

Review

The anaphylatoxins bridge innate and adaptive immune responses in allergic asthma

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Abstract

The complement system has long been recognized for its role as a lytic effector system that protects against microbial pathogens, as well as for its role in mediating acute and chronic inflammatory responses. Many of the inflammatory sequelae of complement activation can be related to the complement cleavage fragments C3a and C5a, the so-called anaphylatoxins (ATs). Cloning and subsequent gene targeting of their corresponding receptors, as well as generation of specific C3a and C5a inhibitors, have fueled new interest in studies aimed at defining the roles of the anaphylatoxins in inflammatory diseases. Traditionally, the anaphylatoxins have been considered mediators of end-stage effector mechanisms. However, recent data from animal models of allergic asthma suggest that C3a and C5a provide a critical link between innate and adaptive immunity. This review is aimed at outlining our current knowledge of when and where anaphylatoxins contribute to and control the development of allergic asthma. The accumulated data suggest a model in which C3a and C5a play important but opposing roles during allergen-induced T-cell polarization: C3a promotes Th2 responses, whereas C5a prevents Th2 polarization. During the effector phase, both anaphylatoxins trigger the inflammatory response and contribute to bronchoconstriction.

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1. Asthma epidemiology and pathogenesis

The world-wide prevalence and severity of allergic asthma have increased dramatically in recent decades. In the US alone, 15 million people suffer from asthma (Anonymous, 1998). The mechanisms underlying these epidemiological shifts remain obscure (Wills-Karp et al., 2001). Further, the development of effective therapies has lagged behind the growing prominence of the disease, and asthma morbidity and mortality continue to rise. Both epidemiologic and therapeutic investigation have been hindered by the lack of a detailed understanding of asthma pathogenesis. Recent advances have been driven by the insight that asthma is, in essence, an inflammatory disorder. The cardinal features of allergic asthma include airway hyperresponsiveness (AHR) to a variety of specific and nonspecific stimuli, excessive airway mucus production, pulmonary eosinophilia, and elevated concentrations of serum IgE. Although asthma is multifactorial in origin, it is generally accepted that it arises as a result of inappropriate immunological responses to

common environmental antigens (Ag) in genetically susceptible individuals. Specifically, a multitude of evidence suggests that CD4⁺ T-cells producing Th2 cytokines (IL-4, IL-5, IL-13) play a pivotal role in disease pathogenesis (Wills-Karp, 1999). Although extensive research is ongoing into the processes underlying the development of deleterious immune responses to the ubiquitous, otherwise harmless, antigens that drive the expression of allergic asthma, these mechanisms remain a mystery. Recent data from animal models and humans provide evidence that the complement system is strongly activated during the disease and that complement cleavage fragments are important in disease pathogenesis (Karp et al., 2000; Bautsch et al., 2000; Humbles et al., 2000; Drouin et al., 2001, 2002; Walters et al., 2002a; Krug et al., 2001).

2. The complement system

Recent years have brought growing mechanistic awareness of the profound influence of the innate immune system on adaptive immune responses. The complement system, a phylogenetically ancient part of the

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innate immune system, is no exception. In addition to its long-recognized role as a lytic effector system that protects against microbial pathogens, it is clear that the complement system regulates adaptive immunity at many levels. First, the complement system is an important regulator of B cell activation, providing an important mechanism for pathogen (or “danger”) recognition by B lymphocytes (Fearon and Carroll, 2000). Second, the engagement of complement receptors on antigen-presenting cells (APCs) leads to potent effects on the production of immunoregulatory cytokines such as IL-12 (Karp et al., 1996; Marth and Kelsall, 1997). Third, while the anaphylatoxins (ATs; the complement activation products C3a, C5a and C5adesArg) have long been appreciated for their effects on myeloid cell migration, activation, and effector functions, it has recently become clear that these molecules can also regulate the functions of APC, and lymphocytes (Wittmann et al., 1999; Braun et al., 2000; Karp et al., 2000; Tsuji et al., 2000; Ottonello et al., 1999).

