-) groups were significantly different with regard to *P. aeruginosa* colonization (p<0.0001, Fisher's exact test) and lung function (p<0.0001, Mann-Whitneys test) with the unfavourable results found in the double-positive group. 127 patients were discordant with respect to IgG and IgA-BPI-ANCA. The results of the A^+G^- group closely resembled those found in the double-positive group. In a similar fashion the A^-G^+ group closely resembled the double-negative group. We concluded that IgA-

BPI-ANCA has better specificity for lung damage and *P. aeruginosa* colonization and that BPI-ANCA measurement in CF patients can be limited to this isotype (table 6). From now on, all results presented refer to IgA-BPI-ANCA. As the highest levels of IgG-BPI-ANCA were seen in patients with severe disease presentation, it is possible that IgG-BPI-ANCA harbours the same information as IgA-BPI-ANCA if analysed with regard to the magnitude of the serum concentration. Presently we do not know at what concentration IgG-BPI-ANCA adds relevant information.

Table 6. Number of patients and median FEV1%pred in patients positive or negative for IgG-and IgA-BPI-ANCA respectively.

	IgA-BPI-ANCA			
	Positive		**Negative**	
	n	**FEV1%pred**	***n***	**FEV1%pred**
IgG-BPI-ANCA Positive	*75*	60	*119*	84
Negative	*18*	54	*100*	87

FEV1%pred, Median forced expiratory volume in 1 second, expressed as proportion of the predicted value, measure of lung function; Comparison between +/+ and -/- groups: p<0.0001 for FEV1%pred, Mann-Whitney's test.

Confirmation of relations between BPI-ANCA, lung function and P. aeruginosa *colonization*

The correlation between lung damage, *P. aeruginosa* colonization and BPI-ANCA was confirmed in both the Swedish and the Danish cohort. The correlation between lung damage and BPI-ANCA was seen in children as well.

The group of patients who were colonized with *P. aeruginosa* but were BPI-ANCA negative, had as a group normal lung function (median FEV1%pred 80%) (figure 11). This finding confirmed what was seen in our first study, and we conclude that BPI-ANCA is found in patients where the *P. aeruginosa* colonization is associated with deterioration in the lung disease, and thus have important clinical implications.

Positive BPI-ANCA in patients not colonized with *P. aeruginosa* is rare and was found only in six patients altogether. Still these patients had significantly lower lung function than the BPI-ANCA negative patients without BPI-ANCA. Chronic colonization is preceded by intermittent colonization and probably also by colonization not detectable by routine sampling and culture. It can be speculated that BPI-ANCA in a not yet colonized patient indicates an early colonization that, according to our findings, will cause serious lung damage.

BPI-ANCA is formed in response to P. aeruginosa colonization during disease course

In order to create a fictive time scale, the patients were divided into groups according to age. The BPI-ANCA levels were higher in the older age groups (figure 12). These findings contrast to Mahadeva *et al.* who found no relation between BPI-ANCA and age.[139] In all age groups there was a close correlation between the

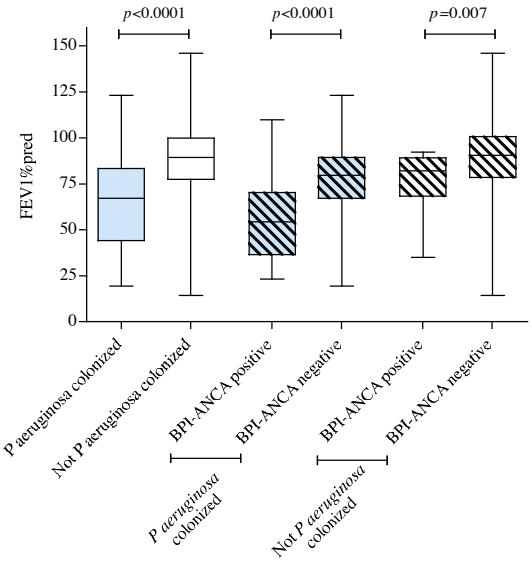

Figure 11. Lung functions according to colonization with *Pseudomonas aeruginosa* and the presence of BPI-ANCA. Patients colonized with *P. aeruginosa* that are BPI-ANCA negative have as a group normal lung function. The Mann–Whitney test was used to compare medians. Medians and inter-quartile ranges are shown.

prevalences of *P. aeruginosa* colonization and BPI-ANCA. More patients were *P. aeruginosa* colonized than BPI-ANCA positive, and more patients were BPI-ANCA positive than those who had severe lung damage. The prevalences of *P. aeruginosa* colonization, BPI-ANCA and severe lung damage increased with age, which gave the impression that colonization had preceded BPI-ANCA formation, which in turn had preceded the development of severe lung damage, see figure 2 in paper II. These findings indicated that BPI-ANCA development was a consequence of *P. aeruginosa* colonization. BPI-ANCA did not, however, always follow *P. aeruginosa* colonization. About one third of the colonized patients did not show a BPI-ANCA response. It seemed that BPI-ANCA could distinguish harmful from harmless colonization.

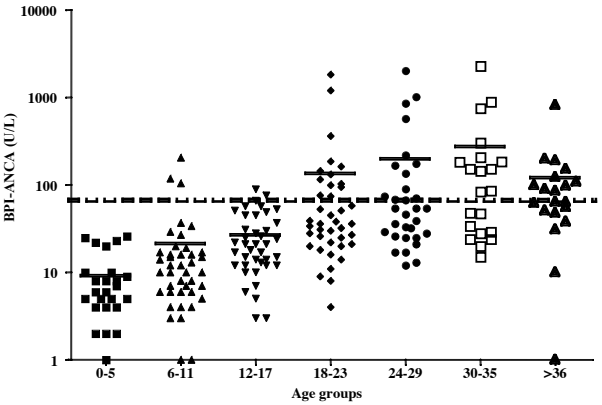

Figure 12. Levels of BPI-ANCA in different age groups (years) in the Danish part of the cohort. The dashed line indicates the reference level for BPI-ANCA (67 U/L).

BPI-ANCA in serum is a stable finding but decreases after lung transplantation

In the Swedish patients, BPI-ANCA was measured at six-month intervals. Significant changes in BPI-ANCA were unusual. Decrease in BPI-ANCA was found in six out of 121 patients, and four of these underwent lung transplantation at the time point in question. In all, seven patients were lung transplant recipients during the study periods, and all of them decreased significantly in BPI-ANCA. One patient was a lung transplant recipient before she was included in the study. At the time of the study she developed chronic rejection and was re-colonized with *P. aeruginosa.* During this period her BPI-ANCA level rose significantly. Increase in BPI-ANCA was found in ten more patients, in one of whom simultaneous acquisition of a new *P. aeruginosa* strain was documented (table 7).

Table 7. Patients whose levels of BPI-ANCA rose during the study period and description of clinical condition.

Gender	Age (years)	Assessement of clinical condition during the inter-sample time.
M	9	Good clinical condition.
F	12	Clinically stable. Chronically colonized.
M	17	Temporarily clinically unstable. Weightloss and repeated iv antibiotics.
M	19	Temporary decline in FEV1 by 1L.
F	20	Temporary increase in need for antibiotics due to recent influenza.
F	21	Clinically unstable with continuous deterioration.
M	24	Chronically advanced lung disease.
F	25	Substantial radiological and clinical progress of lung disease.
M	33	Temporary clinical deterioration and loss of lung function.
F	35	Car accident with thoracic trauma leading to impaired physioterapeutic treatment. Gets chronically *P. aeruginosa* colonized.
F	36	Lung transplanted at age 27. Now debute of chronical rejection and *P. aeruginosa* recolonization.

In summary we found that BPI-ANCA is a stable finding in most CF patient. It seems to develop as a response to *P. aeruginosa* colonization and the removal of bacterial colonization by lung transplantation decreases the levels of BPI-ANCA. ANCA-associated colonization implies higher risk for serious lung damage. The biological events underlying this connection are not known. Either the development of BPI-ANCA is depending on constitution, meaning that patients at risk of developing serious lung damage are the ones prone to produce BPI-ANCA, or is BPI-ANCA developd as a result of colonization with certain pathogenic strains, and is harmful perhaps by blocking BPI, once developed.

MBL pathway of complement and CF (III)

As MBL was proposed as a modifier of CF lung disease, and BPI-ANCA seemed to be correlated to outcome in CF, we were interested in the relation between BPI-ANCA and MBL deficiency.[175,177] To this end, 112 patients were genotyped regarding their *MBL-2* gene. Exon 3 of the MASP-2 encoding gene was also investigated, as an A→G point mutation resulting in exchange of glycine into aspartic acid at position 120 was known to be associated with decreased function of the MBL pathway. The MBL pathway was also measured with a functional assay, and the serum concentrations of MBL and MASP-2 were determined. The correlation of the results with BPI-ANCA and clinical parameters was investigated.

The genes encoding MBL and MASP-2

MBL-2 genotype frequencies were similar in the CF patients and in the 200 healthy blood donors who were investigated as controls. This was no surprise, as a linkage between the CFTR gene on chromosome 7 and the *MBL-2* gene on chromosome 10 was not expected.

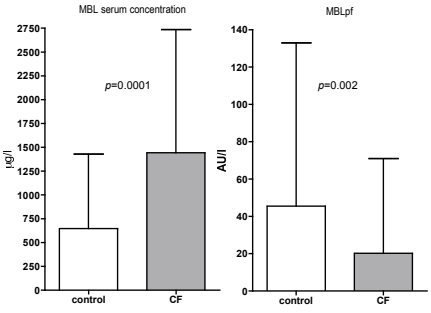

Figure 13. Serum concentrations of mannan binding lectin (MBL) and MBL pathway function (MBLpf) in 200 healthy blood donors (controls) and 112 cystic fibrosis patients. Medians and the 75th percentiles are shown. The Mann-Whitney test was used to compare medians.

The MBL and MASP-2 proteins

The serum levels of MBL correlated as expected with the *MBL-2* genotypes. An unexpected finding was however that the CF patients had higher serum concentrations of MBL than the controls. (Figure 13) This finding was later confirmed in a Danish cohort of CF patients.[181] Furthermore, we found that the MBL concentrations decreased after lung transplantation in four patients (Figure 14). MBL has been reported to be elevated in connection with acute inflammatory conditions.[182,183] In order to estimate the degree of ongoing acute phase reaction, CRP was measured in all CF patients with a high sensitivity method. The median CRP level in the CF patients was 3.1 mg/l, which was slightly elevated compared to 0.9 mg/l in the controls. In clinical practice, the reference interval is usually <5 mg/l, indicating that the CF patients do not show acute phase reaction by the generally appreciated definitions. The elevated serum concentrations of MBL in the CF patients could thus possibly be explained by other factors, perhaps extrahepatic production.

