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Regulation and function of anaphylatoxins and their receptors in allergic asthma

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ABSTRACT

Allergic asthma is a disease of the airways driven by maladaptive T helper 2 (Th2) and Th17 immune response against harmless, airborne substances. The hallmarks of this disease are airway hyperresponsiveness (AHR), eosinophilic and neutrophilic airway inflammation and mucus overproduction. Distinct dendric cell (DC) subsets together with airway epithelial and pulmonary vascular endothelial cells play critical roles in allergen sensing and in driving T cell differentiation towards Th2 and Th17 effector or regulatory T cells (Treg). Previous studies suggested already a pivotal role for the anaphylatoxins (C5a, C3a) in the pathogenesis of allergic asthma. During sensitization for example it is described, that C3a promotes, whereas C5a protects from the development of maladaptive immunity during allergen sensitization. Here we will discuss the role of the anaphylatoxins (C3a, C5a) and their receptors during the pathogenesis of allergic asthma, and specifically in lung DC biology. We will also have a look on canonical and non-canonical complement activation and we will discuss novel concepts on how the adaptive immune system can regulate the function of ATRs also in the context of allergic asthma.

1. Introduction

The last decade has seen major advances in understanding the biology of the complement system, especially with regard to the anaphylatoxins (ATs; i.e. C3a und C5a). Investigators discovered novel roles for the ATs and their receptors (ATRs; i.e. C5aR1, C5aR2, C3aR) in danger transmission and regulation of the innate as well as the adaptive immune system. Originally ATs were simply regarded as pro-inflammatory molecules, but over the years it became more and more evident that in the complex immunological network the AT/ATR system is a central player in modulating immune responses. The AT/ATR system is not only involved in regulating the functions of cells belonging to the innate immune system in lymphoid and non-lymphoid organs, but also regulates the functions of adaptive immune cells, like for human T cells, through the activation of cathepsin (Kolev et al., 2014; Liszewski et al., 2013) or the inflammasome (Arbore et al., 2016; Asgari et al., 2013; Suresh et al., 2016; Triantafilou et al., 2013). Conversely, the AT/ATR system is also influenced and regulated by the innate and adaptive immune system. B cell-derived IL-10 plays for example an important role in regulating the C5a/C5aR1 axis in neutrophils (Kulkarni et al., 2016). Furthermore, the modulation of neutrophil functions by immune complexes (ICs) (Karsten et al., 2012) and putatively by molecules like galectin-3 (Shan et al., 2013) may be a major regulatory pathway of the AT/ATR system.

Here we want to review the role of the AT/ATR system in allergic asthma and discuss novel concepts on how the adaptive immune system can regulate the function of ATRs also in the context of allergic asthma.

2. Intrinsic and extrinsic generation of C3a and C5a

The complement system consists of a network of humoral and cellular proteins, many of which exert their effector functions upon proteolytic cleavage. The complement system can be activated via several intrinsic and extrinsic mechanisms. The central event of the intrinsic complement activation is the formation of the C3 convertase via one of the three canonical complement activation pathways (classical, lectin, alternative), which in turn cleaves C3 into C3a and C3b, followed by the formation of the C5 convertase and subsequent cleavage of C5 into C5a and C5b (Klos et al., 2013). In addition to the intrinsic complement activation, extrinsic activation has been described to occur independent of convertase formation. This pathway relies on the presence of proteases, which autonomously cleave C3 or C5 into their biological

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active fragments C3a/b and C5a/b, respectively, for instance as part of a cross-talk between the contact and the complement system. Here, the kallikrein-related peptidase 14 (KLK14), which is widely expressed in tissues and biological fluids, efficiently cleaves C3 (Oikonomopoulou et al., 2013). Thrombin on the other hand, was shown to substitute for the C3-dependent C5 convertase generating biologically active C5a, thereby linking the coagulation with the complement system (Huber-Lang et al., 2006). In addition, phagocytic cells, such as alveolar macrophages or Kupffer cells in the liver can produce and cleave C5 and are therefore also able to generate C5a independently of a C5 convertase (Huber-Lang et al., 2002; Kumar et al., 2006). Also, cathepsin L is capable to activate C3 in the lysosomes of resting human T cells (Kolev et al., 2014; Liszewski et al., 2013). In the context of allergic asthma, the house dust mite (HDM) *Dermatophagoides farinae*-derived protease (Der p) 1 was found to cleave C3 and C5 into C3a and C5a, respectively (Maruo et al., 1997).

The ATs C3a and C5a have a short half-life in body fluids and are quickly converted into degradation products missing the carboxy terminal arginin residue. This desargination is performed via the activity of plasma carboxypeptidases (Klos et al., 2013). For long the desarginated (desArg) form of C3a has been considered as an inactive peptide (Wilken et al., 1999), and desarginated C5a – compared to C5a – as a molecule displaying a lower potency to activate C5aR1 (Monk et al., 2007). However, accumulating evidences show that both C3a-desArg and C5a-desArg induce potent, even though different, cellular responses compared to their arginated counterparts (Barbu et al., 2015; Reis et al., 2012).

3. Expression and regulation of the anaphylatoxin receptors in the lung

Historically, the expression of C3aR was considered to be mostly restricted to cells of the myeloid lineage, in particular eosinophils, dendritic cells, monocytes/macrophages, mast cells, and basophils (Ames et al., 1996; Dahinden et al., 1991; Martin et al., 1997). However, more recent studies provided evidence speaking for an expression of C3aR also in pulmonary tissue cells, such as endothelial, epithelial, and smooth muscle cells (Drouin et al., 2001; Tschernig et al., 2007). Furthermore, C3aR expression is upregulated in airway epithelial cells and smooth muscle cells in allergic asthma experimental models (Drouin et al., 2001; Mizutani et al., 2009). So far, no data confirm the expression of C3aR in pulmonary DC subsets, even though C3aR has been described in DCs from other organs (Gutzmer et al., 2006).

