

The Role of Complement in the Diagnosis and Management of Allergic Rhinitis and Allergic Asthma

Yves Laumonnier · Inken Schmutde · Jörg Köhl

© Springer Science+Business Media, LLC 2010

Abstract Allergic rhinitis and asthma are common chronic inflammatory diseases of the nasal mucus membranes and the upper airways with a high prevalence in Western countries. In addition to maladaptive T-helper type 2 (Th2) immunity, Th17 cells can drive the inflammatory responses in both diseases. Several reports have shown that the complement system is activated locally and systemically in allergic rhinitis and/or allergic asthma patients. Importantly, recent findings in experimental models of allergic rhinitis and allergic asthma suggest that the complement cleavage products complement 3a and complement 5a and the activation of their corresponding receptors in antigen-presenting cells regulate the development of maladaptive Th2 and Th17 immunity. These findings in experimental asthma are corroborated by genome-wide searches and candidate gene studies in humans. We discuss recent findings in experimental and human allergic airway diseases suggesting that complement may serve as a new diagnostic and therapeutic target for both disorders.

Keywords Innate immunity · Complement · Allergic asthma · Allergic rhinitis · Th2 · Th17

Introduction

Allergic Rhinitis

Allergic rhinitis (AR), or hay fever, is a common inflammatory disorder of the nasal cavity resulting from dysregulated immune responses from harmless environmental antigens in genetically susceptible individuals [1]. The incidence during the past 50 years has dramatically increased in most countries, a development similar to that observed for other allergic diseases. In Western countries, an estimated 500 million people are affected. Clinical symptoms include rhinorrhea, repetitive sneezing, nasal congestion, and postnasal drip, as well as pruritic eyes, ears, nose, and/or throat [1]. The disease develops in response to pollens, feces from house dust mite (HDM), animal dander, mold spores, and indoor and outdoor pollutants [1]. Clinically, early and late phases can be distinguished. The early-phase response is characterized by IgE-triggered histamine production and the release of cysteinyl leukotrienes and prostaglandins as well as several proinflammatory chemokines and cytokines; the late-phase response is characterized by the recruitment of eosinophils, T-helper type 2 (Th2) lymphocytes, and macrophages [2].

Allergic Asthma

Allergic asthma (AA) is a common chronic inflammatory disease of the lower airways with a high prevalence in Western countries [3]. Similar to allergic rhinitis (AR), the disease is a worldwide problem, with an estimated 300 million cases. It develops as a severe lung disease in response to exposure to inhaled allergens, air pollutants, chemicals, and diverse environmental factors. The clinical manifestations of asthma include wheezing, breathlessness, chest tightness, and coughing. The pathophysiology includes bronchoconstriction; airway hyperresponsiveness; mucus cell hyperplasia; elevated IgE

Y. Laumonnier · I. Schmutde · J. Köhl (✉)
Institut für Systemische Entzündungsforschung,
Universität zu Lübeck,
Ratzeburger Allee 160,
23538 Lübeck, Germany
e-mail: joerg.koehl@uk-sh.de

Y. Laumonnier
e-mail: yves.laumonnier@uk-sh.de

I. Schmutde
e-mail: inken.schmutde@uk-sh.de

production; and pulmonary infiltration of leukocytes such as eosinophils, lymphocytes, and mast cells, similar to AR. Intensive investigations have been focusing on the immunologic basis of asthma. It is now generally believed that asthma arises as a result of dysregulated immune responses in which CD4⁺ Th2 cells play a central role in disease pathogenesis and pathology [4]. In addition to Th2 immunity, Th17 cells have been found in experimental and human asthma, in particular in patients with high neutrophilia (see later section) [5]. Long-term asthma can lead to airway remodeling and airway fibrosis [6, 7].

The Complement System and the Anaphylatoxins

The complement system is an ancient danger-sensing system that recognizes exogenous threats such as conserved microbial motifs, as well as endogenous threats, including altered-self molecules and apoptotic cells [8]. Danger-sensing molecules that activate the complement system comprise soluble C-type lectins such as mannan-binding lectin (MBL); ficolins; the C-type lectin-like molecule C1q; and, as recent evidence suggests, properdin. In addition to this direct recognition of conserved danger motifs, C1q binds strongly to another class of innate danger sensors (ie, natural immunoglobulins). Once bound to danger motifs, C1q, MBL, ficolins, and properdin initiate activation of proteolytic cascades that results in the cleavage of the central molecule of the complement system, C3, followed by cleavage of C5. Together with other cleavage products of C3 (ie, C3b, iC3b, C3dg, C3d, and C3c), the smaller cleavage products of C3 and C5, C3a and C5a, form a set of soluble mediators that bind distinct cell-surface receptors expressed on a variety of target cells. The interaction of C3a and C5a with their cognate receptors induces pleiotropic effector functions, translating the danger information from the fluid phase into defined cellular responses.

The anaphylatoxins (ATs) are potent inflammatory mediators targeting a broad spectrum of immune and nonimmune cells. C3a and C5a regulate vasodilation, increase the permeability of small blood vessels, and induce contraction of smooth muscles. In macrophages, neutrophils, and eosinophils, C3a and C5a can trigger oxidative burst. Basophils and mast cells react upon AT stimulation with release of histamine. In eosinophils, C3a and C5a regulate the production of eosinophil cationic protein, their adhesion to endothelial cells, as well as their migration. C3a further promotes serotonin release from guinea pig platelets and modulates synthesis of interleukin (IL)-6 and tumor necrosis factor- α from activated B and T cells, basophils, and mast cells, the latter of which also migrate toward a C3a gradient [9].

It is obvious that control mechanisms have evolved that regulate the activity of these powerful bioactive molecules.

Indeed, AT activity in the circulation and in tissues is tightly controlled by carboxypeptidases, which rapidly cleave off a C-terminal arginine residue. While the resulting cleavage product, C5adesArg, retains 1–10% of the inflammatory activity of C5a, C3adesArg is devoid of any proinflammatory activity.

