C5aR and TLR crosstalk

Regulatory effect of anaphylatoxin C5a on human dendritic cells

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General introduction and scope of this thesis
**Immune system**

Our immune system is essential to maintain tissue homeostasis, and to protect our body from constant exposure to encountered pathogens, such as invading bacteria, viruses and parasites. Dysregulation of immune activation can lead to severe diseases, excessive tissue damage and autoimmunity. To prevent this from happening, our immune system has to recognize invading pathogens, needs to discriminate between self and foreign structures, and has to be tightly regulated. Dendritic cells (DCs) and the complement system are two essential parts of our immune system, that are both involved in pathogen recognition and elimination. They belong to the so called innate immune system, which can recognize and eliminate a wide variety of potential dangerous compounds and pathogens, and present these to the adaptive immune system\(^1\). The adaptive immune system is specialized in recognition of specific pathogen-derived antigens, and plays an important role in pathogen elimination and the generation of immunological memory\(^1\). Although not part of the adaptive immune system itself, both DCs and the complement system form an important bridge between innate and adaptive immunity\(^2\),\(^3\).

Increased levels of both DCs and complement activation products are observed during infection and in several autoimmune diseases, indicating that crosstalk between these two arms of innate immunity is likely to occur. Until recently, however, these two arms of innate immunity have mostly been studied separately, and data on the interplay between complement and DC activation, especially in humans, is limited. The potential use of complement interfering compounds for treatment of several diseases is gaining more interest\(^4\)-\(^8\), emphasizing the importance of understanding the effect of complement on DC activation. In this thesis, we aim to clarify the effect of C5aR and TLR crosstalk on the activation of human DCs.

**Dendritic cells**

Together with macrophages (MØs) and B cells, DCs belong to the group of professional antigen-presenting cells (APCs). DCs can be found through the entire body, both residing in tissues, and in the bloodstream. Upon infection, circulating DCs migrate into the affected tissue, where they can recognize pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) via specific pattern recognition receptors (PRRs)\(^9\),\(^10\). Triggering of PRRs on DCs induces DC maturation, resulting in the production of inflammatory cytokines, increased expression of co-stimulatory molecules, and the expression of chemokine receptors. In addition, DCs internalize and process
encountered pathogens, which results in the presentation of antigens in major histocompatibility complex (MHC) class I and II molecules on the cell surface. The expression of chemokine receptors by DCs is necessary to migrate to T cell areas of secondary lymphoid organs. Antigen presentation, cytokine production, and the expression of co-stimulatory molecules by DCs are three essential steps in initiating appropriate and antigen-specific T cell response. First, antigen-containing MHC molecules on activated DCs interact with antigen-specific T cell receptors on naïve CD4+ and CD8+ T cells, leading to activation of only antigen-specific T cells. Second, co-stimulatory molecules are recognized by receptors present on T cells, and provide activation and survival signals to T cells. In absence of co-stimulatory molecules, T cells are not properly activated and become nonresponsive to antigens. This is important to maintain self-tolerance and to prevent autoimmunity. Third, DC can dictate the polarization of naïve antigen-specific T cells by the type of cytokines that they produce. Upon recognition of pathogenic bacteria, viruses, and fungi, DCs produce IL-12, which induces the formation of IFN-γ producing Th1 cells. These promote the activation of CD8+ cytotoxic T cells and the activation of MØs, thereby inducing direct killing of infected cells and pathogen clearance, respectively. Parasitic helminths, on the other hand, prime DCs to induce Th2 polarization. Th2 cells induce IgE class switching of B cells and the recruitment of mast cells, eosinophils, and basophils, which are important during parasite elimination. T cell polarization is not restricted to Th1 and Th2 subsets. Production of TGF-β, IL-23 and IL-6, for example, leads to the induction of Th17 cells, which play an important role in the defense against extracellular bacteria and fungi by enhancing neutrophil responses. Another Th subset, the follicular T helper cells (Tfh), are formed upon the production of several inflammatory cytokines, namely IL-6, IL-12, IL-21 and IL-27. Tfh cells promote humoral immunity (the production of antibodies) by providing help to antigen-specific B cells, resulting in B cell activation and differentiation into memory B cells and antibody producing plasma cells.

