chapter 1

General introduction

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1. Introduction

Since 1796, the year Dr. Edward Jenner observed that milkmaids infected by cowpox were relatively impervious to the very dangerous smallpox and performed his famous vaccination experiments on his gardeners 8-year old son (figure 1), medical research has yielded an impressive amount of knowledge on the regulation of human defense mechanisms against infection. The inflammation that results from the immune response to a foreign pathogen, and forms an intricate part of this defense mechanism, was already defined by Aulus Cornelius Celsus in the year 25 A.D. with its four classic signs: rubor, dolor, calor, tumor (figure 2).

Nowadays the term ‘inflammation’ is used more widely to describe the body’s reaction to damage, including many diverse events associated with this reaction, ranging from phagocytosis of invading pathogens to joint and gut damage resulting from immune cells that are out of control.

Modern molecular biology has provided us with tools that enhance our insight into the regulation of many inflammatory processes. The use of genetically modified mice in experimental models of inflammatory- (e.g. Crohn’s disease) and infectious- (e.g. pneumococcal pneumonia) diseases, has shed light on the pivotal role of intercellular mediators of inflammation. Especially pro- and anti-inflammatory cytokines, and their receptors, but also chemokines and coagulation factors were shown to be involved during inflammation; the sheer number of participants involved in regulating the inflammatory process amounts to a complex picture. Targeting these factors has become a popular therapeutic angle for modulating inflammation. This is exemplified by the successful use of Tumor Necrosis Factor (TNF)-blocking antibodies in patients with Crohn’s disease. However, medication side effects, disease
relapse and enhanced susceptibility to infectious disease remain unsurpassable problems, and thus this strategy still has many drawbacks.

Surprisingly, many of the signals that are exchanged between inflammatory cells, use similar intracellular pathways in order to relay their signal to the nucleus and evoke a cell response. These signal transduction pathways mediate gene expression and immune functions. Recently, a group of sequentially activated protein kinases, the Mitogen Activated Protein Kinase (MAPK) family, was found to be involved in the majority of inflammatory processes. By targeting elements of this intracellular signal transduction pathway family, stress signals may be inhibited, thus modulating inflammation. Conversely, the role of a particular signal transduction element may be further explored by inhibiting this element in an inflammatory model or disease.

Although much has been learned about MAPKs from in vitro cell systems, little is known about their role in inflammation in vivo. In this introduction possible intracellular targets for modulating several inflammatory disease states will be discussed: low-grade endotoxemia, a model for acute systemic inflammation, Crohn’s disease, a chronic inflammatory disease, and pneumococcal pneumonia, an acute inflammatory infectious disease.

2. The Mitogen Activated Protein Kinase pathways

Members of the MAPK family form evolutionary well conserved cascades of signaling proteins that are found in all eukaryotes. These cascades couple signals from the extracellular environment to the appropriate cellular response. The typical MAPK cassette consists of three MAPK family members that activate each other consecutively. At the base of the cascade is an executor MAPK, which is activated upon phosphorylation of both a threonine and tyrosine residue by a MAPK kinase (M KK). M KK is in turn activated by an upstream M KK kinase (M KKK). Once activated, the executor MAPKs can phosphorylate and activate other kinases or for example transcription factors (figure 3). The influence of MAPKs on transcription factors couples the extracellular environment to the gene expression profile of the cell and thus enables the cell to adapt its transcriptional makeup to clues from its surroundings. Another important example of a cellular process that is regulated by MAPKs is protein
There are at least three distinct MAPK signaling modules: the p38 MAPK, p42/44 MAPK and c-Jun N-terminal Kinase (JNK) pathway. These MAPKs largely control the cell’s response to inflammation and stress.

