Infectious diseases and fibrotic disorders: Potential novel targets
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CCAAT/enhancer binding protein δ: Friend or foe in inflammation?
CCAAT-enhancer-binding protein delta (C/EBPδ) is a member of the C/EBP family of transcription factors, which contains six unique members, C/EBPα, C/EBPβ, C/EBPδ, C/EBPγ, C/EBPε and C/EBPζ (see figure 1a). All these members, except C/EBPζ, consist of an N-terminal transactivation domain, a DNA binding domain and a C-terminal basic leucine zipper domain that allows homo- or hetero-dimerization of the different members, which is a prerequisite for DNA binding. DNA binding occurs at a promoter region with the following sequence (although substantial variations are tolerated): RTTGGYAY, where R is A or G, and Y is C or T (see figure 1b). Even though the (patho)physiological role of C/EBPδ is not fully established yet, recent data suggest that C/EBPδ is of pivotal importance in inflammation and proliferation/apoptosis pinpointing to an important role for C/EBPδ in (among others) infectious disease and fibroproliferative disorders.

**C/EBPδ in inflammation**

Originally C/EBPδ has been identified as a transcription factor rapidly upregulated during the acute phase response. Indeed, expression of C/EBPδ is typically low in most cell types but is rapidly induced by a variety of extracellular stimuli like among others, lipopolysac-

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**Figure 1:** (a) Domain organization and protein size of the six known C/EBP family members. aa: amino acids; BRLZ: basic-region leucine zipper; pink regions: activation and regulatory domains. Domain organization obtained using SMART.69;70 (b) The predicted structure of C/EBP bZIP dimer bound to the consensus sequence of C/EBP where R is A or G, and Y is C or T3. Picture credits: Thomas Splettstoesser.
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Charide (LPS), interleukin (IL)-1, IL-6, interferon γ (IFN-γ) and tumor necrosis factor-α (TNF-α). IL-6-mediated induction of C/EBPδ during the acute phase response in hepatocytes is mediated via activation of signal transducer and activator of transcription 3 (STAT3) and SP1 transcription factor (SP1), which interact with a specific recognition site present in the proximal promoter region of C/EBPδ. IL-6-mediated induction of C/EBPδ during the acute phase response in hepatocytes is mediated via activation of signal transducer and activator of transcription 3 (STAT3) and SP1 transcription factor (SP1), which interact with a specific recognition site present in the proximal promoter region of C/EBPδ. Although the activation of STAT3 under such conditions is transient, C/EBPδ expression levels are maintained for a longer period of time. This is achieved, at least in part, by binding of C/EBPδ to its own promoter. Also the phosphorylation, sumoylation and acetylation of C/EBPδ influence its intracellular localization and transcriptional induction of different genes during the acute phase response.

After induction of C/EBPδ by proinflammatory extracellular stimuli, C/EBPδ on its turn further induces the expression of cytokines and chemokines during the inflammatory response. Indeed, C/EBPδ expression levels are correlated with increased expression levels of pro-inflammatory cytokines in LPS stimulated HUVECs and C/EBPδ regulates COX-2 expression in different cell types. Interestingly, C/EBP-binding motifs have been identified in the functional regulatory regions of various genes expressed by cells of the myelomonocytic lineages, including those encoding the inflammatory cytokines IL-6, IL-1β, and TNF-α, other cytokines/chemokines such as IL-8, monocyte chemoattractant protein-1 (MCP-1), IL-12, genes encoding proteins important for macrophage or granulocyte functions such as, inducible nitric oxide synthase, myeloperoxidase, and neutrophil elastase, the gene encoding the granulocyte colony-stimulating factor (G-CSF), and the macrophage colony-stimulating factor (M-CSF) receptor, the G-CSF receptor, and granulocyte-macrophage (GM)-CSF receptor genes. Finally, a recent paper suggests that C/EBPδ directly regulates TLR4 expression therefore contributing to the inflammatory response upon LPS activation. Consequently, C/EBPδ is generally accepted to act as a pro-inflammatory transcription factor. This hypothesis is strengthened by recent observations that macrophage C/EBPδ is essential in Fcγ receptor-mediated inflammatory cytokine and chemokine production. Indeed, C/EBPδ deficient macrophages fail to induce a full TNF-α, MIP-2 and MIP-1α response induced by IgG Immune complexes. Another interesting finding in this respect is the fact that low dose LPS stimulation of macrophages induced C/EBPδ expression subsequently leading to higher IL-6, MCP-1 and endothelin-1 (a strong vasoconstrictive cytokine) levels. Similarly to low dose LPS, C/EBPδ also potentiates IL-6 expression in macrophages upon high dose LPS stimulation. In contrast however, Lu and...
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Hu actually show no difference in IL-6 and MCP-1 expression levels upon macrophage stimulation with high dose LPS.\(^{34,35}\)

