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A complex role for complement in allergic asthma

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Abstract

Allergic asthma is a chronic inflammatory disease of the upper airway. It is well appreciated that maladaptive Th2 immunity promotes the allergic phenotype, the underlying mechanisms of which remain elusive. The disease is associated with activation of complement, an ancient danger-sensing component of the innate immune system. Different models of experimental allergic asthma suggest that the small complement fragments of C3 and C5, the anaphylatoxins C3a and C5a, not only promote proallergic effector functions during the allergic effector phase but regulate the development of Th2 immunity during allergen sensitization. The available data support a concept in which C5a is dominant during allergen sensitization and protects against the development of maladaptive Th2 immunity. By contrast, C3a and C5a appear to act synergistically and drive allergic inflammation during the effector phase. In this article, we will review the recent findings in the field to judge the benefit of complement targeting in allergic asthma.

Keywords

allergic asthma; anaphylatoxin; complement; dendritic cell; innate immunity; Th2 cytokine

Allergic asthma is a chronic airway inflammatory disease that arises as a result of inappropriate immune responses to common environmental antigens (Ags) in genetically susceptible individuals [1]. The incidence, morbidity and mortality of asthma have increased dramatically over the past three decades, particularly in industrialized countries, and are associated with enormous healthcare costs [2,3]. In 2008, the WHO estimated that 300 million people were suffering from asthma worldwide, causing 250,000 deaths each year.

Symptoms of asthma include recurrent episodes of wheezing, coughing, chest tightness and shortness of breath in response to innocuous aeroallergens, such as dust mite feces, animal dander, windblown pollen and air pollutants. Although the mechanisms underlying the initiation, development and maintenance of pulmonary allergy in asthmatics remain elusive, a dysregulated Th2-mediated adaptive immune response is well accepted to causally

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associate with the major pathophysiologic features of asthma, including bronchoconstriction, airway hyperresponsiveness (AHR) and airway inflammation. Cytokines produced by Th2 cells, such as IL-4, IL-5 and IL-13, orchestrate pulmonary allergic responses, involving the development, migration and activation of inflammatory cells, production of allergen-specific antibody, mucus-cell hyperplasia, and an increase in vascular permeability and airway reactivity, all of which contribute to asthma pathogenesis and pathology [4].

In recent years, a new area of active investigation has focused on the role of innate immune components in the regulation of Th2-biased adaptive immunity in asthma. The complement system, a phylogenetically ancient danger-sensing component of the innate immune system, is no exception [2]. It comprises a network of more than 30 proteins and has long been recognized as a lytic effector system that protects the host from microbial invaders. Upon recognition of pathogen-associated molecular patterns (PAMPs), complement can be activated through three separate pathways – the classical pathway, the alternative pathway and the lectin pathway, leading to downstream proteolytic cleavage and generation of activated complement factors that exert immunobiological functions (Figure 1). Furthermore, C3 and C5 can be directly cleaved by proteases derived from the clotting cascade and the fibrinolytic system, as well as by phagocyte-derived proteases [5]. The most appreciated mechanism leading to the activation of the classical pathway is through binding of immune complexes to C1q, although C1q can also bind to PAMPs and apoptotic cells [6]. The alternative pathway starts with a nucleophilic attack of the internal thioester of C3 by a wide range of molecules expressed on microbial surfaces. In addition to specific activation, C3a is continuously hydrolyzed in small amounts in the circulation ('C3 tickover'). Recent data also suggest that properdin functions as a danger-sensing molecule that activates C3 directly, thus providing 'specificity' to C3 activation by the alternative pathway [7]. The lectin pathway is initiated when mannose-binding lectin (MBL) or ficolins recognize and bind carbohydrate structures on either pathogens or apoptotic cells. Although each pathway is activated by different danger signals, all of them converge at the level of C3, leading to C3 cleavage by C3 convertases to generate C3a and C3b. C3b becomes part of the C5 convertases that further cleave C5 into C5a and C5b. C5b nucleates the formation of the membrane attack complex (MAC), whereas C3a and C5a are released and promote inflammatory responses, among others (see later and [6]).