The ATs bind to a family of three receptors, so-called AT receptors, which belong to the superfamily of G-protein-coupled receptors. The AT receptor family comprises the C3a receptor (C3aR), C5a receptor (C5aR), and C5a receptor-like 2 (C5L2). All share high-sequence homology and are closely related to other chemotactic receptors, such as the N-formyl-methionine-leucine-phenylalanine receptor, ChemR23, and the chemokine receptors CXCR1 and CXCR2. Despite their similarity, the AT receptors differ in ligand specificity, signal transduction capacity, and function.

The C3aR specifically binds C3a with a K_d of about 1 nM but does not recognize its desarginated form or C5a, whereas C5a and C5adesArg bind to two distinct receptors (C5aR and C5L2). C5L2 binds C5a with the same high affinity as C5aR but has a 20-fold higher affinity for the C5a metabolite, C5adesArg, than C5aR [9]. Binding of C5a to C5aR activates cells through the recruitment of G protein subtypes, resulting in increased intracellular calcium concentration, mitogen-activated protein kinase, phosphoinositide 3-kinase, and PKB/Akt activation, among other pathways. In contrast, C5L2 is uncoupled from G proteins due to the lack of critical intracellular amino acid motifs such as DRY and NPXXY. This lack of signaling, together with its constitutive recycling and the internalization of its ligands suggests that C5L2 may function as a decoy receptor regulating the bioavailability of C5a and thus limiting the C5aR-dependent cell activation. In support of this view, C5L2 ligation was found to limit the proinflammatory properties of C5a, providing a negative feedback mechanism to control C5a-mediated inflammatory responses in an *in vivo* model of immune complex disease. Mechanistically, C5L2 was recently shown to negatively modulate C5aR signaling through the β -arrestin pathway [10]. In contrast, recent reports have demonstrated signaling functions of C5L2 under physiologic and pathological conditions. Chen et al. [11] demonstrated that C5L2 is required for optimal biological activities of C3a and C5a, indicating a positive modulation of C5L2 in C3a- and C5a-mediated cellular responses leading to inflammation [11]. In a murine model of moderate sepsis, C5L2-deficient mice (C5L2 knockouts) exhibited an improved survival rate similar to C5aR knockouts, suggesting that C5L2 exerts proinflammatory effects similar to C5aR signaling [12].

Complement Activation in Allergic Airway Diseases

Allergic conditions are associated with complement activation through the three complement activation pathways [6]. In

asthmatic conditions in which allergen-specific preexisting antibodies exist, the classical pathway is activated. Alternative pathway activation can be initiated directly by nucleophilic attack of C3 by allergen polysaccharide structures. In addition, proteases released by inflammatory cells or cells from the mucosal epithelial cells, or direct protease activity of allergens can also drive the generation of anaphylatoxins. Proteases from *Dermatophagoides* spp (in particular, Der p 3 and Der f 3) can cleave C3 and C5 into their active fragments in vitro [13] and promote the generation of C5a/C3a during allergen sensitization [14]. Ragweed extracts have been found to induce complement activation in the sera of allergic patients and healthy controls; however, generation of C3a is much stronger in the circulation of allergic patients [15]. Environmental stimuli also trigger complement activation. Airborne pollutants such as diesel exhaust particles or airborne particulate matter can activate C3 in serum and airway epithelium, respectively [16]. Acute ozone exposure is associated with elevated C3a levels in bronchoalveolar lavage fluid (BAL) in mice [6, 17]. Similarly, airborne allergens such as HDM or animal dander are known triggers of the alternative pathway.

Evidence for complement activation during AR is scant. Complement activation at the nasal mucosa has been described in immediate allergic reaction to pollens (birch and Timothy grass pollen), culminating in the generation of ATs [18]. Ragweed allergen challenge also promotes the generation of C3 degradation products in the nasal mucosa of adolescents with AR [19]. Complement receptor 3 (CR3) is expressed together with Toll-like receptors (TLRs) in human sinonasal tissues [20]. Furthermore, CR3, TLRs, and C5aR are expressed in healthy and allergic myeloid and nonmyeloid cells of the nasal compartment, but the allergic mucosa shows higher C5aR expression levels than healthy mucosa. C3aR, expressed in healthy epithelial cells, submucosal glands, and nerves, is additionally expressed in the endothelium of surrounding sinuses and muscle layers in severe, persistent AR [21]. The nasal and respiratory tract epithelia express complement regulatory molecules such as membrane cofactor protein (CD46), decay-accelerating factor (DAF, CD55), and CD59 [22, 23], suggesting complement activation is tightly controlled in the nasal mucosa. However, it is not known if or how such cellular complement regulators are modulated during allergy.

Role of Complement in the Effector Phase of Allergic Asthma and During Allergen Sensitization

The past few years have seen various studies highlighting the proinflammatory roles of ATs in the effector phase of allergic airway diseases. C3a and C5a are potent chemoattractants for cell populations involved in the development of the asthmatic response, including eosinophils and mast cells. In addition, C5a is a potent chemoattractant for

macrophages, neutrophils, and basophils. Furthermore, C5a stimulates the activation of infiltrating granulocytes, leading to a rapid release of proinflammatory mediators such as leukotrienes, histamine, cytokines, and chemokines. Finally, C3a and C5a contribute to smooth muscle cell contraction and mucus production and enhance vascular permeability [6].

The Anaphylatoxins Regulate the Development of Th17 Cells in Allergic Airway Diseases

In addition to their proinflammatory properties, the ATs are important regulators of adaptive immune responses via their impact on antigen-presenting cells (APCs), in particular on dendritic cells (DCs) and macrophages. As discussed below, several recent publications suggest that C3a and C5a regulate the development of maladaptive Th2 and Th17 immune responses during allergen sensitization.

