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Complement Receptors in Inflammation

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1. Introduction

Complement was first discovered in 1889 as a bactericidal protein, distinct from heat stable antibodies present in normal serum. Since that time, it has been shown that the complement system is a biochemical cascade, comprised of more than 30 fluid phase and membrane-associated proteins, normally present as inactive forms. The complement system can be activated by different sequential cascades of enzymatic reactions (described below) in which proteins are sequentially cleaved and activated. The resulting effector molecules, C3a and C5a (also known as anaphylatoxins) are the most potent complement activation products, showing diverse activities on many cell types ranging from chemoattraction to apoptosis. The main target cells carry specialized complement receptors through which anaphylatoxins participate in host defense, inflammatory processes, and immune responses.

The complement system plays an essential role in innate immunity by defending the host against bacterial, viral, and parasitic invasion. Complement proteins promote opsonisation and/or phagocytosis and intracellular killing of these pathogens by immune effector cells such as macrophage and neutrophils. Complement proteins, particularly those of the classical pathway, aid in the processing of immune complexes and in protection against the development of immune complex diseases such as systemic lupus erythematosus (SLE). Recently, it has become evident that the complement system also regulates adaptive immunity involving B and T cells that help in the elimination of pathogens. Furthermore, the engagement of complement receptors on antigen-presenting cells (APC) and other immune cells leads to production of immunoregulatory cytokines. Not only is complement involved in innate and adaptive immunity, it is also involved in pathological conditions. For example, in allergic disease complement proteins participate in the development of an inflammatory reaction. The complement pathways are also activated in patients with sepsis, allergic rhinitis, allergic asthma, and allergic skin conditions such as urticaria. This chapter will outline how complement proteins, C3a and C5a, and their complement receptors regulate inflammation.

2. Complement system

2.1 Complement activation pathways

Pattern recognition receptors (PRRs) in the complement system such as specific antibody, C1q, C3, mannose-binding lectin (MBL), and ficolins recognize exogenous as well as endogenous pattern-associated molecular patterns (PAMPs) leading to the activation of complement (Wills-Karp, 2007). The complement system can be activated by four different pathways: the classical, alternative, lectin and extrinsic protease pathway. Although each of these pathways is activated by distinct PRRs, they all culminate in activation of C3, the central step in complement activation.

2.1.1 Classical pathway

The classical pathway is activated when immune complexes are formed. These immune complexes are formed when antibodies (released during a humoral immune response (immunoglobulin (Ig)G or IgM)) bind to pathogens or other foreign and non-self antigens. The Fc portion of the antigen-antibody complex is engaged by the C1q molecule of the C1 complex (a multimeric complex consisting of C1q, C1r and C1s molecules) leading to activation of C1s and C1r. C1 then cleaves C4 and C2 to form C3 convertase (C4bC2a; Figure 1) (Wagner and Frank, 2010).

C3 convertase enzyme activates C3, the most abundant complement protein found freely in blood plasma, by proteolytic cleavage. This reaction results in the generation of: (1) complement proteins C3a, C4a and C5a; (2) membrane attack complex (MAC) consisting of C5b, C6, C7, C8 and C9; and (3) opsonisation molecule C3b (Wagner and Frank, 2010; Wills-Karp, 2007).

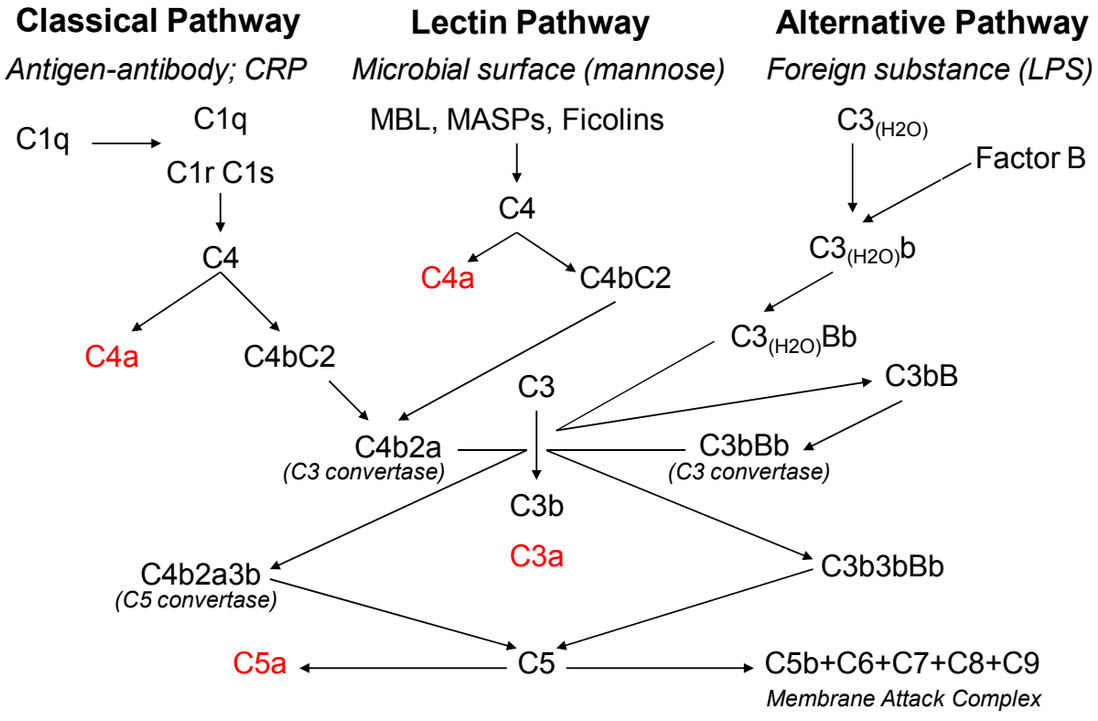

Fig. 1. Complement activation pathways

2.1.2 Lectin pathway

The lectin pathway is initiated when PRRs, MBL, H-, M- and L-ficolin, recognize and bind sugar moieties on yeast, bacteria, parasites and viruses. In the circulation, these PRRs associate with MBL-associated serine protease 1 (MASP1), MASP2 and MASP3, and a truncated MASP2 known as MAP19. Binding of the MBL-MASP complex to pathogen results in the cleavage of C4 and C2 and the generation of a C3 convertase (C4bC2a; Figure 1), similar to that of the classical pathway (Wagner and Frank, 2010).

2.1.3 Alternative pathway

The alternative pathway is triggered by carbohydrates, lipids and proteins found on pathogen. Slow and constant hydrolysis of circulating C3 (C3 tickover) and its interaction with complement factors B, and D leads to formation of a C3 convertase (C3bBb; Figure 1) (Wagner and Frank, 2010).

2.1.4 Extrinsic protease pathway

In addition to the above three pathways, a C3 independent pathway can also activate the complement system. An extrinsic protease pathway involves direct cleavage of C3 and C5 by a series of proteolytic enzymes released by neutrophils and macrophages; and factors such as Kallikrein, plasmin and factor XIIa. Thrombin, a coagulation factor, can directly cleave C5 to generate biologically active C5a in C3-deficient mice in which C5 convertase cannot be formed (Ricklin et al., 2010).

2.2 Complement proteins C3a, C4a and C5a

The complement proteins, C3a, C4a and C5a, are components of the complement system that are produced from C3, C4 and C5, respectively, as part of the complement activation cascade. C3a is a 9 kiloDalton (kDa) peptide fragment released during selective proteolytic cleavage of the C3 α chain by a C3 convertase of the classical or the alternative pathway. C4a is a 8.7 kDa peptide released from the α chain of C4 by C2a cleavage in an early step of the classical pathway. C5a is an 11 kDa peptide released from the α chain of C5 by action of either classical or alternative pathway C5 convertase (Ember and Hugli, 1997).

Although C3a, C4a and C5a are genetically related, they have marked structural differences in their sequences. Only 13 residues are conserved between C3a, C4a and C5a molecules from several species examined. Six of the conserved residues are cysteines whose side chains participate in forming three intrachain disulfide linkages that stabilize the folded alpha-helical peptide chain. The three disulfide bonds in C3a are Cys 22-Cys 49, Cys 23-Cys 56 and Cys 36-Cys 57. The four-helix bundle of C5a is stabilized by Cys 21-Cys 47, Cys 22-Cys 54 and Cys 34-Cys 55 disulfide bonds. Further structural similarities include a flexible carboxyl terminal tail –L-G-L-A-R in C3a, -A/V-G/H-L-A/Q-R in C4a, and –M/I/V-Q-L-G-R in C5a, which forms a helical turn connected by a short loop, which is important for effector functions of these proteins (Ember and Hugli, 1997).

C3a, C4a and C5a are potent inflammatory peptides with diverse activities on many cell types. They act as chemoattractants for immune cells such as neutrophils, eosinophils, mast cells, and monocytes recruiting to sites of injury or inflammation. They also regulate vasodilation, increase the permeability of blood vessels, and induce smooth muscle contraction. Anaphylatoxin stimulation can induce oxidative burst in neutrophils, histamine release from mast cells and basophils, production of eosinophil cationic protein (ECP) from

eosinophils, and production of pro-inflammatory cytokines from monocytes, B cells and T cells. Characterization of the inflammatory activities of complement proteins indicate relative activities in the order of C5a>C3a>C4a on most tissues examined (Hugli, 1981).
The complement proteins generated as a result of the complement activation damage host tissues. It is therefore obvious that control mechanisms are needed to tightly regulate these potent peptides and maintain homeostatic balance. Indeed, once C3a and C5a are cleaved from C3 and C5 respectively, they are rapidly degraded by plasma enzyme carboxypeptidases. The resulting C3a desArg lacks any pro-inflammatory activity; however, C5a desArg exhibits a reduced inflammatory activity of 1-10% compared with C5a. Interestingly, C3a desArg, also known as acylation stimulating protein (ASP), has been described as possessing metabolic hormone activity that drives triglyceride synthesis and glucose update. This suggests a regulatory role of C3a desArg in cell apoptosis and lipid metabolism (Cianflone et al., 1989).

3. Complement receptors for C3a and C5a: C3aR, C5aR and C5L2

The complement proteins C3a and C5a exert their pleiotropic effects by binding to a family of three receptors belonging to the G protein-coupled receptor (GPCR) superfamily. These receptors are the C3a receptor (C3aR), the C5a receptor (C5aR) and the C5a receptor-like 2 (C5L2).

