General discussion and summary
CHAPTER 12

SUMMARY
Chapter 1 gives an overview of the functions of C/EBPδ that are currently known, focusing on the role of C/EBPδ in inflammation, cell proliferation and apoptosis, which are the most relevant processes for the studies presented in this thesis. The knowledge on C/EBPδ in infectious disease specifically is extended in the different chapters that follow in part 1 of this thesis. In chapter 2 the role of C/EBPδ during experimental pneumococcal pneumonia has been studied. We show that C/EBPδ aggravates the outcome of pulmonary S. pneumoniae infection in a Platelet-Activating Factor Receptor (PAFR)-dependent manner. C/EBPδ−/− mice were protected during pneumococcal pulmonary infection which was evident from lower bacterial dissemination and increased survival. Subsequently, in chapter 3 we investigated the role of C/EBPδ during K. pneumoniae-induced pneumonia to elucidate whether C/EBPδ is also detrimental during gram-negative pneumonia. We show however that C/EBPδ is beneficial during pulmonary infection with K. pneumoniae. Indeed, Klebsiella-induced mortality was significantly increased among C/EBPδ−/− mice and we hypothesize that the lack of macrophage mobilization in C/EBPδ−/− mice is a key mechanism causing the higher mortality rates. Shortly after publication of chapter 3 another study about the role of C/EBPδ in pulmonary inflammation was published with seemingly contradictory results. Chapter 4 discusses the different results on C/EBPδ of chapter 2 and 3 in the perspective of the publication in this recent paper and deliberates about the possible role of C/EBPδ during lung infection and inflammation. We conclude that C/EBPδ is an important player in inflammation but its role seems complex and is far from understood and therefore we should be careful to extrapolate murine data to human disease and to the subsequent clinical implementation.

Chapter 5 aims to elucidate whether C/EBPδ plays an important role during E. coli-induced urinary tract infection and describes the effect of C/EBPδ deficiency in a murine model for acute urinary tract infection. We show that C/EBPδ does not play an important role during this infection as evident from similar bacterial clearance rates in both wildtype and C/EBPδ−/− animals. The last chapter of part I focus on the role of C/EBPδ in renal fibrosis using a murine model. In this chapter (Chapter 6) we show that C/EBPδ is protective during renal fibrosis as is evident from enhanced tubular injury, collagen deposition in the interstitial area, and higher expression of transforming growth factor-β in C/EBPδ−/− mice as compared to wildtype mice. Several possible mechanisms (e.g.: macrophage influx and the balance in proliferation and apoptosis) responsible for this protective effect are investigated with no positive result however.
GENERAL DISCUSSION AND SUMMARY

The second part of this thesis extents our knowledge on the role of different PARs during fibrotic disease. Both PAR-1 and PAR-2 have been described to play an important role in cellular processes leading to excessive matrix accumulation in different organs like (among others) lung and liver (described in Chapter 7). Subsequently, Chapter 8 shows the results of an experiment in which PAR-1 signaling was blocked before and after the induction of lung fibrosis. Inhibiting PAR-1 signaling was effective in limiting the development of pulmonary fibrosis, even when administration of the antagonist was started 7 days after induction of fibrosis. The results of this chapter are highly interesting especially as pharmacological treatment of lung fibrosis even after the onset of fibrosis is clinically very relevant. In Chapter 9 we aimed to elucidate whether PAR-4 also contributes to pulmonary fibrosis. We observed that wildtype and PAR-4\(^{-/-}\) mice developed fibrosis, but there were no differences in the severity of fibrosis or in the expression of fibrotic markers like collagen and fibronectin in PAR-4\(^{-/-}\) mice compared with wildtype controls. Overall, these results demonstrate that PAR-4 does not play a significant role during pulmonary fibrosis. Chapter 10 aims to assess the contribution of PAR-1 to the development of (burn)wound-induced dermal fibrosis and attempts to elucidate the underlying mechanism that leads to the thickening of the skin upon PAR-1 activation. We show that PAR-1 contributes to the development of skin fibrosis in a mouse model of bleomycin-induced skin fibrosis as evident from reduced dermal thickening and extracellular matrix (ECM) deposition in PAR-1\(^{-/-}\) animals as compared to wildtype controls. We suggest therefore that PAR-1 potentiates the fibrotic response by inducing fibroblast proliferation and ECM production. Next, in chapter 11, we aimed to elucidate the role of PAR-2 on the development of (burn)wound-induced skin fibrosis. Genetic ablation of PAR-2 exerted potent antifibrotic effects and reduced dermal thickening, fibroblast proliferation and ECM deposition during bleomycin-induced skin fibrosis. Using in vitro experiments we were able to show that PAR-2 on HDF potentiates the fibrotic response by inducing proliferation and ECM production and we hypothesize that these processes are the key mechanisms by which PAR-2 contributes to skin fibrosis in vivo.