Pro-inflammatory effector functions of our immune system are required to initiate proper immune activation, however, our immune system needs to be tightly regulated to prevent overactive immune activation, excessive tissue damage, and autoimmunity. Strong Th17 and Tfh responses, for example, have been associated with several autoimmune diseases. Regulatory T cells play an important role in tissue homeostasis and immune regulation. They are both formed during T cell development (natural Tregs), and upon the production of the immunosuppressive cytokines TGF-β and IL-10 by DCs. Induced regulatory T cells secrete the anti-inflammatory cytokine IL-10,
which inhibits the activity of several immune cells, including APCs, monocytes, and T cells. Although immune regulation is important to maintain tissue homeostasis and to prevent uncontrolled immune activation, excessive immune regulation can interfere with the effector phase of the immune response and is, therefore, undesired. Summarizing, DCs play an important role in T cell activation, polarization and immune regulation.

Toll-like receptors

DCs express different types of PRRs, which are essential to detect both extracellular and intracellular PAMPs and DAMPs. PRRs expressed by DCs include Toll-like receptors (TLRs), NOD-like receptors, C-type lectin receptors and RIG-I-like receptors. Since each PRR has its own specific ligand(s), activation of DCs by different pathogens induces the triggering of a different (combination of) PRR(s), resulting in pathogen-specific DC activation. Ten different TLRs have been described in humans, of which TLR4 is by far the best studied. TLR4 has many different ligands, including lipopolysaccharide (LPS) from Gram-negative bacteria (Table 1). Other TLRs are specialized in, for example, recognition of single or double stranded RNA and DNA from viruses.

Table 1. Toll-like receptors and their corresponding physiological and synthetic ligands

<table>
<thead>
<tr>
<th>TLR</th>
<th>Physiological ligands</th>
<th>Synthetic ligands</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR1-2</td>
<td>Triacyl-lipopeptides (Gram-negative bacteria)</td>
<td>Pam3CSK4</td>
</tr>
<tr>
<td>TLR2-6</td>
<td>Diacyl-lipopeptides (Gram-positive bacteria, mycoplasm); oxidated phospholipids, Amyloid-β (host)</td>
<td>Oxidated lipopeptides, Amyloid-β (host)</td>
</tr>
<tr>
<td>TLR2</td>
<td>Lipopeptides (bacteria, fungi, viruses, parasites); ECM components, HMGB1 (host)</td>
<td>Poly I:C</td>
</tr>
<tr>
<td>TLR3</td>
<td>dsRNA (viruses)</td>
<td></td>
</tr>
<tr>
<td>TLR4</td>
<td>Lipopolysaccharide (LPS) (Gram-negative bacteria, viruses); oxidated phospholipids, ECM components, Hsp*, Amyloid-β, β-defensin-2 (host)</td>
<td>MPLA</td>
</tr>
<tr>
<td>TLR5</td>
<td>Flagellin (Flagellated bacteria)</td>
<td></td>
</tr>
<tr>
<td>TLR7/8</td>
<td>ssRNA (viruses, bacteria, host)</td>
<td>Imidazoquinoline compounds, R848</td>
</tr>
<tr>
<td>TLR9</td>
<td>CpG-DNA (viruses, bacteria, parasites, host)</td>
<td>CpG-ODNs</td>
</tr>
<tr>
<td>TLR10</td>
<td>Unknown</td>
<td></td>
</tr>
</tbody>
</table>

TLR, Toll-Like receptor; ECM, extracellular matrix; HMGB1, high mobility Groep Box 1; Hsp, heat-shock proteins. As reviewed by Takeuchi and Akira (2010), Kawai and Akira (2010), Zhang et al. (2017). *May be due to contamination with Escherichia coli products.
and bacteria (TLR3, TLR7/8 and TLR9) or recognition of lipoproteins (TLR2, TLR1-2 and TLR2-6 heterodimers) (Table 1)\(^9,10,19\). In addition, almost all TLRs can recognize specific DAMPs (Table 1)\(^10,19\).

TLR4 activation induces the recruitment of the adaptor molecules MyD88 and TRIF to the cytosolic domain of the receptor (Figure 1). Both MyD88 and TRIF signaling induce NF-κB activation and signaling via mitogen-activated protein kinases (MAPKs), such as ERK, p38 and JNK. Signaling via interferon regulatory factor (IRF) 3 is restricted to TRIF-induced signaling, whereas IRF5 signaling only has been described downstream of MyD88\(^10\). IRF5, MAPKs and NF-κB signaling induce the production of inflammatory cytokines, such as IL-6, TNF-α, IL-12, IL-23 and IL-10, while IRF3 signaling leads to the production of type I interferons (IFNs), and the expression of co-stimulatory markers\(^10,20,21\). TLR4 is unique in activating both the adaptor molecule MyD88 and TRIF; activation of the other TLRs induces the recruitment of only one of these adaptor molecules\(^10\).
Human circulating DC subsets

During homeostasis, DCs both reside in tissues as well as in the circulation. The recruitment of circulating DCs into the affected tissue is strongly increased during tissue inflammation. DCs are not a homogenous population and can be divided in specific DC subsets. Four main DC subsets have been distinguished in human circulation (Figure 2), which all originate from hematopoietic stem cells (HSC). Some of these DC subsets in addition are precursors for tissue residing DC subsets.