2.1. p38 Mitogen Activated Protein Kinase

P38 MAPK, the mammalian homologue of the yeast HOG (high osmolarity glycerol response) kinase, is ubiquitously expressed throughout the human body. The four known p38 MAPK genes encode four different 38 kDa signal transduction proteins: p38α, β, γ and δ. Each homologue is differentially expressed and has distinct, although often overlapping, functions. Deficiency of p38α can result in embryonic lethality; it plays a critical role in developmental erythropoiesis through regulation of erythropoietin expression. P38 MAPK was first described as a protein that was phosphorylated in response to lipopolysaccharide (LPS). Independently, p38 MAPK was found to be the target of a novel class of medication, the cytokine-suppressive anti-inflammatory drugs. Besides LPS, many other cellular stresses (e.g. UV, osmotic shock) and inflammatory stimuli (TNF, IL-1β, PMA) activate p38 MAPK, via upstream kinases MKK3/4/6. These kinases phosphorylate p38 MAPK at threonine 180 and tyrosine 182 in the TGY motif, resulting in p38 MAPK activation, leading to phosphorylation of downstream transcription factors, e.g. ATF-2, and kinases such as MAPKAPK-2. Inhibition of p38 MAPK, for example with the commonly used SB203580, reduces pro-inflammatory cytokine production in monocytes/macrophages, neutrophils and T-lymphocytes. Furthermore, p38 MAPK is involved in various other inflammation related events, such as neutrophil activation, apoptosis, and NO synthase induction. Hence, p38 MAPK is thought to be a prime candidate for new anti-inflammatory/anti-cytokine therapies. In this thesis, we investigate whether p38 MAPK is actually involved in inflammation in vivo, and whether p38 MAPK is a valid target for anti-inflammatory therapy in humans (chapters 2, 3 and 6).
Figure 3. The p42/44 MAPK, JNK and p38 MAPK pathway. The activity of the MAPK pathways is controlled in a three-tiered cassette that consists of a MAPK, which is activated upon phosphorylation of both a threonine and tyrosine residue by a MAPK kinase (MKK). MKK is in turn activated by an upstream MKK kinase (MKKK). Once activated, MAPKs can phosphorylate and activate other kinases or transcription factors.
Chapter 1

2.2. p42/44 Mitogen Activated Protein Kinase

The second MAPK cascade terminates at p42/44 MAPK. This MAPK consist of two isoforms transcribed from the same gene, p44 MAPK (or Extracellular Regulated Kinase (ERK)-1) and p42 MAPK (or ERK-2). These kinases are activated by mitogenic stimuli such as growth factors (EGF, PDGF), phorbol esters and insulin\(^{17,18}\). Typically, upon binding of the mitogen to its receptor the signal is transduced into the cell via sequential activation of the small GTPase Ras, Raf, MEK1/2 (MAPK or ERK Kinase, a MKK). Activated p42/44 MAPK in turn phosphorylates downstream transcription factors such as Elk-1/Ets. p42/44 MAPK is involved in normal cell growth, differentiation and survival. However, involvement of p42/44 MAPK was also observed in malignant disease, often accompanied by Ras mutations\(^{19}\). High levels of activated p42/44 MAPK have been found in acute myelogenous leukemia cells and breast cancer cells, and therapeutic targeting of the p42/44 MAPK pathway in these conditions has been suggested\(^{20,21}\). Next to the classic role of p42/44 MAPK in mitogen induced growth and differentiation, p42/44 MAPK is also involved in inflammatory cytokine production. Active p42/44 MAPK was observed to be a prerequisite for T-lymphocyte IL-10 production\(^{22}\), and LPS induced TNF\(\alpha\) and IL-1\(\beta\) production\(^{23-25}\). In chapter 9, we explore the role of p42/44 in the inflammatory cytokine response to *Streptococcus pneumoniae*.