3.1 C3a receptor (C3aR)

C3aR is a membrane glycoprotein of approximately 54 kDa (Ames et al., 1996). This receptor displays high affinity for C3a with a dissociation constant (K_d) of about 1 nM, but not for C3a desArg or C5a (Crass et al., 1996). Human platelets express a high molecular weight (95-105 kDa) variant of C3aR that binds C3a with K_d of 8×10^{-10} M.

3.1.1 Structure

As a GPCR, C3aR contains seven transmembrane domains within its 482 amino acids sequence. C3aR distinctively possesses a large second extracellular loop between the fourth and fifth transmembrane domain that is indispensable for ligand binding. This loop contains 175 amino acid residues; in most GPCR the corresponding extracellular loop is 30-40 amino acids long. Sulfation of tyrosine 174 in this loop is essential for binding C3a (Chao et al., 1999). The genes encoding C3aR have been mapped to p13.2-3 of chromosome 12 in humans and 6F1 in mouse (Hollmann et al., 1998). C3aR displays 50-60% homology between various species, with 65% sequence identity between human and murine counterparts (Hollmann et al., 1998).

3.1.2 Expression

C3aR is expressed on cells of myeloid origin, including monocytes/macrophages, neutrophils, eosinophils, basophils, mast cells, dendritic cells and microglia. Additionally, C3aR is also expressed on non-myeloid cells, such as astrocytes, endothelial cells, epithelial cells, smooth muscle cells, and activated T cells. One paper has described the receptor's expression on human tonsillar B cells; while others have confirmed the absence of C3aR on human B cells. The receptor is also expressed in tissues from lung, liver, kidney, brain, heart, muscle and, testis.

3.1.3 Biological role

C3aR mediates chemotaxis of eosinophils (Daffern et al., 1995), mast cells (Nilsson et al., 1996), dendritic cells (Gutzmer et al., 2004) and monocytes, but not neutrophils (Daffern et al., 1995). It can also trigger oxidative burst in macrophages, neutrophils and eosinophils (Burg et al., 1996). In addition, basophils (Bischoff et al., 1990) and mast cells (Johnson et al., 1975, Venkatesha et al., 2005) undergo degranulation and release histamine upon C3a-C3aR interaction. C3aR stimulates production of ECP from eosinophils (Takafuji et al., 1994), as well as upregulation of β_2-integrins and shedding of L-selectins, thereby promoting eosinophil adhesion to endothelial and epithelial cells (Jagels et al., 2000). Human monocytes and mast cells exhibit increased intracellular calcium (Ca^{2+}) levels when stimulated with C3a (Venkatesha et al., 2005). C3a also stimulates smooth muscle contraction (Stimler et al., 1983), lysozyme release from immune cells (Showell et al., 1982), platelet aggregation (Becker et al., 1978), and triglyceride synthesis in adipocytes (Baldo et al., 1993).

3.1.4 Signaling

Upon C3a binding to the C3aR, intracellular signal transduction is promoted via heterotrimeric guanosine triphosphate (GTP)-binding proteins (G proteins). C3aR mediates its effect on immune cells via coupling to the pertussis toxin (PTX)-sensitive and –insensitive G proteins $G\alpha_i$ and $G\alpha_{16}$, respectively. In endothelial cells, C3aR also couples to PTX-insensitive $G\alpha_{12}$ and $G\alpha_{13}$ (Schraufstatter et al., 2002). Downstream signaling events involve activation of phosphoinositol-3-kinase gamma (PI3K-γ), which in turn activates phospholipase C (PLC)β and PLCγ. This leads to generation of inositol triphosphate (IP3) and diacylglycerol (DAG), leading to Ca^{2+} mobilization and phosphokinase C (PKC) activation, respectively. PI3K can also activate Raf/mitogen-activated protein kinase (MAPK)/extracellular-signal regulated kinase (ERK) kinase (MEK)/ERK cascade inducing expression of various pro-inflammatory cytokines and chemokines.

3.2 C5a receptor (C5aR or CD88)

First cloned in 1991 (Boulay et al., 1991, Gerard and Gerard, 1991), C5aR is a membrane glycoprotein of approximately 42 kDa (Richardson et al., 1998), displaying high affinity for C5a and C5a desArg. Human C5aR binds C5a with a K_d of 1 nM, and with 10 to 100-fold lower affinity to C5a desArg (K_d of 412-660 nM) whereas C3a and C3a desArg are not recognized (Monk et al., 2007).

3.2.1 Structure

Similar to C3aR, C5aR is a seven transmembrane GPCR that belongs to the rhodopsin-like family. Based on the analysis of transmembrane domain sequences, C5aR is clustered with other chemoattractant receptors, such as C3aR, formyl peptide receptor family, ChemR23, platelet activating factor (PAF) receptor, IL-8 receptor, and bradykinin receptor (Methner et al., 1997, Samson et al., 1998). The C5aR gene is localized to q13.3-13.4 of human chromosome 19 (Gerard et al., 1998). Murine C5aR exhibits 65% sequence identity with its human counterpart (Gerard et al., 1992). The N terminus of C5aR is required for high affinity binding of C5a, but not for receptor activation (DeMartino et al., 1994, Mery and Boulay, 1993). A second distinct binding site is formed by charged residues in the second and third extracellular loops and the external faces of the transmembrane helical bundle and

hydrophobic residues in the core of the C5aR. Unlike the N terminal binding site, the second site is responsible for receptor activation (Gerber et al., 2001). Current evidence suggests that at least three different discontinuous regions exist within the C5a molecule for interaction with C5aR (Huber-Lang et al., 2003).

3.2.2 Expression

C5aR is expressed on cells of myeloid origin such as neutrophils, eosinophils, basophils, mast cells, dendritic cells and monocytes, as well as on non-myeloid cells, including bronchial and alveolar epithelial cells, endothelial cells, Kupffer cells, stellate cells, astrocytes and microglial cells (Monk et al., 2007).

3.2.3 Biological role

C5aR is a powerful chemoattractant receptor for monocytes (Pieters et al., 1995), neutrophils (Webster et al., 1980), eosinophils (DiScipio et al., 1999), basophils (Lett-Brown and Leonard, 1977), mast cells (Hartmann et al., 1997), B cells (Kupp et al., 1991) and T cells (Nataf et al., 1999). It also stimulates mast cell degranulation (Subramanian et al., 2011), mast cell chemotaxis in specific mast cell subtypes (Hartmann et al., 1997, McCloskey et al., 1999), oxidative burst in granulocytes and the production of reactive oxygen species (ROS) in neutrophils (Guo et al., 2003), secretion of lysosomal enzymes from macrophages (McCarthy and Henson, 1979) and polymorphonuclear (PMN) cells as well as the secretion of pro-inflammatory mediators from monocytes, eosinophils (Takafuji et al., 1994) and mast cells (Hartmann et al., 1997). C5a is also responsible for upregulation of vascular adhesion molecules such as E-selectin, inter-cellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) after systemic activation of complement (Albrecht et al., 2004, Jagels et al., 2000). Our preliminary findings suggest that C5a can promote human mast cell adhesion to extracellular matrix protein, as well as human mast cell migration *in vitro*, indicating a critical role of C5a/C5aR in inflammation.

3.2.4 Signaling

C5aR signaling depends on heterotrimeric G proteins. C5aR mainly couples to $G\alpha_{i2}$, a PTX-sensitive G protein (Skokowa et al., 2005). However, ectopically expressed C5aR, and C5aR on cells of the hematopoietic lineage, can also couple to PTX-insensitive $G\alpha_{16}$ (Amatruda et al., 1993, Monk and Partridge, 1993). C5a-C5aR interaction leads to activation of several components of signaling pathway, including PI3K-γ, PLCβ2, and phospholipase D (PLD) (Rabiet et al., 2007). C5aR can activate the transcription factor, cyclic AMP (cAMP) response element-binding protein (CREB), by phosphorylation at the convergence of two pathways, PI3K/Akt and ERK (Perianayagam et al., 2006). CREB activation has been proposed to be a part of the mechanism by which C5a can delay neutrophil apoptosis and prolong an inflammatory response (Perianayagam et al., 2004). In neutrophils, C5a causes downstream activation of p21-activated kinases (PAK), which are downstream effectors of cdc42 and rac (small guanosine triphosphate (GTP)-binding proteins (GTPases)), as well as Gγ subunits (Huang et al., 1998). PAK family members are involved in altering cell morphology/chemotaxis, activation or potentiation of several distinct MAPK cascades and the activation of nuclear factor (NF)-kB in macrophages. p38 MAPK is activated by PAK1/PAK2 and, in turn, activates MAPK-activated protein kinase 2 (MAPKAP-K2). In primary macrophages from MAPKAP-K2-deficient mice, chemotaxis to C5a is impaired.

Furthermore, the p38 MAPK inhibitor, SB203580, can inhibit C5a-induced migration in a mouse acute lung injury model (Rousseau et al., 2006).
C5aR also couples directly or indirectly to a small range of other intracellular proteins. The Wiskot–Aldrich syndrome protein (WASP) was detected as a binding partner of the C-terminus of C5aR using a yeast two-hybrid assay. WASP binding was strongly potentiated in the presence of active cdc42, suggesting that the association occurs after C5aR activation (Tardif et al., 2003). WASP is a multifunctional protein with a role in the regulation of actin dynamics, and so could be involved in the chemotactic response to C5a. In human erythroleukaemia cells, signal transducers and activators of transcription (STAT3) phosphorylation can be stimulated by C5a in a PTX-insensitive manner, most likely through $G\alpha_{16}$ and the Ras/Raf/MEK/ERK and janus kinase (JAK) pathways (Lo et al., 2006). In contrast, STAT3 phosphorylation occurs only through an ERK pathway in C5a-stimulated neutrophils (Kuroki and O'Flaherty, 1999).

3.3 C5a receptor-like 2 (C5L2)

First discovered in 2000 as a putative orphan receptor (GPR77), (Ohno et al., 2000) C5L2 has since been identified as a second C5a receptor. It is a 37 kDa protein and binds C5a with high affinity (K_d of 2.5 nM). Unlike C5aR, C5L2 binds C5a desArg with a 20-30 fold higher affinity (Cain and Monk, 2002).