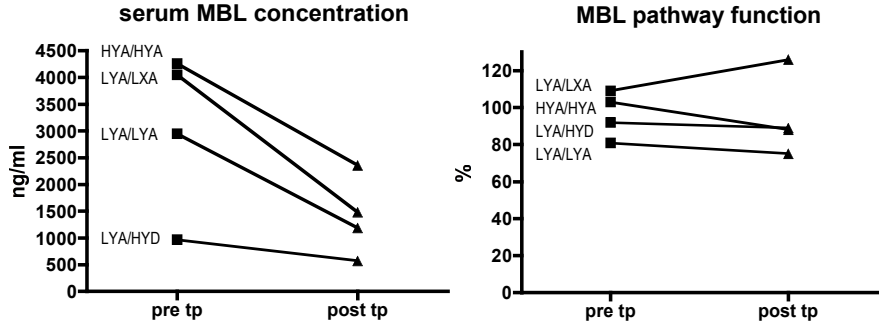

Figure 14. Serum concentrations of mannan-binding lectin (MBL) and MBL pathway function (MBLpf) in four patients who underwent lung transplantation. *MBL-2* genotypes are given.

A report on MBL production in intestinal biopsies from patients with coeliac disease suggested local production of MBL by macrophages.[197] This led us to investigate isolated monocytes from five CF patients, all with MBL sufficient genotypes, concerning the expression of the *MBL-2* gene using real time PCR. However, no such expression was found.

Also for MASP-2, the circulating levels were higher in CF patients than in controls. Both CF patients and controls heterozygous for the MASP-2 gene mutation had decreased levels of circulating MASP-2 compared to patients with no mutation. Another interesting finding was that among the controls, MASP-2 heterozygous individuals had higher levels of MBL than others with the same *MBL-2* genotype, perhaps reflecting a compensatory MBL production.

The function of the MBL pathway

Paradoxically, when the function of the MBL pathway was measured, higher values were found in the healthy controls despite their lower serum MBL levels (figure 13). This discrepancy between the levels of circulating MBL and the function of the MBL pathway has not been reported earlier for CF or any other disease. The impression was that CF could be associated with overproduction of MBL that may be dysfunctional. This notion was underscored by the fact that despite substantial decreases in the MBL serum levels after lung transplantation, no corresponding decreases in the MBL pathway functions were seen. Another possibility is that in the assay used, MBL is not the limiting factor for the MBL activation pathway. MASP-2 gene mutation was associated with decreased function of the MBL pathway in CF patients, but not in controls, again showing an unexplained decreased function of the MBL pathway in the sera of CF patients compared to controls.

MBL pathway and outcome in CF

No difference in lung function between MBL-sufficient and MBL-deficient patients was found, nor was any difference in lung function between patients with MASP-2 gene mutation and wild types found. Only in patients colonized with *S. aureus*, was there a correlation between lung damage and MBL deficiency, although this finding was made *post hoc*. MBL binds to *S. aureus* with high affinity, perhaps explaining the importance of the MBL pathway in individuals colonized with this species.[86,153] No MBL or MASP-2 genotypes were overrepresented in the patients with lung transplants. In contrast to what we expected, we found that chronic colonization with *P. aeruginosa* was more common in patients with MBL-sufficient genotypes. In conclusion, no prognostic disadvantage of MBL deficiency, except for in *S. aureus* colonized patients, was found in the investigated cohort. No correlation between MBL deficiency and BPI-ANCA was found. These results implied that host factors might not be decisive for outcome in CF lung disease, and thus we went on to investigate bacteria related factors.

Induction of cell death and inflammation by P. aeruginosa (IV)

In paper IV we hypothesized that *P. aeruginosa* isolates from BPI-ANCA positive and BPI-ANCA negative patients respectively, had different biological properties, and thus contribute to disease progression in different ways.

Our hypothesis was based on the fact that chronic colonization with *P. aeruginsa* has different implications for disease development in different patients. It was not known what factors that determine the development. The generation of BPI-ANCA seemed to be an event closely correlated to disease progression, although we did not know if BPI-ANCA was a result of an unfortunate interaction between host and pathogen or if BPI-ANCA once formed contributed to the tissue damage (or perhaps both).

Pyocyanin is mainly produced by isolates from BPI-ANCA negative patients

Pyocyanin is a green-blue pigment that is exclusively produced by *P. aeruginosa*. In CF isolates, however, the green phenotype yielded by pyocyanin is rarely found. When inspecting the phenotypes of the collected clinical isolates we found that the strains from BPI-ANCA negative patients in most cases had green phenotypes, while the strains from the BPI-ANCA positive patients in most cases had white (non-pyocyanin producing) phenotypes. The reference strain PAO1 is originally an environmental isolate, which has a distinct green phenotype. In CF *P. aeruginosa* colonizes the airways for years or even decades and during this time the bacteria adapt to the environment by down-regulating virulence factors in order to escape the immune defence and also develop resistance against antibiotics. The phenotypical resemblance between the non-BPI-ANCA associated strains and the environmental

isolate leads us to conclude that BPI-ANCA associated strains have gone further in the process of adapting to the CF airways than the non-BPI-ANCA associated strains (figure 15).

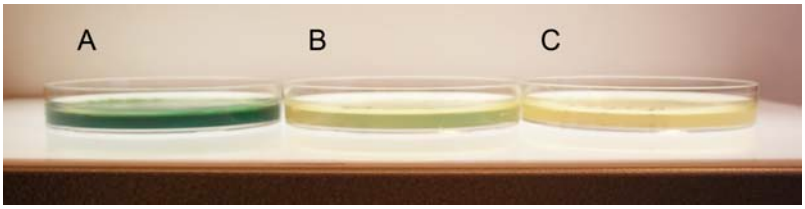

Figure 15. Cultures of *Pseudomonas aeruginosa* grown of ISO–sensitest agar (Oxoid, UK); A, the reference strain PAO1, showing a green phenotype; B, clinical CF isolate with green phenotype; C, clinical CF isolate with white phenotype.

Cell death and induction of IL-8 release in epithelial cells.

Supernatants containing substances released from the bacteria during overnight growth in LB-medium were collected and used for stimulation of epithelial cells. The number of apoptotic and necrotic cells were then investigated using flow cytometry. PAO1 was the most powerful inducer of cell death, both necrosis and apoptosis, and thereafter the non-BPI-ANCA associated strains. The BPI-ANCA associated strains induced no cell death compared to control. The same pattern was seen for IL-8 induction by the different groups of bacteria. PAO1 induced the most and the BPI-ANCA associated strains induced the least IL-8 release from the epithelial cells. (Here, the largest differences between the groups were seen when the epithelial cells were stimulated with whole cell bacteria, measuring the effects of the bacterial surface structures)

These results seemed at first paradoxical. As BPI-ANCA in CF is associated with severe disease and poor prognosis, we were surprised to find that these strains did not induce any cell death in epithelial cells, while the non-BPI-ANCA associated strains did, and that the non-BPI-ANCA associated strains gave a more powerful pro-inflammatory signal. They are however in accordance with the fact that *P. aeruginosa* down regulates virulence factors during long term colonization, which is beneficial for the microbe, but obviously a disadvantage for the host.[23,70,71]

Cell death in PMNs

PMNs were isolated from five healthy donors, and the cells were challenged with supernatants from *P. aeruginosa* isolates over night. In the PAO1 stimulation, flow cytometry was performed at repeated time points during the experiment. We then found that PAO1 induced direct necrosis in the PMNs with no preceding apoptosis. When the clinical isolates were investigated after 18 hours, the strains with green phenotypes had induced increased necrosis in the PMNs. The PMNs stimulated with

supernatants from white phenotype strains showed an increased number of apoptotic cells. Again the isolates that most closely resembled the environmental isolate showed the strongest pro-inflammatory properties.

The fact that the CF patients with the most severe disease carry bacteria with the least ability to induce inflammation underscores the fact that an adequate immune response is necessary to clear a bacterial invasion. From the point of view of the bacteria, the possibilities of propagation increase if they can become “invisible” to host defence.

Conclusions and future perspectives

The clinical relevance of BPI-ANCA

In our first study on BPI-ANCA in CF, we found that the earlier described correlation between BPI-ANCA and lung damage was valid only in *P. aeruginosa* colonized patients. In the large cohort, BPI-ANCA was associated with worse lung function also in the non-colonized patients, perhaps reflecting a not yet detectable colonization. According to our findings, this colonization would, however, be likely to cause serious lung damage to the patient. We also found that the patients who were BPI-ANCA negative and colonized had as a group normal lung function. These findings lead us to hypothesize that the BPI-ANCA response in CF patients could be correlated to disease progression.

The clinical utility of BPI-ANCA is dependent on the test result being a stable phenomenon, so that the information from one sample is representative for an adequate time period. When samples were drawn and analysed at six-month intervals we found that this was the case. We also confirmed that high levels of BPI-ANCA were almost invariably connected with extensive lung damage and *P. aeruginosa* colonization.

Great efforts are being made to find factors that predict prognosis in CF, mostly through the search for modifier genes. To predict prognosis in chronic progressive diseases is important in order to motivate demanding therapy. In CF, prognostic factors could serve as motivators, as intense physiotherapy and intravenous antibiotic courses are time-consuming and demanding for the patient and the family. Prognostic factors could also help to identify patients for pre-transplantation investigation, avoiding emergency surgery.

Prospective investigation of 46 patients for up to nine years indicated that BPI-ANCA was indeed a prognostic marker, which added information besides lung function data.

It is now five years since we began to collect serum samples for BPI-ANCA in the large cohort. Follow-up of these more than 300 patients would determine the prognostic value of BPI-ANCA.

The P. aeruginosa colonization and BPI-ANCA

When studying our cohorts of CF patients at all ages, we found that BPI-ANCA seems to be a consequence of *P. aeruginosa* colonization, so that some but not all patients who become colonized develop BPI-ANCA.

Rises and decreases in the ANCA level were rare. Decrease in BPI-ANCA was seen almost exclusively in patients who underwent lung transplantation. These patients had most of their bacterial load removed when their native lungs were removed and replaced by sterile lungs. **The decreases in BPI-ANCA seen in all transplanted**

patients support the hypothesis that *P. aeruginosa* colonization is a requirement for the BPI-ANCA response.

Increases in BPI-ANCA were not clearly connected with any event, although clinical deterioration was common in these patients during the time periods in question. The use of BPI-ANCA as a maker of current disease activity seems however to be limited.

