Absence of the Complement Anaphylatoxin C3a Receptor Suppresses Th2 Effector Functions in a Murine Model of Pulmonary Allergy

Scott M. Drouin, David B. Corry, Travis J. Hollman, Jens Kildsgaard and Rick A. Wetsel

J Immunol 2002; 169:5926-5933; doi: 10.4049/jimmunol.169.10.5926
http://www.jimmunol.org/content/169/10/5926

References
This article cites 57 articles, 24 of which you can access for free at:
http://www.jimmunol.org/content/169/10/5926.full#ref-list-1

Subscription
Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Absence of the Complement Anaphylatoxin C3a Receptor Suppresses Th2 Effector Functions in a Murine Model of Pulmonary Allergy

Scott M. Drouin,* David B. Corry,†‡ Travis J. Hollman,* Jens Kildsgaard,‡§ and Rick A. Wetsel*†

Asthma is a chronic inflammatory disease of the lung resulting in airway obstruction. The airway inflammation of asthma is strongly linked to Th2 lymphocytes and their cytokines, particularly IL-4, IL-5, and IL-13, which regulate airway hyperresponsiveness, eosinophil activation, mucus production, and IgE secretion. Historically, complement was not thought to contribute to the pathogenesis of asthma. However, our previous reports have demonstrated that complement contributes to bronchial hyperreactivity, recruitment of airway eosinophils, IL-4 production, and IgE responses in a mouse model of pulmonary allergy. To define the complement activation fragments that mediate these effects, we assessed the role of the complement anaphylatoxin C3a in a mouse model of pulmonary allergy by challenging C3aR-deficient mice intranasally with a mixed Ag preparation of Aspergillus fumigatus cell culture filtrate and OVA. Analysis by plethysmography after challenge revealed an attenuation in airway hyperresponsiveness in C3aR-deficient mice relative to wild-type mice. C3aR-deficient mice also had an 88% decrease in airway eosinophils and a 59% reduction in lung IL-4-producing cells. Consistent with the reduced numbers of IL-4-producing cells, C3aR-deficient mice had diminished bronchoalveolar lavage levels of the Th2 cytokines, IL-5 and IL-13. C3aR knockout mice also exhibited decreases in IgE titers as well as reduced mucus production. Collectively, these data highlight the importance of complement activation, the C3a anaphylatoxin, and its receptor during Th2 development in this experimental model and implicate these molecules as possible therapeutic targets in diseases such as asthma. The Journal of Immunology, 2002, 169: 5926–5933.

1 This work was supported by National Institutes of Health Grants AI25011 (to R.A.W.) and AI10223 (to S.M.D.) and by American Heart Association National Grant-in-Aid 9950394 (to R.A.W.).

Address correspondence and reprint requests to Dr. Rick A. Wetsel, Institute of Molecular Medicine for the Prevention of Human Diseases, University of Texas-Houston, † Department of Biochemistry and Molecular Biology, University of Texas-Houston Medical School, and Departments of *Medicine and ‡Immunology and §Biology of Inflammation Center, Baylor University College of Medicine, Houston, TX 77030.

Received for publication July 22, 2002. Accepted for publication September 9, 2002.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Asthma is a complex disease of the lung characterized by airflow obstruction, airway hyperresponsiveness (AHR), and airway inflammation. Although asthma is multifactorial in origin, the genetic predisposition for the development of an IgE-mediated response to common aerolergens is the strongest identifiable predisposing factor for the development of asthma (1). The inflammatory process is thought to arise from inappropriate responses to commonly inhaled allergens (2, 3) with subsequent exposure to allergens initiating a secondary humoral response. Following an allergen provocation, the allergic reaction can be divided into both early (within minutes) and late (hours after exposure) inflammatory and bronchospastic responses. The early response is associated with mast cell degranulation and is characterized by increases in mucosal edema, airway smooth muscle tone, and airway narrowing (4). The late-phase response begins 3–6 h after allergen challenge, and the airway narrowing is associated with migration of neutrophils, eosinophils, and lymphocytes from the blood into lung parenchyma and airway epithelium (4–6).