Activation of the complement system occurs by three different pathways: the classical pathway, the lectin pathway and the alternative pathway (for review see Walport, 2001a,b). All three pathways lead to C3 cleavage resulting in the two activation products C3a and C3b. Downstream cleavage of C5 generates C5a and C5b, the latter of which nucleates formation of the membrane attack complex (MAC). A variety of biological outcomes are downstream of C3 and C5 activation. In addition to microbial lysis, these include: (a) activation of granulocytes and endothelia by sublytic quantities of the MAC; (b) deposition of C3 fragments on membranes and/or particles (e.g. antigen–antibody complexes, microbes) leading to phagocytosis; immune complex clearance; clearance of apoptotic bodies; B-cell activation; and alterations in immune cell signal transduction, adhesion, activation, and cytokine production; and (c) AT-mediated effects. Most of these activities depend on the engagement of specific complement receptors. These include regulator of complement activation family members (CR1, CR2, MCP, DAF, etc.), the β -integrins CR3 and CR4, and the AT receptors C3a-receptor (C3aR), C5a receptor (C5aR; CD88) and C5L2.

3. Anaphylatoxins: Mode of generation in asthma, and effector functions

How complement is activated in asthma, whether during Ag priming or during the effector phase of the response remains unclear. AT may be generated by several different pathways, including: (a) activation through the classical pathway as a result of allergen–Ab complex formation (necessarily an event downstream of Ag presentation unless due to natural Ab); (b) activation of the lectin pathway through engagement of carbohydrate structures on allergens; (c) alternative pathway activation on allergen surfaces; (d) cleavage of C5 and C3 by proteases released from inflammatory cells; and, most interestingly (e) direct cleavage of C3 and

C5 by allergen proteases (Nagata and Glovsky, 1987; Castro et al., 1991; Maruo et al., 1997). Thus the biochemical properties of allergens themselves may play an important role in generation of AT at the airway interface with the environment, a place where they would be uniquely positioned to direct the immune response. Although this is an intriguing hypothesis, it does not explain why complement levels are preferentially elevated in the lungs of asthmatic individuals, as both normals and asthmatics are exposed to similar levels of these allergens. Clearly much remains to be learned about the exact mechanisms of complement activation in the asthmatic lung.

AT were initially defined functionally by their activities on small blood vessels, smooth muscle, mast cells, and peripheral blood leukocytes. The AT have a plethora of proinflammatory actions, including: (a) promotion of leukocyte chemotaxis; (b) enhancement of neutrophil-endothelial cell adhesion; (c) upregulation of vascular permeability; (d) induction of granule secretion by phagocytes; and (e) induction of the production and release of a variety of proinflammatory cytokines (e.g. IL-1, IL-6, TNF- α , and IL-8) (Ember et al., 1998). The potent effects of the AT on the trafficking and activation of effector cells of the allergic response is likely to be of particular relevance to asthma. Notably, C5a is chemotactic for macrophages, activated B and T lymphocytes (Ottonello et al., 1999), (Nataf et al., 1999), and both AT are chemotactic for eosinophils and mast cells (Ember et al., 1998; Schulman et al., 1988). In addition to recruiting leukocytes into the airways, the AT also stimulate their effector mechanisms, leading to rapid release of histamine from basophils and mast cells (Eglite et al., 2000), and to upregulation of eosinophil cationic protein synthesis (Takafuji et al., 1994; Takafuji et al., 1996). Further, AT can also induce smooth muscle contraction (see Section 6).

4. Anaphylatoxin receptors

Once generated, C3a and C5a exert their effects through specific receptors, C3aR or C5aR (CD88), both belonging to the group of seven transmembrane (7TM) receptors (Crass et al., 1996; Ames et al., 1996; Gerard and Gerard, 1991; Boulay et al., 1991). In leukocytes, C3aR and C5aR mediate their effects via coupling to the pertussis-sensitive and -insensitive G-proteins $G_{\alpha i}$ and $G_{\alpha 16}$, respectively (Norgauer et al., 1993; Zwirner et al., 1997; Buhl et al., 1995; Vanek et al., 1994). The C3aR also couples to $G_{\alpha 12}$ and $G_{\alpha 13}$ in endothelial cells (Schraufstatter et al., 2002).

