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Short review

Regulation of human mast cell and basophil function by anaphylatoxins C3a and C5a

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ABSTRACT

Allergic diseases such as asthma result from inappropriate immunologic responses to common environmental allergens in genetically susceptible individuals. Following allergen exposure, interaction of dendritic cells (DC) with $CD4^+$ T cells leads to the production of Th2 cytokines, which induce B cells to synthesize IgE molecules (sensitization phase). These IgE molecules bind to their high affinity receptors (FcεRI) on the surface of mast cells and basophils and their subsequent cross-linking by allergen results in the release of preformed and newly synthesized mediators, which cause bronchoconstriction, lung inflammation and airway hyperresponsiveness (AHR) in asthma (effector phase). The complement components C3a and C5a levels are increased in the lungs of patients with asthma and are likely generated via the actions of both allergen and mast cell proteases. *In vivo* studies with rodents have shown that while C3a facilitates allergen sensitization in some models C5a inhibits this response. Despite this difference, both anaphylatoxins promote lung inflammation and AHR *in vivo* indicating that cells other than DC and T cells likely mediate the functional effects of C3a and C5a in asthma. This review focuses on the contribution of C3a and C5a in the pathogenesis of asthma with a particular emphasis on mast cells and basophils. It discusses the mechanisms by which anaphylatoxins activate mast cells and basophils and the associated signaling pathways via which their receptors are regulated by priming and desensitization.

1. Role of mast cells in asthma

Allergic diseases such as rhinitis and asthma are the most prevalent respiratory diseases in industrialized societies affecting ~20% and ~7% of the US population, respectively [1,2]. These diseases are caused by an overzealous immune response to allergens in which immunoglobulin E (IgE) and mast cells play critical roles. It is therefore not surprising that tremendous efforts have been directed towards developing therapy based on the modulation of IgE and its receptor, FcεRI. A recent exciting development in mast cell research has been the approval by the U.S. Food and Drug Administration of a humanized monoclonal antibody omalizumab for the treatment of allergic diseases. Omalizumab binds free IgE molecules and the resulting complexes are removed from the circulation. Over time, IgE comes off its receptors on mast cells and they lose their ability to respond to allergen [3,4]. Omalizumab is difficult to manufacture, is expensive, effective on a subset of allergic patients and may not be sufficient alone to prevent hyperresponsiveness [5]. Another approach has been to target the intracellular signaling pathway via which IgE–FcεRI activates mast cells. Given that Syk kinase plays a central role in FcεRI signaling, a number of Syk inhibitors have been developed [6]. One compound, R112, was the first Syk inhibitor to enter clinical studies [7]. These findings suggest that other pathways that also activate mast cells could be targeted for the development of asthma therapeutics.

As discussed in this review, the complement components C3a and C5a are involved in the pathogenesis of asthma and their effects have variously been proposed to involve dendritic cells, T cells, airway epithelial cells and smooth muscle cells [8–16]. Although a number of excellent reviews have recently been published on the roles of C3a and C5a in asthma [17–21], the possible involvement of mast cells and basophils have not been discussed in detail. It is noteworthy that murine bone marrow-derived mast cells (BMMC) and rat basophilic leukemia RBL-2H3 cells, which have been extensively used to study FcεRI signaling in mast cells, do not express G protein coupled receptors (GPCRs) for C3a and C5a [22–24]. The purpose of this brief review article is to summarize what is known about the activation and regulation of human mast cells and basophils by C3a and C5a. This review is particularly timely as basophils, which express C3a and C5a receptors, have recently been shown to have previously unrecognized role in the development and maintenance of allergic diseases [25–27]. Thus, understanding the molecular mechanism by which anaphylatoxins activate mast cells and basophils and delineating the signaling pathway via which

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their functions are regulated may provide a novel therapeutic target for asthma and other allergic diseases.

2. Roles of complement component C3a in the pathogenesis of allergic asthma

The complement system forms an important part of innate immunity against bacteria and other pathogens. As a system of 'pattern recognition molecules', foreign surface antigens and immune complexes initiate a proteolytic pathway leading to the formation of a lytic membrane attack complex. The anaphylatoxins C3a and C5a are generated as byproducts of complement activation, and they interact with cell surface GPCRs in target cells to mediate a variety of inflammatory responses [28–30]. Recent studies have shown that C3a and C5a levels are elevated in bronchoalveolar lavage (BAL) fluid after segmental allergen challenge in asthmatic but not healthy subjects [9,31,32]. Furthermore, plasma C3a and C5a levels are elevated in acute exacerbations of asthma [31] and C3a receptor is unregulated in subjects who died with asthma compared with subjects who died from other causes [33]. Additionally, single nucleotide polymorphism in C3 or C3a receptor (C3aR) gene increases susceptibility to asthma [34]. In animal models, complement activation modulates both AHR and airway inflammation [35,36]. Furthermore, deletion of C3aR gene or administration of C3aR inhibitors attenuates both AHR and lung inflammation [9,37–40]. Collectively, these findings demonstrate an important role of C3aR in the pathogenesis of asthma.