This classification of asthma has led to the concept that the immediate response after allergen challenge is mediated by mast cells, whereas T lymphocytes and eosinophils are the predominant effector cells in the late asthmatic reaction. The role of the mast cell becomes evident when challenging WBB6F1/W−/− mast cell-deficient mice with OVA (7). These mice fail to develop AHR even though lung eosinophil levels and serum IgE responses are comparable to wild-type responses. Research efforts have also demonstrated that CD4+ T cells, which produce a Th2 pattern of cytokines, play a pivotal role in the pathogenesis of this disease (8). Through the release of cytokines such as IL-4, IL-13, and IL-5, Th2 cells are thought to contribute to bronchial hyperreactivity and mucus hypersecretion as well as orchestrate the recruitment and activation of eosinophils (9, 10). Moreover, Tcell-deficient, RAG−/− mice, which lack Th2 cells, fail to develop AHR and to recruit eosinophils into the airways after challenge with Ag (11). IL-4 also plays a prominent role in allergy by initiating B lymphocyte differentiation and production of IgE Abs (12, 13).

Traditionally, asthma was considered a simple type I hypersensitivity reaction, and, as such, the complement system was not considered important in its pathogenesis (14). However, data from mice deficient in the third component of the complement system (C3) revealed diminished AHR, lung eosinophil levels, and IL-4 production in Ag-challenged lungs as well as reduced Ag-specific IgE and IgG1 responses, and clearly demonstrated that these features of asthma are significantly attenuated in the absence of C3 (15). Evidence has also surfaced suggesting the importance of the complement-derived C3a anaphylatoxin in asthma with elevated levels of the C3a peptide being detected in the lungs of asthmatic patients (16–19). Considering that C3 is the parent molecule of...
C3a and that known plant, fungal, and insect allergens can cleave C3 and release C3a (20), these findings suggest that the C3a anaphylatoxin may be a key participant in asthma pathogenesis.

As an anaphylatoxin, C3a triggers contraction of smooth muscle, increases permeability of small blood vessels, and regulates vasodilatation (21, 22). However, many studies have described a much broader ability of C3a to regulate effector functions of white blood cells and pulmonary cell types typically associated with asthma. C3a stimulates release of histamine from basophils (23) and mast cells (24) and regulates synthesis of eosinophil cationic protein and adhesion to endothelial cells by eosinophils (25–27).

C3a is also a chemotactic molecule for both eosinophils (28) and mast cells (29). Within the lung, the functional importance of C3a has been established in studies examining the inflammatory effects of intrabronchial instillation of C3a in guinea pig lungs. Instillation of this anaphylatoxin induces respiratory distress characterized by contraction of the smooth muscle walls in bronchioles and aggregation of leukocytes in pulmonary vessels (30). Moreover, synthetic peptides, based on the C-terminal sequence of C3a, can also mimic these properties (31), and the addition of inhibitors to cobra xypeptides N, a known regulator of C3a activity, potentiates the respiratory distress stimulated by instillation of C3a (32, 33).

The C3a peptide regulates these inflammatory functions by interacting with its receptor, C3AR, which belongs to the rhodopsin family of seven transmembrane G protein-coupled receptors (34). The C3AR is present on many cell types associated with asthma such as eosinophils (35), mast cells (36), T lymphocytes (37), and B lymphocytes (38), as well as lung bronchial epithelial and smooth muscle cells (39). Involvement of this receptor in rodent models of pulmonary allergy has also been demonstrated with reports showing that C3AR mediates AHR in C3AR-deficient mice (18) and immediate airway bronchoconstriction in C3AR-deficient guinea pigs (40). Furthermore, elevated expression of this receptor has been documented on bronchial smooth muscle cells in mouse lung challenged with Ag (39). Although these studies are significant and implicate the C3AR as a regulator of airway tone in asthma, the reported data have not addressed the role of the C3AR within the context of Th2 responses, IgE regulation, or mucus production, which are also major characteristics of asthma. Accordingly, to elucidate the role of C3a and its receptor in asthma, we have studied a murine model of pulmonary allergy using a mixed Ag preparation of Aspergillus fumi
gatus cell culture filtrate and OVA in mice deficient for the C3AR. Data from these mice reveal an attenuated allergic response very similar to C3-deficient animals with diminished AHR, eosinophil recruitment, Th2 cytokine production, and mucus secretion in the lung, as well as reduced Ag-specific IgE and IgG1 responses. These results demonstrate a requisite contribu
tion of the C3AR to allergic lung disease and provide evidence that the C3AR has a novel role in Th2 development in pulmonary allergy.