The downstream pathways have been intensively studied in human PMN, in which C5aR signals through $G_{\alpha i}$ and activates phosphoinositide-3-kinase γ (PI3K γ , Fig. 1A). PI3K γ belongs to a family of lipid kinases involved in generating distinct phosphoinositides that are important second messengers for intracellular signaling. C5a appears to activate PI3K γ by two distinct pathways signaling through: (1) $G_{\alpha i}$, which activates the small GTPase Ras; and (2) G $\beta\gamma$. In

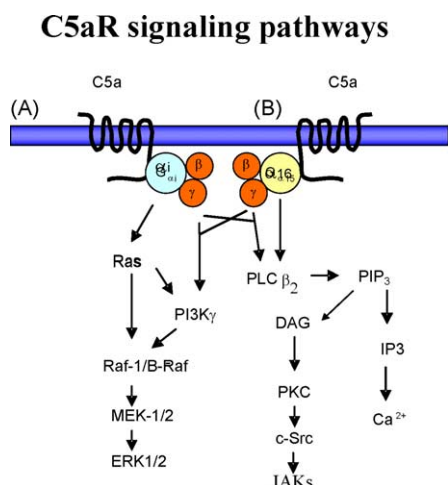


Fig. 1. C5aR signaling pathways. (A) Coupling to G α_i (blue, PTX sensitive); and (B) coupling to G α_{16} (yellow, PTX insensitive).

turn, Ras or PI3K γ can activate the Raf/mitogen-activated protein kinase (MAPK)/extracellular-signal regulated kinase (ERK) kinase (MEK)/ERK cascade, which provides a link between C5aR ligation to gene expression through effects on transcription factor activation and translocation to the nucleus. In monocytes and macrophages, coupling of C5aR to both G α_i and G α_{16} has been described. The G α_{16} subunit and the $\beta\gamma$ -subunit (Fig. 1A and B) can activate phosphoinositide-specific phospholipase C (PLC) subtype β , which is a multidomain phosphodiesterase that generates the second messenger inositol 1,4,5-trisphosphate (IP $_3$) and diacylglycerol (DAG). These latter molecules mediate Ca $^{2+}$ mobilization and the activation of protein kinase C (PKC). Downstream of PKC, signal transducer and activator of transcription 3 (STAT3) can be activated by C5a (through Janus kinase (Jak) phosphorylation) (Lo et al., 2003), suggesting a role for C5a in cell proliferation. In fact, C5a (and C3a) were found to be essential in liver regeneration (Strey et al., 2003). In further support of this view, C5aR signaling induces epidermal growth factor (EGF) receptor phosphorylation in endothelial cells (Schraufstatter et al., 2002). EGF is a strong mitogen for human airway smooth muscle (ASM) cells via activation of the ERK MAPK pathway (Krymskaya et al., 2000).

Recently, the orphan receptor C5L2 has been described to be a second receptor for C5a (Ohno et al., 2000; Cain and Monk, 2002; Okinaga et al., 2003). Tissue expression of mRNA for human and mouse C5L2 is broad and corresponds well to the expression of CD88. However, surface expression of human C5L2 and of human CD88 does not necessarily match mRNA expression. In fact, CD88 is strongly expressed on unstimulated neutrophils whereas C5L2 is not (Otto et al., 2004). Interestingly, C5adesArg binds to C5L2 with a tenfold higher affinity than to CD88 (Cain and Monk, 2002; Okinaga et al., 2003). In contrast to CD88, C5L2 is uncoupled from G-proteins. C5L2

ligation does not result in degranulation, increase in intracellular Ca $^{2+}$, or receptor internalization, suggesting that C5L2 signaling does not follow the classical pathways of chemoattractant receptors. In fact, 7TM have recently been found to use signaling pathways independent of G-proteins (Marinissen and Gutkind, 2001).