The primary cytolytic activity of complement derives from the nonenzymatic function of the MAC. Binding of C5b by two other complement components, C6 and C7, forms an amphiphilic complex that is able to insert into microbial lipid bilayers. Subsequent binding of C8 initiates C9 polymerization, which generates a membrane-spanning channel and leads to osmotic lysis of microbes. Besides being an efficient lytic effector, a variety of biological functions have been elicited following complement activation. Granulocytes and endothelial cells can be activated by sublytic quantities of the MAC [8]. In addition, deposition of C3b or C4b onto membranes can opsonize microbes, allowing their recognition by phagocyte receptors, such as complement receptors 1–4, and leading to phagocytosis and clearance of microbes, as well as B-cell activation. Furthermore, the low-molecular-weight anaphylatoxins (ATs) C3a and C5a generated during complement activation possess many proinflammatory and immunoregulatory properties critical for the development and the modulation of allergic immune responses. First, C3a and C5a recruit leukocytic effector cells of the allergic inflammatory response. C5a is a strong chemoattractant for macrophages, basophils, neutrophils and possibly T lymphocytes, whereas both ATs are chemotactic for eosinophils and some mast cell populations [9]. Second, C3a and C5a stimulate the activation of the infiltrating granulocytes, leading to rapid production and release of various proinflammatory mediators such as histamine, leukotrienes and platelet-activating factor, as well as proinflammatory cytokines and chemokines including IL-1, IL-6

and TNF- α . Third, C3a and C5a are also able to induce smooth muscle contraction, promote mucus secretion and enhance vascular permeability [5].

In addition to its proinflammatory effector functions, complement regulates adaptive immunity at many levels [1,6]. With regard to allergic asthma, complement exerts critical immunoregulatory roles, modulating the initiation and development of adaptive immunity to either promote or suppress pulmonary allergy. In particular, complement components C5 and C3, and their cleavage products C5a and C3a, regulate the magnitude of adaptive immune responses via ligation of their respective receptors expressed on antigen-presenting cells (APCs) and T lymphocytes, as well as on pulmonary structures and stromal cells. These immune responses involve many pathophysiological features of asthma, including inflammatory cell infiltration, mucus secretion, increase in vascular permeability and smooth muscle contraction [1,8,10].

In this review, we will discuss:

- Mechanisms of complement activation under asthmatic conditions
- Complement genetics associated with asthma development
- Mechanisms underlying the complement-mediated regulation of Th2 immunity during allergen sensitization
- The contribution of complement to the proallergic environment in established allergic asthma
- The potential role of complement as a novel therapeutic target in allergic asthma

Activation of complement under asthmatic conditions

Under asthmatic conditions, several pathways can lead to activation of either the entire complement cascade or the specific cleavage of C3 and/or C5. First, C3 and C5 can be cleaved through the classical complement activation pathway following the formation of allergen–antibody complexes. Unless recognized by natural antibodies, this pathway will be activated in an established asthmatic environment when allergen-specific antibodies have been generated. Second, the alternative pathway of complement activation can be initiated directly on the surface of allergen, resulting in AT production. Third, recognition of allergen polysaccharide structures can activate the lectin pathway and fix complement. Last, proteases released from inflammatory cells or derived from allergens can cleave C3 and C5 directly [5]. Indeed, several studies have demonstrated that aeroallergens play an important role in the generation of ATs in the airways. Nagata and Glovsky have shown that extracts of several allergens including house dust mite (HDM), *Aspergillus fumigatus* and perennial ryegrass induce *in vitro* production of ATs in serum in a dose- and time-dependent manner [11]. Interestingly, crude extracts of HDM and HDM-derived proteases from group 3 allergens of *Dermatophagoides* species (*Der p3* and *Der f3*) strongly activate complement and cleave C3 and C5 into their active fragments [12,13].