2.3. c-Jun N-terminal Kinase

JNK consist of at least three genes (*JNK1/2/3*) that each encode proteins with 2-4 different splice variant, resulting in 46kDa and 54kDa proteins\(^7\). Whereas JNK 1 and 2 are ubiquitously expressed, JNK3 is mainly expressed in the brain. Similar to p38 MAPK, JNK is activated by cellular stress (UV, osmoshock, ionizing radiation) and inflammatory cytokines (TNF, IL-1), and together they have been dubbed the Stress-Activated Protein Kinases (SAPKs). Upon activation through upstream kinases MKK4/7\(^{26}\), JNK controls -among others- AP-1 transcription activity through phosphorylation of c-Jun\(^{27}\). Recently, a scaffold protein (JIP) was found to be an important factor in the JNK pathway, connecting MKK4/7 to JNK\(^{28}\). Specific JNK inhibitors have only recently become available\(^{29,30}\), and so far the role of JNK in inflammation remains enigmatic. However, targeted disruption of JNK1 and 2 in mice learned
that they play an important role in T-cell growth and differentiation\textsuperscript{31,32}. The role of JNK in T cells proliferation and differentiation will be discussed more extensively further on.

Thus, the MAPK pathways are implicated in a variety of immune functions, ranging from initiation of the innate immune response to activation of the adaptive immunity. We will now discuss how our current knowledge of MAPKs may relate to LPS-induced systemic inflammation in humans.

3. Endotoxemia

The innate immune response is tasked with the first line of defense against invading pathogens. One of the unique antimicrobial features of the innate immunity is the ability to recognize highly conserved pathogen motifs, enabling it to mount a swift defensive reaction against an array of microbes\textsuperscript{33}. Endotoxin, a lipopolysaccharide that forms part of the cell membrane of all Gram-negative bacteria, is recognized by most inflammatory cells. It is known to induce strong and rapid inflammation, and is thought to be a major contributor to the toxic sequelae of Gram-negative sepsis\textsuperscript{4}.

3.1. LPS signaling

Although LPS is normally located in the cell membrane, poorly recognizable by the immune system, it can also be shed by bacteria, e.g. upon destruction of the pathogen. In plasma, free floating LPS is bound to LPS-binding protein (LBP)\textsuperscript{34,35}. This LPS-LBP complex is recognized by the cell surface molecule CD14, present on inflammatory cells (monocytes/macrophages, granulocytes, but not lymphocytes)\textsuperscript{36}. CD14 itself has no cytosolic domain, however, the LPS signal can be transduced through the Toll-like receptor 4 (TLR4)\textsuperscript{37}. TLR4 is able to transduce the LPS signal independent of CD14, however, CD14 greatly facilitates this process\textsuperscript{38}. Subsequently, TLR4 is able to initiate intracellular signal transduction events in order to relay the LPS signal towards the nucleus and evoke a cell response. All major MAPK family members are known to be rapidly activated upon stimulation of inflammatory cells with LPS. Upon stimulation with LPS, phosphorylation, and
enhanced enzymatic activity has been documented for p38 MAPK, p42/44 MAPK and JNK, \textit{in vitro}^{12,23,39,40}. Whether LPS induces MAPK activation \textit{in vivo} is currently unknown, and this was investigated in humans and in mice as described in chapters 2, 3 and 8.

Intravenous infusion of a moderate amount of LPS (4ng/kg) into humans, induces low-grade endotoxemia and is used as a human model for systemic inflammation. In this model, typical symptoms of endotoxemia appear within 90 minutes after infusion of LPS, and include generalized malaise, myalgia, nausea and sometimes vomiting. Usually a monophasic fever is observed that is preceded by chills\textsuperscript{4}. Many inflammatory cascades that are associated with endotoxemia are well documented. LPS infusion induces cytokine and chemokine release, activation of leukocytes, activation of the coagulation and fibrinolytic systems, and of the vascular endothelium\textsuperscript{4}.