5. Complement and asthma: experimental models

The role of complement in the pathogenesis of allergic asthma has not been studied in detail yet, despite knowledge of the fact that several pathophysiologic features of allergic asthma (e.g. smooth muscle contraction, increased vascular permeability, mucus secretion and recruitment of inflammatory cells) have long been known to be consonant with well-defined effects of the AT (Köhl and Bitter-Suermann, 1993; Gerard and Gerard, 1994; Ember et al., 1998). The fact that the complement system is highly activated in the human asthmatic lung is well-documented (Humbles et al., 2000; Krug et al., 2001; van de Graaf et al., 1992). However, recent awareness of the key immunoregulatory role of complement in infectious and other inflammatory disease models has fueled a new interest in its role in asthma and allergic disease.

5.1. The role of C5 and C5a

A recent study by Karp et al. (2000) pursued genetic susceptibility loci in a murine model that mimics the pathophysiology of human asthma. In this ovalbumin (OVA)-driven model of asthma, A/J mice are susceptible, exhibiting AHR along with mucus metaplasia, and an inflammatory response characterized by marked pulmonary eosinophilia and elevated IgE levels after Ag challenge. C3H/HeJ mice are resistant, do not develop AHR or elevations in IgE production and exhibit a reduced eosinophilic inflammatory response. Susceptibility to the development of allergen-induced AHR in this model is associated with a Th2 dominant cytokine pattern, whereas resistance is associated with Th1 cytokine production. IL-12 is a cytokine that is critical for the development of Th1 responses in most systems (Murphy and Reiner, 2002). As might therefore be expected, allergen-induced AHR is prevented or ablated by exogenous IL-12 in susceptible strains (Gavett et al., 1995), whereas neutralization of IL-12 renders resistant strains susceptible (Keane-Myers et al., 1998).

Microarray analysis of pulmonary gene expression and single nucleotide polymorphism-based genotyping, combined with quantitative trait locus analysis, identified C5 as a susceptibility locus for allergen-induced AHR (Karp et al., 2000). Notably, A/J mice are deficient in C5 whereas C3H/HeJ mice are C5 sufficient (Wetsel et al., 1990). Given the facts that: (i) C3 fragments (C3b, iC3b) inhibit production of IL-12 by APCs (Karp et al., 1996; Marth and Kelsall, 1997; Sutterwala et al., 1997); and (ii) C5a promotes

monocyte/macrophage production of TNF- α , IL-1 β , and IL-6, the effects of ablating C5a-mediated signaling in monocytic cells were examined in a mechanistic search for the link between C5 and susceptibility to allergen-induced AHR. To model the *in vivo* lack of access to C5a in the macrophages of susceptible mice, ablation of C5a-mediated signaling was studied in human monocyte/macrophages cultured in the absence of serum. C5aR signaling through CD88 and C5L2 was blocked with a C5aR antagonist (Heller et al., 1999). Of note, blockade of the C5aRs caused marked, dose-dependent inhibition of IL-12 production from primary human monocytes. Such blockade also inhibited the secretion of TNF- α , albeit less potently. Interestingly, while inhibition of C5aRs signaling had no overall effect on bacterially driven IL-10 production, IFN- γ -mediated suppression of IL-10 production was reversed (Karp et al., 2000). The suppression of IL-12 production seen *in vitro* in human monocytes, was found to be recapitulated *ex vivo* in murine macrophages from sensitive and resistant strains. Peritoneal (PE) macrophages from C5^{-/-} A/J mice produce significantly less IL-12 than those obtained from C5^{+/+} C3H/HeJ mice. Further, stimulation with C5a significantly increases IL-12 secretion by peritoneal macrophages (expressing mainly CD88) from A/J (but not C3H/HeJ mice); and IL-12 production is blocked by the C5aRA in C3H/HeJ peritoneal macrophages. Moreover, the impact of C5aR blockade on T-cell skewing *in vitro* translates into an increased Th2 cytokine pattern (associated with increased airway inflammation) after CD88 blockade in a murine model of house dust mite induced pulmonary allergy (Köhl