DCs are a heterogeneous group of APCs composed of several subsets, some of which drive the development of the aberrant adaptive immune response in AA [4]. Two DC populations can be found in the bronchial airways: myeloid DCs (mDCs) and plasmacytoid DCs (pDCs). While mDCs initiate and drive the maladaptive immune response, pDCs exert regulatory properties by directly suppressing mDC-mediated activation of CD4⁺ T effector cells or by inducing regulatory T cells (Tregs) [4]. Both populations reside in the human airways under steady-state conditions. The proportion of mDCs markedly increases after allergen challenge [24]. Importantly, several studies suggest that the ratio between mDCs and pDCs is critical for the development of the dysregulated Th cell response [14, 25] and that C5a regulates this ratio, thereby modulating the development of the allergic phenotype during allergen sensitization [14, 26]. In addition to bronchial airways, the presence and recruitment of mDCs have been shown in ovalbumin-mediated models of experimental AR and in the nasal mucosa of humans suffering from HDM-mediated AR [27]. In the latter context, mDCs express high levels of costimulatory molecules and localize together with CD4⁺ T cells. In support of the crucial role of mDCs in experimental AR, depletion of CD11c⁺ DCs was found to significantly reduce the allergic characteristics. The presence of pDCs and their role in AR is unclear. Other DC subtypes, such as CD1a⁺ Langerhans cells, can be found in AR [28]. Recently, a high frequency of blood CD1a⁺ CD141 (BMDC3)⁺ cells has been reported in patients suffering from AR [29]. Taken together, these data suggest that DCs not only drive the allergic phenotype in AA but also in AR.

During the past few years, IL-17-producing CD4⁺ T cells have received much interest in autoimmune disease and recently in allergy research. Several reports suggest that in addition to Th2 cells, Th17 cells contribute to the development

of allergic inflammation [30, 31]. In humans, several reports show strong IL-17 production in the airways of asthmatics, particularly in patients with severe asthma that correlates with neutrophil numbers [5]. In experimental asthma, IL-17 [32] and Th17 cells [33] have been shown to drive neutrophil recruitment into the airways, an effect that is enhanced by IL-23 [34]. Similar to AA, patients suffering from AR have elevated IL-17 levels [35] and a higher frequency of Th17 cells [36]. Several recent studies have highlighted an important dual role for C5a in Th17 cell development. When mDCs lack C5aR, they produce the Th17 cell-promoting cytokines IL-6, IL-23, and transforming growth factor- β in response to ovalbumin/TLR2 challenge (Fig. 1a) [37]. Confirming these observations, genetic deletion of C5a or pharmacologic targeting of C5aR results in increased numbers of pulmonary Th17 cells (Fig. 1b) [38]. Importantly, Th17 development in experimental asthma is not only regulated by C5aR but by the second C5a receptor, C5L2. Adoptive transfer of HDM-pulsed, BM-derived mDCs from C5L2-deficient mice induces not only Th2 but also Th17 effector cells via an IL-23-dependent mechanism (Fig. 1b) [39]. In contrast to C5a, which blocks Th17 cell development via an IL-23-dependent mechanism, C3a drives IL-23 production and promotes the development of Th17 cells [38]. Although these data suggest that C3a may serve as a target for asthma therapy, one needs to take into account that recent data suggest that the effects seen in a C3aR-deficient environment are not due to the lack of C3aR but rather to the dominant signaling through the C5aR [26]. In contrast to DCs, C5aR signaling in macrophages is required for optimal Th17 cell development (Fig. 1c and d). C5a triggers IL-6 production via TLR-dependent and independent pathways. IL-6 and transforming growth factor- β together promote the development of Th17 cells. This pathway seems to be of major importance for the inflammatory response in experimental arthritis [40]. The role of C5aR signaling in pulmonary macrophages in AA is not known.

In addition to its impact on Th17 cells, C5a may also regulate the development of Tregs, which are a heterogeneous population of T cells that can suppress adaptive immune responses in allergic diseases [41]. Under steady-state conditions, the lack of C5aR signaling in mDCs results in de novo Treg differentiation, at least in vitro (Fig. 1a) [37]. Thus, C5aR signaling in APCs can modulate Th cell differentiation at several levels.

Genetic Linkage Studies Suggest That Complement Contributes to the Development of Allergic Rhinitis and Allergic Asthma

Several family-based studies provide evidence that AA and AR are multifactorial diseases induced by gene–environment interactions [42]. During the past decade, asthma susceptibility genes have been identified by genome-wide searches and

candidate gene studies. Such studies identified *ADAM33* (a disintegrin and metalloproteinase 33), *DPP10* (dipeptidyl peptidase 10), *PHF11* (plant homeodomain finger protein 11), *PTGDR* (prostanoid DP receptor), and *ORMDL3* (orosomucoid 1-like 3) [43]. Several of these genes also have been linked to AR; however, some genetic polymorphisms have been found that are exclusively associated with AR [44]. Recent studies have associated the transcription factor forkhead box P3, which is crucial for the development of Tregs, with AR in various populations [45, 46].

Several genes encoding complement proteins have been associated with experimental asthma [47] and human AA [43]. Genome-wide screens have identified a linkage of asthma and related traits to the chromosomal regions containing *C5* (9q34) and *C5ar1* (19q13.3) genes. A positive association between single nucleotide polymorphisms in *C3* and *C3ar1* genes and the development of atopic asthma has been demonstrated [48, 49]. In contrast, polymorphisms in the *C5* gene positively correlate with protection from asthma [49], which is in accordance with findings in experimental asthma. A single nucleotide polymorphism association between decay-accelerating factor, an important regulator of C3 convertase, and Japanese cedar seasonal AR and HDM-induced asthma has been identified in a Japanese population [50]. Several other proteins involved in the complement activation also have been studied. The pattern-recognition molecule L-ficolin has been associated with recurrent respiratory infections and allergic respiratory diseases in children [51], but polymorphisms in the *MBL2* gene do not seem to be associated with the development of AA, as shown in independent studies in Japan and China [6]. Several genome-wide searches conducted to identify susceptibility genes for AR found the chromosomal region 9q33.2–9q34.3 (containing the *C5* gene) to be associated with the development of AR in European and Japanese populations [52, 53].