Several clinical studies report strong AT production under asthmatic conditions [14–16]; for example, C3a levels in allergen-challenged lung lobes were significantly higher than in allergen diluent (sham)-challenged lobes of asthmatic individuals [14]. In another study, Krug and coworkers showed increased bronchoalveolar lavage (BAL) levels of both C3a and C5a 24 h following segmental allergen challenge of asthmatic individuals, whereas elevations in normal individuals were only minor [15]. Importantly, BAL levels of C3a in this population of asthmatics appeared to be significantly higher than their C5a levels.

In addition to allergen-mediated mechanisms, environmental stimuli can trigger complement activation. Diesel exhaust particles can activate complement through the alternative pathway and lead to C3 cleavage in human serum [17]. More importantly, it has been indicated that exposure to airborne particulate matter can induce AHR through the deposition and activation of C3 in the airway epithelia [18]. Furthermore, acute ozone exposure can induce AHR and neutrophil infiltration in mice together with elevated C3 levels in BAL fluid. Depletion or inhibition of complement prevents the development of ozone-induced AHR and airway neutrophilia, suggesting an important role of complement activation under such pathophysiological conditions [19]. Tobacco smoke has been shown to directly activate the alternative pathway of complement through cleavage of the internal thioester in C3 [20]. It has also been suggested that C3 production is stimulated by Th2 cytokines such as IL-4 and IL-13, which can induce C3 mRNA expression by human and mouse airway epithelial cells [1].

Taken together, these studies provide evidence that minor complement activation occurs in the lung of healthy individuals, supporting the view of low-level C5a receptor (C5aR) signaling as a means to maintain inhalation tolerance at the mucosal surface (see data from animal studies later). Under conditions of ongoing allergic inflammation, C3a and C5a are likely to act as proinflammatory mediators owing to their effects on resident and infiltrating cells, such as eosinophils, neutrophils and mast cells.

Complement genetics associated with the development of allergic asthma

Allergic asthma is a multifactorial disorder, the development of which is triggered by complex interactions between environmental and genetic components. Although changes in the environment contribute to the increased incidence of asthma in Western societies, it has long been suggested that genetic predisposition plays an important role in asthma pathogenesis by influencing the susceptibility of individuals to various changes in environmental exposures that collectively lead to the expression of asthmatic phenotypes [21]. Previous investigations have shown that asthma is partially hereditary in nature, but the particular patterns of inheritance are not clear [22,23]. Family and twin studies suggest a multigenic model for the pathogenetic mechanisms of asthma with substantial heterogeneity among families [24–26]. Various phenotypic expressions among individuals, genetic heterogeneity across populations and complex genetic–environmental interactions have hindered progress in the discovery of asthma candidate genes. Tremendous work carried out over the past 15 years has resulted in the successful identification of several asthma susceptibility genes through genome-wide searches and candidate gene studies, including a disintegrin and metalloproteinase 33 (*ADAM33*), dipeptidyl peptidase 10 (*DPP10*), plant homeodomain finger protein 11 (*PHF11*), prostanoid DP receptor (*PTGDR*) and, more recently, orosomucoid 1-like 3 (*ORMDL3*), the third member of a gene family of unknown function that encodes transmembrane proteins anchored in the endoplasmic reticulum [21,27].

With regard to complement genes, genome-wide screens for asthma susceptibility loci have identified linkage of asthma and related traits to the chromosomal regions containing both the *C5* (9q34) as well as the *C5ar1* (19q13.3) genes [28,29]. In support of the opposing roles of C3 and C5 in animal studies, an association between a single nucleotide polymorphism (SNP) in the *C3* gene and atopic asthma in both children and adults in a Japanese population has been reported. By contrast, SNPs in the human *C5* gene have been associated with protection against both childhood and adult asthma. The same authors also found a significant association between a SNP in the C3a receptor (*C3ar1*) gene and childhood asthma [29]. In line with this study, a significant association between a three-SNP haplotype in the *C3* gene and asthma, IgE levels and the ratio of IFN- γ /IL-13 levels in serum in an

Afro-Caribbean population [30] has been reported. In contrast to C3 and C5 and their cognate receptors, polymorphisms in MBL do not seem to be associated with the development of allergic asthma. Two independent studies in Japan [31] and China [32] did not find evidence for genetic associations between MBL2 polymorphisms and the development of allergic asthma and atopy in children.