et al., manuscript in preparation). The fact that only one of the two C5aRs, i.e. CD88, was blocked suggests a model in which: (1) CD88 signaling plays a key role in protection from allergen-induced Th2 skewing by mediating IL-12 production; and/or (2) C5L2 signaling promotes Th2 skewing, e.g. by inhibition of IL-12 production. Such a model would suggest that the ratio of CD88 and C5L2 expression on APC and/or CD4⁺ T-cells, and/or the ratio of C5a/C5desArg, would determine the effect of complement system activation on Th cell differentiation (Fig. 2). This model could also explain data showing that C5a can downregulate bacterially driven IL-12 release (Braun et al., 2000; Wittmann et al., 1999; Karp et al., 2000).

These results may provide insight into other models of genetic deletion or antibody-mediated inhibition of C5, C5a, or the C5aR. Notably, such models have suggested a central role for C5 (and C5aR signaling) in the pathogenesis of collagen-induced arthritis, DTH responses, and endotoxic shock, as well as in resistance to *Listeria monocytogenes* and to blood-stage malaria infection (Tsuji et al., 2000; Czermak et al., 1999; Ji et al., 2002; Sam and Stevenson, 1999; Gervais et al., 1989). While C5a has pleiotropic effects, all of these models depend on or are exacerbated by IL-12, and downmodulated by IL-10.

Taken together, these studies suggest that C5a normally serves to facilitate IL-12 production by APC, and that C5a deficiency leads to skewing towards Th2 immune responses due to deficient IL-12 production by such cells. The exact mechanisms by which C5a leads to polarization of the T-cell response remain to be defined.

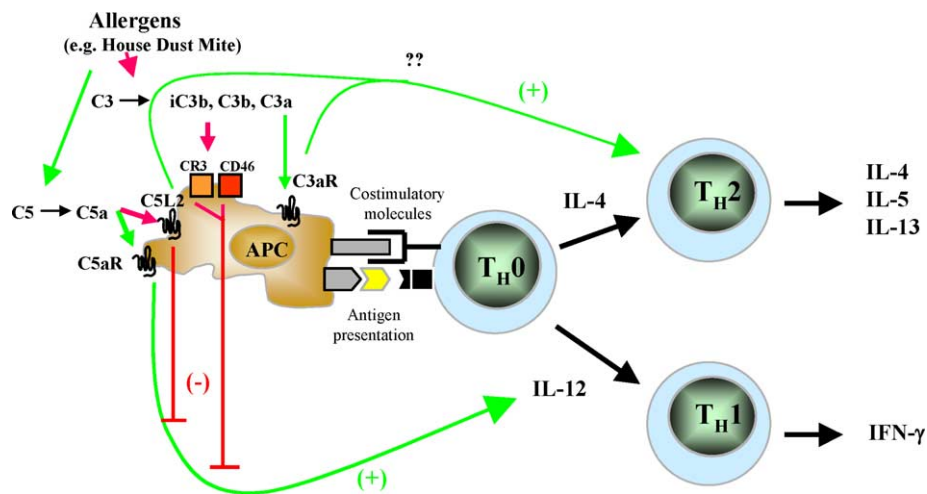


Fig. 2. Proposed effects of C3 cleavage products and C5a on T-cell polarization in allergic asthma. C3 and C5 may be secreted from pulmonary resident cells (e.g. epithelial cells, macrophages) and/or may be derived from plasma leakage. Proteases derived from allergens (e.g. house dust mite, *Aspergillus fumigatus*) or released from activated macrophages/mast cells are able to cleave C3/C5. Once generated, C3a, C5a, C3b and iC3b cleavage products bind to their corresponding receptors on APC. The available data suggest a model in which C5aR signaling through CD88 renders APC competent for IL-12 production and drives a Th1 response whereas signaling through C5L2 inhibits a Th1 response and/or promotes a Th2 response. Conversely, the C3 cleavage products C3b and iC3b can suppress IL-12 production by APC, and favor the development of a Th2 response. Further, C3a appears to promote a Th2 response, the mechanisms of which are yet unknown. C3a may induce or modulate Th2 cytokine release from basophils/mast cells or Th2 polarization during allergen sensitization (on APC), and/or may interact with activated T-cells to promote the maintenance of a Th2 response. Through these opposing actions, C3a (and C3b/iC3b) and C5a play a major role in skewing the T-cell response towards a Th2 phenotype and, consequently, play a major role in the development of allergic airway response.