Materials and Methods

Mice

The C3AR-deficient (C3AR−/−) mice used have been described previously (41). The C3AR−/− mice were backcrossed eight generations onto the C57BL/6 background, and their wild-type littermates (C3AR+/+) were used as controls. This work was conducted in accordance with institutional and National Institutes of Health guidelines and care.

Sensitization and challenge protocol

The Ag preparation is a mixture of A. fumigatus cell culture filtrate prepared free of living organisms as described (42) and OVA (Sigma-Aldrich, St. Louis, MO). OVA added to the preparation permitted detection of Ag-specific IgE, IgG1, and IgG2a levels after AHR measurements to ascertain immune responses during the course of disease. Two batches of A. fumi
gatus cell culture filtrate with equivalent activities (lot nos. 5323R1, 5325) were aliquoted at a concentration of 6 mg/ml and stored at −70°C for use in all the experiments described herein. The LPS content of the Ag extracts was <0.2 EU/100 μg of Ag when tested with the Limulus amebocyte lysate test (BioWhittaker, Walkersville, MD) (42). Four-week-old C3AR−/− and C3AR+/+ mice were sensitized i.p. with the mixed Ag preparation on days 1, 5, 9, and 13 followed by two intranasal (i.n.) challenges on days 17 and 19. In all cases, 25 μg of OVA and 35 μg of A. fumi
gatus culture filtrate were diluted in PBS and delivered in a total volume of 50 μl for the i.p. or i.n. administration. All mice receiving i.n. challenges were anesthetized with isoflurane and held upright until all of the Ag administered into their nostrils was inhaled. PBS controls shown for each experiment were C3AR+/+ or C3AR−/− mice that were sensitized with the Ag and then challenged with PBS.

AHR measurements

Airway responsiveness to i.v. acetylcholine (ACh) challenge was measured as previously described (43). In brief, mice were anesthetized i.p. with etomidate (Abbott Laboratories, Abbott Park, IL) at 20 μg/g body weight and locally with lidocaine (Sigma-Aldrich). The tracheas were surgically exposed, cannulated with a blunt-ended, 20-gauge angiocatheter, and con
tected to a rodent ventilator (Harvard Apparatus, Holliston, MA). Mice were ventilated with 100% oxygen at a rate of 150 breaths per min and a tidal volume of 9 μl/g. Following paralysis with pancuronium bromide (4 μg/g; Genesia Laboratories, Irvine, CA), i.v. access was established using a 27-gauge needle placed into a tail vein, and mice were placed into a rodent plethysmograph capable of determining tidal volume, airflow, and trans
thoracic resistance continuously (Buxco Electronics, Sharon, CT). Airway responses were expressed as the provocative concentration of ACh, in micrograms per gram body weight, required to double baseline trans
thoracic resistance (PC200). Lower PC200 values represent greater airway reactivity.

Bronchoalveolar (BAL) lavage measurements

Airway inflammation was assessed by BAL. Lungs were lavaged three times with 1 ml of PBS. Between 2.2 and 2.4 ml of BAL fluid was recovered from each lung, and total cell numbers were determined using a hemacytometer. Differential cell counts were obtained from BAL cells spun onto slides with a cytocentrifuge (Thermo Shandon, Pittsburgh, PA), and treated with Wright-Giemsa stain (Sigma-Aldrich). Five-hundred cells were classified based on characteristic morphology revealed after staining. Absolute numbers of specific cell types (neutrophils, lymphocytes, macrophages, and eosinophils) were calculated from the recovered BAL vol
tume, total cell count, and percent abundance of specific cells.

Lung histology

After AHR measurements, lungs were inflated with 0.5 ml of formalin (Sigma-Aldrich) and the trachea was tied off. The lungs were then removed from the chest cavity and placed in formalin overnight. The lungs were then dehydrated, embedded in paraf
im, cut into 5-μm sections, and stained with H&E. Inflammatory infiltrates and lung architecture were assessed using light microscopy.