The expression of C5aR1 is better defined. Initial observations, made decades ago, showed that C5aR1 is in the lung widely expressed in myeloid, but not in lymphoid cells (Klos et al., 2009). More recent studies, using reporter mouse models confirmed these early observations about the expression profile of C5aR1 in lung DCs (Dunkelberger et al., 2012; Karsten et al., 2015). To be more specific, among the different DC subsets, C5aR1 was shown to be expressed by $CD103^{-}CD11b^{+}$ conventional (c)DCs and monocyte-derived dendritic cells (moDCs). However, C5aR1 expression could be observed neither in the $CD103^{+}$ $CD11b^{-}$ cDCs nor in plasmacytoid dendritic cells (pDCs). This was confirmed by an independent study relying on surface staining of cells using an anti-C5aR1 antibody (Nakano et al., 2015). Both approaches showed a higher expression level of C5aR1 in moDCs compared to $CD11b^{+}$ cDCs. However, the modulation of C5aR1 during the course of allergic asthma has not been investigated yet.

The pulmonary expression of C5aR2 is ill defined. However, mRNA has been detected in the lung and its expression is increased in a rat experimental sepsis model (Gao et al., 2005). Additionally, the expression of C5aR2 has been reported in rat and human neutrophils, macrophages and DCs (Klos et al., 2013).

The expression of ATRs in lymphoid cells is still debated. Initial reports showed that both, C5aR1 and C3aR, are expressed at steady state by splenic $CD4^{+}$ T cells in mice (Strainic et al., 2008). In contrast, two recent studies found no C5aR1 expression in murine $CD4^{+}$ T cells, neither *in vivo* nor *in vitro* upon activation or under steady state conditions (Dunkelberger et al., 2012; Karsten et al., 2015). However, in human $CD4^{+}$ T helper (Th) cells, autonomous C3 and C5 production, cleavage and subsequent activation of distinct complement receptor pathways drives the differentiation towards IFNγ-producing Th1 cells (Arbore et al., 2016; Liszewski et al., 2013), mediated by a C3b-dependent CD46 and a C5a-driven C5aR1 activation (Cardone et al., 2010; Le Friec et al., 2012). Recently, the Kemper lab uncovered that NLRP3 inflammasome activation in human $CD4^{+}$ T cells is critical for Th1 commitment and that the C5a/C5aR axes control this activation. In their publication they showed that in human $CD4^{+}$ T cells autocrine IL-1β secretion is essential for Th1 commitment and IFNγ production. This IL1-β secretion is dependent on the NLRP3 inflammasome activation, whose assembly requires non-canonical C5 cleavage and C5aR1 stimulation (Arbore et al., 2016). Although a marked recruitment of Th2/Th17 cells is a hallmark of the inflammatory response in allergic asthma, evidence is lacking to support the idea that the expression of C3aR or C5aR1 could be modulated in these conditions by $CD4^{+}$ effector T cells.

Compelling evidence shows that both canonical and non-canonical generation of C3a and C5a may occur in asthmatic lungs. ATs are present in the bronchoalveolar lavage of allergic asthma patients (Krug et al., 2001) as well as in mice in experimental asthma models. Additionally, C3/C3b deposition in airway epithelium and connective tissues has been observed (Walters et al., 2002). Furthermore, the extrinsic generation of ATs by airborne allergen proteases, and alveolar macrophages, as described above also plays a role in the lung. The impact of ATs on the development of allergic asthma has been investigated in most studies using experimental mouse models, in which AT/ATR components have been targeted genetically or pharmacologically.

Ablation of C3aR resulted in a reduction of the AHR, independently from species and the allergen (Bautsch et al., 2000; Drouin et al., 2002; Humbles et al., 2000; Roy et al., 2013; Zhang et al., 2009). In contrast, airway inflammation, Th2/Th17 cytokine production, mucus secretion, and IgE production were reduced in some models (Bautsch et al., 2000; Humbles et al., 2000; Lim et al., 2012; Roy et al., 2013) but not all of them (Drouin et al., 2002; Zhang et al., 2009), depending on species as well as on the model and immunization route used (Schmudde et al., 2013a). Furthermore, in an OVA-induced allergic asthma model, the pharmacological targeting of C3aR resulted in reduction of the AHR, together with a reduction of IL-1β secretion in the lung (Mizutani et al., 2009).

Several studies indicated that targeting the C5a/C5aR1 axis during the sensitization phase of allergic asthma resulted in a marked increase in the allergic asthma phenotype (Drouin et al., 2006; Karp et al., 2000; Kohl et al., 2006; Lajoie et al., 2010; McKinley et al., 2006; Zhang et al., 2009). In line with these observations, cowshed dust extracts, which activate C5a generation, dampen the development of allergic asthma when administered during the sensitization (Stiehm et al., 2013). Altogether, these studies point toward a protective role for C5a/C5aR1 during the sensitization phase. In contrast, ablating the C5a/C5aR1 axis during the effector phase reduces the AHR and the airway inflammation (Kohl et al., 2006; Staab et al., 2014).

Lastly, C5aR2 deficiency has been shown to result in a reduction of the AHR, Th2 cytokine production, mucus secretion and IgE pro-