5.2. The role of C3 and C3a

C3aR^{-/-} mice (BALB/c x 129) are protected from OVA-driven AHR (Humbles et al., 2000). Of note, the pattern and magnitude of inflammatory cell infiltration was similar in the lungs of allergen-exposed C3aR wildtype and knockout mice, suggesting that, in contrast to current dogma, C3 plays a major role in the allergic response that is independent of its chemoattractant properties. Similarly, it was shown that early-phase bronchoconstriction depends on C3aR pathway activation as well. Guinea pigs with a naturally occurring deficiency in the C3aR exhibit a significant decrease in bronchoconstriction as compared to wild-type controls (Bautsch et al., 2000). As in the mouse model, no change in the inflammatory response occurred. Further, AHR induced by airway exposure to a non-antigenic environmental stimulus (urban ambient air-derived particulate matter) or atypical RSV infection is also dependent on C3, as C3^{-/-} mice failed to develop AHR (Walters et al., 2002b; Polack et al., 2002). Of note, not all studies have been able to separate the chemotactic from immunoregulatory effects of C3. Both, suppressed pulmonary Th2 responses and reduced eosinophil and neutrophil infiltrates were described in C3^{-/-} (compared with C3^{+/+}) mice challenged with *Aspergillus fumigatus* and OVA (Drouin et al., 2001). Similar results were found using a C3aR^{-/-} strain by the same authors (Drouin et al., 2002). The differences in the linkage between inflammation and Th2 polarization seen in different C3aR^{-/-} models may be explained by (a) the different antigens that were used and/or (b) by the different backgrounds to which these C3aR^{-/-} mice were back-crossed (BALB/c versus C57BL/6), given the multigenic nature of allergic asthma. These studies suggest that C3 (in particular C3a) is important for the development of pulmonary Th2 responses and AHR. The data are consistent with an upstream immunoregulatory role for C3a, i.e. the promotion of T-cell polarization towards a Th2 phenotype, in addition to a role as a chemoattractant and mediator of pulmonary inflammation (Fig. 2).

Altogether, these data strongly support a role for AT in the pathogenesis of allergic asthma. While C3a and C5a have similar functions as chemoattractants (for mast cells and eosinophils), these studies highlight a likely divergent role in their immunoregulatory effects. Specifically, these data suggest that C3a and C5a have directly opposing immunoregulatory effects, with C3a production driving Th2 immune responses and C5a inhibiting the development of such responses (Fig. 2). The effects of the complement system on asthma pathogenesis are thus likely to be quite complex, with different complement activation products having similar or opposing actions depending on where and when they impact upon the immunopathogenetic process. It is tempting to hypothesize that AT regulate the immunopathogenesis of allergic asthma at two levels, at least: (1) modulation of Th1/Th2 polarization at the time and place of antigen presentation, and/or maintenance of such polarization

at sites of disease (i.e. the lung); and (2) regulation of allergic effector mechanisms (smooth muscle contraction, airway inflammation, mucus secretion) in the lung.

6. Modulation of Th1/Th2 polarization by AT: potential mechanisms

The molecular mechanisms underlying the Th1/Th2 polarization of CD4⁺ T-cells have been reviewed recently (Murphy and Reiner, 2002). A critical upstream event in this process is the cytokine milieu present during the priming of naive T-cells. Dendritic cells (DCs) represent the principal APC for naive T-cells. Immature DC (iDC) reside in tissues, where they actively survey their environment. In order to process and present a given Ag, DC need to undergo activation and maturation (e.g. by microbial products or ligands on activated T-cells). With maturation, DCs migrate to secondary lymphoid organs where they are able to activate Ag-specific naive T-cells. While Ag dose, costimulation, and genetic modifiers can all alter the path of differentiation of activated, naïve T-cells, cytokines of the IL-12 family (IL-12, IL-23 and IL-27) and IL-4 have clearly been shown to be critical determinants of the polarization of Th1 and Th2 cells, respectively. IL-12 is made by DCs and other APC. The relevant source of IL-4 remains unclear. Differentiated effector T-cells traffic to nonlymphoid organs and tissue. There, functional Th1 polarization appears to be maintained by local production of cytokines of the IL-12 family and by IL-18 (Brombacher et al., 2003). Activation of Th1 memory cells appears to be restricted to the new IL-12 family member IL-23.