In summary, some studies suggest that variants in the complement pathway genes affect susceptibility to asthma. However, further studies in additional populations are needed in order to determine the general importance of variants in complement genes to asthma susceptibility.

Role of complement during allergen sensitization

Role of C5/C5a

The ATs C5a and C3a have long been recognized as potent proinflammatory mediators contributing to allergic reactions. Recent studies indicate that ATs regulate the interaction of APCs and immune effector cells, thus directing the development of adaptive immune responses. Following identification of C5 as a susceptibility locus for experimental allergic asthma, further investigations confirmed the observation that C5-deficient mice are more susceptible to the development of AHR and pulmonary inflammation in response to allergen exposure than wild-type (WT) mice [33,34]. More importantly, previous studies from our laboratory have provided the first explanations for this unexpected phenomenon. We have demonstrated that *in vivo* ablation of C5aR signaling prior to initial allergen sensitization leads to the induction of, and a marked increase in, Th2-biased immune responses in mouse models of allergic asthma. By contrast, C5aR blockade in already sensitized mice results in suppressed airway inflammation and AHR. Our data suggest a dual role for C5a/C5aR signaling in allergic asthma, which is protective during allergen sensitization but proallergic in an established inflammatory environment [35].

Mechanistically, C5aR signaling has been shown to exert critical immunoregulatory functions at the interface of the dendritic cells (DCs) and T cells that control the development of unwanted adaptive immunity. DCs are essential APCs; five subsets of DC populations have been identified in lymphoid tissues based on their distinct expression of surface markers, at least two of which can be found in the lung (myeloid DCs [mDCs] and plasmacytoid DCs [pDCs]) [36]. Pulmonary mDCs function as the major APCs in mouse models of allergic asthma, preferentially inducing Th2-biased immune responses in the airways upon allergen exposure. By contrast, pulmonary pDCs are thought to be tolerogenic and able to induce respiratory tolerance [37,38]. C5aR signaling in the lung controls the accumulation of pulmonary mDCs and pDCs, keeping the mDC:pDC ratio low, which facilitates the suppressive effect of pDCs on the mDC-mediated activation of T cells [35]. In addition, C5aR signaling in pulmonary mDCs negatively regulates the production of CC chemokine ligand (CCL)17 and CCL22, which are chemoattractants that recruit Th2 effector cells to sites of inflammation. Decreased levels of CCL17 and CCL22 lead to inhibition of Th2 cell homing into the lung [35]. Furthermore, data obtained with C5-deficient A/J mice support a model in which C5aR signaling in pulmonary mDCs maintains mDC susceptibility to suppression by naturally occurring regulatory T cells (Tregs), which prevents mDCs from activating naive T cells [1,39].

Although the available data suggest that C5aR signaling plays a critical role at the DC/T-cell interface to control the development of allergic inflammation in the airways, the molecular mechanisms underlying this C5aR-mediated regulation of pulmonary DC functions are still unclear. Further insights into the regulatory role of C5 on distinct pulmonary DC populations result from a recent study comparing the mechanisms underlying asthma-

susceptible C5-deficient A/J mice and resistant C3H mice. A/J mice favor allergen uptake by mDCs, leading to upregulation of costimulatory molecules and production of a Th2- and Th17-promoting cytokine profile. By contrast, in C3H mice, allergens are preferentially taken up by tolerogenic pDCs [40]. These data suggest that C5 and possibly C5a regulate maladaptive immunity in asthma through an impact on pDCs. In support of this view, Zhang *et al.* have shown that C5a–C5aR signaling protects against the development of maladaptive Th2 immunity in allergic asthma by regulating the accumulation of pulmonary pDCs expressing costimulatory molecules B7-H1 (PD-L1) and B7-DC (PD-L2), which can modulate the function of mDCs as well as regulatory T cells (Figure 2) [41]. In accordance with previous observations, the authors found increased eosinophilic inflammation, Th2 cytokine production and IgE response in C5aR-deficient mice upon airway HDM challenge, suggesting a protective role of C5aR signaling. The B7 molecules provide crucial second signals to promote or inhibit the activation of DCs and T cells at their interface. It is well appreciated that B7-2 signaling is important for the development of allergen-induced allergic responses *in vivo* [42]. Furthermore, B7-DC, but not B7-H1, has been associated with pulmonary allergy during the effector phase of asthma [43]. Importantly, recent studies demonstrate a critical role for B7-H1 and B7-DC signaling in pulmonary pDCs to control Th2 cytokine production from effector T cells in response to HDM stimulation. The important role of B7-H1 and B7-DC in peripheral T-cell tolerance has also been shown in chronic viral infections and tumors [41,44].