\subsection*{3.2. The cytokine response}

Cytokines are small proteins that are produced by inflammatory cells upon encountering a wide variety of immunologic and infectious stimuli. Once released into the cell’s surrounding, cytokines are potent mediators of inflammation, interacting with other cytokines and inflammatory cells in a complex network. Alternatively, cytokines can also act in a membrane-bound form. A common but arbitrary classification divides the numerous cytokines into proinflammatory cytokines (e.g. TNF\textalpha, IL-1\beta, IFNy: promote inflammation), anti-inflammatory cytokines (e.g. IL-4 and IL10; reduce inflammation and inhibit proinflammatory cytokine production), and cytokine inhibitors that are thought to protect the body from excessive inflammation (e.g. soluble TNF and IL-1 receptor antagonist(IL-1ra)).

Upon intravenous infusion of LPS in humans, a transient rise in TNF\textalpha plasma levels is noted within 30–45 minutes, usually peaking at 90 minutes. Subsequently, IL-6 and IL-10 plasma levels transiently rise. Noteworthy, endotoxemia-induced IL-6, IL-10 and IL-1ra production were shown to be (partly) dependent on the initial induction of TNF\textalpha\textsuperscript{41-43}.

The production of TNF\textalpha by inflammatory cells was one of the first pro-inflammatory mechanisms documented to be regulated by a member of the MAPK family. Lee \textit{et al.}
reported that a specific p38 MAPK inhibitor, SB 202190, a compound from the pyridinyl-imidazole class, inhibited LPS-induced TNFα production in monocytes. In recent years it became clear that p38 MAPK, JNK and p42/44 MAPK are all involved in LPS-induced TNFα production; the degree of involvement of each MAPK seems to rely on the cell type and condition tested\textsuperscript{12,30,44}. p38 MAPK seems to feature most prominently as a TNFα production regulator, especially in monocytes and neutrophils\textsuperscript{12,14}. Often several MAPKs cooperate in regulating cytokine production: full TNFα gene induction relies on activation of all three major MAPK pathways in macrophages\textsuperscript{45}, and a cooperative effect of p38 MAPK and p42/44 MAPK was also found in TNFα and IL-6 production in alveolar macrophages\textsuperscript{44}. Inhibition of p42/44 MAPK led to decreased levels of TNFα in monocytes\textsuperscript{24}. On the other hand, inhibition of p42/44 MAPK in primary murine peritoneal macrophages did not inhibit LPS induced TNF production\textsuperscript{25}, suggesting that MAPKs have a cell-type and condition-dependent role. We further explored this hypothesis in chapter 8, and investigated the role of p38 MAPK in cytokine production in several cell types.

Several rodent studies indicate that also in vivo p38 MAPK is necessary for LPS-induced TNFα production\textsuperscript{46-48}. In vivo, inhibition of p38 MAPK was found to inhibit endotoxic shock induced TNFα production and mortality in mice\textsuperscript{46}. In a model for pulmonary inflammation, TNFα accumulation appeared to be dependent on active p38 MAPK\textsuperscript{48}. Mice deficient in MAPKAPK-2, a downstream target for p38 MAPK, are endotoxin tolerant, although this resistance seems to depend on reduced TNFα translation rather than transcription\textsuperscript{49}. Thus it seems that LPS-induced cytokine release from monocytes/macrophages and neutrophils can be inhibited, at least in part, by members of the MAPK pathway in vitro and in animals. How these data relate to the human situation, is currently unknown. In chapter 3 we investigate the effect of inhibition of p38 MAPK on the cytokine response during human endotoxemia.