It will be of major interest to determine whether the AT modulate the expression of other members of the IL-12 family (and of IL-18) to obtain a more comprehensive understanding of CD4⁺ T-cell polarization and/or maintenance by C3a and C5.

Functional AT receptors are expressed on bone marrow-derived iDCs and have been found to induce several effector functions: (a) DCs isolated from rat respiratory tract tissue, or generated in vitro from human monocytes, migrate in response to C5a (Mc William et al., 1996); (b) human Langerhans cells (a type of dermal iDC) express C5aR and respond chemotactically to C5a (Morelli et al., 1997); (c) Langerhans cell C5aR expression increases during maturation and tissue trafficking (Morelli et al., 1996); (d) human dermal DCs, and monocyte-derived iDCs, express C5aR and C3aR (Kirchhoff et al., 2001); (e) C5a induces calcium fluxes in such dermal DCs, whereas C3a does not; (f) expression of both AT receptors is downregulated on such dermal DC by TNF- α (Yang et al., 2000); and (g) both human and murine monocyte-derived iDCs and mature DCs (mDCs) respond to C5a as determined by chemotaxis and Ca²⁺ mobilization (Yang et al., 2000). Thus, the interaction between C5a has potent functional effects on both mDCs and iDCs.

Low basal expression of C5aR has been reported on primary, unstimulated T-cells, something that was strikingly up-regulated upon mitogen stimulation in a time- and dose-dependent manner (Nataf et al., 1999). T-cells have also been shown to express C5aR mRNA, and migrate to nanomolar concentrations of C5a (Werfel et al., 2000). Further, Werfel et al. (2000) showed expression of C3aR on blood- or skin-derived CD4+ and CD8+ T-cell clones derived from birch pollen-sensitized patients with atopic dermatitis but not on unstimulated T-cells. The fact that functional AT receptors are expressed on DCs and activated T-cells, as well as tissue macrophages, suggests a variety of direct ways by which AT can modulate Th1/Th2 differentiation, as well as maintain the Th1/Th2 phenotype of effector T-cells (Fig. 2).

6.1. The role of airway smooth muscle cells in allergic asthma

The traditional view of airway smooth muscle (ASM) cells as a purely contractile tissue is rapidly changing. It is now clear that ASM play an important role not only in regulating the bronchomotor tone in asthma, but also in the perpetuation of airway inflammation and in the remodeling of airways. Growth factors, cytokines/chemokines have been found in BAL of asthmatics that can regulate ASM proliferation by different signal transduction mechanisms. The ERK MAPK, PI3K and the Jak/STAT pathways appear to be key positive regulators of ASM mitogenesis (Zhou and Hershenov, 2003). Further, ASM function as important resident immunomodulatory cells in the lung, contributing substantially to pulmonary infiltration with inflammatory cells. Activation of the ERK MAPK (and p38MAPK) pathways by 7TMR signaling and activation of the Jak/STAT pathway by different cytokines (TNF- α , IL-1 β , IL-4, IL-13) appear to be of major importance for the immunomodulatory activities of ASM, inducing release of cytokines/chemokines such GM-CSF, IL-6, IL-8, eotaxin and RANTES (Hirst, 2003). The ERK MPK pathway in ASM is activated not only by 7TMR signaling, but also after ligation of cytokine receptors such as the type II IL-4R or IL-13R α I (Laporte et al., 2001). On the other hand, activation of Jak/STAT signaling pathways is not a unique feature of cytokine receptors. There is now compelling evidence of the importance of Jak/STAT activation in 7TMR signaling (Marrero et al., 1995; Mellado et al., 1998; Guillet-Deniau et al., 1997a). Activation of the Jak2-STAT3 pathway has been demonstrated for CCR2B (Mellado et al., 1998) and 5-HT2a receptors (Guillet-Deniau et al., 1997b). Recently, C5a was also demonstrated to induce STAT3 tyrosine phosphorylation (by a Jak-2-dependent mechanism) and STAT3 serine phosphorylation by an (ERK MAPK-dependent mechanism) in erythroleukemia cells (HEL) (Lo et al., 2003). Thus, there is now growing evidence that ASM respond to ligands that ligate 7TM and cytokine receptors and that the downstream pathways of these receptors form