Measurement of lung mucin levels

For determination of lung mucin levels, BAL fluid was collected as de
scribed, and cells were removed by centrifugation. Lung mucin levels were measured as previously reported (44) using the mucin-binding lectin ja
calin (45). Wells of microtiter plates (Innolun IV; Fisher Scientific, Pitts
burgh, PA) were precoated with serial 2-fold dilutions of mucin standard (Sigma-Aldrich) and BAL samples diluted in PBS for 2 h at 37°C and then blocked with 0.2% I-block (Applied Biosystems, Foster City, CA) in PBS containing 0.05% Tween 20. Plates were washed with PBS containing 0.05% Tween 20 and then incubated with 5 μg/ml biotinylated ja
calin (Vector Laboratories, Burlingame, CA) diluted in PBS for 1 h at 37°C. Plates were washed and incubated with a 1/500 dilution of streptavidin and provided evidence that the C3AR has a novel role in Th2 development in pulmonary allergy.
IV; Fisher Scientific) that had been precoated with polyclonal Abs against either mouse IL-4 or mouse IFN-γ (R&D Systems). Serial 2-fold dilutions were prepared, and the plates were incubated undisturbed for 8 h at 37°C. Wells were washed with PBS to remove cells and incubated with biotinylated secondary polyclonal Abs against mouse IL-4 or mouse IFN-γ (R&D Systems). After 1 h, wells were washed and incubated for 1 h with 100 μl of streptavidin-conjugated alkaline phosphatase (Jackson ImmunoResearch Laboratories) in PBS with 0.05% Tween 20 and 5% FBS. Color was developed with 5-bromo-4-chloro-3-indolyl phosphate in 0.1 M 2-amino-2-methyl-1-propanol buffer (Sigma-Aldrich) suspended in 0.6% agarose. After solidification of the agar, individual blue spots were counted by inverted microscopy (9). For both ELISPOT assays, cytokine-producing cells could be measured to 50 cells/ml.

Levels of IL-5 and IL-13 were determined in the BAL fluid to further assess the Th2 response in this model. BAL fluid was collected as previously described, and cells were removed by centrifugation. The BAL supernatants were then assayed for the presence of IL-5 and IL-13 by ELISA (R&D Systems) as per the manufacturer’s instructions, and the assays were sensitive to 2 ng/ml. For the Ag-specific Ig measurements, serum IgE titers were determined by two-site sandwich ELISA using an ELISA kit (BD PharMingen, San Diego, CA) as per manufacturer’s specifications, and the assay was sensitive to 2 ng/ml. For the Ag-specific Ab analyses, wells of microtiter plates were precoated with 40 μl of OVA in PBS and blocked with 5% BSA in PBS with 0.05% Tween 20. Serum diluted 1/500 was then added and incubated for 1 h at 37°C. Plates were washed, and biotinylated isotype-specific Abs (anti-IgE, IgG1, and IgG2a; Jackson ImmunoResearch Laboratories) were added for an additional hour of incubation. Plates were washed again and streptavidin-conjugated alkaline phosphatase (Jackson ImmunoResearch Laboratories) was added for 30 min at room temperature. After a final wash, plates were developed with 0.5 M nitrophenyl phosphate in 0.1 M 2-amino-2-methyl-1-propanol buffer (Sigma-Aldrich). Data are expressed as OD₄₀₅.

Statistical analysis

Statistical analysis was performed using the Prism software package (GraphPad, San Diego, CA). Statistical significance of all results were assessed using the two-tailed, unpaired Student’s t test.