The relation between P. aeruginosa phenotype and the formation of BPI-ANCA

P aeruginosa possesses a number of pro-inflammatory virulence factors, such as pili, flagella, LPS and pyocyanin that are necessary for invading the host tissues in order to establish colonization. In their interaction with host cells and molecules these virulence factors induce an inflammatory response that enables the host to defend itself against the invader by orchestrating an immune response. In the unique milieu of the CF airways, the bacteria adapt to their environment during years or decades of colonization, often under the pressure of antibiotic treatment. The virulence factors are then down-regulated and the bacteria become invisible to host defence and thus impossible to fight. Biofilm formation, QS and alginate production are other virulence factors of *P. aeruginosa* that by contrast with the pro-inflammatory virulence factors, make the microorganisms inaccessible to host defence as well as to antibiotics.

P. aeruginosa is known to change phenotypes during the years of colonization of the CF airways. The early phenotypes include the expression of pili, flagella and pyocyanin. These virulence factors are later down-regulated and in the late phenotypes the bacteria produce alginates and go into a biofilm, QS mode of growth. This change in phenotypes is obviously beneficial for long-term bacterial survival and proliferation. Although not inducing a full-scale immune response, the slow but progressive proliferation of these late phenotype strains causes tissue damage of the CF airways by steadily increasing the bacterial load and maintaining a net influx of PMNs.

Gram-negative bacteria can shed parts of their outer membrane, outer membrane vesicles (“blebs”) constituitively.[198] Since these blebs comprise LPS and other envelope antigenic components they may be important for delivery of antigenic material to antigen-presenting cells. BPI has the ability to determine the delivery of whole bacteria to PMNs and purified LPS complexes to mononuclear cells.[117] Recent data on the interaction of BPI, blebs and professional antigen-presenting cells, monocyte-derived macrophages and dendritic cells (H. Schultz, Iowa City Iowa, USA, personal communication), suggest that generation of BPI-ANCA might be the result of BPI being presented erroneously to the adaptive immune system in conjunction with bacterial antigens. The requirement of presenting a combination of a TLR-4 mediated signal and a foreign antigen in the phagosome of dendritic cells in order to achieve further processing of the foreign antigen to the MHC class II

complex[199] could be a mechanism by which BPI bound to the endotoxin of outer membrane vesicles becomes presented to the adaptive immune system as a non-self antigen, the BPI-ANCA epitope (figure 16). Once formed, BPI-ANCA can contribute to disease development by blocking the antibacterial effects of BPI.

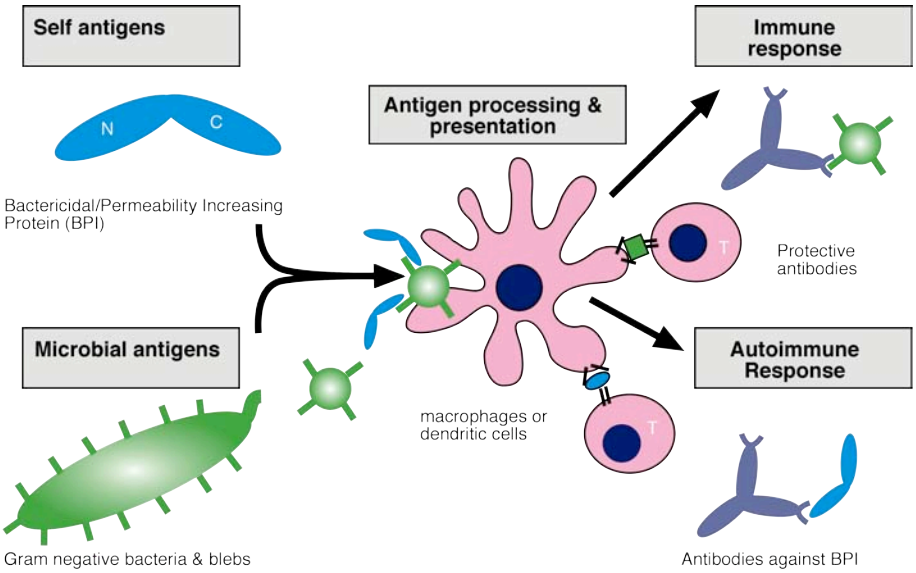

Figure 16. Bactericidal/permeability increasing protein (BPI) binds to lipopolysaccharides on outer membrane vesicles (blebs) shed by Gram-negative bacteria. The BPI-coated blebs are engulfed by dendritic cells, processed as a foreign antigen. The presentation of BPI to T-cells will evoke an autoimmune response. Modified from H. Schultz, Iowa, USA, with permission.[200]

In paper IV, we found that the BPI-ANCA associated isolates of *P. aeruginosa* were non-pyocyanin producing, this being shown by a white phenotype. Accordingly, we also found that these isolates were less powerful inducers of inflammation. **Our interpretation of these findings is that BPI-ANCA is a phenomenon connected to late *P. aeruginosa* phenotypes in CF lung disease.**

Future investigations in this area will include further characterization of *P. aeruginosa* isolates from CF patients with and without BPI-ANCA. First of all, every isolated strain from each of the selected patient needs to be investigated. To study the isolates with a gene array approach would give valuable information about the differences between BPI-ANCA and not BPI-ANCA associated isolates, and perhaps a clue to the association between BPI-ANCA and poor prognosis. If we assume that the inflammatory differences between the strains depend on pyocyanin,

it would be interesting to quantify the pyocyanin production from each isolate in order to see if the inflammatory response is dose-dependent.

Macrophages are important in the resolution of inflammation. So far we have only investigated the interaction between bacteria and PMNs. It is however plausible that tissue damage in CF lung disease caused by accumulating PMNs is aggravated by dysfunctional macrophages. The role of *P. aeruginosa* in activation or inhibition of macrophages is poorly understood and needs to be investigated.

The MBL pathway of complement and CF

MBL deficiency has been reported to be associated with poor prognosis in CF. No such correlation was found in the present investigation. The conflicting data on MBL deficiency and poor outcome in CF can be due to the improvement in treatment that has occurred during the time that has elapsed between the earlier studies and ours. This may also explain why other described associations between decreased expression of certain factors involved in immune responsiveness and poor outcome in CF not were reproducible. It is possible that CF patients undergoing modern treatment are less vulnerable and that minor immunodeficiencies have larger impact in predisposed populations. [201]

In paper III we found that in the CF patients the serum levels of both MBL and MASP-2 were higher than expected. We also saw that the MBL levels decreased substantially following lung transplantation in four cases, suggesting that the elevated levels are secondary to the inflammatory state of the CF lung disease. For MBL, this finding was in accordance with MBL being reported to be elevated in inflammatory conditions, such as traumatic injuries and glomerulonephritis.[182-184] Elevated levels of MASP-2 in inflammatory conditions have not been reported before, and the relevance of this needs further investigation.

In conflict with the finding of elevated serum levels of MBL and MASP-2, we found decreased function of the MBL pathway in the CF sera compared to controls. In the patients whose MBL serum levels decreased after lung transplantation there was no corresponding decrease in the MBL pathway function. There is no obvious explanation to these findings, and they raise a number of questions about the regulation of the production of MBL and the MASPs. Is there an upregulation of MBL in the hepatocytes during inflammatory conditions? The additional MBL seen in the CF patients could also be the result of local production at the site of inflammation. Elucidation of this matter would give valuable information on the function of MBL, as the high prevalence of MBL deficiency in the population suggests additional functions of MBL to complement activation.

The functional assay of the MBL pathway measures the formation of membrane attack complex following complement activation by MBL binding to mannan-coated onto the wells of an ELISA plate. Falsely low values of the MBL pathway function can be obtained when the function of the classical pathway is low. We have, however, no reason to believe that the CF patients would have innate or acquired

deficiencies of the components of the classical pathway. **It is thus plausible that MBL is produced in excess due to CF lung disease and that this additional MBL circulates in a dysfunctional form.**

At this time point we can only speculate about the nature of this dysfunctional MBL. It could be partly degraded due to complement activation. Another speculative idea is that the balance in the production of the MASPs is disturbed, and that the dysfunctional MBL molecules circulate in complex with MASP-1, MASP-3 or Map-19 instead of MASP-2. Future investigations will include measurement of MBL and MBL pathway function in other diseases, such as SLE, vasculitis and chronic obstructive pulmonary disease, to see if dysfunctional MBL occurs in these diseases as well. To search for extrahepatic production of MBL would be interesting in order to learn more about local inflammatory response and perhaps other functions of MBL.

Immunologiska aspekter på lungskadan vid cystisk fibros

(Popularised summary in Swedish)

Cystisk fibros (CF) är en ärftlig sjukdom som oftast debuterar i de tidiga barnaåren. Symtom uppkommer framför allt i lungor och luftvägar, i form av segt slem och kronisk kolonisation med bakterier. Även mag-tarmkanalen drabbas, vilket gör att CF-patienter ofta har svårt att tillgodogöra sig näringsämnen. Prognosen vid CF har förbättrats radikalt under de senaste decennierna tack vare förbättrad behandling i form av antibiotika, sjukgymnastik och nutrition. Lungtransplantationer har också förbättrat överlevnaden. CF-patienter födda på 1990-talet förväntas överleva sin 40-årsdag.

CF uppkommer på grund av avsaknad av en jonkanal som kallas cystic fibrosis transmembrane regulator (CFTR). Denna jonkanal ska normalt finnas på slemhinneceller och ska möjliggöra transport av vatten och elektrolyter från blodbanan ut till luftvägarna och mag-tarmkanalen. Om CFTR saknas rubbas balansen på slemhinneytan och det blir svårare för immunförsvaret att bekämpa bakterier i luftvägarna.

Pseudomonas aeruginosa är den bakterie som orsakar lungskada hos flest patienter med CF. Kolonisationen med *P. aeruginosa* vid CF är mycket svår att utrota med antibiotika och den kan pågå under decennier. Bakterierna har alltså lång tid på sig att anpassa sig till miljön och därigenom öka sina chanser att överleva. Kronisk kolonisation med *P. aeruginosa* innebär som regel sämre prognos vid CF. Det finns dock patienter som bär på bakterien under mycket lång tid utan att utveckla lungskada.

I kontakten med luftvägarnas slemhinneceller kan *P aeruginosa* på olika sätt inducera en signal som aktiverar individens immunförsvar. Bland annat frisätter bakterierna ett ämne som heter pyocyanin. Det är ett blågrönt färgämne som har förmåga att få slemhinnecellerna att frisätta signalsubstanser. Detta leder till en mängd förändringar i vävnaden, vilka syftar till att bekämpa bakterierna. Bland annat uppkommer ansamling av vita blodkroppar och ökad genomblödning, det vill säga inflammation.