3.3.1 Structure

C5L2 consists of 337 amino acids with asparagine 3 as a potential glycosylation site (Ohno et al., 2000). In the conserved transmembrane regions, C5L2 shares 58% sequence identity with C5aR and 55% with C3aR (Lee et al., 2001). Unlike C5aR, C5L2 uses critical residues in its N terminal domain for binding only to C5a desArg. In addition to C5a and C5a desArg binding, C5L2 has been considered a binding partner for C3a, C3a desArg (Kalant et al., 2005), C4a and C4a desArg; however the available data is not very convincing (Johswich et al., 2006, Okinaga et al., 2003).
Although C5L2 has the conventional structure of a GPCR, studies have found that C5L2 does not couple to G proteins. This may be due to a structural difference in the third transmembrane domain. In GPCR, a highly conserved DRY motif in the third transmembrane domain is important for its interaction with the corresponding G proteins. The DRY motif is DRC in C3aR and DRF in C5aR, but DLC in C5L2. Moreover, the third transmembrane domain of C5L2 is truncated. Mutation of DLC in human C5L2 to DRC has been shown to increase coupling to $G\alpha_{16}$ in co-transfected human embryonic kidney (HEK)293 cells and induce a functional response (Okinaga et al., 2003). Interestingly, no functional response occurs in rat basophilic leukemia cells (RBL-2H3) using a C5L2-mutant where the DRY-motif and two additional regions typically involved in G protein coupling are replaced by the corresponding C5aR sequences (Scola et al., 2009). Taken together, these findings strongly suggest that C5L2 completely lacks the potential to couple with G proteins.

3.3.2 Expression

C5L2 is expressed in various tissues of myeloid and non-myeloid origin and transcripts are detected in brain, placenta, ovary, testis, spleen and colon. Surface expression of C5L2 has been detected in lung, liver, heart, kidney, adipose tissue, skin fibroblasts, neutrophils, and

immature, but not mature dendritic cells. In the presence of a C5aR antagonist, binding of C5a has been demonstrated on differentiated myeloblastic HL-60, U937, and epithelial HeLa-cells. C5L2 and C5aR seem to be frequently co-expressed in most cells or tissues (Monk et al., 2007).

3.3.3 Biological role and signaling

C5L2 is an enigmatic receptor as available data suggest opposing roles. On the one hand, C5L2 has been described as a non-signaling decoy receptor for C5a and C5a desArg. In contrast, some studies suggest that C5L2 serves as a signaling functional receptor. In support of a role as a decoy receptor, no mobilization of intracellular Ca^{2+} occurs in C5L2 transfected cells after C5a administration (Cain and Monk, 2002, Okinaga et al., 2003). Moreover, no Ca^{2+} mobilization occurs in neutrophils from C5aR-deficient mice after stimulation with C5a (Hopken et al., 1996) or in C5L2 expressing epithelial and myeloid cell lines (Johswich et al., 2006). In the presence of C5a and C5a desArg, C5L2 transfected RBL-2H3 cells do not degranulate (Cain and Monk, 2002). Further, C5a binding to C5L2 in bone marrow cells derived from C5aR-deficient mice fails to induce any changes in messenger RNA (mRNA) expression (Okinaga et al., 2003).

Nevertheless, there is accumulating evidence that C5L2 is a functional receptor capable of regulating C5aR function *in vitro* and *in vivo*. Neutrophils and macrophages from C5L2-deficient mice produce more tumor necrosis factor (TNF)α and interleukin (IL)6 in response to stimulation with C5a and lipopolysaccharide (LPS) than their wildtype (WT) counterparts. Further, C5a+LPS stimulation drive more IL-6 production from rat neutrophils when C5L2 is blocked (Gao et al., 2005). *In vivo*, C5L2-deficient mice suffer from augmented inflammatory responses and higher numbers of infiltrating neutrophils in a model of pulmonary immune complex injury (Gerard et al., 2005), indicating an anti-inflammatory function of C5L2. The anti-inflammatory role of C5L2 is further supported by a study in which LPS-injected C5L2-deficient mice show higher IL-1β levels and decreased survival rates (Chen et al., 2007). Similarly, C5L2-deficient mice display higher serum concentrations of IL-6 as compared to WT and C5aR-deficient mice in a model of septic peritonitis (Rittirsch et al., 2008).

In contrast, under the same conditions, a strong reduction of other inflammatory mediators, such as IL-1β, macrophage inflammatory protein (MIP)-1α and MIP-2 is observed in C5L2-deficient mice compared to WT mice. Indeed, the plasma concentrations of these mediators are comparable to those found in C5aR-deficient mice. Furthermore, C5L2-deficient mice, like C5aR-deficient mice, or mice in which either of the receptors are blocked by anti-receptor antibodies, show a higher survival rate in mid-grade sepsis (Rittirsch et al., 2008). Contrasting the *in vitro* findings that C5a+LPS-driven IL-6 production by mouse neutrophils is increased when C5L2 is blocked by antibodies (see above), Chen et al. found that IL-6 release is reduced from C5L2-deficient neutrophils (Chen et al., 2007). Moreover, C5a+LPS-induced Mac-1 surface expression on these neutrophils also diminishes, suggesting neutrophils chemotaxis may be impacted. Likewise, whereas C5a or C5a+LPS stimulation can lead to strong ERK1/2- and AKT-phosphorylation in neutrophils from WT mice, there is only a weak effect in the absence of C5L2. Additionally, C5L2-deficient macrophages have an impaired induction of co-stimulatory molecules (CD40, CD86). Even C3a mediated effects such as ERK1/2- and Akt-phosphorylation or F-actin formation on neutrophils are impaired in C5L2-deficient mice (Chen et al., 2007). In addition to these *in vitro* findings,

inflammatory responses are reduced *in vivo* in models of thioglycollate induced peritonitis, thioglycollate induced migration into dorsal air pouches, and ovalbumin (OVA) induced airway hyperresponsiveness (AHR) (Chen et al., 2007). Thus, these studies point to a more complex role of C5L2 in inflammation with C5L2 acting not only as a decoy receptor but also as positive modulator of C5aR and even C3aR. Although C3a desArg does not bind directly to C5L2, overexpression of C5L2 or its downregulation by antisense oligonucleotides influences the effects of C3a desArg (Kalant et al., 2005), suggesting that C5L2 can modulate signaling pathways of other receptors.

Although C5L2 does not appear to signal using the traditional mechanisms employed by GPCRs, several studies suggest that C5L2 has the ability to induce cellular effects. A recent study has found that noradrenaline upregulates C5L2 message and protein in rat astrocytes, and this correlates with an anti-inflammatory response induced by noradrenaline. Transfection of astrocytes by C5L2 down regulates NF-kB activity, whereas antisense oligonucleotides against C5L2 induce the reverse effect (Gavrilyuk et al., 2005). These observations suggest that the presence of C5L2 may exert some inhibitory effects within the cell, although the mechanisms behind such responses are currently unknown.

4. Regulation of complement receptor signaling

GPCR represent the largest family of cell surface receptors in the human genome, allowing extracellular signals to regulate a vast number of physiological events. Following ligand binding, GPCR undergo a conformational change which activates heterotrimeric G proteins. G proteins exist as a complex of Gα and Gβγ subunits. Upon activation, GDP is displaced by GTP from the Gα subunit resulting in disassociation of Gα subunit from the Gβγ subunits. This facilitates the free subunits interactions with various effector molecules and initiates downstream signaling (Pundir and Kulka, 2010). In the immune system, GPCRs play a role in innate, adaptive and pathological responses. In allergic diseases, stimulation of the GPCRs, C3aR and C5aR, by C3a and C5a, respectively produces a diverse array of effector functions, ranging from inflammatory cell migration to pro-inflammatory mediator production, thus contributing towards the pathophysiology of the diseases.

Given that the activation of C3aR and C5aR can induce massive inflammation and tissue destruction, there are mechanisms in place to limit complement activation where and when it occurs. As mentioned before, after generation, C3a and C5a are quickly degraded by plasma carboxypeptidases. The enzyme cleave the C-terminal arginine, resulting in C3a desArg and C5a desArg formation, each having less than 10% of their original biological activity (Bokisch and Muller-Eberhard, 1970). Formation of C3a and C5a is also regulated by either preventing the assembly of C3 convertase or, once it is formed, by inhibiting its activity. This is accomplished through the actions of decay acceleration factor (DAF), C4 binding protein (C4BP) and Factor H (Sarma and Ward, 2011). In addition, desensitization to prolonged or repeated exposure to high agonist concentration is another important mechanism of GPCR regulation. Furthermore, G proteins may terminate their own activation by Gα hydrolysis of GTP, thereby allowing Gα-GDP to reunite with Gβγ and form an inactive heterotrimer (Tsang et al., 1998). As this reaction proceeds at a slow rate (Tsang et al., 1998), additional cofactors come into play that aid in GPCR desensitization. Indeed, there are around 30 isoforms of Regulator of G protein signaling (RGS) proteins, which bind to activated Gα subunit and accelerate their intrinsic GTPase activity (Willars,

2006). RGS13 can regulate human mast cell lines, HMC-1 and laboratory of allergic diseases (LAD)2, response to C5a. RGS13-deficient HMC-1 cells have more cytosolic Ca^{2+} in response to C5a, indicating that RGS13 regulates C5aR-stimulated events in immune cells (Bansal et al., 2008).

GPCR desensitization is also achieved by the G protein-coupled receptor kinase (GRK)-arrestin pathway. There are seven GRKs in humans, GRK1-7, and four arrestins, arrestin 1-4. Most GPCRs are regulated by only four GRKs: GRKs 2, 3, 5, or 6, and two arrestins: arrestin-2 (β-arrestin1) and arrestin-3 (β-arrestin2) (Premont and Gainetdinov, 2007). Agonist-induced desensitization of GPCRs occurs via a multistep process. Just as G proteins recognize activated GPCR, GRKs also recognize activated GPCR, which leads to receptor phosphorylation at various serine/threonine residues on the intracellular loops and the carboxyl-terminal tail (Langkabel et al., 1999). Upon phosphorylation by GRK, GPCR's affinity for arrestin proteins is increased, which prevents the receptor from activating additional G proteins. The β-arrestins interact with clathrin and the adaptor protein complex AP-2 and target the agonist occupied receptors to pre-existing clathrin-coated pits for internalization. Thus GRK phosphorylation and arrestin binding result in termination of GPCR signaling, despite the continued presence of agonist (Santini et al., 2002, Scott et al., 2002).