The Role of Complement in the Diagnosis of Allergic Rhinitis and Allergic Asthma

Clinical diagnosis of AA relies on symptoms such as episodic breathlessness, wheezing, coughing, and chest tightness [54], complemented by measurements of the allergic status, including determination of IgE levels and allergen skin testing. The severity of asthma is assessed by the extent of airway hyperresponsiveness to methacholine, sputum eosinophils counts, and exhaled nitric oxide levels, among other parameters. The diagnosis of AR is based on the allergic history of the patient, complemented by symptoms including rhinorrhea, sneezing, nasal obstruction, and pruritus. Similar to AA, blood allergen-specific IgE level skin tests help to identify the allergen that

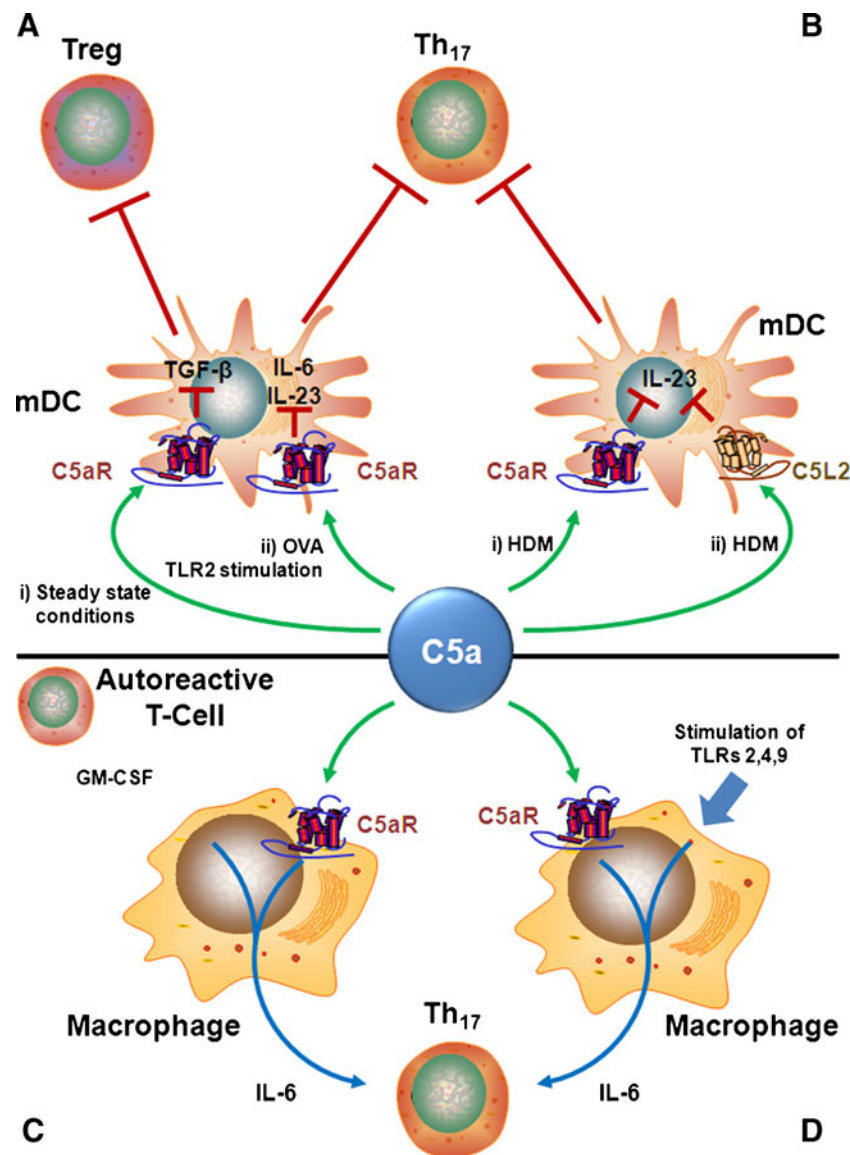


Fig. 1 Impact of complement 5a (C5a) on T-helper type 17 (Th17) cell and regulatory T cell (Treg) differentiation. C5a has opposing regulatory roles in the differentiation of naïve T cells toward Th17 cells depending on the targeted cell type (ie, myeloid dendritic cells [mDCs] or macrophages). **a**, Steady-state conditions in mDCs: C5a downregulates the expression of transforming growth factor (TGF)- β , thereby inhibiting the differentiation of naïve T cells toward Tregs. Upon allergen stimulation (eg, ovalbumin [OVA]) in combination with activation of Toll-like receptor (TLR)2, mDCs produce interleukin (IL)-12, but no IL-6 and IL-23, resulting in Th1 differentiation (not shown). When C5a receptor (C5aR) is absent, mDCs produce high IL-

6, TGF- β , and IL-23, leading to Th17 differentiation [37]. **b**, House dust mite (HDM) triggers similar effects in bone marrow-derived mDCs. Here, absence of (i) C5aR or (ii) C5a receptor-like 2 (C5L2) results in IL-23-dependent Th17 differentiation [39]. For simplification, the induction of Th2 differentiation in the absence of C5aR signaling is not shown. **c**, Activation of C5aR in macrophages in concert with additional stimulatory signals such as granulocyte-macrophage colony-stimulating factor (GM-CSF) released from activated autoreactive T cells in autoimmune arthritis [40], or **d**, activation of TLR2, TLR4, and TLR9 induces the secretion of IL-6 and promotes differentiation toward Th17 cells [69]

accounts for the allergic response [1]. Several studies favor the concept of united airway disease, in which AA and AR are considered two aspects of the same disease instead of being two distinct entities [55]. In line with this view, both disorders show a strong comorbidity. More than 80% of asthmatics suffer from rhinitis episodes, and 10% to 40% of patients with AR report AA symptoms. Onset of AR very

often precedes that of AA. Furthermore, bronchial challenge induces nasal inflammation and vice versa, and endobronchial challenge in rhinitis patients induces a bronchial reaction. In addition, both diseases share similar cell-type activation and release of cytokines and chemokines. Last but not least, many epidemiologic studies have demonstrated a link between AA and AR. Thus, it is not surprising that an integrated approach

has been suggested for diagnosis and treatment of this united airway disease [55].