Results

Effect of C3aR deficiency on AHR after Ag challenge

Mice deficient in the C3aR lack a functional receptor that can bind the complement anaphylatoxin C3a (41). In our laboratory, mouse models of pulmonary allergy have demonstrated that the expression of this receptor is increased on airway smooth muscle after Ag challenge (39) and that AHR, lung inflammation, and immune responses are attenuated in mice deficient in C3, the parent protein of C3a (15). Collectively, these studies suggest that C3a and its receptor, C3aR, are important molecular mediators of pulmonary allergy and, therefore, potential therapeutic targets in the treatment of asthma. To further establish the role that C3aR plays in allergic lung disease, we subjected C3aR-deficient mice to an in vivo mouse model of pulmonary allergy. C3aR⁻/⁻ and C3aR⁺/⁺ mice were challenged with a mixed Ag preparation comprised of A. fumigatus cell culture filtrate prepared free of living organisms (42) and OVA. OVA was specifically added to the preparation to detect Ag-specific Ig levels after AHR measurements and evaluate immune responses during the course of disease. Twenty-four hours after the last challenge, AHR to increasing doses of ACh was evaluated by airway plethysmography. Within this model, mice challenged with the Ag preparation are expected to have greater sensitivity to ACh and, as a result, the provocative concentration of ACh required to induce a 200% increase in airway resistance above baseline will be lower for these mice compared with PBS-challenged controls (9). As shown in Fig. 1, Ag-challenged wild-type mice developed an asthma-like response characterized by an increase in AHR as revealed by enhanced sensitivity when exposed to ACh. In contrast, AHR was abrogated in Ag-challenged C3aR⁻/⁻ mice as shown by the attenuation in ACh sensitivity relative to challenged wild-type littermates. Furthermore, the AHR in the C3aR⁻/⁻ mice approached that observed in PBS-challenged C3aR⁺/⁺ mice that were sensitized with the mixed Ag preparation and then challenged with PBS.

Effect of C3aR deficiency on inflammatory cell recruitment in Ag-challenged lungs

An additional facet of asthma is the pulmonary inflammation characterized by the presence of white blood cells, specifically eosinophils, recruited into the airways (5). Eosinophils are chemotactic to the C3a anaphylatoxin (26), and mice deficient in the C3aR could potentially have reduced eosinophil recruitment into the lung after exposure to Ag. To determine whether the C3aR contributes to eosinophil recruitment, lungs from challenged mice were analyzed after the AHR measurements, and the BAL fluid was examined for influx of white blood cells (Fig. 2). Quantitation of white blood cells from the BAL of PBS-challenged C3aR⁺/⁺ and C3aR⁻/⁻ mice revealed a population of cells comprised predominantly of macrophages with few neutrophils, eosinophils, and lymphocytes. Challenging wild-type mice with the mixed Ag preparation resulted in an increase in all cell types relative to PBS controls, and the BAL cell population consisted mainly of eosinophils and neutrophils. However, challenged C3aR-deficient mice revealed a marked reduction in all inflammatory cells, including an 88% reduction in eosinophils as well as a 79% decrease in airway neutrophils and a 54% decrease in airway lymphocytes. The reduced presence of inflammatory cells in the BAL fluid was further corroborated by H&E stained lung sections from challenged C3aR knockout mice (Fig. 3), which revealed diminished infiltration of eosinophils into the peribronchial region between the pulmonary blood vessels and the airways relative to their wild-type counterparts (Fig. 3). Collectively, these results demonstrate that mice deficient in the C3aR not only have deficits in lung eosinophil recruitment after challenge but also have defects in neutrophil and lymphocyte recruitment as well.
PBS controls shown were C3aR<sup>−/−</sup> and C3aR<sup>+/+</sup> littermates was assessed 24 h after the last Ag challenge. MAC, macrophages; NEU, neutrophils; EOS, eosinophils; LYM, lymphocytes. Results are plotted as means ± SEM (PBS-C3aR<sup>+/+</sup>, n = 10; PBS-C3aR<sup>−/−</sup>, n = 5; Ag-C3aR<sup>+/+</sup>, n = 15; Ag-C3aR<sup>−/−</sup>, n = 15), and significant differences between wild-type and knockout mice are indicated as *, p < 0.05 and **, p < 0.01, as determined by Student’s t test. PBS controls shown were C3aR<sup>+/+</sup> or C3aR<sup>−/−</sup> mice that were sensitized with the mixed Ag preparation and then challenged with PBS.