As opposed to the overwhelming amount of data suggesting that C/EBPδ acts as a pro-inflammatory transcription factor, provocative papers show that C/EBPδ could also exert anti-inflammatory effects. Interestingly, although C/EBPδ binding to the COX-2 promoter induces its expression,\(^{18,19}\) siRNA dependent targeting of C/EBPδ during LPS stimulation of macrophages also increases COX-2 expression. These seemingly contradictory results may be explained by the fact that C/EBPα and C/EBPβ are more efficiently recruited to the COX-2 promoter in the absence of C/EBPδ, and these transcription factors may in turn be more potent COX-2 activators than C/EBPδ.\(^{36}\) Moreover, C/EBPδ also induces the anti-inflammatory cytokine IL-10 upon LPS stimulation of macrophages.\(^{37}\) Overall, the role of C/EBPδ in the regulation of the inflammatory response during infection thus seems to be complex and is not fully understood yet.

The potential C/EBPδ-dependent positive feedback loop in inflammation (i.e. cytokines induce C/EBPδ and C/EBPδ induces cytokine production) would suggest that this might lead to a continuous inflammatory state. To prevent such a potential life-threatening situation, several mechanisms to downregulate C/EBPδ during the inflammatory response have been suggested. The binding of the transcription factor ATF-3 to the C/EBPδ promoter thereby inhibiting C/EBPδ transcription is one of the suggested mechanisms by which C/EBPδ is downregulated.\(^{33}\) Alternatively, Miz1 and FBXW7α seem to be important for the downregulation of C/EBPδ expression during the inflammatory response leading to the resolution of LPS-induced inflammation.\(^{4,30}\)

In line with its proinflammatory role in vitro, C/EBPδ also plays an important role in the inflammatory response in vivo. Indeed, C/EBPδ deficient mice had a prolonged survival as compared to wildtype mice in a double hit LPS model for disseminated intravascular coagulation (DIC).\(^{38}\) In this particular model, C/EBPδ deficiency limited LPS-induced systemic inflammation as reflected by reduced TNF-α and IL-6 levels. This attenuated inflammatory response is likely responsible for the increased survival of the C/EBPδ deficient mice as it is well-known that an overwhelming inflammatory response increases organ damage subsequently leading to multiple organ failure and death. In line with this notion, organ injury markers were significantly lower in C/EBPδ deficient mice as compared to wildtype controls. Moreover, C/EBPδ also plays a role in a specific model of LPS-induced lung injury.\(^{39}\)
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Indeed, C/EBPδ deficient mice show attenuated TNF-α, IL-6 and MIP-2 levels and subsequent lung injury upon intranasal LPS inoculation. Moreover, C/EBPδ is also suggested to potentiate the inflammatory response during E. coli-induced peritonitis. Interestingly however, in this model in which the induction of the inflammatory response is highly important for bacterial clearance, C/EBPδ deficient mice were more susceptible to the bacterial infection leading to increased mortality.

The important role of C/EBPδ in inflammatory disease seems strongly dependent on its role in macrophages. Interestingly, C/EBPδ expression is increased in macrophages during age-related disorders like rheumatoid arthritis, Alzheimer disease and atherosclerosis. During these pathological disorders, macrophage C/EBPδ is suggested to play a key role in the development of the disease. In fact, it has been shown that the increased expression of C/EBPδ in macrophages during rheumatoid arthritis contributes, at least in a murine model, to disease progression. It is thus tempting to speculate that macrophage C/EBPδ may play a more general role in inflammation although clear in vivo proof is lacking. In general the current data strongly suggest that C/EBPδ is an essential component of the inflammatory response, although its exact role in inflammatory related conditions like infectious disease needs further exploration. The first part of this thesis consequently focuses on the role of C/EBPδ in the inflammatory response during infectious disease.