A study by Ahamed et al. has shown that C3a stimulates degranulation and chemokine monocyte chemotactic protein-1 (MCP-1) production in RBL-2H3 cells expressing C3aR. Stimulation with C3a causes phosphorylation of WT C3aR but not of phosphorylation-deficient C3aR (ΔST-C3aR). In addition, C3a stimulation increases degranulation only in RBL-2H3 cells expressing ΔST-C3aR, suggesting that receptor phosphorylation provides an "off" signal for degranulation. Overexpression of GRK2 in C3aR-transfected cells results in increased C3a-induced C3aR phosphorylation. This increase in receptor phosphorylation is associated with a significant inhibition of degranulation. Furthermore, C3a causes a rapid translocation of β-arrestin2 from the cytosol to the membrane in RBL-2H3 cells expressing C3aR but not in cells expressing ΔST-C3aR, indicating that GRK2-induced C3aR phosphorylation is required for β-arrestin recruitment following C3a stimulation (Ahamed et al., 2001). Similarly, in transfected COS cells, overexpression of GRK2, GRK3, GRK5 or GRK6 results in enhanced C3a-induced C3aR phosphorylation (Langkabel et al., 1999). Knockdown of GRK2 or GRK3 expression in HMC-1 and LAD2 cells, causes higher Ca^{2+} mobilization, attenuated C3aR desensitization, and enhanced degranulation, thus indicating GRK2 and GRK3 involvement in C3aR desensitization. On the other hand, GRK5 or GRK6 knockdown in HMC-1 and LAD2 cells has no effect on C3aR desensitization, but instead promotes C3a-mediated degranulation, suggesting a complex role for GRKs in regulating human mast cells (Guo et al., 2011).

A recent study has identified the roles of β-arrestin1 and β-arrestin2 on C3aR desensitization and internalization (Vibhuti et al., 2011). By stably knocking down their expression in HMC-1 cells, this study shows that β-arrestin2, but not β-arrestin1, is required for C3aR desensitization and internalization. Interestingly, β-arrestin1-deficient, but not β-arrestin2-deficient, HMC-1 cells show reduced NF-κB activation and chemokine MIP-1β generation in response to C3a. Similar knock-down study with LAD2 cells shows that the absence of β-arrestin1, but not β-arrestin2, significantly inhibits C3a-induced degranulation. This demonstrates that β-arrestin1 and β-arrestin2 play a distinct role on C3aR desensitization, internalization and mediator generation in human mast cells (Vibhuti et al., 2011).

Upon ligand binding, C5aR undergoes rapid phosphorylation of the six serine residues located in the carboxyl-terminal tail (Giannini et al., 1995). This phosphorylation is a critical step in the termination of the C5aR signaling since a phosphorylation-deficient C5aR mutant triggers sustained intracellular signaling leading to increased C5a-induced superoxide production by HL-60 cells (Christophe et al., 2000). A study by Braun et al. shows that a persistent complex between activated/phosphorylated C5aR and β-arrestins is necessary for receptor desensitization and internalization. WT C5aR and β-arrestin1-transfected RINm5F cells exhibit significant β-arrestin1 recruitment to the plasma membrane when stimulated with C5a. Moreover, in resting RINm5F cells, the C5aR is mainly located in the plasma membrane, and both β-arrestin1 and β-arrestin2 are evenly distributed throughout the cytoplasm. Upon addition of C5a, β-arrestin1 and β-arrestin2 rapidly move from the cytosol to the plasma membrane, co-localize with C5aR, promote endocytosis of C5aR and remain associated to C5aR-containing vesicles. Furthermore, this study also indicates that C5aR is mainly internalized via the classical clathrin-dependent pathway (Braun et al., 2003). Interestingly, an observation by Bamberg et al. reveals that in resting neutrophils C5L2 is distributed throughout the cytoplasm co-localized with β-arrestin, while C5aR is expressed on their surface. Following stimulation with C5a, association of both C5aR and C5L2 with β-arrestin is greatly increased. Cells treated with an anti-C5aR antibody when activated with C5a fail to translocate C5L2 to β-arrestin, indicating that the activation of C5L2 is a consequence of C5aR activation. Moreover, C5L2-β-arrestin complex is a negative regulator of C5aR signaling in neutrophils (Bamberg et al., 2010).

Thus, a number of mechanisms have evolved to efficiently regulate complement activation; however, these regulatory mechanisms seem to fail in various clinical and experimental situations underlining the important role of complement proteins and their complement receptors in the pathogenesis of inflammatory diseases. These include airway inflammation and asthma (Wagner et al., 1999), sepsis (Gao et al., 2005), multiple organ dysfunction (Mastellos et al., 2005), hyperactute graft rejection (Link et al., 1999), ischemic injuries of various organs (Arumugam et al., 2004), autoimmune disorders such SLE (Karp, 2005) or multiple sclerosis (Rus et al., 2005), neurological diseases such as stroke (Yanamadala and Friedlander), and cancer (Yan et al., 2008).

5. Diseases associated with complement function

5.1 Allergic inflammation: Allergic asthma

Asthma is a chronic inflammatory disorder of the airways characterized by variable airflow obstruction, and associated increase in airway responsiveness to a variety of stimuli. The clinical features of asthma include dyspnea, wheezing and coughing. It is thought to be mediated primarily by allergen-specific $CD4^+$ T cells, T helper (Th)2 cytokines, and allergen-specific IgE, leading to pulmonary inflammation and AHR. Complement is well recognized as an important factor in the pathophysiology of asthma.

In allergic asthma and allergic rhinitis conditions, the complement system is activated by several pathways leading to the cleavage of C3 and/or C5 into their active fragments. First, the presence of preexisting allergen-specific antibodies in asthmatic patients can activate the classical pathway through formation of immune complexes. Second, the alternative pathway can be initiated by nucleophilic attack of C3 directly on the surface of allergen or by Factor B (Taube et al., 2006). Third, the recognition of PAMPs and danger-associated molecular patterns (DAMPs), such as nucleic acids on apoptotic cells (Elward et al., 2005) or

allergen polysaccharide, (Bito, 1977, Zimmermann et al., 1989) can activate the lectin pathway. Fourth, proteases released from inflammatory cells (Huber-Lang et al., 2002) or direct protease activity of allergens (Maruo et al., 1997) can drive the generation of C3a and C5a. Indeed, a variety of allergens have been shown to activate the complement and generate C3a and C5a in the airways. Ragweed extracts activate the complement cascade in both allergic individuals and healthy controls; however, generation of C3a is much stronger in allergic patients (Gonczi et al., 1997, Hidvegi et al., 1995). Ragweed allergen challenge also promotes C3a generation in the nasal mucosa of patients with allergic rhinitis. House dust mite (HDM), *Aspergillus fumigatus* and perennial ryegrass extracts induce serum C3a and C5a production in a dose and time-dependent manner (Nagata and Glovsky, 1987). Proteases from Dermatophagoides spp. (in particular, Der p 3 and Der f 3) can cleave C3 and C5 into their active fragments (Castro et al., 1991, Maruo et al., 1997). During an ongoing allergic reaction the Th2 cytokine IL-4 induces C3 mRNA production in human and murine epithelial cells (Khirwadkar et al., 1993).

Collectively, these studies provide strong evidence that major complement activation, and C3a and C5a generation occurs in asthmatic individuals. C3a and C5a are important contributing factors in the pathophysiology of asthma, because of their ability to recruit and activate inflammatory immune cells such as mast cells, eosinophils, macrophages, neutrophils, and basophils, increase vascular permeability, stimulate smooth muscle contraction, and trigger mast cell degranulation (Wust et al., 2006). Several studies have reported marked anaphylatoxins production under asthmatic condition. In the serum of asthmatic patients, an increase in C3a and C5a levels have been observed after allergen-induced bronchospasm (Arroyave et al., 1977, Smith et al., 1990). The levels of C3a, and to a lesser extent C5a increase in the bronchoalveolar lavage fluid (BALF) of asthmatic patients after segmental allergen provocation. Similarly, allergen-challenged lung lobes show significantly higher C3a levels in comparison to diluent (sham)-challenged lobes from asthmatic individuals (Humbles et al., 2000). In addition, sputum from patients with asthma exhibit increased C5a/C5a desArg concentrations (Marc et al., 2004). Infact, plasma C5a desArg levels reflect allergic disease severity (Bowser et al., 2010). Complement C3a and C4a concentrations increase in plasma of patients with aspirin-induced asthma (Lee et al., 2006). Studies with animal models of AHR have also indicated that C3a and C5a are crucial for asthma pathogenesis (Abe et al., 2001, Bautsch et al., 2000, Drouin et al., 2002, Lukacs et al., 2001).

In addition to allergen-mediated mechanisms, environmental stimuli can also trigger complement activation. Airborne pollutants such as diesel exhaust particles or airborne particulate matter can activate complement through the alternative pathway in human serum and airway epithelium, respectively (Walters et al., 2002). Moreover, acute ozone exposure can induce AHR and neutrophil infiltration, accompanied by elevated C3 levels in BALF in mice (Lambrecht and Hammad, 2009, Park et al., 2004). Cigarette smoke has been shown to directly activate the alternative pathway through cleavage of the internal thioester bond in C3 (Kew et al., 1987). Consistent with these studies, elevated levels of C3 have been found in children from smoking homes compared to those from non-smoking homes (Shima and Adachi, 1996).

Candidate gene and genome-wide screens for asthma susceptibility loci have identified C5 (9q34) (Ober et al., 1998, Wjst et al., 1999) and C5aR (19q.13.3) gene-containing chromosomal regions to be linked to asthma susceptibility (CSGA, 1997, Ober et al., 1998). One study reports an association between a single nucleotide polymorphism (SNP) in the *C3* gene and

atopic asthma in children and adults in a Japanese population. On the other hand, SNPs in the human *C5* gene are associated with protection against both childhood and adult asthma. The authors also report a significant association between a SNP in the C3a receptor (*C3ar1*) gene and childhood asthma (Hasegawa et al., 2004). These observations may explain the opposing role of C3 and C5 in animal-based asthma studies. C3-deficient mice when challenged with an allergen to induce pulmonary allergy exhibit diminished AHR and lung eosinophilia. Furthermore, these mice show markedly reduced IL-4 secreting cells and attenuated allergen-specific IgE response (Drouin et al., 2002). Collectively, these results demonstrate that C3 promotes Th2 effector function in asthma. On the other hand, the A/J mouse strain in which allergic inflammation and AHR can be easily induced, are deficient in C5 (Karp et al., 2000), demonstrating that C5-deficient mice are more susceptible to experimental asthma. Furthermore, in vitro experiments have shown that peritoneal macrophages from C5-deifcient A/J mice produce significantly less IL-12, because IL-12 is critical for promotion of Th1 immune response (Karp et al., 2000), these studies suggest that dysfunction in C5 cleavage may influence susceptibility to asthma. In contrast, a study by Peng et al. has demonstrated the contribution of C5 in the development of airway inflammation, AHR and ongoing allergic response in OVA-sensitized mice (Peng et al., 2005). Pharmacological inhibition of C5 using a monoclonal antibody against C5, BB5.1, markedly attenuates airway obstruction, perivascular and peribronchial infiltration of inflammatory cells, and BALF levels of inflammatory mediators such as eotaxin, regulated upon activation, normal T-cell expressed and secreted (RANTES), TNFα, and matrix metalloproteinase (MMP)9 (Peng et al., 2005).