The mechanism by which C3a regulates AHR and inflammation in asthma is unknown and has been the subject of considerable debate. C3aR is expressed in both antigen-presenting cells (APCs) and activated T cells indicating that C3a may promote asthma by inducing Th2 cytokine production and IgE synthesis [41–44]. Indeed, Drouin et al. [37] reported that in models of *Aspergillus fumigatus* and ovalbumin-induced pulmonary allergy, C3aR-deficiency in mice on C57BL/6 background results in significant decrease in Th2 cytokine production and IgE synthesis. More recently, Zhang et al. [13] showed that in house dust mite (HDM)-induced allergic asthma $C3aR^{-}/^{-}$ mice produce less Th2 cytokine when compared to wild-type mice. These findings are in contrast with previous reports, which showed that C3aR-deficiency in guinea pigs and mice on the BALB/c background are not protected from serum IgE secretion, Th2 cytokine secretion [9,39]. These differences might reflect differences in species and strains of animals, nature of allergen and methods of sensitization used. Despite this, C3aR-deficiency protects animals from allergen-induced AHR and lung inflammation. Furthermore, administration of complement inhibitor in mice after sensitization but before challenge prevented the development of AHR and blocked lung inflammation [36]. Additionally, a small molecule antagonist of C3a receptor, when administered after sensitization but before challenge also significantly inhibited airway inflammation [38]. These findings suggest that although C3a has variable effect on allergen sensitization, its effect on AHR and lung inflammation in animal models of allergic asthma is likely mediated via the activation of C3aR in effector cells such as mast cells and basophils [12,21,36,38].

3. Dual roles of C5a in the pathogenesis of allergic asthma

As described above, development of allergic asthma in animal models can be modulated either at the level of allergen sensitization or the effector phase. Administration of C5aR monoclonal antibody after sensitization but before allergen challenge leads to substantial improvement of AHR and reduction in airway inflammation [38]. These findings are consistent with the idea that C5a also contributes the pathogenesis of allergic asthma via the modification of the effector phase. However, this contention was challenged by Karp et al. [45], who showed that C5-deficient mice are more susceptible to experimental asthma when compared with C5-sufficient mice indicating that C5a may instead play a protective role in the pathogenesis of asthma. Kohl et al. [15] recently utilized three experimental approaches to resolve this paradox. These included (a) administration of anti-C5a receptor (C5aR) monoclonal antibody to the lung, (b) expression of a lung-inducible mutant form of C5a (C5aRA $A8^{\Delta 71-73}$) that acts as a C5aR antagonist and (c) C5aR-deficient mice. They found that blocking or deleting C5aR prior to initial allergen sensitization in murine model of allergic asthma either induces or causes a marked enhancement of Th2-polarized immune responses, airway inflammation, and AHR. These effects result from an increase in the number of myeloid dendritic cells and in the production of Th2-selective chemokines. However, when C5aR was blocked during airway allergen challenge in already Th2-sensitized mice, AHR and lung inflammation were attenuated. Based on these findings, it has been proposed that C5a plays a dual role in allergic asthma; protection from the development of maladaptive Th2 immune responses during allergen sensitization at the level of myeloid dendritic and the production of Th2 cytokines but enhancement of airway inflammation and AHR in an established inflammatory environment [15]. This suggests that, as for C3a, the effect of C5a on asthma likely involves the activation of effector cells such as mast cells and basophils.

3.1. Activation of human mast cells by C3a and C5a

Mast cells are important effector cells that orchestrate the development of AHR and inflammation via their close interaction with airway smooth muscle (ASM), T cells and leukocytes [46–50]. In lungs of asthmatic individuals, mast cells are found in different compartments including bronchoalveolar space beneath the basement membrane, adjacent to blood vessels and scattered throughout the ASM bundles [51,52]. The ability of allergen to cross-link FcεRI on mast cells to induce mediator release is well documented [53–55]. In addition to FcεRI, mast cells express C3a and C5a receptors [56,57,49,21,58], which have been implicated in the pathogenesis of asthma.

Two subtypes of human mast cells were initially recognized based on the composition of their secretory granules. Thus, mast cell granules that contain both tryptase and chymase are designated MC_{TC} whereas those that contain only tryptase are known as MC_T [59]. Interestingly, MC_T cells predominate in the alveolar wall and the epithelium of the lung whereas MC_{TC} cells favor bronchial smooth muscle and glandular regions [60]. Furthermore, MC_T cell number in the respiratory epithelium increases during pollen season [61,62] and markedly elevated levels of MC_{TC} cells are found in bronchial smooth muscle cells of patients with asthma [63]. These findings suggest that different mast cell types may play distinct roles in the pathogenesis of asthma.

Studies performed in the 1980s indicated that while C3a and C5a induce mediator release in human skin mast cells, lung mast cells are unresponsive to these anaphylatoxins [56,64,65]. One possible reason for the discrepancy might reflect the fact that while MC_{TC} cells are the predominant cell type present in the skin they are the minority cell type found in the lung [66,67]. Indeed, Oskeritzian et al. [60] recently showed that MC_T cells in the lung do not express C5aR whereas M_{TC} cells do and that this is correlated with substantial C5a-induced degranulation in MC_{TC} cells. It is noteworthy that RBL-2H3 cells and BMMC, which are thought to be counterparts of human MC_T mast cells do not express C3aR or C5aR and are unresponsive to anaphylatoxins for mediator release [23,24,68,69].

Although the effects of C3a on human lung MC_{TC} cells are unknown, C3aR are expressed in a human mast cell line, HMC-1 cells [70–72], highly differentiated $CD34^{+}$-derived primary human