Polymorfkärniga granulocyter (PMN) är en typ av vita blodkroppar som är viktiga i sammanhanget, då de är först på plats för att frisätta substanser som på olika sätt skadar och dödar bakterierna. En sådan substans är bactericidal/permeability increasing protein (BPI), som verkar genom att dels avdöda bakterier genom att göra hål i bakteriemembranet och dels binda upp och neutralisera bakteriernas toxiner.

Komplementsystemet är en del av immunförsvaret. Det består av ett antal molekyler som samverkar i en kaskadreaktion vilken leder till avdödning av bakterier. Mannanbindande lectin (MBL) är startmolekyl för en av komplementsystemets aktiveringsvägar. Ärftlig brist på MBL är vanligt och förekommer hos ungefär 10 %

av alla svenskar. Det verkar dock inte som att MBL-brist utgör någon ökad risk för infektioner hos i övrigt friska individer. Möjligen kan det vara en nackdel om man har någon kronisk sjukdom eller nedsatt immunförsvar av någon annan anledning. Några studier har visat att CF-patienter med MBL-brist har allvarligare sjukdom än andra.

Autoimmuna sjukdomar uppkommer när det bildas antikroppar riktade mot ett kroppseget ämne. CF är ingen autoimmun sjukdom, men trots det har majoriteten av CF-patienterna antikroppar mot det kroppsegna ämnet BPI. Dessa antikroppar kallas BPI-ANCA (ANCA = anti neutrofil cytoplasmatisk antikropp). Dessa antikroppar tycks framför allt förekomma hos patienter som är koloniserade med *P. aeruginosa* och hos patienter med nedsatt lungfunktion.

I den här avhandlingen har jag studerat sambandet mellan BPI-ANCA, bakteriell kolonisation och lungskada hos CF-patienter. Jag har också studerat om BPI-ANCA och MBL hos CF patienter har någon betydelse för prognosen. Slutligen ville jag titta närmare på de bakterier som koloniserar luftvägarna hos CF patienter och se om bakterier framodlade från luftvägarna på patienter med dålig prognos orsakade ett annorlunda immunsvar än bakterier från patienter med god prognos.

I **delarbete I** studerade vi 46 vuxna CF patienter. På alla patienter mättes BPI-ANCA i serum och lungfunktion och förekomst av kronisk kolonisation med *P. aeruginosa* noterades. Vi fann då en stark koppling mellan BPI-ANCA och nedsatt lungfunktion. Vi fann också att mycket få patienter som inte var koloniserade med *P. aeruginosa* hade BPI-ANCA i serum. Detta var känt sedan tidigare. En grupp av patienter var koloniserade med *P. aeruginosa*, men hade ingen BPI-ANCA. Denna grupp hade normal lungfunktion, vilket var mycket förvånande, eftersom *P. aeruginosa* kolonisation är mycket starkt kopplat till lungskada vid CF. Det fanns alltså anledning att tro att BPI-ANCA var kopplat till lungskada bara hos *P. aeruginosa* koloniserade patienter. Dessa fynd behövde bekräftas i en större patientgrupp.

Delarbete II omfattade 366 CF patienter i åldrarna sex månader till 55 år från Sverige och Danmark. Vi mätte BPI-ANCA i serum och noterade lungfunktion, bakteriell kolonisation och om patienten hade genomgått lungtransplantation. De svenska patienterna följde vi sedan med nya prover och noteringar var sjätte månad i upp till tre år. Dessutom gjorde vi en uppföljning av de 46 patienterna som ingick i delarbete I. Vi fann att:

- BPI-ANCA är stabilt i serum från halvår till halvår hos de allra flesta patienterna.
- BPI-ANCA uppkommer som en följd av *P. aeruginosa*-kolonisation hos en del men inte hos alla CF-patienter.
- efter lungtransplantation (när det mesta av bakteriekolonisationen tas bort) sjunker BPI-ANCA radikalt. I övrigt är det mycket ovanligt att halten BPI-ANCA i serum sjunker.

- patienter med BPI-ANCA i serum har större risk än andra för allvarligt sjukdomsförlopp på några års sikt.

Sammantaget tycktes BPI-ANCA vara en markör för kolonisation med *P. aeruginosa*, bara om denna kolonisation innebär risk för allvarlig lungskada.

I **delarbete III** studerade vi komplementsystemets MBL-aktiveringsväg hos 112 CF patienter. 18 % av patienterna hade MBL-brist. Dessa patienter hade dock inte sämre lungfunktion än de med normala halter av MBL. Ett överraskande fynd var att CF patienterna hade högre halter av MBL i serum än friska kontrollpersoner. Hos fyra patienter som genomgick lungtransplantation medan studien pågick, sjönk MBL-halterna efter transplantationen utan att funktionen försämrades. Det verkade alltså som om CF-patienterna till följd av sin lungsjukdom hade ökad produktion av MBL.

I delarbete II hade vi lärt oss att *P. aeruginsa*-kolonisation hos individer utan BPI-ANCA inte innebar ökad risk för lungskada. I **delarbete IV** frågade vi oss om det var någon skillnad på bakterier från patienter med BPI-ANCA och patienter utan BPI-ANCA i fråga om deras förmåga att inducera inflammation och vävnadsdöd. Vi fann då att bakterier framodlade från de BPI-ANCA-positiva patienterna inducerade ett mindre kraftfullt inflammatoriskt svar när bakterierna fick stimulera odlade slemhinneceller än bakterier från BPI-ANCA negativa patienter. Resultaten visar att de BPI-ANCA-associerade bakterierna troligen har anpassat sig till miljön i CF-patienternas luftvägar genom att göra sig osynliga för immunförsvaret. Så länge bakterierna inducerar ett normalt inflammatoriskt svar har individens immunförsvar möjlighet att ingripa och bekämpa bakterierna, vilket tycks dämpa utvecklingen av individens lungskada.

Sammanfattningsvis har de studier som ligger till grund för den här avhandlingen visat att BPI-ANCA vid CF är associerat med kolonisation med stammar av *P. aeruginosa* som med stor sannolikhet kommer att leda till allvarlig lungskada. Möjligen beror detta på att dessa stammar har anpassat sig till att leva och föröka sig i luftvägarna genom att inducera ett stillsamt inflammatoriskt svar. Resultaten kan få stor betydelse i omhändertagandet av CF-patienter, då BPI-ANCA kan bli ett prognostiskt verktyg. Vi har inte funnit något samband mellan MBL-brist och dålig prognos vid CF.

Mitt varma tack till (Acknowledgements)

Till alla som på olika sätt bidragit till genomförandet av studierna vill jag rikta mitt varma tack, särskilt:

Mårten Segelmark, min handledare, för goda samtal, många och kloka idéer och för att du har lärt mig bli forskare. Det är ett privilegium att få ta del av ditt sprudlande intellekt.

Lennart Truedsson, biträdande handledare och närmaste chef på Klinisk Immunologi, för givande pratstunder, engagemang, skarpögdhet och för att du delar med dig av dina djupa immunologiska kunskaper.

Thomas Hellmark, biträdande handledare, för spännande diskussioner och aldrig sinande tålamod. Och för att boken blev så fin. Och för support när det behövs som bäst. Och för att "njurlab" är så bra. Tusen tack.

CF-teamet i Lund: **Leif Eriksson, Lena Mared, Ragnhild Kornfält, Peter Meyer, Inger Erwander, Louise Lannerfors, Pernilla Neglén, Catarina Isgren, Kristina Ågård** och alla andra för att ni låtit mig vara en del av ert team, lärt mig allt jag kan om CF och outtröttligt inkluderat patienter i studierna. **Charlotta Schaedel** värdefull för hjälp med bland annat CFTR-genotyperna.

CF-teamet i Köpenhamn: särskilt **Tania Pressler**, för trevligt och givande samarbete.

Alla arbetskamrater på Klinisk Mikrobiologi och Immunologi: **Catarina Andersson** för imponerande insatser i sista delarbetet. **Ann-Christine Petersson** för gott Pseudomonas-samarbete. Klinikledningen, i synnerhet **Ingvar Eliasson** och **Susanne Jönsson** för ett forskningsvänligt klimat. Hela kliniken för hjälpsamhet och god stämning. **Lillemor Melander Skattum** för vänskap.

Wieslab/Eurodiagnostica, i synnerhet **Jörgen Wieslander**, för utmärkt hjälp med analyserna och för uppmuntran och tro på projekten.

Övriga medförfattare för gott samarbete, särskilt vill jag minnas **Anders Sjöholm** för sitt engagemang, sin forskarglädje och sin enorma kunskap. Du fattas mig.

Vaskulitgruppen/Njurlab: **Lena Gunnarsson, Elinor Johnsson, Åsa Pettersson, Nermina Jaganjac** för utmärkt laboratoriearbete, och hela gruppen för god forskningsatmosfär och vänskap.

Catharina Svanborg för entusiasm och stöd.

Hans Fischer, biträdande handledare under de första åren, för att du lärde mig hantera celler och bakterier och för glada skratt.

Gertud Hellmer och **Birgitta Gullstrand** för utmärkt laboratoriearbete och trivsam samvaro.

Mina föräldrar, **Lilian och Conny** för hjälp med allt praktiskt och för oändlig kärlek och omtanke som omfattar oss alla. Mina svärföräldrar **Marianne och Henry** för kärlek och stöd.

Slutligen vill jag tacka min älskade familj: **Ola**, min bäste vän i världen, och våra barn **Axel, Alma och Annie.** Ni är bäst.