3.3. Chemokines and chemokine receptors

Chemokines are small proteins with strong chemotactic activity that regulate granulocyte activation and migration to the site of inflammation. They can be divided in a number of subgroups, of which CXC chemokine haven been studied most thoroughly. Members of the CXC chemokine family include IL-8; growth-related oncogenes (GRO)α, GROβ, and GROγ;
and epithelial-derived neutrophil attractant (ENA)\textsuperscript{78,50,51}. Granulocytes express 2 types of CXC chemokine receptors (CXCR) that interact with these mediators: CXCR1, which exclusively binds IL-8, and CXCR2, which, besides IL-8, can also bind GROs and ENA-78\textsuperscript{50,51}. LPS induced effects on CXCR1 and CXCR2 and their ligands have been well documented. Upon infusion with LPS, surface expression of both CXCR1 and 2 on neutrophils is reduced. Recently, Plasma levels of chemokines IL-8, GROα and ENA-78 transiently rise after LPS infusion. Recently, CXCR2 surface expression on granulocytes was shown to be dependent on p38 MAPK, \textit{in vitro}\textsuperscript{52}. Furthermore, inhibition of p38 MAPK diminishes IL-8 production in monocytes, granulocytes and endothelial cells\textsuperscript{53,54}. How these data relate to the human situation is unknown. In chapter 4 we studied the role of p38 MAPK in regulating these neutrophil migratory factors.

\textbf{3.4. Coagulation and inflammation}

Within the array of LPS-induced effects, activation of coagulation and fibrinolysis features prominently. Two hours after infusion of LPS, parameters of activation of the common pathway of the coagulation system can be measured: plasma concentrations of the prothrombin fragment F1+2 and of thrombin-antithrombin (TAT) complexes rise transiently. Interestingly, activation of the coagulation pathway is preceded by a rapid, transient activation of fibrinolysis. LPS-induced fibrinolytic activation starts with the release of tissue-type plasminogen activator (tPA) into the circulation, followed by a rise in plasminogen activator inhibitor type I (PAI-I) levels, indicating that the fibrinolytic response to LPS is highly regulated. The transient generation of active plasmin is confirmed by the detection of elevated plasma concentrations of plasmin-α2-antiplasmin (PAP) complexes. The LPS-induced activated vascular endothelium is the likely source of tPA; other activated vascular endothelium-derived products that can be detected during endotoxemia are soluble E-selectin and von Willebrand factor. Three to four hours after administration of LPS parameters of fibrinolysis activation have returned towards normal levels, while at that time coagulation is still active, resulting in a net pro-coagulant state\textsuperscript{4}.

Little is known about the role of MAPKs in the regulation of coagulation and fibrinolysis during inflammation. Upon exposure of blood mononuclear cells or vascular endothelial cells
Introduction

to LPS or proinflammatory cytokines, tissue factor (TF) expression in upregulated, inducing activation of the coagulation pathway. Both p38 MAPK and p42/44 MAPK have been suggested to regulate membrane TF expression on monocytes, in vitro. However, in this study the experimental design was flawed by the use of high amounts of MAPK inhibitors (up to 100μM). The use of MAPK inhibitors, such as SB203580 and PD98059, in concentrations above 10μM has been known to affect their specificity. Thus, whether MAPKs are actually involved in TF expression is under debate. Thrombin is a known activator of p38 MAPK in HUVEC cells, enhancing both its phosphorylation and kinetic activity in a dose-dependent fashion. Furthermore, inhibition of p38 MAPK reduced thrombin-induced IL-8 and MCP-1 production in HUVEC cells. No effect was noted of inhibition of p42/44 MAPK on thrombin induced chemokine production in HUVEC cells. Using an inhibitor, we investigated the role of p38 MAPK in regulation of inflammation- (i.e. LPS-) induced changes in the coagulation and fibrinolysis cascades in humans (chapter 5).

4. Crohn’s disease

Crohn’s disease is a chronic inflammatory disorder of the gastrointestinal tract, which is thought to arise in genetically susceptible hosts caused by an inappropriate immunologic response against the microflora of the gut. A more profound understanding of the molecular mechanisms underlying the unbridled inflammation is slowly starting to translate into novel strategies in the treatment of Crohn’s disease. Using animal models and genetic approaches, pivotal pro- and anti-inflammatory extracellular mediators (mainly cytokines) have been identified. Extending these data to the human situation has led to successful trials, most notably those using anti-TNF antibodies. Despite the advances in anti-cytokine based approaches, current treatment modalities are still insufficient for many patients. Although remission induction can be achieved in many instances, using conventional (immunosuppressive drugs) and new therapeutics (e.g. anti-TNF antibodies), medication side effects and disease relapse remain problems that need to be resolved.