Interestingly, a Th2-cytokine-driven environment with high levels of IL-4 down-regulates C5aR expression in monocyte-derived DCs [45]. Thus, C5aR expression on DCs might be downregulated in the allergic, Th2 cytokine-dominated environment, thus dampening the negative regulatory effect of C5aR signaling, in particular on pDCs. Indeed, C5aR expression on pulmonary DCs, isolated from mice after repeated HDM challenge, is substantially reduced. By contrast, C3aR expression on pulmonary DCs is markedly increased.

Collectively, these data suggest that intact C5aR signaling in pulmonary DCs acts as an essential immune regulator during allergen sensitization, silencing potentially harmful immunogenic responses toward innocuous allergens. Under conditions where normal C5a generation and/or pulmonary C5aR signaling is ablated, loss of DC modulation will break peripheral tolerance and lead to proliferation, activation and Th2-polarization of naive T cells as well as increased recruitment of Th2 effector cells, eventually resulting in the induction of dysregulated Th2-biased immune responses.

Role of C3/C3a

In contrast to C5a, the role of C3/C3a in the sensitization phase of asthma is less clear. C57BL/6 mice deficient in C3 or C3aR exhibit significant attenuation in AHR, eosinophil infiltration, Th2 cytokine production and Ag-specific IgE responses upon pulmonary exposure of a mixture of *A. fumigates* and ovalbumin (OVA) [46,47]. However, in a model of particulate matter-induced pulmonary allergy, C3-deficient mice on a mixed background develop dramatically reduced AHR as compared with WT mice, but they are not protected from airway inflammation [18]. Similarly, in an OVA-induced asthma model, bronchoconstriction and AHR are markedly decreased in C3aR-deficient mice on a BALB/c background, but IgE production and Th2 cytokine levels in those mice are indistinguishable from WT controls [14]. These data suggest that C3a/C3aR signaling contributes to the development of the allergic phenotype. Differences in mouse strains and/or in the nature of the allergens used for immunization may account for the controversial results generated in different animal models. More recently, Th2-mediated immune responses have been compared side-by-side in C5aR- and C3aR-deficient mice in a well-characterized model of HDM-induced asthma. In this model, the authors found opposing effects of the two AT

receptors, that is, C5aR-deficient mice suffered from increased Th2 immunity, whereas in C3aR-deficient animals it was decreased [41].

The reduced asthmatic phenotype in C3aR-knockouts (KOs) may result from the absence of proallergic C3aR, or a shift toward protective C5aR signaling. The fact that C5aR blockade abolished the reduced Th2-biased immune responses in C3a receptor-deficient mouse strains (C3aR KOs) favors the latter hypothesis. This view is further supported by data showing a negative impact of C3aR on C5aR expression in pulmonary DCs [41]. Interestingly, the reciprocal modulation of C3aR and C5a/C5aR has been demonstrated in previous studies. Settmacher *et al.* have shown that ligand-induced C3aR internalization on granulocytes, monocytes and mast cells is dramatically decreased by costimulation with C5a, which can be blocked by the addition of a neutralizing C5aR antibody [48]. Receptor internalization is an important negative feedback mechanism protecting cells from overstimulation. The inhibitory effect of C5a on C3aR internalization enhances the expression of noninternalized C3aR present on the cell surface, thus increasing the amount and duration of C3aR-mediated signaling in target cells [10]. Interestingly, we observed a negative impact of C5aR signaling on C3aR expression in pulmonary DCs in the HDM-induced asthma model, suggesting an antagonistic effect of C5a on C3aR-mediated activity [41]. More recently, in a mouse model of respiratory syncytial virus disease, airway reactivity, lung eosinophilia and mucus production were found to be significantly increased in C5-deficient mice. Similar to what we had observed with pulmonary DCs from C5aR KOs, C3aR expression in bronchial epithelial and smooth muscle cells of C5-deficient mice was elevated as compared with WT mice, which is necessary for the observed enhanced phenotype since ablation of C3aR signaling in C5-deficient mice substantially attenuated disease expression, suggesting that C5 modulates AHR and eosinophilic inflammation by controlling C3aR expression in the lungs [49].