6-sulfo LacNac DCs (slanDCs) are the most abundant circulating DC subset, representing 0.5-2% of the PBMC fraction. They comprise a very pro-inflammatory DC subset only described in humans, and express almost all TLRs. Increased infiltration of slanDCs is observed at the site of inflammation in several chronic diseases, including psoriasis, systemic lupus erythematosus (SLE), arthritis, inflammatory bowel disease, and multiple sclerosis. In addition, slanDCs were found to accumulate in...
human carcinoma-draining lymph nodes\textsuperscript{37}, and upon HIV-1 infection\textsuperscript{38}. slanDC are also called slan\textsuperscript{+} monocytes, and are as such part of the non-classical monocyte subset (CD14\textsuperscript{-}CD16\textsuperscript{+})\textsuperscript{27}. However, they are considered to be more related to DC subsets in terms of their capacity to induce T cell activation\textsuperscript{30,39}. The other circulating DC subsets are the myeloid DC subsets (or conventional DCs), and the plasmacytoid DCs (pDCs). Myeloid DCs can be divided in two subsets, cDC1 (defined by CD141\textsuperscript{+} in humans and CD8\textsuperscript{+} DCs in mice) and cDC2 (defined by CD1c\textsuperscript{+} DCs in humans, and CD8\textsuperscript{+} DCs in mice). They differ from each other in the expression of transcription factors (IRF8 and IRF4, respectively), TLRs (TLR3 and TLR7/8, respectively) and in their tissue distribution\textsuperscript{40,41}. Both myeloid DC subsets can induce CD4\textsuperscript{+} and CD8\textsuperscript{+} T cell responses, however, in mice, cDC1 induce much stronger CD8\textsuperscript{+} T cell responses compared to cDC2. In human, differences between myeloid DCs in terms of CD8\textsuperscript{+} T cell activation seems to be less clear\textsuperscript{40}. pDCs are the most deviant DC subset compared to the other DC subsets, and are mainly found in the circulation and in lymphoid tissues\textsuperscript{24}. They are specialized in the recognition of viruses via TLR7 and TLR9, and do not express other TLRs. Upon activation, pDCs produce high levels of type I interferons, which leads to the activation of macrophages, myeloid DCs, NK cells and CD4\textsuperscript{+} and CD8\textsuperscript{+} T cells\textsuperscript{24,42}.

Inflammatory DCs comprise yet another DC subset. They are different from the above described human DC subsets by their absence during steady state. Inflammatory DCs are also called monocyte-derived DCs (moDCs), as they specifically arise from monocytes during extravasation at the inflammatory site \textit{in vivo}. Inflammatory DCs are found during several bacterial, viral and fungal infections, as well as in several autoimmune diseases\textsuperscript{23,43}. moDCs can be generated \textit{in vitro} from monocytes using GM-CSF and IL-4, and are commonly used as inflammatory DCs during \textit{in vitro} studies and immunotherapy\textsuperscript{44}.

**The complement system**

Complement activation is important to combat invading pathogens, as it leads to immune cell recruitment (via the production of anaphylatoxins), pathogen opsonization and uptake (via the production of opsonins), and pathogen disruption (by the formation of membrane attack complexes (MACs) on pathogenic surfaces). Complement deficiency results in increased susceptibility to bacterial infections\textsuperscript{45,46}, illustrating the importance of complement activation. However, complement activation needs to be tightly controlled to prevent tissue damage, organ failure and autoimmunity\textsuperscript{45,46}. This is highlighted by the fact that many inflammatory diseases have been associated with excessive
complement activation, including many autoimmune, neurodegenerative and infectious diseases\textsuperscript{7,47,48}.

The complement system consists of more than 30 soluble and membrane-bound proteins. The soluble complement components are mainly produced in the liver, but can also be produced locally by various immune cells, including DCs and T cells\textsuperscript{49-53}. Membrane-bound complement components, which include many of the complement regulators and receptors, are widely expressed among host cells. They are important to facilitate host protection and form an important link between complement and cell-mediated immunity.