In recent years the focus of attention in Crohn’s disease research is shifting from known intercellular signals such as TNFα, IL-10, IFNγ, and IL-12, towards the intracellular molecules that transduce them. The signal transduction cascades of the MAPK family have
been implicated in regulating mediators of inflammation in Crohn’s disease. In this chapter we will focus on the involvement of MAPK family members in processes governing Crohn’s disease pathogenesis and discuss their possible therapeutic value.

4.1. Tumor Necrosis Factor

Crohn’s disease is characterized by chronic inflammation leading to destruction of normal tissue integrity. TNF, a prototype pro-inflammatory cytokine, plays a central role in the initiation and amplification of the inflammatory reaction seen in Crohn’s disease\(^3\). Altering the regulation of TNF production or its efficacy thus seems attractive in Crohn’s disease. Indeed, monoclonal antibodies against TNF have been proven effective in both inducing clinical remission and endoscopic healing\(^{59,60}\). As discussed earlier, members of the MAPK family have a profound influence on the production of TNFα in monocytes/macrophages and neutrophils. In T-lymphocytes, all three MAPK pathways are also involved in transcriptional regulation of TNF production\(^{61}\). Furthermore, in Th1 cells a specific JNK inhibitor (SP600125) reduced CD3/28-induced TNFα production\(^{30}\).

4.2. Interleukin 12 and 18

Poorly controlled activation of Th1-biased CD4+ T lymphocytes is believed to be a major pathogenic mechanism in Crohn’s disease. Interleukin (IL) -12 and IL-18 play an important role in activating naïve T cells and driving them into a Th1 phenotype, leading to increased IFNγ and TNF release. Indeed, elevated levels of IL-12 and IL-18 (initially described as IFNγ inducing factor) have been measured in intestinal lesions from patients with Crohn’s disease\(^{62,63}\), and stimulation of lamina propria cells with IL-18 and IL-12 resulted in an increased IFN-gamma production\(^{64}\).

IL-12 was shown to induce MKK3/6 and p38 MAPK, but not JNK or p42/44 MAPK activation in T cells\(^{65,66}\), suggesting involvement of the p38 MAPK pathway in IL-12 signaling. A well-known participant of IL-12 signaling, Signal Transducer and Activator of Transcription (STAT)-4, can be phosphorylated on serine 721 by MKK6 and p38 MAPK, and
this p38 MAPK pathway dependent phosphorylation was shown to be necessary for STAT4 mediated IL-12 activity. However, IL-12 can also induce effects such as IFNγ production in a STAT4-independent manner. Zhang et al described that IL-12 induced, STAT4 independent, IFNγ production depends on functional p38 MAPK. Finally, T cell stimulation through TLR4, which preferentially induces a Th1 response, induces IL-12p70 in a p38 MAPK dependent fashion. These data are in line with in vivo experiments. Mice with a targeted MKK3 disruption, resulting in diminished p38 MAPK activation, show defective IL-12 production in antigen presenting cells (macrophages and dendritic cells), leading to decreased IFNγ production by naïve T cells. Contrasting these data, is a report describing p38 MAPK inhibition induced augmentation of IL-12 production by human monocytes/macrophages upon stimulation with LPS and IFNγ. These data, again, suggest that p38 MAPK function in cytokine production is cell type specific.

