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C5aR and TLR Crosstalk: Regulatory Effect of Anaphylatoxin C5a on Human Dendritic Cells

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English summary

Our immune system is crucial to combat invading pathogens and to maintain tissue homeostasis. Both the complement system and dendritic cells (DCs) are essential parts of our immune system and form an important bridge between innate and adaptive immunity. Although both are activated at the site of infection and in autoimmune diseases, these two arms of the innate immune system have mostly been studied separately.

DCs are activated upon recognition of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) via specific pattern recognition receptors (PRRs), including Toll-like receptors (TLRs). DC activation leads to the production of pro-inflammatory cytokines, increased expression of co-stimulatory markers and migration of DCs to secondary lymphoid tissues. In addition, DCs take up pathogens and immune complexes, resulting in the presentation of antigens on their cell surface. Together, these induced properties of DCs determine T cell activation and differentiation, which is essential for the induction of a proper and pathogen specific immune response. On the negative side, the presence of immune complexes containing self-antigens can contribute to undesired immune activation, as observed in autoimmune diseases, emphasizing that immune activation needs to be tightly regulated.

Complement activation leads to immune cell recruitment, pathogen opsonization and uptake, and pathogen disruption. Thereby, the complement system contributes to pathogen elimination. C5a is a well-known chemoattractant produced upon complement activation, which only recently has been implicated to modulate antigen-presenting cell (APC) activity in mice. Human DCs can produce complement components and express receptors that recognize complement activation products, such as receptors for C5a (C5aRs), indicating that C5a may affect human DC activity. Data on the interplay between C5a and DC activation is, however, limited, especially in human. The emerging interest in the use of C5/C5a modulating compounds to interfere with the effector function of C5a in various diseases, including auto-immune diseases, emphasizes the need to further elucidate the impact of C5a on DC activation. The scope of this thesis is to investigate how C5aR and TLR crosstalk modulates human DC activation.

The impact of C5aR and TLR crosstalk on the production of inflammatory cytokines and co-stimulatory molecule expression by human monocyte-derived DCs (moDCs) was investigated in **chapter 2**. C5a reduced the production of

TLR4-induced pro-inflammatory cytokines IL-6, IL-12, IL-23 and TNF-α. The inhibitory effect of C5a on TLR4-induced cytokine production by human moDCs was very much depending on simultaneous activation of C5aRs and TLRs. In addition, C5a promoted pro-inflammatory cytokine production by human DCs in the absence of additional stimuli (PAMPs), revealing that the inhibitory effect of C5a on human DCs is dependent on the presents of pathogens. C5aR and TLR crosstalk had no effect on the expression of co-stimulatory molecules by moDCs, indicating that the inhibitory effect of C5aR and TLR crosstalk is not general. C5aR and TLR crosstalk is not restricted to TLR4 signaling, as C5a also inhibits pro-inflammatory cytokine production induced upon TLR7/8 and TLR2 activation.

Immune regulation is important to prevent uncontrolled and overwhelming immune activation. As described above, C5a regulates TLR-induced pro-inflammatory cytokine production in human DCs, suggesting that C5a may contribute to immune regulation. Studies on C5aR and TLR crosstalk in mouse APCs, however, reported both regulatory as well as stimulatory effects of C5a on TLR-induced DC activation. These combined findings highlight the importance of further elucidating the effect of C5aR and TLR crosstalk on human DCs. The effect of C5aR and TLR crosstalk on other human DC subsets and involved signaling transduction pathways was, therefore, further investigated in **chapter 3**. C5aR was almost exclusively expressed on 6-sulfo LAcNAc DCs (slanDCs), indicating that especially slanDCs are prone to regulation by C5a. slanDCs are a very pro-inflammatory DC subset accumulating in several autoimmune diseases. We revealed that C5a inhibited TLR-induced pro-inflammatory cytokine production by slanDCs.

C5a accelerates TLR-induced ERK/p38-CREB1 signaling, resulting in IL-10 induction and negative feedback signaling (**chapter 3**). CREB1 and IL-10 demonstrated to be key in the regulatory effect of C5a on TLR-induced production of the pro-inflammatory cytokines IL-23 and TNF-α by DCs, whereas the inhibitory effect of C5a on TLR-induced IL-12 production only partly depends on CREB1-mediated IL-10 induction. In addition, the effect of C5aR and TLR crosstalk in DCs on subsequent T cell activation was assessed in **chapter 3**. In line with the observed inhibition of pro-inflammatory cytokine production, C5a-priming of DCs reduced Th1 and cytotoxic $CD8^+$ T cell immune responses. These findings underline the existence of functionally relevant regulatory feedback mechanisms between the complement system and human DCs.

Whereas in the first chapters we mainly focused on the effect of C5a on TLR-induced cytokine production, we addressed the full extent of C5aR and TLR

crosstalk in human DCs using whole transcriptome analyses in **chapter 4**. C5aR and TLR crosstalk induced a core regulatory network in human moDCs, with a central role for FOXO1 and FOXO3 transcription factors. Although CREB1 plays an important role in C5a-mediated inhibition of TLR-induced pro-inflammatory cytokines (as observed in chapter 3), the effect of C5a on the whole transcriptome upon C5aR and TLR crosstalk was mainly CREB1-independent in human DCs. Instead, motif enrichment analysis revealed a prominent role for the transcription factor IFN regulatory factor 4 (IRF4) upon C5aR and TLR crosstalk.

In **chapter 5**, we performed whole transcriptome analyses on DC activated by C5a in the absence of additional stimuli. Analysis of biological processes affected by C5a was suggestive for a role of C5a in Fc-receptor mediated phagocytosis and endosomal maturation. Given that both complement activation and the presence of immune complexes is associated with autoimmunity, we further assessed the effect of C5a on DC-mediated immune complex uptake. We revealed that C5a specifically acts on DCs to inhibit Fc-gamma receptor mediated uptake of larger immune complexes, revealing yet another regulatory effect of C5a on the activation of human DCs.

The findings in this thesis are summarized and discussed in **chapter 6**. Taken together, we discovered a potent role for C5a in regulating DCs and DC-mediated immunity in human. This regulatory effect of C5a may be of great importance to prevent the development of autoimmunity and to regulate inflammation during infections. Since C5a also has other functions apart from modulating DC activity, we suggest that the balance between the different effector functions of C5a determines the overall effect of C5a during inflammation. Of note, our findings suggest that the use of C5/C5a modulating compounds may not only have desirable effects like damping complement-mediated immunity and influx of immune cells, but at the same time may also affect local DC activation and adaptive immune responses. This highlights the importance of careful consideration of the use of C5/C5a interfering compounds.