Two reports have investigated the activation of complement in AR. Andersson et al. [18] determined C3a and C5a levels in the BAL fluid of patients suffering from seasonal AR. Allergen challenge with birch or Timothy grass pollen extract resulted in strong complement activation significantly correlating with the amount of allergen used for challenge (Table 1). In another study, strong C3bBbP production was found in patients exposed to ragweed allergen. Interestingly, the threshold dose required to induce allergic symptoms correlated strongly with maximum complement activation, suggesting that the allergen activates complement in the nasal lavage fluid. More recently, factor B as well as C3 and C5 gene expression were found to be upregulated in chronic rhinosinusitis and allergic fungal rhinosinusitis [56]. Not surprisingly, elevated C3a and C5a levels have been reported in the BAL fluid of asthmatic patients in whom the lungs had been segmentally challenged with allergens [57, 58]. Moreover, it has been shown that complement activation is not restricted to the lung but that severe acute asthma episodes are associated with increased C3a plasma levels that decrease upon successful treatment [59]. More recently, a positive correlation between allergic disease severity as determined by Asthma Quality Life Questionnaire scores and circulating C5adesArg levels has been reported [60•]. Although the small sample sizes in most studies somewhat limit the power of the findings, they

suggest that determination of C3a and C5a in BAL fluid and in blood samples may prove useful as additional diagnostic parameters to judge the severity of united airway diseases and to control treatment success. At the least, such findings warrant further study to evaluate the full potential of complement measurements for AR and AA diagnosis and treatment.

Targeting the Complement System in Allergic Airway Diseases

Allergic respiratory disorders share similar treatment approaches. Patients suffering from acute exacerbation of AA are treated with a combination of β 2-agonists, anticholinergic agents, and corticosteroids. AR treatment includes second-generation oral and intranasal H1-antihistaminics, intranasal glucocorticosteroids, and nasal decongestants.

Data from experimental AA, genome-wide association studies, and candidate gene studies, as well as the association of pulmonary and systemic complement activation with the severity of AA and AR suggest that complement may serve as an attractive novel target for allergy therapy. Indeed, blockade of complement activation with the mouse membrane complement inhibitor complement receptor-related gene y (Crry) [61], targeting C3 [16, 62], C5 [63], the C3aR [57, 64, 65],

Table 1 Complement cleavage products in bronchoalveolar lavage fluid or plasma from patients with allergic rhinitis or allergic asthma

Disorder	Parameter measured in BAL	Concentration in controls	Concentration in allergic patients	Study (year)	Comments
Rhinitis	C3a ^a	177.2±54 ng/mL	1378.3±840.4 ng/mL	Andersson et al. [18] (1994)	Patients with seasonal allergic rhinitis or nonatopic controls were challenged with birch and Timothy pollen extract; strong correlation between C3a and C5a levels and the severity of the nasal allergic response
	C5a ^b	4.4±3.1 ng/mL	12.3±7.5 ng/mL		
	C3bBbP	2.23 U/mL	9.73 U/mL	Mezei et al. [19] (2001)	High levels of C3bBbP in BAL fluid of adolescents allergic to ragweed following allergen challenge; strong positive correlation between the threshold dose that induced symptoms and maximum complement activation
Asthma	C3a ^a	64 ng/mL	123 ng/mL	Humbles et al. [57] (2000)	High levels of C3a in BAL fluid of asthmatics in response to segmental pulmonary allergen challenge
	C3a ^a C5a ^b	3.4 ng/mL 1.2 ng/mL	29 ng/mL 16.4 ng/mL	Krug et al. [58] (2001)	Minor AT levels in BAL fluid of healthy individuals; high AT levels in BAL fluid of patients with mild asthma after segmental allergen challenge; strong correlation between increased AT levels and eosinophil and neutrophil numbers
	C3a (plasma) ^a	166 ng/mL	a) 256 ng/mL b) 197 ng/mL	Nakano et al. [59] (2003)	C3a plasma levels are higher in patients with severe acute asthma episode (a) than in patients with stable chronic asthma (b); C3a levels significantly decrease 7 d after treatment
	C3a (plasma) ^a	449.4±100 ng/mL	901.1±316.4 ng/mL	Bowser et al. [60•] (2010)	C3a levels are higher in adult outpatients with allergic respiratory diseases. C5a levels correlate with allergic respiratory disease severity
	C5a (plasma) ^b	3.92±1.82 ng/mL	3.67±1.86 ng/mL		

^a C3a and C3adesArg

^b C5a and C5adesArg

AT anaphylatoxin; BAL bronchoalveolar lavage; C3a complement 3a; C5a complement 5a

or the C5aR [14, 66, 67] during the allergic effector phase, decreased the allergic phenotype in different models of experimental AA. Based on such encouraging results, complement inhibition has been considered an attractive new therapeutic approach. Unfortunately, in a phase 2a asthma trial in 2004, the oral C5aR antagonist NGD-1000 developed by Neurogen Corp. (Branford, CT) did not improve the asthmatic phenotype in asthmatics.