**Effect of C3aR deficiency on airway mucin level in Ag-challenged lungs**

Mucus hypersecretion is another characteristic feature contributing to the airway obstruction in asthma. Overproduction of mucus in the airways is the result of increased proliferation of goblet cells and their increased production of mucin subsequent to challenge with allergen (1). Measurement of BAL mucin levels in Ag-challenged C3aR<sup>−/−</sup> mice revealed a significant 52% reduction in airway mucin levels compared with the Ag-challenged controls (Fig. 4) and indicate that the C3aR participates in airway mucin production. Furthermore, this data is consistent with the attenuated AHR and airway eosinophil recruitment observed in challenged C3aR-deficient mice and strongly suggests that the C3aR contributes to much of the pulmonary inflammation in this model.

**Effect of C3aR deficiency on IL-4 and IFN-γ production in Ag-challenged lungs**

Previous reports have described elevated levels of Th2 cytokines in asthma (1) and the importance of the Th2 response in mouse models of pulmonary allergy (9, 11). Considering these observations and that challenged C3aR<sup>−/−</sup> mice had a 54% decrease in levels of airway lymphocytes (Fig. 2), we performed additional studies to understand the role of the C3aR with respect to T cell effector function. To assess T cell activation in C3aR<sup>−/−</sup> mice, IL-4 and IFN-γ production in the lung was quantitated by ELISPOT and evaluated as a marker of Th2 vs Th1 responses after challenge (Fig. 5, top panel). Consistent with this model, wild-type mice challenged with the mixed Ag preparation exhibited a pronounced Th2 response in the lung as demonstrated by the elevated levels of cells expressing IL-4 and lack of cells expressing IFN-γ. In contrast to the elevated numbers of IL-4-producing cells in the wild-type animals, cells expressing IL-4 were reduced 59% in the lungs of Ag-challenged C3aR<sup>−/−</sup> mice. Lungs from Ag-challenged C3aR-deficient and wild-type mice did not have measurable IFN-γ-producing cells above the background limits for the assay, and analysis of BAL fluid by ELISA also revealed no detectable quantities of IFN-γ protein in either challenged wild-type or C3aR-deficient animals (data not shown).

To further support the IL-4 ELISPOT observations, levels of IL-5 and IL-13 were measured because production of these cytokines are also indicative of a Th2 response (1). Analysis by ELISA revealed no detectable cytokine levels in the BAL fluid from PBS-challenged C3aR<sup>+/+</sup> and C3aR<sup>−/−</sup> animals. Challenging wild-type mice with the mixed Ag preparation resulted in increased IL-5 and IL-13 levels (Fig. 5, bottom panel). Challenging C3aR<sup>−/−</sup> mice with the mixed Ag preparation did not have a pronounced effect on IL-5 expression in the airways, and the IL-5 levels detected in the BAL fluid of these mice was below the sensitivity limits of the assay. Challenging C3aR<sup>−/−</sup> mice did increase BAL IL-13 levels above PBS controls. However, IL-13 levels in the knockout mice were still reduced 77% compared with Ag-challenged wild-type animals. These results and the reduced numbers...
of IL-4-producing cells demonstrate that the C3aR is involved in IL-4, IL-5, and IL-13 regulation in the lung and indicate that the C3a anaphylatoxin and its receptor are potentially linked to adaptive immunity by promoting Th2 responses in this model.

**Effect of C3aR deficiency on Ag-specific Ig levels**

The preceding data suggest that the dramatic reduction in the airway responses and pulmonary inflammation observed in Ag-challenged C3aR−/− mice is due to a defect in Th2 function. To gain further insight into the mechanisms linking complement to adaptive immunity, we determined total and Ag-specific Ab responses in wild-type and C3aR−/− mice. Increased production of IgE Abs during the course of asthma is another characteristic of the disease (46), and examination of total serum IgE in Ag-challenged C3aR+/+ and C3aR−/− mice revealed elevated levels of IgE compared with their PBS controls. However, total IgE levels in challenged C3aR-deficient mice were significantly reduced by 40% compared with the similarly challenged wild-type mice (Fig. 6, top panel). Ag-specific IgE, IgG1, and IgG2a isotypes were also examined in C3aR−/− mice (Fig. 6, bottom panel), and although C3aR knockout and wild-type mice had comparable levels of IgG2a, Ag-specific IgE and IgG1 levels were decreased 70% and 64%, respectively, in the Ag-challenged C3aR−/− animals. These results are consistent with the premise that IgE and IgG1 production, which is controlled by Th2 cells, is reduced in C3aR−/− mice.