References

1. Collins FS. Cystic fibrosis: molecular biology and therapeutic implications. Science. 1992;256:774-779.
2. Lannefors L, Lindgren A. Demographic transition of the Swedish cystic fibrosis community--results of modern care. Respir Med. 2002;96:681-685.
3. Slieker MG, Uiterwaal CS, Sinaasappel M, et al. Birth prevalence and survival in cystic fibrosis. Chest. 2005;128:2309-2315.
4. Fanconi G, Uehlinger E, Knauer C. Das Coeliakiesyndrom bei angeborener zystischer Pancreasfibromatose und Bronchiektasien. Wiener Medizinische Wochenschrift. 1936.
5. Anderson D. Cystic fibrosis of the pancreas and its relation to celiac disease. A clinical and pathological study. Am J Dis Child. 1938;56:344-399.
6. Dickey LB. Pulmonary disease, associated with cystic fibrosis of the pancreas. Dis Chest. 1950;17:151-171.
7. Di Sant'Agnese P, Darling RC, Perara GA, Shea E. Abnormal electrolyte composition of sweat in cystic fibrosis of the pancreas. AMA Am J Dis Child. 1953;86:618-619.
8. Riordan JR, Rommens JM, Kerem B, et al. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science. 1989;245:1066-1073.
9. Frederiksen B, Lanng S, Koch C, Hoiby N. Improved survival in the Danish center-treated cystic fibrosis patients: results of aggressive treatment. Pediatr Pulmonol. 1996;21:153-158.
10. Trezise A. Exquisite and multilevel regulation of CFTR expression. In: Bush A et al, eds. Cystic Fibrosis in the 21st century. Vol. 34. Basel: Karger; 2006:11-20.
11. http://www.genet.sickkids.on.ca/cftr/app. Cystic Fibrosis Mutation Database. (Cited 2007 Jan 23)
12. Welsh MJ, Smith AE. Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. Cell. 1993;73:1251-1254.
13. Worldwide survey of the delta F508 mutation--report from the cystic fibrosis genetic analysis consortium. Am J Hum Genet. 1990;47:354-359.
14. Rowntree RK, Harris A. The phenotypic consequences of CFTR mutations. Ann Hum Genet. 2003;67:471-485.
15. McKone EF, Goss CH, Aitken ML. CFTR Genotype as a Predictor of Prognosis in Cystic Fibrosis. Chest. 2006;130:1441-1447.
16. Schaedel C, de Monestrol I, Hjelte L, et al. Predictors of deterioration of lung function in cystic fibrosis. Pediatr Pulmonol. 2002;33:483-491.
17. Welsh MJ, Ramsey BW. Research on cystic fibrosis: a journey from the Heart House. Am J Respir Crit Care Med. 1998;157:S148-154.
18. Mall M, Boucher RC. Pathogenesis of Pulmonary disease in cystic fibrosis. In: Bush A et al, eds. Cystic Fibrosis in the 21st century. Vol. 34. Basel: Karger; 2006:116-121.
19. Boucher RC. New concepts of the pathogenesis of cystic fibrosis lung disease. Eur Respir J. 2004;23:146-158.
20. Matsui H, Grubb BR, Tarran R, et al. Evidence for periciliary liquid layer depletion, not abnormal ion composition, in the pathogenesis of cystic fibrosis airways disease. Cell. 1998;95:1005-1015.
21. Smith JJ, Travis SM, Greenberg EP, Welsh MJ. Cystic fibrosis airway epithelia fail to kill bacteria because of abnormal airway surface fluid. Cell. 1996;85:229-236.

22. Terheggen-Lagro SW, Rijkers GT, van der Ent CK. The role of airway epithelium and blood neutrophils in the inflammatory response in cystic fibrosis. J Cyst Fibros. 2005;4 Suppl 2:15-23.
23. Adamo R, Sokol S, Soong G, Gomez MI, Prince A. Pseudomonas aeruginosa flagella activate airway epithelial cells through asialoGM1 and toll-like receptor 2 as well as toll-like receptor 5. Am J Respir Cell Mol Biol. 2004;30:627-634.
24. Saiman L, Prince A. Pseudomonas aeruginosa pili bind to asialoGM1 which is increased on the surface of cystic fibrosis epithelial cells. J Clin Invest. 1993;92:1875-1880.
25. Pier GB, Grout M, Zaidi TS. Cystic fibrosis transmembrane conductance regulator is an epithelial cell receptor for clearance of Pseudomonas aeruginosa from the lung. Proc Natl Acad Sci U S A. 1997;94:12088-12093.
26. Godaly G, Bergsten G, Hang L, et al. Neutrophil recruitment, chemokine receptors, and resistance to mucosal infection. J Leukoc Biol. 2001;69:899-906.
27. Sadikot RT, Blackwell TS, Christman JW, Prince AS. Pathogen-host interactions in Pseudomonas aeruginosa pneumonia. Am J Respir Crit Care Med. 2005;171:1209-1223.
28. Mizgerd JP. Molecular mechanisms of neutrophil recruitment elicited by bacteria in the lungs. Semin Immunol. 2002;14:123-132.
29. McGarvey LP, Dunbar K, Martin SL, et al. Cytokine concentrations and neutrophil elastase activity in bronchoalveolar lavage and induced sputum from patients with cystic fibrosis, mild asthma and healthy volunteers. J Cyst Fibros. 2002;1:269-275.
30. 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.
31. Karpati F, Hjelte FL, Wretlind B. TNF-alpha and IL-8 in consecutive sputum samples from cystic fibrosis patients during antibiotic treatment. Scand J Infect Dis. 2000;32:75-79.
32. Armstrong DS, Hook SM, Jamsen KM, et al. Lower airway inflammation in infants with cystic fibrosis detected by newborn screening. Pediatr Pulmonol. 2005;40:500-510.
33. Zhang Z, Louboutin JP, Weiner DJ, Goldberg JB, Wilson JM. Human airway epithelial cells sense Pseudomonas aeruginosa infection via recognition of flagellin by Toll-like receptor 5. Infect Immun. 2005;73:7151-7160.
34. Greene CM, Carroll TP, Smith SG, et al. TLR-induced inflammation in cystic fibrosis and non-cystic fibrosis airway epithelial cells. J Immunol. 2005;174:1638-1646.
35. Becker MN, Sauer MS, Muhlebach MS, et al. Cytokine secretion by cystic fibrosis airway epithelial cells. Am J Respir Crit Care Med. 2004;169:645-653.
36. Look DC, Stoll LL, Romig SA, Humlicek A, Britigan BE, Denning GM. Pyocyanin and its precursor phenazine-1-carboxylic acid increase IL-8 and intercellular adhesion molecule-1 expression in human airway epithelial cells by oxidant-dependent mechanisms. J Immunol. 2005;175:4017-4023.
37. Haslett C. Granulocyte apoptosis and its role in the resolution and control of lung inflammation. Am J Respir Crit Care Med. 1999;160:S5-11.
38. Bianchi SM, Dockrell DH, Renshaw SA, Sabroe I, Whyte MK. Granulocyte apoptosis in the pathogenesis and resolution of lung disease. Clin Sci (Lond). 2006;110:293-304.
39. Allen L, Dockrell DH, Pattery T, et al. Pyocyanin production by Pseudomonas aeruginosa induces neutrophil apoptosis and impairs neutrophil-mediated host defenses in vivo. J Immunol. 2005;174:3643-3649.

40. Saba S, Soong G, Greenberg S, Prince A. Bacterial stimulation of epithelial G-CSF and GM-CSF expression promotes PMN survival in CF airways. Am J Respir Cell Mol Biol. 2002;27:561-567.
41. Usher LR, Lawson RA, Geary I, et al. Induction of neutrophil apoptosis by the Pseudomonas aeruginosa exotoxin pyocyanin: a potential mechanism of persistent infection. J Immunol. 2002;168:1861-1868.
42. Hubeau C, Puchelle E, Gaillard D. Distinct pattern of immune cell population in the lung of human fetuses with cystic fibrosis. J Allergy Clin Immunol. 2001;108:524-529.
43. Khan TZ, Wagener JS, Bost T, Martinez J, Accurso FJ, Riches DW. Early pulmonary inflammation in infants with cystic fibrosis. Am J Respir Crit Care Med. 1995;151:1075-1082.
44. Rao S, Grigg J. New insights into pulmonary inflammation in cystic fibrosis. Arch Dis Child. 2006;91:786-788.
45. Rosenfeld M, Gibson RL, McNamara S, et al. Early pulmonary infection, inflammation, and clinical outcomes in infants with cystic fibrosis. Pediatr Pulmonol. 2001;32:356-366.
46. Pressler T, Frederiksen B, Skov M, Garred P, Koch C, Hoiby N. Early rise of anti-pseudomonas antibodies and a mucoid phenotype of pseudomonas aeruginosa are risk factors for development of chronic lung infection--a case control study. J Cyst Fibros. 2006;5:9-15.
47. Machen TE. Innate immune response in CF airway epithelia: hyperinflammatory? Am J Physiol Cell Physiol. 2006;291:C218-230.
48. Conway SP, Denton M. Stapylococcus aureus and MRSA. In: Bush A et al, eds. Cystic Fibrosis in the 21st century. Vol. 34. Basel: Karger; 2006:153-159.
49. Govan JRW. Other Gram-negative organisms. In: Bush A et al, eds. Cystic Fibrosis in the 21st century. Vol. 34. Basel: Karger; 2006:145-152.
50. Griffith DE. Nontuberculos mycobacterial lung disease in patients with cystic fibrosis. In: Bush A et al, eds. Cystic Fibrosis in the 21st century. Vol. 34. Basel: Karger; 2006:160-165.
51. Que C, Geddes D. Respiratory fungal infections and allergic bronchopulmomary aspergillosis. In: Bush A et al, eds. Cystic Fibrosis in the 21st century. Vol. 34. Basel: Karger; 2006:166-172.
52. Bjarnsholt T, Givskov M. The role of quorum sensing in the pathogenicity of the cunning aggressor Pseudomonas aeruginosa. Anal Bioanal Chem. 2006;387:409-414.
53. De Boeck K. Improving standards of clinical care in cystic fibrosis. Eur Respir J. 2000;16:585-587.
54. Kappler M, Kraxner A, Reinhardt D, Ganster B, Griese M, Lang T. Diagnostic and prognostic value of serum antibodies against Pseudomonas aeruginosa in cystic fibrosis. Thorax. 2006;61:684-688.
55. Hoiby N, Flensborg EW, Beck B, Friis B, Jacobsen SV, Jacobsen L. Pseudomonas aeruginosa infection in cystic fibrosis. Diagnostic and prognostic significance of Pseudomonas aeruginosa precipitins determined by means of crossed immunoelectrophoresis. Scand J Respir Dis. 1977;58:65-79.
56. Burns JL, Gibson RL, McNamara S, et al. Longitudinal assessment of Pseudomonas aeruginosa in young children with cystic fibrosis. J Infect Dis. 2001;183:444-452.
57. Cabral DA, Loh BA, Speert DP. Mucoid Pseudomonas aeruginosa resists nonopsonic phagocytosis by human neutrophils and macrophages. Pediatr Res. 1987;22:429-431.