Although the reasons for this failure remain elusive, data obtained with experimental models of asthma provide evidence that C5a and C5aR signaling in asthma has a dual role in AA. Clearly, C5a drives the asthmatic phenotype during the allergic effector phase. However, as discussed previously, C5a regulates adaptive immune responses at several levels. At least in experimental asthma, the absence of C5aR or C5L2 increases DC-driven development of Th2 [14] and Th17 [38, 39] effector cells. Thus, the failure of the Neurogen trial may be linked to the protective role of C5a in AA. More recently, Alexion Pharmaceuticals (Cheshire, CT) reported the first results of a clinical trial with the C5-blocking antibody eculizumab, approved for therapeutic use in patients with paroxysmal nocturnal hemoglobinuria. Eculizumab treatment attenuated allergen-induced airway responses in patients suffering from mild asthma [68]. At this point, complement has not been targeted in experimental or human AR.

Conclusions

During the past decade, we have learned that the complement system plays a complex role in the development and perpetuation of allergic airway diseases. The most surprising finding is that the proinflammatory mediator C5a serves as an important immune regulator at the interface between innate and adaptive immunity. Based on findings in experimental allergy models, it might be detrimental to block complement at the level of C5a in people at risk of developing allergic airway diseases. Such treatment might shift the delicate balance between tolerance and immunity toward the development of Th2 and Th17 immunity, resulting in manifest AR or AA. Once an allergic airway disease has been established, ATs are generated locally in the airways and the circulation and correlate significantly with disease severity. Thus, AT levels in BAL fluid or blood may serve as powerful indicators of disease severity and may help judge the efficacy of current therapeutic approaches. Bedside tests that allow quick and easy determination of AT concentrations may be helpful in this regard. Furthermore, blocking C5 in an established allergic environment may be beneficial. Although the first data from the Alexion trial are encouraging, appropriately controlled multicenter studies are needed to judge the potential of C5 and AT receptors blockade in allergic airway diseases and to assess whether the data obtained

in experimental asthma can be translated into human asthma. In line with this view, it will be of major importance to evaluate the immunoregulatory properties of C3a and C5a in human asthmatics.

Also, an urgent need exists for experimental models of allergic airway diseases mimicking the human situation by exclusively using allergens relevant to human asthma. This includes immunization through the airways without the use of additional adjuvants. Furthermore, it will be critical to conduct studies in chronic models of experimental asthma to assess the impact of complement on airway remodeling. Eventually, such studies will help to better define the contribution of the protective as well as the proallergic effects of complement in allergic airway diseases and will help us better judge the benefit of targeting complement in such diseases.

Acknowledgement The authors wish to thank the following institution for providing support for some work cited in this review: DFG SFB/TR22 project A21 to Dr. Köhl. The authors also wish to thank Una Doherty for proofreading and for excellent assistance in the preparation of this manuscript.

Disclosure No potential conflicts of interest relevant to this article were reported.

References

Papers of particular interest, published recently, have been highlighted as:

- Of importance

1. Bousquet J, Khaltaev N, Cruz AA et al.: Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). *Allergy* 2008, 63 Suppl 86: 8–160.
2. Dykewicz MS, Hamilos DL: Rhinitis and sinusitis. *J Allergy Clin Immunol* 2010, 125: S103–S115.
3. Bateman ED, Hurd SS, Barnes PJ et al.: Global strategy for asthma management and prevention: GINA executive summary. *Eur Respir J* 2008, 31: 143–178.
4. Lambrecht BN, Hammad H: Biology of lung dendritic cells at the origin of asthma. *Immunity* 2009, 31: 412–424.
5. Al-Ramli W, Prefontaine D, Chouiali F et al.: T(H)17-associated cytokines (IL-17A and IL-17F) in severe asthma. *J Allergy Clin Immunol* 2009, 123: 1185–1187.
6. Zhang X, Köhl J: A complex role for complement in allergic asthma. *Expert Rev Clin Immunol* 2010, 6: 269–277.
7. Köhl J, Wills-Karp M: Complement regulates inhalation tolerance at the dendritic cell/T cell interface. *Mol Immunol* 2007, 44: 44–56.
8. Köhl J: The role of complement in danger sensing and transmission. *Immunol Res* 2006, 34: 157–176.
9. Klos A, Tenner AJ, Johswich KO et al.: The role of the anaphylatoxins in health and disease. *Mol Immunol* 2009, 46: 2753–2766.
10. Bamberg CE, Mackay CR, Lee H et al.: The C5a receptor (C5aR) C5L2 is a modulator of C5aR-mediated signal transduction. *J Biol Chem* 2010, 285: 7633–7644.