**Complement activation and regulation**

The classical view on complement activation is based on the induction of three main pathways, which are activated via immune complexes (classical pathway), pathogenic mannose structures (mannose-binding lectin pathway) and via spontaneous C3 hydrolysis (alternative pathway) (Figure 3)\textsuperscript{1,45}.

**Figure 3. The complement system.** Complement activation by the classical, mannose-binding lectin and alternative pathway, as well as convertase-independent complement activation. Adapted figure from Huber-Lang et al.\textsuperscript{55}. 

\[ \text{Classical (Immune complexes)} \]

- C1q
- C1r, C1s

\[ \text{Lectin (Mannose structures)} \]

- MBL
- MASP-2

\[ \text{Alternative (Hydrolysis)} \]

- C3b

\[ \text{Convertase-independent} \]

- C3
- C5 and C3
- C5

- Trypsin
- Elastase
- Cathepsin L
- Thrombin
- Plasmin
- Factor Xa
- Factor XIIa
- Granzyme B
- Kallikrein
- FSAP
- Cathepsin D
- C5b-9
Activation of these three pathways is tightly regulated and converge at the level of C3 cleavage by pathway-specific C3 convertases. This subsequently leads to the formation of C5 convertases, C5 activation, and downstream terminal complement activation. More recently, it has become clear that complement activation is not limited to these three complement pathways; also convertase-independent activation of C3 and C5 contributes to complement activation [54-63]. Since C3 and C5 can be produced locally by immune cells [49-52, 64, 65], convertase-independent complement activation is suggested to play an important role in local immune cell activation [54].

Terminal complement activation results in the formation of membrane attack complexes (MAC; C5b-9) on pathogenic surfaces, inducing pathogen lysis. Host cells are protected against complement-mediated destruction both by binding of soluble regulators as well as by the expression of membrane-bound regulatory complement proteins [66]. Furthermore, complement activation leads to the formation of opsonins (C3b, iC3b, C4b), and anaphylatoxins (C3a, C4a and C5a) (Figure 3). Pathogens are opsonized with C3b, iC3b and C4b, and can be recognized by membrane-bound complement receptors (CR1, CR2 and CR3) present on phagocytes [3]. This facilitates the uptake of invading pathogens, which results in pathogen clearance, and antigen presentation to the adaptive immune system. Opsonization also improves the uptake of immune complexes [3, 46].

The anaphylatoxins C5a and C3a are well-known for their function as chemoattractants. C5a is the most potent chemoattractant and induces the recruitment of many different immune cells to inflamed tissue, among which neutrophils [67-69], eosinophils [67, 69], monocytes [70], and human DCs [30, 71]. In addition, release of C5a increases blood vessel permeability, chemokine release from neutrophils, and the expression of adhesion molecules on endothelial cells [72], thereby further facilitating immune cell recruitment into inflamed tissue and local inflammation. Compared to C5a, C3a is a weaker chemoattractant, and is mainly involved in the attraction of eosinophils and mast cells [67, 73]. The chemoattractant function of C4a is at the moment still unclear [74]. To prevent overwhelming immune cell recruitment, the activity of anaphylatoxins is regulated by the presence of plasma carboxypeptidases [75]. Cleavage of anaphylatoxins C3a and C5a by carboxypeptidases results in the formation of C5a desArg and C3a desArg [66, 75]. These inactivated anaphylatoxins have been described as less active compared to their activate counterparts [70, 76]. C5a desArg, however, can still induce the recruitment of immune cells [70, 77]. Both endothelial cells and immune cells, such as APCs, recognize anaphylatoxins via specific receptors [78], named C3aR, C5aR1 (CD88) and C5aR2 (previously
called C5L2). These are seven transmembrane-spanning receptors, of which only C3aR and C5aR1 are G-protein coupled receptors. Stimulation of C3aR and C5aR1 induces Ca\(^{2+}\) influx and can induce the activation of several different signaling transduction pathways. For a long time, C5aR2 was thought to only function as a decoy receptor, thereby regulating C5a availability to C5aR1. More recently, however, C5aR2 was stated to actively inhibit C5aR1 induced signaling\(^{79,80}\). The effect of C5a on APC activity will be further explained below.

**Complement and diseases**

Many pathological conditions and diseases are associated with altered complement activation. Deficiencies in complement components MBL and C3, as well as deficiencies in terminal complement components, for example, are associated with predisposition to bacterial or pyrogenic infections\(^{45}\). In addition, a strong correlation exists between deficiencies in complement components C1q, C4 and C2 and the development of SLE\(^{81}\), whereas defects in complement regulators are linked to the development of atypical hemolytic uremic syndrome (aHUS) and paroxysmal nocturnal hemoglobinuria (PNH)\(^{7,45,66}\). This highlights complement interference as an interesting strategy to treat various complement-mediated diseases.