The paradoxical effects of C5 and C5a observed in allergic asthma may be due to C5 behaving differently during allergen sensitization as opposed to during established allergic inflammation. Evidence to this effect was provided by Kohl et. al in a study utilizing two models of pulmonary asthma: a) OVA sensitization and challenge of mice leading to inhalation tolerance; and b) HDM sensitization and challenge of mice which induce Th2 sensitization, airway inflammation, and AHR. Ablation of C5aR signaling by pharmacological targeting prior to initial pulmonary OVA challenge, using a neutralizing anti-C5aR monoclonal antibody, induces significant production of Th2 cytokines (IL-5, IL-13, and IL-10), high serum IgE levels, marked influx of inflammatory cells (eosinophils, neutrophils, and lymphocytes) into BALF, mucus production, and AHR. Similar results are observed when C5aR is blocked prior to initial pulmonary HDM challenge. Together, this data suggests that the presence of C5a in the airways at the time of initial allergen encounter serves to prevent the development of Th2-mediated immune response (Kohl et al., 2006).

Protection against allergic sensitization by C5aR activation is a complex process involving alterations in the function of APCs, in particular dendritic cells. Dendritic cells strategically located in the upper layers of the epithelium and lamina propria of the airways, are specialized for the recognition and internalization of PAMPs. Once loaded with allergen, dendritic cells migrate to draining lymph nodes and present antigen to naïve T cells, which under appropriate polarizing cytokine signals, differentiate into effector T cells (Banchereau and Steinman, 1998). Dendritic cells are composed of several subsets, some of which drive the development of an aberrant adaptive immune response. The two dendritic cell subsets which have been found in the bronchial airways are myeloid dendritic cells (mDCs) and plasmacytoid dendritic cells (pDCs) (GeurtsvanKessel and Lambrecht, 2008). Pulmonary mDCs function as the major APCs in mouse models of allergic asthma, preferentially

inducing Th2 immune response towards inhaled allergen (Lambrecht et al., 2000). Pulmonary pDCs are immature dendritic cells, which induce respiratory tolerance by directly suppressing mDC-mediated activation of $CD4^+$ T effector cells or by inducing regulatory T cells (T_{regs}, a heterogeneous population of T cells that can suppress allergic immune responses) (GeurtsvanKessel and Lambrecht, 2008).

Several studies suggest that the ratio between mDCs and pDCs is critical for the development of the dysregulated Th cell response and that C5a regulates this ratio, thereby modulating the development of the allergic phenotype during allergen sensitization. $CD4^+$ T cells from mice challenged with HDM allergen in presence of an anti-C5aR monoclonal antibody produce higher levels of Th2 cytokines in co-culture with mDCs, but not with pDCs. Importantly, in the same setting, pDCs suppress the mDC-induced production of Th2 cytokines (Kohl et al., 2006). Furthermore, exposure to HDM leads to an increased number of mDCs, whereas the number of pDCs remains unaffected. These findings demonstrate that C5aR signaling controls the accumulation of pulmonary mDCs and pDCs in lungs, keeping the mDC:pDC ratio low, which facilitates the suppressive effects of pDCs on the mDC-mediated activation of T cells. In addition, C5aR blockade positively regulates the production of thymus and activation regulated chemokine (TARC) and macrophage-derived chemokine (MDC), thus promoting the recruitment of Th2 effector cells into the lungs (Kohl et al., 2006). Data obtained from C5-deficient mice indicate that C5aR signaling is required to keep mDC susceptible to suppression T_{regs}, thereby controlling the immunogenic effect of mDC (Lewkowich et al., 2005).

A study by Zhang et al. demonstrates that regulation of co-stimulatory molecules on mDCs and pDCs is an important mechanism underlying the protective impact of C5aR signaling on the development of the Th2 immune response. HDM exposure increases the frequency of pulmonary mDCs expressing B7-H1 and B7-DC. In vivo ablation of C5aR signaling dramatically reduces the frequency of pDCs expressing B7-H1 and B7-DC. In the presence of C5aR signaling, blockade of B7-H1 or B7-DC results in enhanced Th2 cytokine production by T cells, suggesting that B7-H1 and B7-DC are critical for pDC-driven regulation of mDCs, and protection against allergic sensitization (Zhang et al., 2009). Interestingly, C5aR expression on dendritic cells might be downregulated in the Th2 cytokine-dominated environment, thus dampening the negative regulatory effect of C5aR signaling, in particular on pDCs. Indeed, high levels of IL-4 downregulates C5aR expression in monocyte-derived dendritic cells (Soruri et al., 2003).

In the absence of C5aR, C5a binds C5L2, which has a similar distribution in lung tissue as C5aR. The pro-allergic role of C5L2 in experimental asthma has been demonstrated recently. OVA-sensitized C5L2-deficient mice exhibit reduced AHR, inflammatory cell infiltration, and airway inflammation (Chen et al., 2007). Similarly, HDM-sensitized C5L2-deficient mice show significantly attenuated AHR and airway inflammation that is associated with decreased mucus production, Th2 cytokine production, and serum IgE levels (Zhang et al., 2010), confirming the pro-inflammatory role of C5L2 in the development of asthma. The available data further indicates that C5L2 also has a regulatory impact on mDCs. Adoptive transfer of C5L2-deficient mDCs into WT mice results in decreased eosinophilic and lymphocytic inflammation and a decreased IgE response. Importantly, C5L2-deficient mDCs still promote production of large amounts of Th2 cytokines from lung cells; however, in contrast to their WT counterparts, the production of Th1 and Th17 cytokines is also enhanced, and is associated with an increased neutrophil influx. Moreover, HDM

stimulation of C5L2-deficient mDCs results in production of the Th17-promoting cytokine IL-23. Collectively, this suggests a complex role of C5L2 in asthma; controlling the development of Th1 and Th17 cells in response to allergen challenge as well as driving the Th2 immune response (Zhang et al., 2010).

Th17 are IL-17A–producing subset of $CD4^+$ T cells which have received much interest recently in allergy research. Several reports suggest that in addition to Th2 cells, Th17 cells contribute to the development of allergic inflammation. Sputum IL-17A mRNA is significantly elevated in asthmatic patients, and correlates with increase in sputum neutrophil counts (Bullens et al., 2006). Bronchial biopsies taken from patients with severe asthma show massive infiltration of IL-17 producing Th17 cells (Pene et al., 2008). In experimental asthma, IL-17A (Hellings et al., 2003) and Th17 (McKinley et al., 2008) cells have been shown to promote bronchial influx of neutrophils and induce airway inflammation and AHR. Th17 cells not only mediate neutrophilic airway inflammation, they also upregulate airway eosinophilia, together with IL-23 (Wakashin et al., 2008). Recent studies have highlighted an important dual role for C5a in Th17 cell development. C5aR-deficient mDCs show elevated production of Th17 promoting cytokines (transforming growth factor (TGF)-β, IL-6, and IL-23) and Th17 cell differentiation (Weaver et al., 2010). Furthermore, A/J mice (lacking C5) develop severe AHR associated with elevated levels of IL-17A and Th2 cytokines in comparison to C3H/HeJ strain of mice (mice manifesting less severe AHR). HDM challenge induces increased frequency of Th17 cells, as well as IL-17A in lungs of A/J mice. *In vivo* blockade of IL-17A with an anti-IL-17A antibody reduces AHR and BALF neutrophilia in HDM challenged A/J mice. Furthermore, an inverse relationship is found between the serum C5a concentration and the frequency of Th17 cells and IL-17A staining in lungs of HDM challenged A/J mice. Pharmacological inhibition of C5aR in BALB/c mice leads to enhanced influx of Th17 cells into the lungs after HDM challenge, and increased production of IL-17A per cell. Also, dendritic cells from these mice produce more HDM-driven IL-23. Taken together this suggests that C5a/C5aR complex controls IL-17A by limiting IL-23 production (Lajoie et al., 2010). As described above, Th17 development in experimental asthma is not only regulated by C5aR but by C5L2 as well (Zhang et al. 2010).

In addition to its impact on Th17 cells, C5a may also regulate the development of T_{regs} (Palomares et al., 2010). Lack of C5aR signaling in mDCs results in T_{regs} differentiation (Lajoie et al.. 2010). Thus, C5aR signaling in APCs can modulate Th cell differentiation at several levels in allergic asthma.

The development of asthma is not only regulated by C5a-C5aR/C5L2 interactions, but also by C3a-C3aR; however the role is less clear. In contrast to C5a, several studies suggest that C3a mainly contributes to the pathogenesis of asthma. When challenged with allergen, C3-deficient mice exhibit diminished AHR and lung eosinophilia (Drouin et al., 2001). C3aR-deficient mice challenged with *Aspergillus fumigatus* and OVA show attenuated/decreased AHR, airway eosinophilia, lung IL-4 producing cells, BALF levels of Th2 cytokines, serum IgE, and mucus production (Drouin et al., 2002). Similarly, C3a is also involved in the development of the late asthmatic response and AHR; the mechanism being related to the production of IL-1β in the lung of OVA-challenged mice in response to C3a (Mizutani et al., 2009). However, in a model of particulate matter-induced pulmonary allergy, C3-deficient mice develop dramatically reduced AHR, but they are not protected from airway inflammation (Walters et al., 2002). Similarly, OVA-sensitized C3aR-deficient mice show markedly reduced bronchoconstriction and AHR, but IgE production and Th2 cytokine levels remains the same when compared to WT mice (Humbles et al., 2000).

Differences in mice strains, nature of the allergens used for sensitization and route of allergen administration may account for the conflicting results generated in different animal models. The study by Zhang at al. compared Th2 immune responses in C5aR- and C3aR-deficient mice side-by-side. As mentioned earlier, in the absence of C5aR signaling HDM-challenged mice suffer from an enhanced Th2 immune response. However, C3aR-deficient mice are protected against a Th2 immune response under same settings, delineating the opposing roles of C5aR and C3aR signaling in asthma. Interestingly, blocking C5aR-mediated signaling in C3aR-deficient mice significantly increases AHR, airway eosinophilia, and Th2 cytokines production, indicating that the decreased Th2 immune response in the absence of C3aR signaling results from a shift towards protective C5aR signaling (Zhang et al., 2009). Cross-talk between the two receptors is further supported by the fact that C5a negatively regulates C3aR internalization. Pulmonary C5aR-deficient dendritic cells exhibit dramatically higher expression of C3aR in comparison to WT dendritic cells. In contrast, C5aR expression significantly decreases in HDM-pulsed WT dendritic cells, but less so in C3aR-deficient dendritic cells, further confirming that there is a reciprocal regulation between C5aR and C3aR (Settmacher et al., 1999).