Taken together, these data suggest an intimate cross-regulation between C3aR and C5aR signaling, which has a potential regulatory role in the pulmonary inflammatory processes of allergic asthma. The data support a model in which reciprocal modulation of C3aR and C5aR expression in pulmonary DCs results in reciprocal suppression of C3aR- and C5aR-mediated biological functions during allergen sensitization. Such a model is in line with the opposing phenotype that we have observed in C3aR KOs and C5aR KOs upon pulmonary HDM exposure. By contrast, C3aR and C5aR signaling synergize during the asthmatic effector phase, possibly through reciprocal augmentation of receptor expression to activate effector cells, particularly infiltrating granulocytes and mast cells, thus promoting the expression of allergic responses in an established inflammatory environment. Of note, these data further emphasize a critical cell-specific mechanism underlying AT receptor-mediated regulation of asthma pathogenesis (i.e., the same AT receptor activation in different cell types elicits distinct effects, contributing to either the enhancement or the inhibition of pulmonary adaptive immune responses to inhaled allergens). Furthermore, AT receptor signaling in pulmonary structural cells, such as bronchial epithelial cells, smooth muscle cells and fibroblasts, may also have an impact on the development of the asthmatic phenotype. Although accumulating data have demonstrated an important role for airway epithelial cells in regulating the maturation, differentiation and activation of DCs and T cells, little is known about the contribution of ATs in this process.

Since all previous studies aimed at elucidating the possible regulatory impact of C3/C3a have been conducted in animals with genetic alterations, they do not allow differentiation between the effects that occur during allergen sensitization or during the effector phase of asthma. Experiments supporting a role for C3a in regulating DC/T-cell interactions at the time of allergen sensitization have shown that DCs express the C3aR and that local production of C3 by bone marrow-derived DCs has a strong impact on DC activation, regulating differentiation and proliferation of naive T cells during allospecific T-cell

responses [50]. In addition, DCs that either lack C3aR or were treated with a C3aR antagonist have been found to elicit impaired T-cell priming against expressed alloantigen in a mouse transplant model, which is associated with decreased expression of costimulatory molecules and MHC class II on DCs [51]. Elevation in the levels of intracellular cyclic adenosine monophosphate, a strong suppressor of proinflammatory cytokines, has been suggested as a potential mechanism underlying the defective Ag uptake and T-cell stimulation mediated by DCs deficient in either C3 or C3aR [52]. Moreover, C3aR signaling has been demonstrated to modulate Th2 cell-mediated allergic responses to epicutaneously delivered allergen via regulation of IL-12 secretion by DCs [53]. Together, these data provide evidence for a regulatory role of C3a at the DC/T-cell interface to influence the nature and/or the magnitude of adaptive T-cell responses upon allergen exposure. Further studies are needed to directly evaluate the effects of C3a specifically at the time of initial sensitization in response to aeroallergens introduced through the airways.