There are many compounds in clinical development to interfere with undesired effector functions of complement\(^{8,77}\). Treatment of PNH and aHUS by the use of a monoclonal antibody directed against C5, called eculizumab, is very successful in humans, and has been approved for the treatment of patients since 2007\(^{7,8}\). In addition, treatment with C5 or C5a modulating compounds improved disease severity in murine models for several autoimmune diseases\(^{4,5,77}\), and C5a is associated with several autoimmune diseases, including SLE\(^{82,83}\), psoriasis\(^{71}\), rheumatoid arthritis\(^{84,85}\), allergic asthma\(^{86}\) and inflammatory bowel disease\(^{5}\). Also transplant rejection is associated with elevated C5a levels\(^{87,88}\). The potential use of C5/C5a modulating compounds for treatment of autoimmune diseases is gaining more interest\(^{4,8}\), and their use for the treatment of RA and psoriasis already reached clinical trials\(^7\). The involvement of C5a in these diseases is, however, still poorly understood. C5a interference may for example affect APC activation and subsequent adaptive immune responses, as will become clear below. Long term complement inhibition in these diseases may, furthermore, lead to increased susceptibility to bacterial infections\(^8\).
Table 2. Stimulatory effect of C5a on antigen presenting cell activation and adaptive immunity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>species</th>
<th>cell type</th>
<th>stimulus</th>
<th>Cytokine production</th>
<th>Co-stimulatory molecules</th>
<th>T cell activation</th>
<th>Clinical relevance</th>
<th>ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>C5aR deficiency</td>
<td>murine</td>
<td>BMDC</td>
<td>OVA</td>
<td>n.d.</td>
<td>n.d.</td>
<td>↓ T cell response, ↓ IFN-γ</td>
<td>C5aR deficiency reduces Th1 response to T. gondii and in EAE and herpes keratitis models</td>
<td>[50]</td>
</tr>
<tr>
<td></td>
<td>murine</td>
<td>BMDC</td>
<td>LPS</td>
<td>↓ IL-1, IL-10</td>
<td>↓ MHCI, CD80, CD86</td>
<td>↓ T cell response, ↓ IFN-γ</td>
<td>n.d.</td>
<td>[49]</td>
</tr>
<tr>
<td></td>
<td>murine</td>
<td>BMDC</td>
<td>allograft</td>
<td>n.d.</td>
<td>n.d.</td>
<td>↓ T cell response, ↓ IFN-γ</td>
<td>C5aR deficiency prolongs renal allograft survival and decreases cell infiltration</td>
<td>[95]</td>
</tr>
<tr>
<td></td>
<td>murine</td>
<td>peritoneal MØ</td>
<td>OVA</td>
<td>n.d.</td>
<td>↓ CD80, CD40</td>
<td>n.d.</td>
<td>n.d.</td>
<td>[50]</td>
</tr>
<tr>
<td>C5aR antagonist</td>
<td>murine</td>
<td>BMDC</td>
<td>OVA</td>
<td>↓ IL-1, IL-23</td>
<td>↓ MHCI, CD80, CD86, CD40</td>
<td>↓ T cell response, ↓ IFN-γ</td>
<td>n.d.</td>
<td>[50]</td>
</tr>
<tr>
<td></td>
<td>murine</td>
<td>BMDC</td>
<td>LPS</td>
<td>↓ IL-1, IL-10</td>
<td>↓ MHCI, CD80, CD86</td>
<td>↓ T cell response, ↓ IFN-γ</td>
<td>n.d.</td>
<td>[49]</td>
</tr>
<tr>
<td></td>
<td>murine</td>
<td>n.d.</td>
<td>allograft</td>
<td>↓ IL-1, TNF-α, MIP-2</td>
<td>n.d.</td>
<td>n.d.</td>
<td>C5aR inhibition improves renal graft survival</td>
<td>[88]</td>
</tr>
<tr>
<td>C5a treatment</td>
<td>murine</td>
<td>BMDC</td>
<td>LPS</td>
<td>↑ IL-1, TNF-α, ↓ IL-10</td>
<td>↑ MHCI, CD86, CD40</td>
<td>↑ T cell response, ↑ IFN-γ</td>
<td>n.d.</td>
<td>[49]</td>
</tr>
<tr>
<td></td>
<td>human</td>
<td>moDC</td>
<td>LPS</td>
<td>↑ IL-6, TNF-α, IL-12</td>
<td>↑ HLA-DR, CD86, CD40</td>
<td>↑ IFN-γ, TNF-α, IL-17</td>
<td>n.d.</td>
<td>[102]</td>
</tr>
<tr>
<td></td>
<td>murine</td>
<td>peritoneal MØ</td>
<td>LPS</td>
<td>↑ IL-1, TNF-α</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>[94]</td>
</tr>
<tr>
<td></td>
<td>human</td>
<td>monocyte*</td>
<td>LPS</td>
<td>↑ IL-6, TNF-α, no effect on IL-10</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>[103]</td>
</tr>
</tbody>
</table>