11. Chen NJ, Mirtsos C, Suh D et al.: C5L2 is critical for the biological activities of the anaphylatoxins C5a and C3a. *Nature* 2007, 446: 203–207.
12. Rittirsch D, Flierl MA, Nadeau BA et al.: Functional roles for C5a receptors in sepsis. *Nat Med* 2008, 14: 551–557.
13. Maruo K, Akaike T, Ono T et al.: Generation of anaphylatoxins through proteolytic processing of C3 and C5 by house dust mite protease. *J Allergy Clin Immunol* 1997, 100: 253–260.
14. Köhl J, Baelder R, Lewkowich IP et al.: A regulatory role for the C5a anaphylatoxin in type 2 immunity in asthma. *J Clin Invest* 2006, 116: 783–796.
15. Hidvegi T, Schmidt B, Varga L et al.: In vitro complement activation by ragweed allergen extract in the sera of ragweed allergic and non-allergic persons. *Immunol Lett* 1995, 48: 65–71.
16. Walters DM, Breyse PN, Schofield B, Wills-Karp M: Complement factor 3 mediates particulate matter-induced airway hyperresponsiveness. *Am J Respir Cell Mol Biol* 2002, 27: 413–418.
17. Park JW, Taube C, Joetham A et al.: Complement activation is critical to airway hyperresponsiveness after acute ozone exposure. *Am J Respir Crit Care Med* 2004, 169: 726–732.
18. Andersson M, Michel L, Llull JB, Pipkorn U: Complement activation on the nasal mucosal surface—a feature of the immediate allergic reaction in the nose. *Allergy* 1994, 49: 242–245.
19. Mezei G, Varga L, Veres A et al.: Complement activation in the nasal mucosa following nasal ragweed-allergen challenge. *Pediatr Allergy Immunol* 2001, 12: 201–207.
20. Lane AP, Truong-Tran QA, Myers A et al.: Serum amyloid A, properdin, complement 3, and toll-like receptors are expressed locally in human sinonasal tissue. *Am J Rhinol* 2006, 20: 117–123.
21. Jun SW, Kim TH, Lee HM et al.: Overexpression of the anaphylatoxin receptors, complement anaphylatoxin 3a receptor and complement anaphylatoxin 5a receptor, in the nasal mucosa of patients with mild and severe persistent allergic rhinitis. *J Allergy Clin Immunol* 2008, 122: 119–125.
22. Varsano S, Frolkis I, Shapiro H, Ophir D: Human nasal epithelium adsorbs complement C3-related fragments and expresses cell membrane complement regulatory proteins. *Laryngoscope* 1996, 106: 599–604.
23. Varsano S, Frolkis I, Rashkovsky L et al.: Protection of human nasal respiratory epithelium from complement-mediated lysis by cell-membrane regulators of complement activation. *Am J Respir Cell Mol Biol* 1996, 15: 731–737.
24. Bratke K, Lommatzsch M, Julius P et al.: Dendritic cell subsets in human bronchoalveolar lavage fluid after segmental allergen challenge. *Thorax* 2007, 62: 168–175.
25. de Heer HJ, Hammad H, Soullie T et al.: Essential role of lung plasmacytoid dendritic cells in preventing asthmatic reactions to harmless inhaled antigen. *J Exp Med* 2004, 200: 89–98.
26. Zhang X, Lewkowich IP, Köhl G et al.: A protective role for C5a in the development of allergic asthma associated with altered levels of B7-H1 and B7-DC on plasmacytoid dendritic cells. *J Immunol* 2009, 182: 5123–5130.
27. KleinJan A, Willart M, van Rijt LS et al.: An essential role for dendritic cells in human and experimental allergic rhinitis. *J Allergy Clin Immunol* 2006, 118: 1117–1125.
28. Till SJ, Jacobson MR, O'Brien F et al.: Recruitment of CD1a⁺ Langerhans cells to the nasal mucosa in seasonal allergic rhinitis and effects of topical corticosteroid therapy. *Allergy* 2001, 56: 126–131.
29. Lundberg K, Greiff L, Borrebaeck CA, Lindstedt M: FcεpsilonRI levels and frequencies of peripheral blood dendritic cell populations in allergic rhinitis. *Hum Immunol* 2010, 71: 931–933.
30. Nakae S, Komiyama Y, Nambu A et al.: Antigen-specific T cell sensitization is impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses. *Immunity* 2002, 17: 375–387.
31. Schnyder-Candrian S, Togbe D, Couillin I et al.: Interleukin-17 is a negative regulator of established allergic asthma. *J Exp Med* 2006, 203: 2715–2725.
32. Hellings PW, Kasran A, Liu Z et al.: Interleukin-17 orchestrates the granulocyte influx into airways after allergen inhalation in a mouse model of allergic asthma. *Am J Respir Cell Mol Biol* 2003, 28: 42–50.
33. McKinley L, Alcorn JF, Peterson A et al.: TH17 cells mediate steroid-resistant airway inflammation and airway hyperresponsiveness in mice. *J Immunol* 2008, 181: 4089–4097.
34. Wakashin H, Hirose K, Maezawa Y et al.: IL-23 and Th17 cells enhance Th2-cell-mediated eosinophilic airway inflammation in mice. *Am J Respir Crit Care Med* 2008, 178: 1023–1032.
35. Ciprandi G, De AM, Murdaca G et al.: Serum interleukin-17 levels are related to clinical severity in allergic rhinitis. *Allergy* 2009, 64: 1375–1378.
36. Ciprandi G, Filaci G, Battaglia F, Fenoglio D: Peripheral Th-17 cells in allergic rhinitis: New evidence. *Int Immunopharmacol* 2010, 10: 226–229.
37. • Weaver DJ, Jr., Reis ES, Pandey MK, et al.: C5a receptor-deficient dendritic cells promote induction of Treg and Th17 cells. *Eur J Immunol* 2010, 40: 710–721. *This is the first study demonstrating that the lack of C5aR in DCs regulates steady-state Treg formation. Furthermore, it shows that C5aR-deficient DCs favor Th17 cell development when stimulated with a TLR2 ligand in the presence of antigen by an IL-23-dependent mechanism.*
38. Lajoie S, Lewkowich IP, Suzuki Y, et al.: Complement-mediated regulation of the IL-17A axis is a central genetic determinant of the severity of experimental allergic asthma. *Nat Immunol* 2010, 11: 928–935.
39. Zhang X, Schmudde I, Laumonnier Y et al.: A critical role for C5L2 in the pathogenesis of experimental allergic asthma. *J Immunol* 2010, 185: 6741–6752.
40. Hashimoto M, Hirota K, Yoshitomi H et al.: Complement drives Th17 cell differentiation and triggers autoimmune arthritis. *J Exp Med* 2010, 207: 1135–1143.
41. Palomares O, Yaman G, Azkur AK et al.: Role of Treg in immune regulation of allergic diseases. *Eur J Immunol* 2010, 40: 1232–1240.
42. Swarr DT, Hakonarson H: Unraveling the complex genetic underpinnings of asthma and allergic disorders. *Curr Opin Allergy Clin Immunol* 2010, 10: 434–442.
43. Vercelli D: Discovering susceptibility genes for asthma and allergy. *Nat Rev Immunol* 2008, 8: 169–182.
44. Davila I, Mullol J, Ferrer M et al.: Genetic aspects of allergic rhinitis. *J Investig Allergol Clin Immunol* 2009, 19 Suppl 1: 25–31.
45. Suttner K, Depner M, Wetzke M et al.: Genetic variants harbored in the forkhead box protein 3 locus increase hay fever risk. *J Allergy Clin Immunol* 2010, 125: 1395–1399.
46. Zhang L, Zhang Y, Desrosiers M et al.: Genetic association study of FOXP3 polymorphisms in allergic rhinitis in a Chinese population. *Hum Immunol* 2009, 70: 930–934.
47. Karp CL, Grupe A, Schadt E et al.: Identification of complement factor 5 as a susceptibility locus for experimental allergic asthma. *Nat Immunol* 2000, 1: 221–226.
48. Barnes KC, Grant AV, Baltadzhieva D et al.: Variants in the gene encoding C3 are associated with asthma and related phenotypes among African Caribbean families. *Genes Immun* 2006, 7: 27–35.
49. Hasegawa K, Tamari M, Shao C et al.: Variations in the C3, C3a receptor, and C5 genes affect susceptibility to bronchial asthma. *Hum Genet* 2004, 115: 295–301.
50. Kawai T, Takeshita S, Imoto Y et al.: Associations between decay-accelerating factor polymorphisms and allergic respiratory diseases. *Clin Exp Allergy* 2009, 39: 1508–1514.