Role of complement during the effector phase

The potent proinflammatory functions of ATs are well known to be consonant with the cardinal pathophysiological features of asthma. Indeed, mice deficient in C3 or C3aR are protected from the development of AHR and/or Th2 cell-mediated inflammatory responses, including airway eosinophilia, elevated levels of Th2 cytokines and Ag-specific IgE in various models of pulmonary allergy and asthma [14,18,46,47]. Furthermore, pharmacological targeting of C3aR or C5/C5aR during the effector phase of experimental asthma significantly suppresses the disease-associated allergic phenotype in response to airway challenges with HDM, OVA or *A. fumigatus* extract [35,54,55]. These studies clearly indicate that ATs act as positive regulators in the development of Th2-biased adaptive immunity in an established inflammatory environment. Mechanistically, AT may promote the production of Th2 cytokines through the recruitment and activation of eosinophils, basophils, neutrophils and mast cells. In addition, cross-talk between allergen-specific IgG-mediated production of IL-13 from mast cells and C3a [56], as well as the induction of IL-13 by C5a and C5a_{desarg}, have been described [57]. Interestingly, in OVA-induced experimental asthma, C3aR blockade using the C3aR agonist SB290157 suppressed the increased expression of the proinflammatory cytokine IL-1 β , which was shown to promote airway obstruction, AHR and neutrophil infiltration in this model [58]. In addition to their impact on cytokines, the ATs can induce the contraction of airway smooth muscle by the induction of prostanoids, leukotrienes and platelet-activating factor from mast cells and/or eosinophils. Interestingly, C3a-mediated platelet-activating factor release has been reported as an important mechanism in peanut-induced anaphylaxis [59]. Thus, diverse immunobiological activities of ATs in the effector phase enhance the inflammatory scenario in allergic asthma. Of importance, reagents that specifically block C5, C5a or C5aR are already in clinical trials for their potential therapeutic effect on chronic inflammatory disorders [60,61].

Expert commentary & five-year view

In the past decade, an important role for complement in the pathogenesis of allergic asthma has been uncovered. Several studies have demonstrated complement activation in human and experimental asthma. Based on the proinflammatory properties of the complement effector molecules C3a and C5a, a proallergic effect of complement has been anticipated. In support of this view, C5a has been shown to drive production of cysteinyl-leukotrienes from human lung fragments following an anaphylactic reaction [62]. Furthermore, blockade of complement activation with the mouse membrane complement inhibitor complement receptor-related gene γ (*Crry*) [63], targeting C3 [18,46], C5 [55,64], C3aR [14,47,65] or C5aR [35,54,64] during the allergic effector phase decreased the allergic phenotype in

different models of experimental allergic asthma. Based on such encouraging results, complement inhibition has been considered an attractive new therapeutic approach. Unfortunately, in a Phase IIa asthma trial in 2003, the oral C5aR antagonist NGD-2000-1 developed by Neurogen Corp. (Branford, CT, USA) did not improve the asthmatic phenotype as determined by lung capacity measurement (forced expiratory volume in 1 s) [101]. Although the reasons for this failure remain elusive, several data obtained from experimental models of asthma provide evidence that C5a and C5aR signaling in asthma can be a double-edged sword. Clearly, C5a drives the asthmatic phenotype during the allergic effector phase. However, in addition to this well-recognized proinflammatory effect, C5a regulates adaptive immune responses at several levels [6]. At least in experimental asthma, targeting of C5aR results in the enhancement of DC-driven development of Th2 effector cells, even in an established allergic environment [35]. Thus, the failure of the Neurogen trial may be linked to the Janus-faced role of C5a in asthma. More recently, Alexion Pharmaceuticals (CT, USA) have presented the first data from a clinical trial in which C5 was blocked by the anti-C5 antibody eculizumab, which is approved for therapeutic use in patients suffering from paroxysmal nocturnal hemoglobinuria. Interestingly, after a single dose of eculizumab, they found attenuated allergen-induced airway responses in patients suffering from mild asthma [66].

In contrast to C5a and C5aR, promising drug candidates that could be used to block C3a or C3aR in human asthma trials are lacking. The most frequently used C3aR antagonist, SB290157 [67], may have C3aR agonistic properties under certain conditions [68] and does not appear suitable as a lead compound for human use.

In future studies, it will be important to: first, assess whether the data obtained in experimental asthma can be translated into human asthma, in particular, the immunoregulatory properties of C5a on adaptive immune responses need to be evaluated in human asthmatics; and second, to improve the models of experimental asthma in order to get closer to the human situation. Experimental asthma models should use allergens that are relevant for human asthma and animals should be immunized preferentially through the airways. Furthermore, it will be very important 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 proallergic effects of complement in pulmonary allergy, and will help better judge the benefit of targeting complement in allergic asthma.