Recently, it has been shown that AHR, lung eosinophilia and mucus production are significantly increased in C5-deficient mouse model of Respiratory Syncytial Virus disease. C3aR expression in bronchial epithelial and smooth muscle cells of these mice are elevated as compared with WT mice. Ablation of C3aR signaling in C5-deficient mice significantly attenuates the disease phenotype, suggesting that C5 plays a crucial role in modulating AHR and eosinophilic inflammation by affecting expression of C3aR in the lungs (Melendi et al., 2007). C3a also has an impact on Th17 cell development. C3aR-deficient mice exhibit diminished AHR, fewer Th17 cells and decreased IL-17 compared to WT counterparts, which correlates with reduced IL-23 production. This suggests that unlike C5a, C3a mediates IL-23 production, IL-17 production and susceptibility to AHR (Lajoie et al., 2010).

It is clear that in experimental models of allergic asthma, C5a-C5aR signaling seems to protect against the development of Th2 immune response during allergen exposure, whereas C3a-C3aR signaling contributes to the development of maladaptive immune responses. However, the strong evidence that suggests C5a and C3a synergistically contribute to the development of allergic inflammation and asthma can not be overlooked. As mentioned earlier, the contradictory nature of this evidence may be due to the fact that once allergic inflammation is established (effector phase of allergic asthma), both C3a and C5a act on circulating and tissue resident inflammatory immune cells such as mast cells, eosinophils, basophils, and lymphocytes leading to the induction of a pro-allergic immune response. Indeed, pharmacological targeting of C3aR and C5aR during the effector phase suppresses AHR, airway inflammation and Th2 cytokines production (Baelder et al., 2005). Thus, complement C3a and C5a, and their receptors display diverse activities during the course of disease progression. Reagents that specifically targets C3a, C3aR, C5, C5a or C5aR could serve as potential therapy for asthma.

5.2 Sepsis

Sepsis is a life-threatening medical condition characterized by dysregulated systemic inflammatory responses followed by immunosuppression. Despite advances in medical health care, sepsis remains one of the leading causes of death, accounting for more than 1.5 million deaths annually in Europe and North America. Systemic inflammation in sepsis can

be triggered by various infectious agents, including bacteria (leading cause of sepsis), fungi, parasites and viruses. Over recent years, efforts to better understand the pathophysiology of sepsis, has given rise to enough convincing evidence to suggest that the activation of the complement system and production of C3a and C5a occurs in sepsis.

Infectious agents activate the complement system through their interaction with C1q, MBL, or ficolins leading to local and/or systemic complement activation with subsequent production of C3a and C5a. Indeed, patients with sepsis syndrome show elevated plasma or serum levels of C3a/C3a desArg, C4a and C5a/C5a desArg (Bengtson and Heideman, 1988, Cole et al., 2002, Nakae et al., 1994, Nakae et al., 1996, Selberg et al., 2000, Solomkin et al., 1985, Weinberg et al., 1984), and circulating levels of C3a and C5a are inversely proportional to patient survival (Groeneveld et al., 2003, Hack et al., 1989, Hecke et al., 1997, Selberg et al., 2000). In humans, C5a levels can rise as high as 100 nM (Ward, 2004).

In vivo generation of C3a and C5a and their inflammatory effects in sepsis have been studied using three major animal models: a) intravenous injection of an exogenous toxin (e.g. anaphylatoxins); b) intravenous infusion of exogenous bacteria; and c) cecal ligation and puncture (CLP). Infusion of C5a into rabbits and rats produces the typical septic shock symptoms, including a rapid drop in mean arterial pressure and reduced circulation of granulocytes, monocytes and platelets in peripheral blood. Infusion of *Escherichia coli (E. coli)* in rabbits leads to severe septicemia with enhanced levels of C5a in the plasma, indicating that the degree of complement activation determines the severity of sepsis (Bergh et al., 1991). Administration of an anti-C5a desArg antibody to *E. coli*-infused primates confers protection against mortality and attenuates systemic manifestations of sepsis (Stevens et al., 1986), highlighting the importance of C5a in the septic shock pathology. Most of the studies dealing with the role of C5a in pathology of sepsis have been done using the rat and rodent models of CLP. This model of sepsis closely mimics the pathophysiology of sepsis in humans. Intravenous infusion of a neutralizing polyclonal antibody to rat C5a in CLP rats is highly protective, causing reduced evidence of multiorgan failure and resulting improved survival rates (from 0% survival in the unprotected to 50% survival in rats treated with an anti-C5a antibody). Moreover, CLP rats treated with an antibody against C5a show profound reduction (98% compared to unprotected rats) in bacterial colony forming units (CFUs), indicating that C5a is associated with the development of bacteremia in sepsis (Czermak et al., 1999).

Successive studies with anti-C5a antibody-treated CLP rats and mice have further revealed that the survival rate of these animals depends on amount of antibody infused at the time of CLP, as well as the time of administration of the antibody. For instance, the infusion of 600 g of antibody targeted against the mid region of C5a remarkably improves survival in CLP rats (83-90% survival rate compared to 23% survival rate in CLP rats receiving pre-immune IgG or 100-220 g of anti-C5a antibody). Moreover, when 600 g of antibody is given at time 0 of CLP, the survival rate is 90%; when infusion is delayed until 6 hr. post CLP, survival is around 60%; and when delayed until 12 hr. post CLP, the survival drops to 40% (Huber-Lang et al., 2001). These results suggest that neutralization of C5a during a specific time window after the onset of sepsis may be efficacious in the treatment of sepsis.

In sepsis, excessive production of C3a and C5a subsequently leads to dysfunction of neutrophils. Blood neutrophils display suppressed chemotactic responsiveness (Goya et al., 1994, Solomkin et al., 1981), depressed enzyme release (Goya et al., 1994, Solomkin et al., 1981, Utoh et al., 1989), alteration of intracellular pH (Sachse et al., 2000), and a defective respiratory burst (Czermak et al., 1999, Goya et al., 1994, Solomkin et al., 1981), resulting in

impaired bactericidal activity. For instance, during experimental sepsis, blood neutrophils show a decreased ability to bind C5a, impaired chemotactic response to C5a and a loss of H_2O_2-generating capacity. Exposure of rat neutrophils to C5a induces a defect in phagocytic function (Huber-Lang et al., 2002). Treatment of CLP rats with an anti-C5a antibody reverses these functional defects in neutrophils (Huber-Lang et al., 2002). Early after CLP, blood neutrophils from septic mice exhibit enhanced expression of the CC chemokine receptors (CCR1, CCR2, and CCR5) (Speyer et al., 2004), which may contribute to a stronger neutrophilic inflammatory response in sepsis. Moreover, CLP causes blood neutrophils to show an increased expression of β1 integrins (CD29) and β2 integrins (CD18), indicating a hyperresponsiveness to the receptors for these integrins (Guo et al., 2002). Collectively, this demonstrates that neutrophils develop an exaggerated response to various inflammatory mediators in the early stages of sepsis.

Besides neutrophil dysfunction, C5a also affects other components of innate immunity leading to exacerbation of septicemia and immunosuppression. Systemic activation of complement in CLP model of sepsis induces C5a-dependent apoptosis of thymocytes (Guo et al., 2000), inducing a significant loss of thymus in first few days following CLP (Riedemann et al., 2002). C5aR expression on thymocytes rises rapidly after CLP and increased binding of C5a to CLP thymocytes can be found as early as 3 hr. post CLP. Administration of an anti-C5a neutralizing antibody at the time of CLP preserves the thymic mass and abolishes the apoptosis of thymocytes (Riedemann et al., 2002). Moreover, CLP in mice induces significant apoptosis of adrenomedullary cells 24 hr. post CLP, which is diminished after dual blockade of both C5aR and C5L2 (Flierl et al., 2008), indicating a role of C5a in multiorgan apoptosis during sepsis. C5a also promotes septic cardiomyopathy (Niederbichler et al., 2006) and consumptive coagulopathy (Laudes et al., 2002). The intensity of the coagulopathy of sepsis is greatly attenuated in CLP rats (for example, clotting times are minimally prolonged, thrombocytopenia is reduced and plasma levels of fibrin split products as well as thrombin-antithrombin complexes are greatly reduced) as a result of neutralization of C5a after CLP (Laudes et al., 2002).

It seems clear that excessive C5a produced during sepsis has harmful effects, as described above and it is obvious that the effects are mediated via the interaction of C5a with its receptors. Indeed, experimental studies in animals with CLP suggest a dynamic balance between C5aR and C5L2 on immune cells and in organs. C5aR expression is markedly increased in lung, liver, kidney, and heart early in septic mice (Riedemann et al., 2002). C5L2 mRNA is highly expressed in liver and thyroid of CLP rat, and weakly expressed in CLP rat brain, spleen, kidney, large intestine, eye as well as lung alveolar macrophages and peripheral blood neutrophils (Gao et al., 2005). Increased expression of C5aR and C5L2 in lung, liver, heart, and kidney after CLP-induced sepsis strongly implies the role of complement receptors in multiple organ failure.