So far little is known about IL-18 signal transduction in Th cells. Similar to IL-1, IL-18 utilizes an IL-1R-Associated Kinase (IRAK) mediated pathway, and thus MAPK involvement is very well conceivable. Indeed, IL-18 induces activation of p38 MAPK and JNK in a T cell line, and p42/44 MAPK in a NK cell line. IL-18 induced NK cytolytic activity was shown to be dependent on p42/44 MAPK activation, and IL-18 induced IFNγ production could be inhibited with p38 and p42/44 MAPK inhibitors.

4.3. Th1 differentiation and Interferon γ production

IL-2 is an important factor in driving T cell proliferation and activation. Signal transduction events in CD4+ T cells following exposure to IL-2 include p38 MAPK and JNK phosphorylation. However, reports concerning the functional significance of p38 MAPK and JNK activity in IL-2 signaling are contradictory, and it is unclear whether MAPKs are critically involved in IL-2 induced T cell proliferation. Whether IL-2 production in T-cells is under control of JNK is under debate. JNK1 and JNK2 deficient T cells produce normal levels of IL-2. In contrast, JNK1/2 and MKK4 deficient T cells were observed to produce reduced levels of IL-2 upon CD28 costimulation.
Differentiation of naïve CD4+ T cells into Th1 is regulated by JNK1/2. JNK1 deficient naïve CD4+ T cells preferentially differentiate into Th2 cells, and thus JNK1 seems to be a negative regulator of Th2 differentiation\(^\text{31}\). T cells defective in JNK2 produced less IFN and differentiation into Th1 was impaired, while differentiation into Th2 was not\(^\text{32}\). For Th2 differentiation and IL-4 production, p42/44 MAPK activation seems to be a prerequisite\(^\text{78}\).

Th1 differentiation is also under control of p38 MAPK. Using a specific inhibitor of p38 MAPK and by generating transgenic mice that overexpress a dominant-negative p38 MAP kinase, Rinçon \textit{et al.} showed that inactivation of p38 MAPK led to reduced IFN\(\gamma\) production in Th1 cells, but had no effect on IL-4 production in Th2 cells\(^\text{79}\). In line with these results, Th1 cells from transgenic mice overexpressing MKK6, leading to constitutively activated p38 MAPK, showed increased IFN\(\gamma\) production\(^\text{79}\). Furthermore, Zhang \textit{et al} observed that p38 MAPK, but not p42/44 MAP or JNK is required for IL-12 induced IFN\(\gamma\) production in activated T cells and Th1 cells\(^\text{65}\). Correlating with these studies, CD4+ T cells from M KK3 deficient mice exhibit impaired IFN\(\gamma\) production\(^\text{69}\). Recently it was shown that TCR
dependent and independent IFNγ production in Th1 cells depend on p38 MAPK and JNK via GADD45 a protein involved in cell cycle and differentiation\textsuperscript{80-82}. Thus both p38 MAPK and JNK are a critical determinant of Th1 differentiation.

Both the production of, and response to many known extracellular mediators that are critical in the pathogenesis in Crohn’s disease involve one or more MAPK pathways. Furthermore, MAPKs seem to be involved in the differentiation of Th1 cell, a critical process in Crohn’s disease pathogenesis. Accordingly, activation of several MAPK members was reported in biopsies from inflamed mucosa from Crohn’s disease patients\textsuperscript{83}. Furthermore, in a murine model of rheumatoid arthritis, a disease with many similarities with Crohn’s disease, inhibition of JNK or p38MAPK was shown to be beneficial\textsuperscript{46,84}.

The above summarized data suggest that targeting members of the MAPK family might be beneficial in patients with Crohn’s disease. However, to data no reports are available on the effect of MAPK inhibitors in patients with Crohn’s disease, or in animal models of this disease. Hence, we employed a MAPK inhibitor (CNI-1493; a combined JNK and p38 MAPK inhibitor), to investigate the safety and efficacy of this approach in combating Crohn’s disease (chapter 6). Furthermore, we studied the role of p38 MAPK in a rodent model for Crohn’s disease, TNBS colitis (chapter 7).