**Discussion**

In this report, we demonstrate that all features of the experimental allergic asthma model, which include AHR, lung and airway recruitment of eosinophils, mucus hypersecretion, Th2 activation and recruitment to lung, and IgE and IgG1 secretion by B cells, are...
either abrogated or severely reduced in the genetic absence of the C3aR. These results indicate that the C3aR is a major contributor to allergic lung disease and demonstrate for the first time that this complement anaphylatoxin receptor impacts Th2 development and effector functions. Moreover, these data provide compelling new evidence that complement, and more specifically the complement C3a anaphylatoxin and its receptor, mediates much of the pulmonary inflammation that occurs in allergic lung disease and suggest that C3a on binding C3aR may play a significant role in bridging innate and adaptive Th2 immune responses in asthma.

The AHR findings from this study are consistent with previous reports examining the role of C3 or C3a in rodent models of pulmonary allergy. ACh is a cholinergic agonist that can induce bronchoconstriction in mice (49), and the increased sensitivity of airway smooth muscle to ACh is thought to originate from the release of spasmodic substances during the effector phase of asthma which cause bronchoconstriction and airway inflammation (reviewed in Ref. 50). When comparing the airway responses of C3aR-deficient mice with C3-deficient mice in the A. fumigatus/OVA model (15) or with C3aR-deficient rodents in the OVA model (18, 40), Ag-challenged animals deficient in C3 or C3aR had problems mounting immediate bronchospastic responses or late-phase AHR relative to their wild-type controls. Although the level of reduction in the airway response varies between the different reports, these studies collectively highlight the ability of C3a to regulate airway tone in these rodent models. Given these findings, one could postulate that production of complement components in the airways (51) could provide a local source of C3. After challenge with an allergen, proteolytic cleavage of C3 from classical, lectin, or alternative pathway activation or from proteases present in the A. fumigatus extract (20) or released from activated mast cells (52) could generate C3a in the airways leading to subsequent bronchial smooth muscle contraction. Considering that elevated levels of the C3a peptide have been detected in the BAL of human asthma patients (16–19) and that the C3aR has been detected on airway smooth muscle in human lung (39), C3a and its receptor are implicated as regulators of AHR in asthma. However, it currently is unclear whether C3a modulates AHR directly by binding C3aR on airway smooth muscle cells or indirectly by stimulating release of mediators of bronchoconstriction from other cells bearing C3aR.

In addition to the airway responses, both C3- and C3aR-deficient strains had similar deficits in IL-4 expression and IgE serum levels in the A. fumigatus/OVA model (15), and, although not previously reported, Ag-challenged C3-deficient mice also have decreases in IL-5 and IL-13 expression comparable to the C3aR-deficient mice (data not shown). Curiously though, white blood cell recruitment into the airways was dramatically different between the two knockouts. Specifically, C3-deficient mice had a 42% reduction in lung eosinophil levels whereas C3aR-deficient mice had an 88% reduction when compared with their wild-type controls. One possible explanation for this difference comes from studies examining the role of Mac-1 (CD11b/CD18) in pulmonary allergic disease (53). Widely known as an adhesion molecule involved in leukocyte diapedesis, Mac-1 also binds C3bi (54), another complement activation fragment derived from C3. When challenged with Ag, mice deficient in Mac-1 exhibit increased eosinophil recruitment into the airways and lung parenchyma relative to wild-type controls. These findings are in contrast to the observations described in this study where challenged C3aR-deficient mice have dramatically reduced levels of airway eosinophils relative to wild-type controls. Knowing that C3-deficient mice lack both the C3a and C3bi peptides, one could postulate that the reduction in airway eosinophils that results from the lack of C3a in challenged C3-deficient mice is compromised by the concomitant absence of C3bi.