In vitro exposure of neutrophils to C5a reduces surface C5aR expression suggesting that following interaction C5a/C5aR complex undergo internalization, suggesting a possible cause for compromised neutrophil function (Huber-Lang et al., 2002). During experimental sepsis C5aR content on blood neutrophils reduces 2.5-fold 24 hr. after CLP and increases steadily thereafter. On contrary, C5L2 content on blood neutrophils increases significantly 24 and 36 hr. after onset of CLP. Moreover, confocal microscopy on blood neutrophils from CLP rats show cytoplasmic distribution of C5aR, but not C5L2, indicating that C5a/C5L2 complex do not undergo internalization as C5a/C5aR after CLP (Gao et al., 2005), and that C5aR and C5L2 are independently regulated during sepsis. In presence of a cyclic peptide

antagonist (C5aRa) to the C5aR, the binding of C5a to mice peritoneal neutrophils is diminished, and the *in vitro* chemotactic response of neutrophils to C5a is decreased, C5a-induced defect in the oxidative burst of neutrophils is reversed, and the lung vascular permeability index is markedly diminished. CLP mice treated with C5aRa show improved survival rates (from 10% survival in the sham to 60% survival in mice treated with C5aRa) (Huber-Lang et al., 2002). Using a combination of C5aR and C5L2 blocking antibodies, and mice lacking one of the two C5a receptors, considerable improvement in survival following CLP has been shown in a study by Rittirsch et al. In mid-grade CLP, WT, C5aR-deficient and C5L2-deficient mice show 31%, 80% and 100% survival by day 7, respectively, indicating harmful roles for both C5a receptors in the CLP model of sepsis. All WT mice show 100% and 80% survival after treatment with anti-C5aR and anti-C5L2 serum, respectively, compared to 40% survival in mice treated with preimmune serum. In high-grade CLP, none of the WT mice treated with preimmune serum, C5L2-deficient mice treated with anti-C5aR serum and C5aR-deficient mice survived. Interestingly, when C5L2-deficient mice are treated with anti-C5aR serum the survival rate improves significantly (80%). Similarly, treatment of C5aR-deficient mice with anti-C5L2 serum greatly improves survival (87% compared to 17% for C5aR-deficient mice treated with preimmune serum or 0% for WT mice treated with anti-C5L2 serum). Dual blockade of C5aR and C5L2 by a polyclonal antibody is also protective against the lethal outcome after high-grade CLP. Moreover, if the high-grade CLP mice are treated with anti-C5aR and anti-C5L2 serum after a delay of 12 hr. or 24 hr. post CLP there is no protective effect against mortality. The combined blockade of the C5a receptors during sepsis is most effective when given before the onset of sepsis. In summary, both C5aR and C5L2 contribute cooperatively to mortality in sepsis. Evaluation of plasma cytokine and chemokine concentrations after CLP shows a reduction in the levels of IL-1β, MIP-1α, and MIP-2 in C5aR-deficient and C5L2-deficient mice in comparison to WT mice. However, the plasma concentration of IL-6 is significantly increased in C5L2-deficient CLP mice in comparison to WT mice. In contrast, plasma IL-6 is markedly reduced in C5aR-deficient CLP mice when compared to WT mice, as observed by Gao et al. (Gao et al., 2005), suggesting that the sepsis-induced cytokine and chemokine production may occur in a sequential fashion which requires C5a engagement of both C5a receptors. Another important observation obtained from this study is that C5L2-deficient CLP mice have greatly reduced levels of high-mobility group protein 1 (HMGB1, a late mediator in sepsis) in plasma. Interestingly, CLP in C5aR-deficient CLP mice has no effect on plasma HMGB1 levels when compared to WT CLP mice. Furthermore, C5a-stimulated macrophages from WT and C5aR-deficient mice produce significant amounts of HMGB1, whereas C5a-stimulated macrophages from C5L2-deficient mice produce very little HMGB1 (Rittirsch et al., 2008), supporting the fact that C5a mediates the pathophysiology of sepsis by acting on C5a receptors and that C5L2 has a functional role in sepsis.

Recently, a study has shown that γδT cells are involved in the pathogenesis of sepsis by producing large amounts of IL-17 (Xu et al., 2010), express C5aR, and the expression of C5aR increases in mice following sepsis. Neutralization of C5a partially prevents the upregulation of C5aR on γδT cells in septic mice. Furthermore, C5a promotes the IL-17 expression by γδT cells which can be attenuated by blocking PI3K/Akt signaling pathway, demonstrating that C5a acts on C5aR expressed on γδT cells, resulting in the pathophysiology of sepsis (Han et al.).

In summary, C5a binding to C5aR and C5L2 receptors seem to contribute to cytokine storm, associated multiple organ dysfunction and subsequent lethal outcome in the setting of experimental sepsis. C5aR and C5L2 both contribute synergistically to the harmful events in

sepsis. A maximal beneficial effect can be achieved by the blockade or absence of both receptors, which might have implication in complement-based therapy for inflammatory diseases.

5.3 Chronic urticaria

Chronic urticaria is a debilitating skin condition characterized by the near daily occurrence of pruritic wheals for at least six weeks (Kaplan, 2004). While the pathogenesis of chronic urticaria is not completely understood, mast cell and basophils degranulation and histamine release are believed to be of central importance. Recent studies suggest that this activation of mast cells and basophils could in part be initiated by the C3a and C5a or these complement proteins can augment allergen-antibody mediated cell activation. Indeed, heating serum from patients with chronic urticaria, which heat-inactivates complement proteins, reduces the ability of serum to induce histamine release from basophils. Similarly, decomplemented sera deficient in C5 is incapable of releasing histamine from dermal mast cells (Kikuchi and Kaplan, 2002).

C5a may play a key role in the pathogenesis of chronic urticaria as it can degranulate mast cells and basophils following its interaction with the C5aR present on these cells (Fureder et al., 1995). C5a can also chemoattract neutrophils, basophils, eosinophils and mast cells, which are present in chronic urticaria lesions. Korosec et al. showed that patients with chronic urticaria have enhanced basophil activation in response to C5a. However, C5aR antagonist-treated serum from these patients show decreased histamine release from basophil. Furthermore, the release of histamine from basophils by anti-FcεRI autoantibodies can be augmented by C5a activation (Korosec et al., 2009). Taken together these studies suggest that complement proteins and their receptors contribute towards the pathology of chronic urticaria.

5.4 Cancer

The complement system has also been well recognized as a promoter of tumor development. The deposition of complement component C3 is associated with the tumor vasculature in mice; C3-deficient mice show reduced tumor growth. The anaphylatoxin C5a promotes the growth of malignant tumors in a mouse model of cervical carcinoma. C5aR-deficient mice treated with a C5a antagonist exhibit reduced tumor growth, as well as enhanced infiltration of tumor tissue with $CD8^+$ cytotoxic T cells in comparison to WT mice, suggesting that C5a promotes tumor growth by inhibiting the response of $CD8^+$ cytotoxic T cells against tumors. Elimination of $CD8^+$ cytotoxic T cells from C5aR-deficient increases the rate of tumor growth, confirming that C5a modulates the $CD8^+$ T cells-mediated anti-tumor immune responses (Markiewski et al., 2008).

The interplay between $CD8^+$ cytotoxic T cells and myeloid-derived suppressor cells (MDSCs) play a significant role in determining the fate of tumors. MDSCs are a heterogeneous population of regular myeloid cells, also referred to as immature counterparts of monocytes and neutrophils, which express C5aR. C5aR is partially internalized in tumor-associated MDSCs, suggesting that MDSCs are constantly exposed to and activated by C5a generated in the tumor microenvironment. C5a chemoattracts MDSCs to the tumor, partly by upregulating adhesion molecules on MDSCs. C5a also enhances the production of ROS and reactive nitrogen species (RNS) from MDSC, which are known to suppress $CD8^+$ cytotoxic T cell anti-tumor responses. C5aR-deficient mice or mice treated

with a C5aR antagonist show reduced numbers of MDSCs within the tumor tissue, increased numbers of $CD8^+$ cytotoxic T cells and decreased tumor growth. MDSCs from C5a-deficient mice are unable to inhibit T cell proliferation in vivo (Markiewski et al., 2008). These observations highlight the potential of anaphylatoxins and their receptors as novel targets for anti-cancer immunotherapy.

5.5 Ischemia-reperfusion injury

Ischemia-reperfusion (I/R) injury is defined as cellular injury occurring after the reperfusion of previously vascularised tissue following an extended period of ischemia. The augmentation of tissue injury after reperfusion results from an intense inflammatory response that develops simultaneously with tissue reperfusion (Eltzschig and Collard, 2004). Several pathological conditions can lead to I/R injury including myocardial infarction, stroke, hemorrhagic shock, severe trauma, and organ transplantation resulting in associated morbidity and mortality (Eltzschig and Collard, 2004).

Numerous studies have shown that ischemic tissue activates the complement system, which remarkably contributes to the development of tissue damage by enhancing inflammation (Hart et al., 2004). The first evidence for involvement of complement in I/R injury was proposed by Hill and Ward in 1971 (Hill and Ward, 1971). During I/R injury the complement system can be activated by the classical, alternative, and lectin pathways. For instance, skeletal muscle injury resulting from I/R likely occurs through the complement activation via the classical and lectin pathways (Weiser et al., 1996). Lectin pathway may be involved in myocardial (Jordan et al., 2001) and gastrointestinal I/R-induced complement activation (Hart et al., 2004). However, the amplification of complement activation in gastrointestinal I/R occurs through the alternative pathway (Hart et al., 2004). Alternative pathway of complement activation may contribute to renal I/R injury in mice (Thurman et al., 2003).

Studies suggest that complements C3a and C5a are major complement factors responsible for the induction of the reperfusion-associated inflammatory response. C3a and C5a activate endothelial cells and inflammatory leukocytes. C5a upregulates the expression of adhesion molecules on human umbilical vein endothelial cells (Foreman et al., 1996) and induces release of various cytokines, including IL-1, IL-6, MCP-1, and TNFα (Schindler et al., 1990). C5aR expression is upregulated following cold I/R injury in a mouse model of syngenic kidney transplantation, suggesting that C5aR may contribute to tissue damage, tubular apoptosis and dysfunction of donor organs. Furthermore, upregulation of C5aR expression in cadaveric kidneys correlates with cold ischemia time. Ablation of C5aR signaling during cold ischemia has a protective effect on kidney graft survival (Lewis et al., 2008). Animals treated with a C5aR antagonist show dramatically reduced accumulation of neutrophils in the post-ischemic livers and sustain less injury during reperfusion (Arumugam et al., 2003). In a mouse model of I/R muscle injury, mice treated with a C5aR antagonist exhibit decreased levels of circulating creatinine kinase, lactate dehydrogenase, alanine and aspartate aminotransferase, creatinine, blood urea nitrogen, muscle edema, lung and liver myeloperoxidase, and lung TNFα following hind limb injury (Woodruff et al., 2004).

Studies targeting C5a/C5aR complex have further confirmed the role played by C5a in the pathogenesis of I/R injury. Blocking C5aR signaling using an anti-C5aR antibody markedly decreases leukocyte adherence, microvascular permeability in the ischemic myocardial area (Zhang et al., 2007), myocardial neutrophil infiltration and arteriolar endothelial injury

(Park et al., 1999). Knocking down C5aR expression with small interference (si)RNA preserves renal function from I/R injury, reduces chemokine production and neutrophil infiltration (Zheng et al., 2008). Treatment with an anti-C5 neutralizing antibody reduces apoptosis and necrosis in heart allografts (Ferraresso et al., 2008).