58. Pedersen SS, Kharazmi A, Espersen F, Hoiby N. Pseudomonas aeruginosa alginate in cystic fibrosis sputum and the inflammatory response. Infect Immun. 1990;58:3363-3368.
59. Baker NR, Svanborg-Eden C. Role of alginate in the adherence of Pseudomonas aeruginosa. Antibiot Chemother. 1989;42:72-79.
60. Leid JG, Willson CJ, Shirtliff ME, Hassett DJ, Parsek MR, Jeffers AK. The exopolysaccharide alginate protects Pseudomonas aeruginosa biofilm bacteria from IFN-gamma-mediated macrophage killing. J Immunol. 2005;175:7512-7518.
61. Hentzer M, Teitzel GM, Balzer GJ, et al. Alginate overproduction affects Pseudomonas aeruginosa biofilm structure and function. J Bacteriol. 2001;183:5395-5401.
62. Stapper AP, Narasimhan G, Ohman DE, et al. Alginate production affects Pseudomonas aeruginosa biofilm development and architecture, but is not essential for biofilm formation. J Med Microbiol. 2004;53:679-690.
63. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science. 1999;284:1318-1322.
64. Davies DG, Parsek MR, Pearson JP, Iglewski BH, Costerton JW, Greenberg EP. The involvement of cell-to-cell signals in the development of a bacterial biofilm. Science. 1998;280:295-298.
65. Singh PK, Schaefer AL, Parsek MR, Moninger TO, Welsh MJ, Greenberg EP. Quorum-sensing signals indicate that cystic fibrosis lungs are infected with bacterial biofilms. Nature. 2000;407:762-764.
66. Bjarnsholt T, Jensen PO, Rasmussen TB, et al. Garlic blocks quorum sensing and promotes rapid clearing of pulmonary Pseudomonas aeruginosa infections. Microbiology. 2005;151:3873-3880.
67. Camilli A, Bassler BL. Bacterial small-molecule signaling pathways. Science. 2006;311:1113-1116.
68. Mattick JS. Type IV pili and twitching motility. Annu Rev Microbiol. 2002;56:289-314.
69. DiMango E, Zar HJ, Bryan R, Prince A. Diverse Pseudomonas aeruginosa gene products stimulate respiratory epithelial cells to produce interleukin-8. J Clin Invest. 1995;96:2204-2210.
70. Prince A. Flagellar activation of epithelial signaling. Am J Respir Cell Mol Biol. 2006;34:548-551.
71. Kiska DL, Gilligan PH. Pseudomonas. In: Murray PR, ed. Manual of Clinical Microbiology. Vol. 1 (8). Washington DC: ASM Press; 2003:719-728.
72. Lau GW, Hassett DJ, Ran H, Kong F. The role of pyocyanin in Pseudomonas aeruginosa infection. Trends Mol Med. 2004;10:599-606.
73. Lau GW, Ran H, Kong F, Hassett DJ, Mavrodi D. Pseudomonas aeruginosa pyocyanin is critical for lung infection in mice. Infect Immun. 2004;72:4275-4278.
74. Kong F, Young L, Chen Y, et al. Pseudomonas aeruginosa pyocyanin inactivates lung epithelial vacuolar ATPase-dependent cystic fibrosis transmembrane conductance regulator expression and localization. Cell Microbiol. 2006;8:1121-1133.
75. Denning GM, Wollenweber LA, Railsback MA, Cox CD, Stoll LL, Britigan BE. Pseudomonas pyocyanin increases interleukin-8 expression by human airway epithelial cells. Infect Immun. 1998;66:5777-5784.
76. Slieker MG, Sanders EA, Rijkers GT, Ruven HJ, van der Ent CK. Disease modifying genes in cystic fibrosis. J Cyst Fibros. 2005;4 Suppl 2:7-13.
77. Davies JC. Modifier genes in cystic fibrosis. Pediatr Pulmonol Suppl. 2004;26:86-87.

78. Cutting GR. Modifier genetics: cystic fibrosis. Annu Rev Genomics Hum Genet. 2005;6:237-260.
79. Drumm ML, Konstan MW, Schluchter MD, et al. Genetic modifiers of lung disease in cystic fibrosis. N Engl J Med. 2005;353:1443-1453.
80. Wright JM, Merlo CA, Reynolds JB, et al. Respiratory epithelial gene expression in patients with mild and severe cystic fibrosis lung disease. Am J Respir Cell Mol Biol. 2006;35:327-336.
81. Rosenfeld M, Emerson J, Williams-Warren J, et al. Defining a pulmonary exacerbation in cystic fibrosis. J Pediatr. 2001;139:359-365.
82. Garside JP, Kerrin DP, Brownlee KG, Gooi HC, Taylor JM, Conway SP. Immunoglobulin and IgG subclass levels in a regional pediatric cystic fibrosis clinic. Pediatr Pulmonol. 2005;39:135-140.
83. Jones MM, Seilheimer DK, Pollack MS, Curry M, Crane MM, Rossen RD. Relationship of hypergammaglobulinemia, circulating immune complexes, and histocompatibility antigen profiles in patients with cystic fibrosis. Am Rev Respir Dis. 1989;140:1636-1639.
84. Pressler T, Mansa B, Jensen T, Pedersen SS, Hoiby N, Koch C. Increased IgG2 and IgG3 concentration is associated with advanced Pseudomonas aeruginosa infection and poor pulmonary function in cystic fibrosis. Acta Paediatr Scand. 1988;77:576-582.
85. Smith JA, Owen EC, Jones AM, Dodd ME, Webb AK, Woodcock A. Objective measurement of cough during pulmonary exacerbations in adults with cystic fibrosis. Thorax. 2006;61:425-429.
86. Aaron SD, Ramotar K, Ferris W, et al. Adult cystic fibrosis exacerbations and new strains of Pseudomonas aeruginosa. Am J Respir Crit Care Med. 2004;169:811-815.
87. Ratjen F, Doring G. Cystic fibrosis. Lancet. 2003;361:681-689.
88. Westaby D. Cystic fibrosis: Liver disease. In: Bush A et al, eds. Cystic Fibrosis in the 21st century. Vol. 34. Basel: Karger; 2006:251-259.
89. Finnegan MJ, Hinchcliffe J, Russell-Jones D, et al. Vasculitis complicating cystic fibrosis. Q J Med. 1989;72:609-621.
90. Hodson ME. Vasculitis and arthropathy in cystic fibrosis. J R Soc Med. 1992;85 Suppl 19:38-40.
91. Bourke S, Rooney M, Fitzgerald M, Bresnihan B. Episodic arthropathy in adult cystic fibrosis. Q J Med. 1987;64:651-659.
92. Janeway C, Travers P, Walport M, Shlomchik M.Immunobiology (6). New York: Garland Science Publishing; 2005.
93. Sheoenfeld Y, Gershwin ME, Meroni PL.Autoantibodies (Second edition). Oxford: Elsevier; 2007.
94. Bach JF. Infections and autoimmune diseases. J Autoimmun. 2005;25 Suppl:74-80.
95. Guilherme L, Kalil J. Rheumatic fever: from sore throat to autoimmune heart lesions. Int Arch Allergy Immunol. 2004;134:56-64.
96. Caporale CM, Papola F, Fioroni MA, et al. Susceptibility to Guillain-Barre syndrome is associated to polymorphisms of CD1 genes. J Neuroimmunol. 2006;177:112-118.
97. Rashid T, Ebringer A. Ankylosing spondylitis is linked to Klebsiella-the evidence. Clin Rheumatol. 2006.
98. Rasmussen N, Sjolin C, Isaksson B, Bygren P, Wieslander J. An ELISA for the detection of anti-neutrophil cytoplasm antibodies (ANCA). J Immunol Methods. 1990;127:139-145.

99. Segelmark M, Westman K, Wieslander J. How and why should we detect ANCA? Clin Exp Rheumatol. 2000;18:629-635.

100. Cooper T, Savige J, Nassis L, et al. Clinical associations and characterisation of antineutrophil cytoplasmic antibodies directed against bactericidal/permeability-increasing protein and azurocidin. Rheumatol Int. 2000;19:129-136.

101. Yang JJ, Tuttle R, Falk RJ, Jennette JC. Frequency of anti-bactericidal/permeability-increasing protein (BPI) and anti-azurocidin in patients with renal disease. Clin Exp Immunol. 1996;105:125-131.

102. Davies DJ, Moran JE, Niall JF, Ryan GB. Segmental necrotising glomerulonephritis with antineutrophil antibody: possible arbovirus aetiology? Br Med J (Clin Res Ed). 1982;285:606.

103. Faber V, Elling P, Norup G, Mansa B, Nissen NI. An Antinuclear Factor Specific for Leucocytes. Lancet. 1964;14:344-345.

104. van der Woude FJ, Rasmussen N, Lobatto S, et al. Autoantibodies against neutrophils and monocytes: tool for diagnosis and marker of disease activity in Wegener's granulomatosis. Lancet. 1985;1:425-429.

105. Walmsley RS, Zhao MH, Hamilton MI, et al. Antineutrophil cytoplasm autoantibodies against bactericidal/permeability-increasing protein in inflammatory bowel disease. Gut. 1997;40:105-109.

106. Elzouki AN, Eriksson S, Lofberg R, Nassberger L, Wieslander J, Lindgren S. The prevalence and clinical significance of alpha 1-antitrypsin deficiency (PiZ) and ANCA specificities (proteinase 3, BPI) in patients with ulcerative colitis. Inflamm Bowel Dis. 1999;5:246-252.

107. Schultz H, Csernok E, Herlyn K, et al. ANCA against bactericidal/permeability-increasing protein, azurocidin, calprotectin and defensins in rheumatic and infectious diseases: prevalence and clinical associations. Clin Exp Rheumatol. 2003;21:S117-120.

108. Stoffel MP, Csernok E, Herzberg C, Johnson T, Carroll SF, Gross WL. Anti-neutrophil cytoplasmic antibodies (ANCA) directed against bactericidal/permeability increasing protein (BPI): a new seromarker for inflammatory bowel disease and associated disorders. Clin Exp Immunol. 1996;104:54-59.

109. Hertervig E, Wieslander J, Johansson C, Wiik A, Nilsson A. Anti-neutrophil cytoplasmic antibodies in chronic inflammatory bowel disease. Prevalence and diagnostic role. Scand J Gastroenterol. 1995;30:693-698.

110. Heeringa P, Huugen D, Tervaert JW. Anti-neutrophil cytoplasmic autoantibodies and leukocyte-endothelial interactions: a sticky connection? Trends Immunol. 2005;26:561-564.

111. Jennette JC, Xiao H, Falk RJ. Pathogenesis of vascular inflammation by anti-neutrophil cytoplasmic antibodies. J Am Soc Nephrol. 2006;17:1235-1242.

112. Weiss J, Elsbach P, Olsson I, Odeberg H. Purification and characterization of a potent bactericidal and membrane active protein from the granules of human polymorphonuclear leukocytes. J Biol Chem. 1978;253:2664-2672.

113. Weiss J, Olsson I. Cellular and subcellular localization of the bactericidal/permeability-increasing protein of neutrophils. Blood. 1987;69:652-659.