Key issues

- Asthma is associated with complement activation. Allergen proteases can cleave complement proteins directly and generate the anaphylatoxins C3a and C5a.
- Genome-wide screens for asthma susceptibility loci have identified linkage of asthma traits to C3 and C5.
- In experimental models of allergic asthma, C5a protects from the development of maladaptive Th2 immunity during allergen sensitization. Regulation of distinct subsets of pulmonary dendritic cells is critical for this effect. During the course of the disease, the C5a receptor is downregulated on pulmonary dendritic cells.
- C3a and C5a synergistically contribute to the development of the proallergic environment in established allergic asthma owing to their effects on circulating immune cells such as eosinophils and granulocytes.

- In a clinical study, C5 targeting in patients with mild asthma attenuated allergen-induced airway responses, suggesting that C5 may serve as a suitable new target in asthma therapy.

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Figure 1. Complement system sensing of exogenous and endogenous danger in the fluid phase

The immunosensors C1q and MBL are soluble proteins that recognize exogenous and endogenous danger-associated molecular patterns (DAMPs). Recent data suggest that properdin may also serve as an immunosensor for exogenous and endogenous DAMPs. Exogenous DAMPs are mainly of microbial origin. They are often released from injured or apoptotic cells or can be altered self-molecules on the cell surface. The complement-derived danger sensors not only recognize DAMPs but translate the danger information into specific cellular responses by directly interacting with specific receptors on cells or activation of the complement cascade, which generates split products of C3 and C5 that target cells through a panel of complement receptors (danger transmission; lower right). C3 acts as a ubiquitous

danger sensor that becomes activated through nucleophilic attack of its thioester. Once activated, it covalently binds as C3b to its activator surface. Specificity is gained by the environment of the activator surface, that is, the availability of complement regulator molecules, which ultimately decides the fate of the C3b molecule. This can be either termination of further C3 cleavage, or amplification of C3 cleavage to generate huge amounts of C3b and its degradation products iC3b, C3dg and C3a. The latter outcome results in danger transmission through activation of C3 cleavage product-specific receptors (danger transmission: lower right). C3aR: C3a receptor; C5aR: C5a receptor; CD46: Membrane cofactor protein; CR1–4: Complement receptors 1–4; CRIg: Complement receptor of the Ig superfamily; MAC: Membrane attack complex; MBL: Mannan-binding lectin. Modified with permission from [69].

Figure 2. Potential mechanisms underlying the immunoregulatory functions of anaphylatoxin receptor signaling during allergen sensitization

(A) Upon allergen exposure, pulmonary mDCs stimulate naive $CD4^+$ T cells (Th0) through antigen presentation, upregulation of costimulatory molecules and secretion of proinflammatory cytokines, resulting in Th2 proliferation and activation. C5aR signaling in mDCs promotes mDC-mediated activation of naive T cells. C3aR signaling in mDCs is dispensable for mDC-mediated T-cell activation, but modulates the expression of C5aR in pulmonary DCs to regulate T-cell priming indirectly. (B) The absence of C5aR signaling negatively regulates the accumulation of pDCs expressing inhibitory B7-H1 and B7-DC molecules. Such pDCs are required to block the development of Th2-biased immune

responses directly by negatively regulating mDC–T-cell interactions or indirectly by promoting the generation of immunosuppressive Treg cells. The decrease of tolerogenic pDCs results in enhanced polarization and proliferation of Th2 effector cells. In summary, anaphylatoxin receptors play critical roles at the DC/T-cell interface to regulate polarization of the Th2 lineage in response to initial allergen exposure. Solid arrows represent pathways that are triggered when C5aR signaling is intact. Dotted lines represent arrows that are favored in the absence of C5aR signaling. C3aR: C3a receptor; C5aR: C5a receptor; HDM: House dust mite; mDC: Myeloid dendritic cell; pDC: Plasmacytoid dendritic cell; TLR: Toll-like receptor; Treg: Regulatory T cell.