In contrast to C5a, the role of C3a/C3aR in I/R injury is not properly established. Systemic inhibition of C3a with a C3aR antagonist minimally resolves myocardial I/R injury, and neutropenia rather than C3aR antagonism appears to be responsible for C3aR antagonist-associated improvement in myocardial I/R injury (Busche and Stahl, 2010). These results confirm similar observations from previous studies (Ames et al., 2001, Proctor et al., 2004), indicating that C3aR antagonist-mediated neutrophil tissue sequestration during I/R injury may account for the protective effects observed. Overall, the data indicate while C3a/C3aR inhibition in the clinical setting of I/R injury does not appear to be therapeutic, targeting C5a as well as C5aR may be a promising approach to prevent I/R injury.

5.6 Transplantation

The activation products of complement system play an important role in allograft rejection as evidenced by the fact that the lack of C3 in donor kidneys is associated with long-term graft survival in experimental transplantation (Pratt et al., 2002). A study in human kidney transplantation has shown that donor C3 polymorphisms are associated with late graft failure. Thus donor expression of C3 influences the alloimmune response and the fate of the transplantation (Brown et al., 2006). APCs are the source of C3 and macrophages from C3-deficient mice have an impaired potency to stimulate alloreactive T cell response and to drive Th1-biased adaptive immune responses supporting graft survival (Peng et al., 2006, Zhou et al., 2006). It is now clear that this effect of C3 is mediated via the interaction of C3a with the C3aR on APCs. C3a-deficient dendritic cells show reduced surface expression of major histocompatibility complex (MHC) class II and co-stimulatory molecules and elicit a defective T cell priming against alloantigen expressed on the dendritic cells (Peng et al., 2008). Mechanistically, C3a-C3aR interaction on surface of dendritic cells decreases the level of intracellular cAMP, which in turn promotes allergen uptake and T cell priming (Li et al., 2008).

As mentioned in previous section, complement activation is critically involved in I/R injury; C5a and C5aR blockade has been shown attenuate organ damage, improve graft function and transplantation outcome. In human kidney transplants with acute rejection, C5aR expression is increased in renal tissue and in cells infiltrating the tubulointerstitium. Treatment of recipient mice with a C5aR antagonist before transplantation markedly improves renal allograft survival and reduces alloreactive T cell priming. Similarly, inhibition of C5aR in murine model of renal allotransplantation substantially improved graft survival from 11 days to 12 weeks. In addition, C5aR inhibition reduces kidney inflammation, apoptosis, and priming of alloreactive T cells (Gueler et al., 2008). Pharmacological targeting of C5aR during organ preservation significantly improves kidney graft survival (Lewis et al., 2008). Baboons treated with an anti-C5a monoclonal antibody exhibit prolonged pulmonary xenografts survival, indicating that C5a exacerbates pulmonary xenografts injury (Gaca et al., 2006). A recent study by Vieyra et al. have shown that dendritic cell-derived C3a and C5a regulates $CD4^+$ T cell help to $CD8^+$ T cell responses required for murine allograft rejection (Vieyra et al., 2011). These results demonstrate that C3aR and C5aR signaling contribute towards the innate and adaptive inflammatory

responses following solid organ transplantation, suggesting that pharmaceutical targeting of C3aR and C5aR may have an application in transplantation medicine.

5.7 Stroke

Stroke is the second leading cause of death worldwide, which is defined as a rapidly developing loss of brain function(s) due to inadequate blood flow in a localized area. When blood flow is interrupted to part of the brain, brain cells quickly begin to die leading to stroke development. There are many elements that contribute to the development of stroke, of which neuroinflammation is a major one. However, the precise roles of various pro-inflammatory mediators, including cytokines, chemokines and immune cells, are still largely unknown. Increasing evidence suggests that the activation of the complement cascade contributes to pathological inflammatory events in brain (upregulation of adhesion molecules, immune cell activation, chemotaxis, expression of IL-8 and MCP-1 by endothelial cells) (D'Ambrosio et al., 2001), resulting in ischemic insult, neurodegeneration and stroke development.

Following brain I/R injury ROS, RNS as well as oxygen free radicals are generated by activated brain cells and infiltrating immune cells, which stimulate stroke-associated brain injury (Elsner et al., 1994, McColl et al., 2009). Brain cells such as astrocytes, microglia, neurons, and endothelial cells and infiltrating immune cells produce various pro-inflammatory mediators following ischemia, further contributing towards cell death (Yilmaz et al., 2006). Ischemic stroke enhances interaction between endothelial cells, brain cells, and immune cells that may aggravate the injury process (Urra et al., 2009). All these stroke-associated brain pathologies are function of C3a-C3aR and C5a-C5aR interactions (D'Ambrosio et al., 2001). Strong C3a and C5a activation is observed in patients with acute ischemic stroke, which correlates with disease severity (Szeplaki et al., 2009). Regional brain I/R injury induces an inflammatory reaction that involves generation of C3a and C5a, upregulation and enhanced activation of their receptors C3aR and C5aR.

In the brain, C3aR and C5aR are expressed by astrocytes (Gasque et al., 1995, Gasque et al., 1997, Gasque et al., 1998, Lacy et al., 1995, Sayah et al., 1997), glial cells (Davoust et al., 1999, Gasque et al., 1997, Lacy et al., 1995) and neurons (Davoust et al., 1999). Experimental models of permanent and transient middle cerebral artery occlusion (MCAO) have demonstrated an increase in the expression of complement receptors. The expression of C3aR and C5aR is enhanced in mouse ischemic cortex following permanent MCAO. In addition, the expression is also induced on endothelial cells within ischemic core, suggesting that the complement receptors are important in leukocyte recruitment and neuroinflammation (Van Beek et al., 2000). Expression of C3aR and C5aR is significantly increased after transient MCAO in rats (Nishino et al., 1994) and mice (Barnum et al., 2002). In the later study, C5aR expression dramatically increases within 3 hr. after MCAO, whereas C3aR expression reduces to 25% of control animals. By 24 hr. post-occlusion, expression of both receptors is highest. This increased expression at the later time points after occlusion is most likely the result of a massive infiltration of immune cells expressing C3aR and C5aR (Barnum et al., 2002).

Early attempts at complement inhibition using cobra venom factor (CVF) have further revealed that abrogation of the complement system can provide protection for the brain during I/R injury and stroke. Pretreatment of rats with CVF (complement-depleted rats) prior to temporary cerebral ischemia significantly enhances the magnitude of reactive

hyperemia and increases preservation of somatosensory evoked potentials (SSEPs), demonstrating that depleting the complement system can improve blood flow and clinical outcome following cerebral I/R (Vasthare et al., 1998). Similarly, complement depletion via CVF significantly reduces post-ischemic cerebral infarct volume in adult rats and post-hypoxic-ischemic cerebral atrophy in neonates (Figueroa et al., 2005). However, the lack of specificity of CVF did not give any information regarding complement components that are most relevant in the pathogenesis of brain injury and stroke.

Recent studies using genetic knockouts of C3 and C5 and inhibitors of C3a-C3aR, and C5a-C5aR signaling have better our understanding of the role of anaphylatoxins in stroke. C3-deficient mice are protected against cerebral I/R injury, as demonstrated by significant reductions in both infarct volume and neurological deficit score. C3-deficient mice also exhibit diminished granulocyte infiltration and oxidative stress. The administration of a C3aR antagonist reduces stroke volume leading to neurological improvement (Mocco et al., 2006), implicating the involvement of C3a and C3aR in acute stroke. Studies with genetic knockouts of C5 in stroke have yielded conflicting data. C5-deficient animals show increased vulnerability to intracerebral hemorrhage (ICH) (Nakamura et al., 2004) and ischemic stroke (Mocco et al., 2006). In contrast, C5-deficient mice subjected to brain I/R injury exhibits improved functional outcome and less brain damage (Arumugam et al., 2007). A recent study by Rynkowski et al. have shown that blocking C3a-mediated signaling using a C3aR antagonist is protective in a mouse model if ICH (Rynkowski et al., 2009). Similarly, mice treated with C5aR antagonist alone or C3aR and C5aR antagonists exhibit improvement in neurological functions following ICH, suggesting that blockade of C3aR and C5aR represents a promising therapeutic strategy in stroke.

6. Conclusion

The complement system is composed of a network of proteins that play an important role in innate and adaptive immunity. Originally discovered as antimicrobial agents, the main function of C3a and C5a was considered to be the opsonization of pathogens and chemoattraction to remove apoptotic and necrotic cells. However, today complement proteins, C3a and C5a, are considered as crucial immunoregulatory molecules with pleiotropic biological functions on immune cells which help to shape the immune response. Activation of complement system is exquisitely regulated, while improper activation or under certain conditions the effect can lead to adverse consequences. Similar to dysregulation of the adaptive immune system in hypersensitivity reactions, the pathological role of C3a, C5a and their receptors in inflammatory diseases as well as tumor growth is well defined. Due to their strong inflammatory properties, C3a/C3aR and C5a/C5aR are considered attractive pharmacological targets for the development of therapeutic agents. Given that many therapeutic agents targeting the interaction of C3a-C3aR and C5a-C5aR are already under investigation, the advances made in the field of complement and complement receptors discussed in this book chapter will better our understanding of the disease process and help develop new therapeutic approaches to modulate immune response

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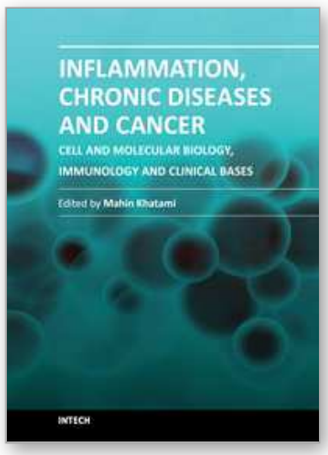

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This book is a collection of excellent reviews and perspectives contributed by experts in the multidisciplinary field of basic science, clinical studies and treatment options for a wide range of acute and chronic inflammatory diseases or cancer. The goal has been to demonstrate that persistent or chronic (unresolved or subclinical) inflammation is a common denominator in the genesis, progression and manifestation of many illnesses and/or cancers, particularly during the aging process. Understanding the fundamental basis of shared and interrelated immunological features of unresolved inflammation in initiation and progression of chronic diseases or cancer are expected to hold real promises when the designs of cost-effective strategies are considered for diagnosis, prevention or treatment of a number of age-associated illnesses such as autoimmune and neurodegenerative diseases as well as many cancers.

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