114. Lennartsson A. Transcriptional control of bactericidal/permeability increasing protein (BPI) and cathepsin G in myeloid cells (dissertation). Department of Haematology, Lund University 2004.

115. Schultz H, Weiss J, Carroll SF, Gross WL. The endotoxin-binding bactericidal/permeability-increasing protein (BPI): a target antigen of autoantibodies. J Leukoc Biol. 2001;69:505-512.

116. Weiss J, Elsbach P, Shu C, et al. Human bactericidal/permeability-increasing protein and a recombinant NH2-terminal fragment cause killing of serum-resistant gram-negative bacteria in whole blood and inhibit tumor necrosis factor release induced by the bacteria. J Clin Invest. 1992;90:1122-1130.
117. Iovine NM, Elsbach P, Weiss J. An opsonic function of the neutrophil bactericidal/permeability-increasing protein depends on both its N- and C-terminal domains. Proc Natl Acad Sci U S A. 1997;94:10973-10978.
118. Lennartsson A, Pieters K, Vidovic K, Gullberg U. A murine antibacterial ortholog to human bactericidal/permeability-increasing protein (BPI) is expressed in testis, epididymis, and bone marrow. J Leukoc Biol. 2005;77:369-377.
119. Elsbach P, Weiss J. Role of the bactericidal/permeability-increasing protein in host defence. Curr Opin Immunol. 1998;10:45-49.
120. Canny G, Cario E, Lennartsson A, et al. Functional and biochemical characterization of epithelial bactericidal/permeability-increasing protein. Am J Physiol Gastrointest Liver Physiol. 2006;290:G557-567.
121. Canny G, Levy O, Furuta GT, et al. Lipid mediator-induced expression of bactericidal/permeability-increasing protein (BPI) in human mucosal epithelia. Proc Natl Acad Sci U S A. 2002;99:3902-3907.
122. Canny GO, Trifonova RT, Kindelberger DW, Colgan SP, Fichorova RN. Expression and function of bactericidal/permeability-increasing protein in human genital tract epithelial cells. J Infect Dis. 2006;194:498-502.
123. Levy O, Canny G, Serhan CN, Colgan SP. Expression of BPI (bactericidal/permeability-increasing protein) in human mucosal epithelia. Biochem Soc Trans. 2003;31:795-800.
124. Reichel PH, Seemann C, Csernok E, et al. Bactericidal/permeability-increasing protein is expressed by human dermal fibroblasts and upregulated by interleukin 4. Clin Diagn Lab Immunol. 2003;10:473-475.
125. Aichele D, Schnare M, Saake M, Rollinghoff M, Gessner A. Expression and antimicrobial function of bactericidal permeability-increasing protein in cystic fibrosis patients. Infect Immun. 2006;74:4708-4714.
126. Charles LA, Caldas ML, Falk RJ, Terrell RS, Jennette JC. Antibodies against granule proteins activate neutrophils in vitro. J Leukoc Biol. 1991;50:539-546.
127. Pereira HA, Spitznagel JK, Winton EF, et al. The ontogeny of a 57-Kd cationic antimicrobial protein of human polymorphonuclear leukocytes: localization to a novel granule population. Blood. 1990;76:825-834.
128. Zhao MH, Jones SJ, Lockwood CM. Bactericidal/permeability-increasing protein (BPI) is an important antigen for anti-neutrophil cytoplasmic autoantibodies (ANCA) in vasculitis. Clin Exp Immunol. 1995;99:49-56.
129. Mahadeva R, Zhao MH, Stewart S, et al. Vasculitis and bronchiectasis in a patient with antibodies to bactericidal/permeability-increasing protein and alpha1-antitrypsin deficiency. Chest. 1997;112:1699-1701.
130. Matsuyama W, Wakimoto J, Watanabe A, et al. Bronchiectasis with myeloperoxidase antineutrophil cytoplasmic antibody and bactericidal/permeability-increasing protein antineutrophil cytoplasmic antibody. Intern Med. 1999;38:813-816.
131. Kobayashi O. Clinical role of autoantibody against bactericidal/permeability increasing protein in chronic airway infection. J Infect Chemother. 1998;4:83-93.
132. Ohtami S, Kobayashi O, Ohtami H. Analysis of intractable factors in chronic airway infections: role of the autoimmunity induced by BPI-ANCA. J Infect Chemother. 2001;7:228-238.

133. Efthimiou J, Spickett G, Lane D, Thompson A. Antineutrophil cytoplasmic antibodies, cystic fibrosis, and infection. Lancet. 1991;337:1037-1038.

134. Zhao MH, Jayne DR, Ardiles LG, Culley F, Hodson ME, Lockwood CM. Autoantibodies against bactericidal/permeability-increasing protein in patients with cystic fibrosis. Qjm. 1996;89:259-265.

135. Aebi C, Theiler F, Aebischer CC, Schoeni MH. Autoantibodies directed against bactericidal/permeability-increasing protein in patients with cystic fibrosis: association with microbial respiratory tract colonization. Pediatr Infect Dis J. 2000;19:207-212.

136. Carlsson M, Eriksson L, Erwander I, Wieslander J, Segelmark M. Pseudomonas-induced lung damage in cystic fibrosis correlates to bactericidal-permeability increasing protein (BPI)-autoantibodies. Clin Exp Rheumatol. 2003;21:S95-100.

137. Carlsson M, Eriksson L, Pressler T, et al. Autoantibody response to BPI predict disease severity and outcome in cystic fibrosis. J Cyst Fibros. doi:10.1016/j.jcf.2006.10.005

138. Dorlochter L, Carlsson M, Olafsdottir EJ, Roksund OD, Rosendahl K, Fluge G. Anti-neutrophil cytoplasmatic antibodies and lung disease in cystic fibrosis. J Cyst Fibros. 2004;3:179-183.

139. Mahadeva R, Dunn AC, Westerbeek RC, et al. Anti-neutrophil cytoplasmic antibodies (ANCA) against bactericidal/permeability-increasing protein (BPI) and cystic fibrosis lung disease. Clin Exp Immunol. 1999;117:561-567.

140. Rotschild M, Elias N, Berkowitz D, et al. Autoantibodies against bactericidal/ permeability-increasing protein (BPI-ANCA) in cystic fibrosis patients treated with azithromycin. Clin Exp Med. 2005;5:80-85.

141. Schultz H, Csernok E, Schuster A, Schmitz TS, Ernst M, Gross WL. Anti-neutrophil cytoplasmic antibodies directed against the bactericidal/permeability-increasing protein (BPI) in pediatric cystic fibrosis patients do not recognize N-terminal regions important for the anti-microbial and lipopolysaccharide-binding activity of BPI. Pediatr Allergy Immunol. 2000;11:64-70.

142. Schultz H, Schinke S, Mosler K, Herlyn K, Schuster A, Gross WL. BPI-ANCA of pediatric cystic fibrosis patients can impair BPI-mediated killing of E. coli DH5alpha in vitro. Pediatr Pulmonol. 2004;37:158-164.

143. Sediva A, Bartunkova J, Bartosova J, Jennette C, Falk RJ, Jethwa HS. Antineutrophil cytoplasmic antibodies directed against bactericidal/permeability-increasing protein detected in children with cystic fibrosis inhibit neutrophil-mediated killing of Pseudomonas aeruginosa. Microbes Infect. 2003;5:27-30.

144. Sediva A, Bartunkova J, Kolarova I, et al. Antineutrophil cytoplasmic autoantibodies (ANCA) in children with cystic fibrosis. J Autoimmun. 1998;11:185-190.

145. Schinke S, Fellermann K, Herlyn K, et al. Autoantibodies against the bactericidal/ permeability-increasing protein from inflammatory bowel disease patients can impair the antibiotic activity of bactericidal/permeability-increasing protein. Inflamm Bowel Dis. 2004;10:763-770.

146. Sjoholm AG, Jonsson G, Braconier JH, Sturfelt G, Truedsson L. Complement deficiency and disease: an update. Mol Immunol. 2006;43:78-85.

147. Matsushita M, Fujita T. Activation of the classical complement pathway by mannose-binding protein in association with a novel C1s-like serine protease. J Exp Med. 1992;176:1497-1502.

148. Sorensen R, Thiel S, Jensenius JC. Mannan-binding-lectin-associated serine proteases, characteristics and disease associations. Springer Semin Immunopathol. 2005;27:299-319.

149. Sjöholm AG. Deficiencies of the mannose-binding lectin, the alternative pathway, and the late complement components. In: Rose N et al., eds. Manual of Clinical Laboratory Immunology. Washington DC: ASM Press; 2002:847-854.

150. Garred P, Larsen F, Seyfarth J, Fujita R, Madsen HO. Mannose-binding lectin and its genetic variants. Genes Immun. 2006;7:85-94.

151. Turner MW. Mannose-binding lectin: the pluripotent molecule of the innate immune system. Immunol Today. 1996;17:532-540.

152. Davies J, Neth O, Alton E, Klein N, Turner M. Differential binding of mannose-binding lectin to respiratory pathogens in cystic fibrosis. Lancet. 2000;355:1885-1886.

153. Neth O, Jack DL, Dodds AW, Holzel H, Klein NJ, Turner MW. Mannose-binding lectin binds to a range of clinically relevant microorganisms and promotes complement deposition. Infect Immun. 2000;68:688-693.

154. Madsen HO, Garred P, Thiel S, et al. Interplay between promoter and structural gene variants control basal serum level of mannan-binding protein. J Immunol. 1995;155:3013-3020.

155. Madsen HO, Satz ML, Hogh B, Svejgaard A, Garred P. Different molecular events result in low protein levels of mannan-binding lectin in populations from southeast Africa and South America. J Immunol. 1998;161:3169-3175.

156. Carlsson M, Sjöholm AG, Eriksson L, et al. Deficiency of the mannan-binding lectin pathway of complement and poor outcome in cystic fibrosis: bacterial colonization may be decisive for a relationship. Clin Exp Immunol. 2005;139:306-313.

157. Eisen DP, Dean MM, Thomas P, et al. Low mannose-binding lectin function is associated with sepsis in adult patients. FEMS Immunol Med Microbiol. 2006;48:274-282.

158. Gordon AC, Waheed U, Hansen TK, et al. Mannose-binding lectin polymorphisms in severe sepsis: relationship to levels, incidence, and outcome. Shock. 2006;25:88-93.

159. Hamvas RM, Johnson M, Vlieger AM, et al. Role for mannose binding lectin in the prevention of Mycoplasma infection. Infect Immun. 2005;73:5238-5240.

160. Ip WK, Chan KH, Law HK, et al. Mannose-binding lectin in severe acute respiratory syndrome coronavirus infection. J Infect Dis. 2005;191:1697-1704.