a network that regulates ASM contraction, proliferation and the secretion of immunomodulatory cytokines/chemokines.

7. The role of AT in airway smooth muscle contraction and activation

Among the various effector pathways of the asthmatic response, AT have long been recognized as potent contractile agonists for ileal and tracheal smooth muscle (Regal et al., 1980; Stimler et al., 1980; Kola et al., 1992; del Balzo et al., 1989). However, most data obtained in guinea pigs 20 years ago, supported a model in which C5a-stimulated ASM contraction resulted from indirect effects on histamine release and production of cysteinyl leukotrienes (CysLT) (Stimler et al., 1982). C3a was found to be considerably less potent than C5a and appeared to act primarily by causing the release of cyclooxygenase pathway products (Stimler et al., 1983). The AT thus stimulate release of many of the same mediators from lung tissues that are released by Ag challenge during asthma, and may therefore play an important, if indirect, role in the pathogenesis of allergic bronchospasm. However, no data have yet been published on the effect of AT on ASM contraction in mice.

Indeed, novel data suggest that previous findings in guinea pigs cannot be simply transferred to the mouse system. No contraction occurs in isolated tracheal strips from BALB/c or B10.D2 mice challenged with C3a or C5a, even though C3aR and C5aR are present on murine (and human) ASM cells (Drouin et al., 2001). Further, no airway contraction or airway inflammation occurs after intratracheal instillation of AT to naïve mice, whereas guinea pigs suffer from respiratory distress, strong airway contraction and inflammation (with death in 50% of C5adesArg-treated animals) (Stimler et al., 1980). In mice immunized intratracheally with house dust mite, however, subsequent intratracheal administration of AT does induce ASM contraction and airway inflammation (Köhl et al., manuscript in preparation). These data suggest that AT-mediated attraction and activation of infiltrating cells (e.g. neutrophils, eosinophils and/or monocytes/macrophages) and/or activation of resident pulmonary cells (epithelial cells, mast cells) via upregulated AT receptors is needed for ASM contraction. In addition, AT receptors may be upregulated on ASM and induce contraction directly. In support of this view, Drouin et al. (2001) found C3aR upregulation on ASM in OVA-sensitized mice. The functional consequences of triggering either C3aR or C5a signaling pathways in airway smooth muscle cells remain to be determined.

8. Conclusion

Substantial evidence suggests that complement activation plays an important role in the development of allergic responses. Traditionally, C3a and C5a have been thought to

contribute to the allergic response via their potent proinflammatory and chemotactic activities. New insights into the biology of complement suggest a much more complex role for complement in asthma. Taken together, the accumulated data support the hypothesis that asthma-related polarized T-cell responses, and allergen-induced AHR, arise either as a result of impaired generation of C5a (or C5aR activation) and/or as a result of enhanced C3a generation (or C3aR activation), or both. This imbalance in triggering of the C3aR/C5aR pathways may predispose to deleterious Th2 immune response and disturbances in the local environment of the lung following allergen exposure resulting in the known pathologic features of the asthmatic response. Although much remains to be learned about the complex role played by AT in asthma, identification of the roles of complement in the development of allergic asthma provides a focus for novel therapeutic strategies for an increasingly important disease. C3aR and/or C5aR may provide tractable targets for therapeutic development.

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