51. Cedzynski M, Atkinson AP, St SA et al.: L-ficolin (ficolin-2) insufficiency is associated with combined allergic and infectious respiratory disease in children. *Mol Immunol* 2009, 47: 415–419.
52. Dizier MH, Bouzigon E, Guillaud-Bataille M et al.: Genome screen in the French EGEA study: detection of linked regions shared or not shared by allergic rhinitis and asthma. *Genes Immun* 2005, 6: 95–102.
53. Yokouchi Y, Shibasaki M, Noguchi E et al.: A genome-wide linkage analysis of orchard grass-sensitive childhood seasonal allergic rhinitis in Japanese families. *Genes Immun* 2002, 3: 9–13.
54. Levy ML, Fletcher M, Price DB et al.: International Primary Care Respiratory Group (IPCRG) Guidelines: diagnosis of respiratory diseases in primary care. *Prim Care Respir J* 2006, 15: 20–34.
55. Braunstahl GJ: United airways concept: what does it teach us about systemic inflammation in airways disease? *Proc Am Thorac Soc* 2009, 6: 652–654.
56. Schlosser RJ, Mulligan RM, Casey SE et al.: Alterations in gene expression of complement components in chronic rhinosinusitis. *Am J Rhinol Allergy* 2010, 24: 21–25.
57. Humbles AA, Lu B, Nilsson CA et al.: A role for the C3a anaphylatoxin receptor in the effector phase of asthma. *Nature* 2000, 406: 998–1001.
58. Krug N, Tschernig T, Erpenbeck VJ et al.: Complement factors C3a and C5a are increased in bronchoalveolar lavage fluid after segmental allergen provocation in subjects with asthma. *Am J Respir Crit Care Med* 2001, 164: 1841–1843.
59. Nakano Y, Morita S, Kawamoto A et al.: Elevated complement C3a in plasma from patients with severe acute asthma. *J Allergy Clin Immunol* 2003, 112: 525–530.
60. • Bowser C, Erstein DP, Silverberg JI, et al.: Correlation of plasma complement split product levels with allergic respiratory disease activity and relation to allergen immunotherapy. *Ann Allergy Asthma Immunol* 2010, 104: 42–49. *This study suggests that determination of plasma C3a and C5a might prove useful to monitor disease activity and the efficacy of asthma therapy.*
61. Taube C, Rha YH, Takeda K et al.: Inhibition of complement activation decreases airway inflammation and hyperresponsiveness. *Am J Respir Crit Care Med* 2003, 168: 1333–1341.
62. Drouin SM, Corry DB, Kildsgaard J, Wetsel RA: Cutting edge: the absence of C3 demonstrates a role for complement in Th2 effector functions in a murine model of pulmonary allergy. *J Immunol* 2001, 167: 4141–4145.
63. Peng T, Hao L, Madri JA et al.: Role of C5 in the development of airway inflammation, airway hyperresponsiveness, and ongoing airway response. *J Clin Invest* 2005, 115: 1590–1600.
64. Bautsch W, Hoymann HG, Zhang Q et al.: Cutting edge: guinea pigs with a natural C3a-receptor defect exhibit decreased bronchoconstriction in allergic airway disease: evidence for an involvement of the C3a anaphylatoxin in the pathogenesis of asthma. *J Immunol* 2000, 165: 5401–5405.
65. Drouin SM, Corry DB, Hollman TJ et al.: Absence of the complement anaphylatoxin C3a receptor suppresses Th2 effector functions in a murine model of pulmonary allergy. *J Immunol* 2002, 169: 5926–5933.
66. Abe M, Shibata K, Akatsu H et al.: Contribution of anaphylatoxin C5a to late airway responses after repeated exposure of antigen to allergic rats. *J Immunol* 2001, 167: 4651–4660.
67. Baelder R, Fuchs B, Bautsch W et al.: Pharmacological targeting of anaphylatoxin receptors during the effector phase of allergic asthma suppresses airway hyperresponsiveness and airway inflammation. *J Immunol* 2005, 174: 783–789.
68. • Gail G, Boulet LP, Severino B et al.: The effect of C5 inhibition by eculizumab on allergen-induced asthmatic responses in patients. *Mol Immunol* 2009, 46: 2837–2838. *These are the first preliminary data to indicate that C5 blockade might prove useful as a new therapeutic approach in AA.*
69. Fang C, Zhang X, Miwa T, Song WC: Complement promotes the development of inflammatory T-helper 17 cells through synergistic interaction with Toll-like receptor signaling and interleukin-6 production. *Blood* 2009, 114: 1005–1015.