Likewise, inconsistencies in the lung eosinophil levels and the immune responses exist when comparing data from this study with reports examining pulmonary allergy in C3aR-deficient mice (18) and guinea pigs (40). Although deficits in lung eosinophil recruitment were observed in this study, no significant differences in eosinophil recruitment were detected between OVA-challenged wild-type and C3aR knockout mice or OVA-challenged wild-type and C3aR-deficient guinea pigs. Moreover, total serum IgE levels and BAL levels of IL-4, IL-5, and IL-13 were reported to be similar between challenged wild-type and C3aR knockout mice (18). These responses could possibly be explained by differences in the animals used for the study. The knockout mice used in this study were backcrossed eight generations onto the C57BL/6 background whereas the previously published reports used C3aR-deficient guinea pigs (40) or C3aR knockout mice that were backcrossed three generations on the BALB/c background (18). Alternatively, differences in Ag might also explain these findings. The studies by Humbles et al. (18) and Bautsch et al. (40) used OVA to induce pulmonary allergy whereas this study used extracts from A. fumigatus combined with OVA. In contrast to OVA alone, A. fumigatus is an allergen relevant to human allergic lung disease and has been shown to activate complement and release complement anaphylatoxins in vitro (20).

Studies have also examined whether the complement anaphylatoxin C5a and its receptor, C5aR, are primary mediators in pulmonary allergy models. Recent studies have demonstrated that inhibition of C5a using a C5aR antagonist will reduce late airway responses in OVA-challenged rats (55) and have implicated the gene encoding the fifth component of the complement system (C5), which is the parent protein for C5a, as a susceptibility locus for allergen-induced AHR (56). C5a has also been shown to regulate the production of IL-12 from macrophages (57), a cytokine which suppresses Th2 responses, and the expression of IL-4 and IL-13 from basophils (58), which may contribute to Th2 development in the initial stages of asthma. Because IL-4, IL-13, IgE, and mucus production were not completely inhibited in C3aR-deficient mice, it is possible that the C3aR and C5aR may have overlapping functions in regulating the underlying inflammatory and immune responses in this model. Additional studies directly comparing mice deficient in the C3aR and C5aR will be required to resolve these issues.

Perhaps the most significant findings reported in this study are the combined defects in AHR, eosinophil recruitment, IgE responses, and mucus hypersecretion after Ag challenge. Previous studies have established that IL-4, IL-5, and IL-13 regulate these functions in similar pulmonary allergy models (9, 10) and suggest that the reduced presence of IL-4, IL-5, and IL-13 in the lungs of C3aR-deficient mice are the central cause for these observed defects. Although the C3aR has been detected on human T lymphocytes in vitro (37), expression of the C3aR has not been demonstrated on mouse T lymphocytes nor does the receptor have a known role in regulating T cell responses in vivo. One explanation for these findings is that T lymphocytes lack the C3aR which is critical for the direct development of Th2 responses in this model. Alternatively, one could hypothesize that the C3aR has a vital regulatory role on many cell types that contribute to the pulmonary inflammation and immune responses. The C3aR has been detected on both human and mouse bronchial epithelial and smooth muscle cells (39) and on human B lymphocytes (38), eosinophils (35), and mast cells (36), cell types which are involved in the development of asthma. Although this report demonstrates that the C3aR is important for both Th2 development and experimental allergic lung
disease, further investigation will be required to distinguish the distinct roles played by the C3aR regarding the function of T lymphocytes and other supporting cells in asthma.

In conclusion, the study presented in this report substantiates previous reports describing C3aR-mediated regulation of AHR and documents a novel regulatory role for the C3aR in the development of Th2 responses in a mouse model of pulmonary allergy. These results lay the foundation for additional studies directed at assessing the role of the C3aR on CD4+ T lymphocytes in vitro as well as delineating in vivo the mechanisms by which C3a contributes to airway responses, mucus hypersecretion, lung eosinophil recruitment, and IgE production. Given the important contribution of Th2 cytokine production in asthma, further study of C3a-mediated regulation of T lymphocytes may shed light in the causative events leading to AHR and airway inflammation in this disease.

Acknowledgments

We thank Dr. Irma Gigli for critical evaluation of the data and text as well as Patricia Dillard for technical support. We also thank Dr. Stacey Mueller-Ortiz for assistance with the data collection and Dr. Li Zhen for assistance with the mucin assays.

References