161. Dahl M, Tybjaerg-Hansen A, Schnohr P, Nordestgaard BG. A population-based study of morbidity and mortality in mannose-binding lectin deficiency. J Exp Med. 2004;199:1391-1399.

162. Summerfield JA, Sumiya M, Levin M, Turner MW. Association of mutations in mannose binding protein gene with childhood infection in consecutive hospital series. Bmj. 1997;314:1229-1232.

163. Koch A, Melbye M, Sorensen P, et al. Acute respiratory tract infections and mannose-binding lectin insufficiency during early childhood. Jama. 2001;285:1316-1321.

164. Peterslund NA, Koch C, Jensenius JC, Thiel S. Association between deficiency of mannose-binding lectin and severe infections after chemotherapy. Lancet. 2001;358:637-638.

165. Neth O, Hann I, Turner MW, Klein NJ. Deficiency of mannose-binding lectin and burden of infection in children with malignancy: a prospective study. Lancet. 2001;358:614-618.

166. Bergmann OJ, Christiansen M, Laursen I, et al. Low levels of mannose-binding lectin do not affect occurrence of severe infections or duration of fever in acute myeloid leukaemia during remission induction therapy. Eur J Haematol. 2003;70:91-97.

167. Kilpatrick DC, McLintock LA, Allan EK, et al. No strong relationship between mannan binding lectin or plasma ficolins and chemotherapy-related infections. Clin Exp Immunol. 2003;134:279-284.

168. Davies EJ, Teh LS, Ordi-Ros J, et al. A dysfunctional allele of the mannose binding protein gene associates with systemic lupus erythematosus in a Spanish population. J Rheumatol. 1997;24:485-488.

169. Lee YH, Witte T, Momot T, et al. The mannose-binding lectin gene polymorphisms and systemic lupus erythematosus: two case-control studies and a meta-analysis. Arthritis Rheum. 2005;52:3966-3974.

170. Horiuchi T, Tsukamoto H, Morita C, et al. Mannose binding lectin (MBL) gene mutation is not a risk factor for systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) in Japanese. Genes Immun. 2000;1:464-466.

171. Garred P, Voss A, Madsen HO, Junker P. Association of mannose-binding lectin gene variation with disease severity and infections in a population-based cohort of systemic lupus erythematosus patients. Genes Immun. 2001;2:442-450.

172. Jönsen A. Studies on neuropsychiatric manifestations and genetic factors in systemic lupus erythematosus (dissertation). Department of Rheumathology, Lund University 2006.

173. Barton A, Platt H, Salway F, et al. Polymorphisms in the mannose binding lectin (MBL) gene are not associated with radiographic erosions in rheumatoid or inflammatory polyarthritis. J Rheumatol. 2004;31:442-447.

174. Graudal NA, Garred P, Madsen HO, et al. Variant mannose-binding lectin genotypes and outcome in early versus late rheumatoid arthritis: comment on the article by Ip et al. Arthritis Rheum. 2002;46:555-556.

175. Garred P, Pressler T, Madsen HO, et al. Association of mannose-binding lectin gene heterogeneity with severity of lung disease and survival in cystic fibrosis. J Clin Invest. 1999;104:431-437.

176. Garred P, Pressler T, Lanng S, et al. Mannose-binding lectin (MBL) therapy in an MBL-deficient patient with severe cystic fibrosis lung disease. Pediatr Pulmonol. 2002;33:201-207.

177. Gabolde M, Guilloud-Bataille M, Feingold J, Besmond C. Association of variant alleles of mannose binding lectin with severity of pulmonary disease in cystic fibrosis: cohort study. BMJ. 1999;319:1166-1167.

178. Yarden J, Radojkovic D, De Boeck K, et al. Polymorphisms in the mannose binding lectin gene affect the cystic fibrosis pulmonary phenotype. J Med Genet. 2004;41:629-633.

179. Davies JC, Turner MW, Klein N. Impaired pulmonary status in cystic fibrosis adults with two mutated MBL-2 alleles. Eur Respir J. 2004;24:798-804.

180. Muhlebach MS, MacDonald SL, Button B, et al. Association between mannan-binding lectin and impaired lung function in cystic fibrosis may be age-dependent. Clin Exp Immunol. 2006;145:302-307.

181. Olesen HV, Jensenius JC, Steffensen R, Thiel S, Schiotz PO. The mannan-binding lectin pathway and lung disease in cystic fibrosis-dysfunction of mannan-binding lectin-associated serine protease 2 (MASP-2) may be a major modifier. Clin Immunol. 2006;121:324-331.

182. Ezekowitz RA, Day LE, Herman GA. A human mannose-binding protein is an acute-phase reactant that shares sequence homology with other vertebrate lectins. J Exp Med. 1988;167:1034-1046.

183. Thiel S, Holmskov U, Hviid L, Laursen SB, Jensenius JC. The concentration of the C-type lectin, mannan-binding protein, in human plasma increases during an acute phase response. Clin Exp Immunol. 1992;90:31-35.

184. Skattum L, Akesson P, Truedsson L, Sjoholm AG. Antibodies against four proteins from a Streptococcus pyogenes serotype M1 strain and levels of circulating mannan-binding lectin in acute poststreptococcal glomerulonephritis. Int Arch Allergy Immunol. 2006;140:9-19.

185. Uemura K, Saka M, Nakagawa T, et al. L-MBP is expressed in epithelial cells of mouse small intestine. J Immunol. 2002;169:6945-6950.

186. Seyfarth J, Garred P, Madsen HO. Extra-hepatic transcription of the human mannose-binding lectin gene (mbl2) and the MBL-associated serine protease 1-3 genes. Mol Immunol. 2006;43:962-971.

187. van de Geijn FE, Roos A, de Man YA, et al. Mannose-binding lectin levels during pregnancy: a longitudinal study. Hum Reprod. 2006.

188. Stengaard-Pedersen K, Thiel S, Gadjeva M, et al. Inherited deficiency of mannan-binding lectin-associated serine protease 2. N Engl J Med. 2003;349:554-560.

189. Standardization of Spirometry, 1994 Update. American Thoracic Society. Am J Respir Crit Care Med. 1995;152:1107-1136.

190. Aurora P, Wade A, Whitmore P, Whitehead B. A model for predicting life expectancy of children with cystic fibrosis. Eur Respir J. 2000;16:1056-1060.

191. Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. Eur Respir J Suppl. 1993;16:5-40.

192. Solymar L, Aronsson PH, Bake B, Bjure J. Nitrogen single breath test, flow-volume curves and spirometry in healthy children, 7-18 years of age. Eur J Respir Dis. 1980;61:275-286.

193. Sjöholm AG, Truedsson L, Jensenius JC. Complement pathways and meningicoccal disease- diagnostic aspects. In: Pollard AJ, Maiden MCJ, eds. Methods in Molecular Medicine. Vol. 67. Totowa: Humana Press INC; 2001:529-547.

194. Roos A, Bouwman LH, Munoz J, et al. Functional characterization of the lectin pathway of complement in human serum. Mol Immunol. 2003;39:655-668.

195. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988;16:1215.

196. Ahmadian A, Gharizadeh B, Gustafsson AC, et al. Single-nucleotide polymorphism analysis by pyrosequencing. Anal Biochem. 2000;280:103-110.

197. Boniotto M, Radillo O, Braida L, et al. Detection of MBL-2 gene expression in intestinal biopsies of celiac patients by in situ reverse transcription polymerase chain reaction. Eur J Histochem. 2003;47:177-180.

198. Beveridge TJ. Structures of gram-negative cell walls and their derived membrane vesicles. J Bacteriol. 1999;181:4725-4733.

199. Blander JM, Medzhitov R. Toll-dependent selection of microbial antigens for presentation by dendritic cells. Nature. 2006;440:808-812.

200. Schultz H. From infection to autoimmunity: A new model for induction of ANCA against the bactericidal/permeability increasing protein (BPI). Autoimmunity Reviews. doi: 10.1016/j.autrev.2006.08.005

201. Davies JC, Griesenbach U, Alton E. Modifier genes in cystic fibrosis. Pediatr Pulmonol. 2005;39:383-391.

202. Buranawuti K, Boyle MP, Cheng S, et al. Variants in mannose-binding lectin and tumor necrosis factor alpha affect survival in cystic fibrosis. Journal of Medical Genetics. 2007.

203. Arkwright PD, Pravica V, Geraghty PJ, et al. End-organ dysfunction in cystic fibrosis: association with angiotensin I converting enzyme and cytokine gene polymorphisms. Am J Respir Crit Care Med. 2003;167:384-389.

204. Brazova J, Sismova K, Vavrova V, et al. Polymorphisms of TGF-beta1 in cystic fibrosis patients. Clin Immunol. 2006;121:350-357.

205. Hull J, Thomson AH. Contribution of genetic factors other than CFTR to disease severity in cystic fibrosis. Thorax. 1998;53:1018-1021.

206. Yarden J, Radojkovic D, De Boeck K, et al. Association of tumour necrosis factor alpha variants with the CF pulmonary phenotype. Thorax. 2005;60:320-325.

207. Schmitt-Grohe S, Stuber F, Book M, et al. TNF-alpha promoter polymorphism in relation to TNF-alpha production and clinical status in cystic fibrosis. Lung. 2006;184:99-104.

208. Doring G, Krogh-Johansen H, Weidinger S, Hoiby N. Allotypes of alpha 1-antitrypsin in patients with cystic fibrosis, homozygous and heterozygous for deltaF508. Pediatr Pulmonol. 1994;18:3-7.

209. Mahadeva R, Westerbeek RC, Perry DJ, et al. Alpha1-antitrypsin deficiency alleles and the Taq-I G-->A allele in cystic fibrosis lung disease. Eur Respir J. 1998;11:873-879.

210. Frangolias DD, Ruan J, Wilcox PJ, et al. Alpha 1-antitrypsin deficiency alleles in cystic fibrosis lung disease. Am J Respir Cell Mol Biol. 2003;29:390-396.

211. Grasemann H, Storm van's Gravesande K, Gartig S, et al. Nasal nitric oxide levels in cystic fibrosis patients are associated with a neuronal NO synthase (NOS1) gene polymorphism. Nitric Oxide. 2002;6:236-241.

212. Texereau J, Marullo S, Hubert D, et al. Nitric oxide synthase 1 as a potential modifier gene of decline in lung function in patients with cystic fibrosis. Thorax. 2004;59:156-158.

213. Buscher R, Eilmes KJ, Grasemann H, et al. beta2 adrenoceptor gene polymorphisms in cystic fibrosis lung disease. Pharmacogenetics. 2002;12:347-353.