5. Pneumococcal Pneumonia

Despite potent anti-microbial agents, pneumococcal pneumonia remains an important cause of morbidity and mortality. It accounts for up to 70% of community-acquired pneumonias in hospital, and its incidence seems to be rising\textsuperscript{85,86}. Furthermore, an increase in penicillin-resistant \textit{S. pneumonia} was reported, prompting for research into new therapeutic options and a better understanding of host defense\textsuperscript{87}. In recent years, much has been learned about the cytokine network that forms an intricate part of the host defense mechanism against bacterial pathogens. Pro-inflammatory cytokines, such as TNF, IL-6 and IFNγ, are produced in the lung during pneumococcal pneumonia, and are important for clearance of bacteria from the respiratory tract\textsuperscript{88-90}. Anti-inflammatory cytokines, such as IL-10, impair bacterial clearance and shorten survival\textsuperscript{91}. Generally, the balance between pro- and anti-inflammatory mediators
Chapter 1

of inflammation, is a delicate one. Whereas proinflammatory cytokines are necessary for an adequate immune response to foreign pathogens, excessive inflammation may eventually lead to morbidity and mortality.

The details of the regulatory mechanisms by which MAPKs govern the production of some of these cytokines have been discussed previously in this chapter. p42/44 MAPK can be activated by bacterial cell wall components, e.g. from S. pneumoniae, but was reportedly not involved in (heat killed) Staphylococcus aureus induced TNF production. Bactericidal mechanisms play an important role in clearing the bacteria form the lung. In vitro, neutrophil phagocytosis and production of reactive oxygen species is impaired upon inhibition of p38 MAPK and 42/44 MAPK. How these intracellular processes are related to host defense in vivo remains relatively poorly understood. Hence, we investigate the effect of inhibition of p38 MAPK and p42/44 MAPK on pulmonary cytokine levels and bacterial clearance in a murine pneumococcal pneumonia model (chapters 8 and 9).

6. Aim and outline of this thesis

Targeting members of the MAPK family seems an attractive alternative for present immunomodulatory therapies. However, our current understanding of the role and function of these MAPKs is mainly derived from cell-based models for inflammation, and a hand full of animal experiments. Little is known about their part in human inflammation. Furthermore, in vitro data suggest that (1) MAPKs may have a cell-type and condition-dependent role and (2) MAPKs are pivotal for normal bactericidal effector mechanisms. These findings indicate that the effects of inhibition of MAPKs in vivo may not be as clear cut as some in vitro studies suggest. Further study into the safety and efficacy of MAPK inhibitors during human inflammation seem warranted. The aim of this thesis, therefore, was to elucidate the role of MAPK pathways in inflammation in vivo:

(1) Are the MAPK pathways activated during systemic inflammation in humans?

In chapter 2 activation of the MAPK pathways is analyzed during human endotoxemia.
(2) Can we modulate inflammation in humans targeting a MAPK pathway? 
*Chapter 3* describes the effect of a new p38 MAPK inhibitor on p38 MAPK activity and the cytokine response during low grade human endotoxemia. *Chapter 4* focuses on the role of p38 MAPK on regulating determinants of neutrophil migration and activation during human endotoxemia. *Chapter 5* describes the effect of inhibition of p38 MAPK on endotoxemia-induced changes in the coagulation and fibrinolysis cascades.

(3) Are MAPKs suitable targets for therapy in chronic inflammatory disease? 
In *Chapter 6* the safety and efficacy of CNI-1493, an inhibitor of p38 MAPK and JNK, is described in the treatment of patients with Crohn’s disease. *Chapter 7* focuses on the role of p38 MAPK in TNBS colitis, a murine model for Crohn’s disease.

(4) What is the effect of MAPK inhibition on pneumococcal pneumoniae? 
The effect of inhibition of the p38 MAPK and p42/44 MAPK pathway on (a murine model for) pneumococcal pneumonia is described in *chapters 8 and 9*, respectively.