n.d., no data; BMDC, bone marrow-derived dendritic cell; MØ, macrophages; moDC, monocyte-derived dendritic cells; OVA, Ovalbumin; *monocytes are not defined antigen presenting cells
### Table 3. Inhibitory effect of C5a on antigen presenting cell activation and adaptive immunity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>species</th>
<th>cell type</th>
<th>stimulus</th>
<th>Cytokine production</th>
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<th>T cell activation</th>
<th>Implication</th>
<th>ref</th>
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</thead>
<tbody>
<tr>
<td><strong>C5aR deficiency</strong></td>
<td>murine</td>
<td>n.d.</td>
<td>L. major</td>
<td>n.d.</td>
<td>n.d.</td>
<td>↑ T cell infiltration, ↑ IFN-γ n.d.</td>
<td>C5aR−/− more resistant to L. major infections C5aR−/− mice are more protected against periodontal bone loss, C5aR−/− promotes intracellular clearance of P. gingivalis</td>
<td>[93]</td>
</tr>
<tr>
<td><strong>C5aR antagonist</strong></td>
<td>murine</td>
<td>n.d.</td>
<td>P. gingivalis</td>
<td>↑ IL-12, IL-23, IFN-γ, ↑ IL-6, TNF-α, IL-1β</td>
<td>n.d.</td>
<td>n.d.</td>
<td>C5aR inhibition promotes intracellular clearance of P. gingivalis</td>
<td>[97]</td>
</tr>
<tr>
<td><strong>C5a treatment</strong></td>
<td>murine</td>
<td>peritoneal MØ</td>
<td>LPS</td>
<td>↓ IL-12, IFN-γ, ↓mRNA IL-12 family members</td>
<td>n.d.</td>
<td>↓ Th1, ↓ IFN-γ n.d.</td>
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<tr>
<td></td>
<td>murine</td>
<td>peritoneal MØ</td>
<td>LPS</td>
<td>↓ IL-12, ↓mRNA: p40, p35, TNF-α, NOS, IFN-γ, IL-10</td>
<td>n.d.</td>
<td>n.d.</td>
<td></td>
<td>[96]</td>
</tr>
<tr>
<td></td>
<td>murine</td>
<td>peritoneal MØ</td>
<td>LPS</td>
<td>↓ IL-12</td>
<td>n.d.</td>
<td>n.d.</td>
<td></td>
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<tr>
<td></td>
<td>murine</td>
<td>peritoneal MØ</td>
<td>P. gingivalis</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>C5a promotes P. gingivalis intracellular survival</td>
<td>[100]</td>
</tr>
<tr>
<td></td>
<td>murine</td>
<td>peritoneal MØ</td>
<td>P. gingivalis</td>
<td>↓ IL-12, ↑ IL-6, TNF-α</td>
<td>n.d.</td>
<td>n.d.</td>
<td>C5a attenuates P. gingivalis intracellular clearance</td>
<td>[97]</td>
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<tr>
<td></td>
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<td>peritoneal MØ</td>
<td>LPS</td>
<td>↓ IL-1β</td>
<td>n.d.</td>
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<td></td>
<td>murine</td>
<td>peritoneal MØ</td>
<td>LPS</td>
<td>↓ IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-12, IL-13, IL-17, Eotaxin, GM-CSF, IFN-γ, KC, MCP-1, MIP-1α, MIP-1β, RANTES, TNF-α, ↑ G-CSF, IL-10</td>
<td>n.d.</td>
<td>n.d.</td>
<td></td>
<td></td>
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<tr>
<td>human</td>
<td>moMØ</td>
<td>LPS</td>
<td></td>
<td>↓ IL-6, TNF-α, IL-10</td>
<td>n.d.</td>
<td>n.d.</td>
<td>C5a decreases S. typhimurium survival in MØ</td>
<td>[103]</td>
</tr>
<tr>
<td>human</td>
<td>moDC</td>
<td>SAC/IFN-γ</td>
<td></td>
<td>no effect on IL-12</td>
<td>n.d.</td>
<td>n.d.</td>
<td></td>
<td>[101]</td>
</tr>
<tr>
<td>human</td>
<td>monocyte*</td>
<td>LPS/IFN-γ</td>
<td></td>
<td>↓ IL-12, ↓mRNA IL-12 family members</td>
<td>n.d.</td>
<td>n.d.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>human</td>
<td>monocyte*</td>
<td>LPS/IFN-γ</td>
<td></td>
<td>↓ IL-12</td>
<td>n.d.</td>
<td>n.d.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>human</td>
<td>monocyte*</td>
<td>SAC/IFN-γ</td>
<td></td>
<td>↓ IL-12, mRNA; p40, p35; no effect on TNF-α, IL-10, TGF-β</td>
<td>n.d.</td>
<td>n.d.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n.d., no data; MØ, macrophages; moMØ, monocyte-derived macrophages; SAC, S. aureus Cowan-I. *monocytes are not defined antigen presenting cells
C5aR and TLR crosstalk in APCs