GENERAL DISCUSSION

In the first part of this thesis we intended to obtain further insight in the role of C/EBPδ in the inflammatory response especially during infectious disease. C/EBPδ is generally accepted to act as a pro-inflammatory transcription factor and several studies show that C/EBPδ may act as transcriptional activator of different pro-inflammatory cytokines. Indeed, in response to LPS, administered either systemically or intranasally, C/EBPδ deficiency leads to a dampened inflammatory response as evident from lower cytokine levels (e.g. IL-6 or....
TNF-α) as described, but not shown, in chapter 4 and reference 1 and 2. Although inflammation is a key process in the host response to bacterial infection, excessive and prolonged exposure of different body tissues to inflammation can result in tissue damage, ultimately leading to multiple organ failure and subsequent death. Our finding that C/EBPδ is beneficial during Klebsiella pneumoniae-induced pulmonary infection suggests therefore that this might be due to the regulation of the inflammatory response. Surprisingly, we did not find a role for C/EBPδ in cytokine production that could be responsible for the observed effect, but macrophage migration was however decreased in C/EBPδ−/− mice. On the other hand, we show that the loss of C/EBPδ protects against infection by Streptococcus (S.) pneumoniae. While this result appears at odds with the aforementioned results, this particular phenotype is explained by the role of C/EBPδ in lung epithelial PAFR, the receptor used by S. pneumoniae to enter the organism, expression. Therefore, the relative resistance of C/EBPδ deficiency to S. pneumoniae is unrelated to C/EBPδ’s role in the immune system and no conclusions about the role of C/EBPδ in cytokine production can be drawn from this study as the bacterial loads already skew the response to one side. Strikingly, also during Escherichia (E.) coli-induced acute urinary tract infection no differences in cytokine production were observed between C/EBPδ−/− mice as compared to wildtype mice. This is particularly interesting as a previous study shows that IL-6 mRNA levels were reduced in C/EBPδ−/− mice in response to E. coli-induced peritonitis. Lastly, during renal fibrosis, induced by unilateral ureteral obstruction, we did not find a role for C/EBPδ in the inflammatory response, as measured by macrophage infiltration or cytokine production.

Our results clearly show that the role of C/EBPδ in inflammation is complex and not as straightforward as one might have thought. A possible explanation of the seeming contradictory results may reside in the fact that during bacterial infections different signalling pathways are activated, whereas previously reported results showing a role for C/EBPδ in cytokine regulation are mainly obtained using single pathway activators (e.g.: LPS and IgG Immune complexes). Knowledge about the role of C/EBPδ in the inflammatory response derived from the studies described in this thesis have to be further extended, investigating the seeming contradictory results on inflammation and to integrate results from different studies described elsewhere to get a better view on the precise role of C/EBPδ during inflammation and infectious disease.

In the second part of this thesis we aimed to get more insight into the role of PARs in fibrosis. Several studies already showed that PARs play a key role during fibroproliferative disease of different organs. Indeed, genetic ablation of PAR-1 limits fibrosis in the lung.
and liver\textsuperscript{9}, whereas PAR-2 deficiency protected against the development of lung\textsuperscript{10}, liver\textsuperscript{11} and kidney\textsuperscript{12} fibrosis. The results described in this thesis show that PAR-1 and PAR-2 both contribute to the fibrotic response in the skin and the role of these receptors in fibrosis might well be part of a more general mechanism, rather than a tissue specific mechanism. Interestingly, we also show that pharmacological inhibition of PAR-1 protected against bleomycin-induced pulmonary fibrosis even when administered after the induction of the fibrotic process. This is particularly of interest for the treatment of patients suffering from pulmonary fibrosis as not many treatment options have proven to be highly effective. Further studies are however needed before the inhibition of PAR-1 will be available as a standard treatment option as all data described are obtained using murine models. Although a potential role for PAR-4 in lung fibrosis has been suggested\textsuperscript{13,14}, PAR-4 does not seem to play an important role during the fibroproliferative response, at least for the lung. Whether this holds also true for different organs remains elusive, but contribution of PAR-4 in fibroproliferative disease is definitely not a general mechanism.

REFERENCES
10. Borensztajn, K., P. Bresser, C. van der Loos, I. Bot, B. van den Blink, M. A. den Bakker, J. Daal...