C/EBPδ in cell proliferation and apoptosis

Soon after its discovery, C/EBPδ was also implicated in cell proliferation. Nowadays C/EBPδ is believed to act as a tumor suppressor due to its ability to decrease expression of cell cycle proteins cyclin D1 and cyclin E, while increasing expression of p27. Moreover, C/EBPδ regulates proapoptotic gene expression during mammary gland involution and inhibits the growth of human cell lines from different tumor types in vitro. In line, increased mammary epithelial cell proliferation and ductal hyperplasia is one of the main features observed in C/EBPδ deficient mice. The fact that C/EBPδ expression is reduced in mammary tumor-prone MMTV-c-neu transgenic mice and in carcinogen-induced rodent mammary tumors further strengthens the notion that C/EBPδ acts as a tumor suppressor. Finally, C/EBPδ deficiency on a MMTV-c-neu background potentiates breast tumor formation. In addition to these laboratory studies, more clinical orientated studies also support a tumor suppressor role of C/EBPδ. Indeed, down regulation of C/EBPδ due to hypermethylation is observed in cervical cancer, hepatocellular carcinoma, ductal carcinoma and during acute myeloid leukemia. Moreover, C/EBPδ levels correlate with...
low-grade histology and disease-free survival in meningioma patients, and C/EBPδ is among a 70-gene signature predicting better survival of breast cancer patients. Finally, site-specific CpG methylation in the C/EBPδ promoter is associated with metastatic relapse in breast cancer. Taken together, C/EBPδ expression seems to limit proliferation and to promote apoptosis thereby limiting tumor growth.

Intriguingly, proliferation/apoptosis is not exclusively involved in cancer progression but these processes are also crucial during infectious disease (for instance apoptosis of neutrophils upon bacterial phagocytosis, proliferation of damaged epithelial/endothelial cells or expansion of leucocytes) and fibrosis (evidently fibroblast proliferation is a key process during fibrosis but apoptosis/proliferation of epithelial cells during the repair phase and macrophage apoptosis during the resolution phase are also important during fibrosis). Consequently, we also focused on the role of C/EBPδ in proliferation/apoptosis during infectious disease and fibrotic disorders in the first part of this thesis.

C/EBPδ in other physiological processes

C/EBPδ also seems to play an important role in adipogenesis. Early on during the adipocyte differentiation process, the C/EBP family members C/EBPβ and C/EBPδ are rapidly upregulated and are postulated to be the transcription factors directing the differentiation process. Both transcription factors are important for the induction of both C/EBPα and Proliferator-activated receptor γ2 (PPARγ2) gene expression, which play a dominant role in adipocyte differentiation. Interestingly, TNF-α, a well-known inhibitor of adipocyte differentiation, exerts its inhibitory role mainly through the inhibition of C/EBPδ during the early stages of adipocyte differentiation. This is somewhat peculiar in the perspective of the previously reported fact that TNF-α induces C/EBPδ in different celltypes. However, not only in adipocytes C/EBPδ seems to play an anti-inflammatory role. Next to the above described inhibitory effect of C/EBPδ on COX-2 expression and the induction of IL-10 in macrophages, C/EBPδ has also an inhibitory role in inflammation and proliferation in pancreatic beta cells.

Next to the role of C/EBPδ in adipogenesis, C/EBPδ might also contribute to the pro-inflammatory properties of adipose tissue. Excessive accumulation of adipose tissue, which is called obesity, leads to diseases such as type 2 diabetes, hypertension and cardiac infarction. Interestingly, circulation of low dose LPS seems to be, at least in part, responsible for the development of these disorders. Circulating low-dose endotoxemia potentially
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leads to activation of C/EBPδ and the subsequent pro-inflammatory state seen in chronic metabolic endotoxemia. Because of the central role of C/EBPδ in adipogenesis and its role in inflammation, it is tempting to speculate that C/EBPδ might be an interesting candidate as therapeutic target during these diseases.

C/EBPδ is also widely expressed in the murine nervous system, suggesting that it might play a role in neurological processes. Indeed, although C/EBPδ deficient mice show normal basic neurological functions and have normal spatial learning, genetic ablation of C/EBPδ leads to a selectively enhanced contextual fear response, which was evident from increased conditioned freezing. The C/EBPδ deficient mice showed however equivalent conditioning to the auditory cue test as compared to wild-type controls. C/EBPδ thus seems to play an important role in a specific type of learning. Whether C/EBPδ plays a more general role in the nervous system, for instance affecting meningitis, Alzheimer disease or other neurodegenerative diseases, remains to be established however.

Despite the obvious scientific (and potential clinical) importance of C/EBPδ in metabolic and neurologic disorders, this thesis does not focus on the role of C/EBPδ in these pathologies.
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REFERENCES

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