Although both DCs and the complement system are activated at the site of inflammation and play an important role during infections and in autoimmunity, crosstalk between complement activation products and DCs has only been investigated during the last decade. Although some studies address the direct effect of anaphylatoxins on T cell activation, most research focuses on the effect of anaphylatoxin C5a during DC and MØ maturation by PAMPs. Published data on the effect of anaphylatoxins on APC activation and subsequent adaptive immune responses are, however, contradicting. In addition, most research has been performed in mice and it remains to be shown if these findings can be translated to the human setting (Table 2 and 3). The increased interest in the use of C5/C5a modulating compounds for the treatment of various diseases, emphasizes the importance to understand the crosstalk between these two arms of innate immunity. Especially because DCs can strongly modulate adaptive immunity, and can locally produce complement components, including C5, C5aR and TLR crosstalk in mice

In mice, the effect of C5a on immune activation has been investigated mainly in bone marrow-derived DCs (BMDCs) and peritoneal MØs (Table 2 and 3). C5a promotes co-stimulatory marker expression and enhances LPS-induced IL-12 production in BMDCs, resulting in increased Th1 polarization. In line with this, disabled C5aR signaling during BMDC-T cell interaction, either by the use of C5aR deficient mice or by the use of C5aR antagonists, results in an impaired Th1 immune response. C5a stimulation also leads to increased production of IL-1 and TNF-α in BMDCs, while decreasing IL-10 production. In these studies, the pro-inflammatory effect of C5a on cytokine production is associated with increased PI3K-induced Akt phosphorylation and NF-κB activity, whereas cAMP/PKA signaling is reduced (Figure 4B).

This pro-inflammatory effect of C5a on DC activation may promote adaptive immunity during infections, but can also contribute to the development of autoimmunity in the absence of an infection. During Toxoplasma gondii infection, C5aR deficient BMDCs are indeed impaired to induce Th1 immune responses, resulting in decreased protection during infection. In C5aR deficient mice, impaired Th1 immune responses are observed in models for experimental autoimmune encephalomyelitis and herpes keratitis (Table 2), indicating that C5a indeed promotes disease severity in autoimmune settings. In addition, the pro-inflammatory effect of C5a on DCs interferes with successful organ...
transplantation, for dysregulated C5aR signaling reduces graft rejection and allo-reactive T cells responses in mice. In contrast to the stimulatory effects of C5a, also inhibitory effects of C5a on APC activation and Th1 immunity have been reported (Table 3). Different from the studies described above, all of these studies were performed in mouse peritoneal MØs. C5a decreases the production of many pro-inflammatory cytokines, including TNF-α, IL1-β, IL-6, IL-12, and IL-23, while increasing the expression of IL-10. Decreased pro-inflammatory cytokine production upon C5a stimulation is associated with reduced Th1 polarization and attenuation of Porphyromonas gingivalis clearance. In line with this, C5aR deficiency increases the resistance against Leishmania major infections. In these studies, C5a treatment is associated with both increased ERK1/2 as well as increased PI3K phosphorylation (Figure 4C). The inhibitory effect of C5a on IL-12 production, as well as the stimulatory effect of C5a on IL-10 production, is dependent on ERK1/2 signaling. Also, cAMP levels increase upon C5a treatment in mouse peritoneal MØs. In general, C5a seems to promote pro-inflammatory immune activation of mouse BMDCs, whereas C5a decreases the activation of mouse peritoneal MØs.
**General introduction**

Knowledge on C5aR and TLR crosstalk in human APCs is very limited ([Table 2 and 3](#)). C5a stimulation had no effect on pro-inflammatory cytokine production of human moDCs when added 1 h prior to stimulation with *Staphylococcus aureus* Cowan I (SAC) and IFN-γ. To our knowledge, this is the only study investigating C5aR and TLR crosstalk in human DCs. C5aR expression is present on moDCs and C5a increases the production of TNF-α, IL-6, and IL-12 and the expression of co-stimulatory marker expression by human moDC in the absence of LPS. Although different in experimental set-up (presence of PAMPs compared to the absence of PAMPs), the activation of signal transduction pathways by C5a in these moDCs is in line with observations in TLR-stimulated mouse BMDCs ([Figure 4A and B](#)). In human MØs, similar results have been found as observed in mouse peritoneal MØs. Also here, C5aR and TLR crosstalk is associated with an decrease in IL-6 and TNF-α production, phosphorylation of ERK1/2, and independency of PI3K signaling in relation to cytokine production ([Figure 4C](#)). Although not defined as APCs, it is still worthwhile to mention that C5a also inhibits pro-inflammatory cytokine production in three out of four studies performed in human monocytes ([Table 2 and 3](#)). Among human circulating DC subsets, no C5aR expression is observed on ex vivo isolated myeloid DCs, whereas C5aR expression by pDCs correlates with DC activation status. Apart from expression on moDCs, high C5aR1 expression is also observed on slanDCs. This suggests that in humans, especially moDCs and slanDCs may be susceptible to modulation by C5a. As mentioned before, increased levels of both DCs and C5a are observed in several autoimmune diseases, and DCs play an important role in modulating adaptive immune responses during pathogen infections and in autoimmunity. In addition, the increasing interest in the use of C5/C5a modulating compounds highlights the importance to unravel the effect of C5aR and TLR crosstalk in human DCs. Overall, knowledge on C5aR and TLR crosstalk in human DCs is very limited, and needs further investigation.
Scope of this thesis

The scope of this thesis was to investigate how C5aR and TLR crosstalk affects the activation and eventual function of human moDCs and slanDCs. In chapter 2 we investigated the impact of C5aR and TLR crosstalk on the production of inflammatory cytokines and co-stimulatory molecule expression by human moDCs. C5a reduced the production of TLR-induced pro-inflammatory cytokines. This inhibitory effect of C5a was very much depending on simultaneous activation of C5aRs and TLRs. C5aR and TLR crosstalk had no effect on the expression of co-stimulatory molecules by moDCs.

In chapter 3, we assessed the effect of C5aR and TLR crosstalk on other human DC subsets, and elucidated how C5aR and TLR crosstalk occurred in the underlying intersecting signaling transduction pathways. In addition, we investigated the effect of C5a-primed DCs on subsequent T cell immunity. Analysis of C5aR expression on human DC subsets indicated that especially slanDCs are prone to regulation by C5a. We uncovered that C5a inhibited TLR-induced pro-inflammatory cytokine production by slanDCs. IL-10 induction upon accelerated TLR-induced ERK/p38-CREB1 signaling was demonstrated to be key in the regulatory effect of C5a on pro-inflammatory cytokine production by DCs. In line with these findings, C5a-priming of DCs reduced Th1 and cytotoxic CD8 T cell immune responses.

RNA sequencing analyses were performed to investigate the early effects of C5aR and TLR crosstalk on DC activation in an unbiased fashion in chapter 4. This demonstrated that C5aR and TLR crosstalk not only affects expression of pro-inflammatory cytokines, but induces a core regulatory network in human moDCs. Forkhead box (FOX)01 and FOX03 transcription factors play a central role in this regulatory network. In addition, motif enrichment analysis revealed a prominent role for basic leucine zipper and IFN regulatory factor 4 transcription factors during C5aR and TLR crosstalk.

In chapter 5, RNA sequencing analyses were used to determine the effect of C5a on DC activation in absence of TLR stimulation. Gene ontology term enrichment analysis was suggestive for a role of C5a in Fc-receptor mediated phagocytosis and endosomal maturation. Functional experiments revealed that C5a specifically acts on DCs to inhibit Fc-gamma receptor mediated uptake of immune complexes. Finally, chapter 6 contains a general discussion and summarizes our present findings.
References


