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The Critical Role of Complement Alternative Pathway Regulator Factor H in Allergen-Induced Airway Hyperresponsiveness and Inflammation

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Activation of the alternative pathway of complement plays a critical role in the development of allergen-induced airway hyperresponsiveness (AHR) and inflammation in mice. Endogenous factor H, a potent inhibitor of the alternative pathway, is increased in the airways of sensitized and challenged mice, but its role in regulating inflammation or AHR has been unknown. We found that blocking the tissue-binding function of factor H with a competitive antagonist increased complement activation and tissue inflammation after allergen challenge of sensitized mice. Conversely, administration of a fusion protein that contains the iC3b/C3d binding region of complement receptor 2 linked to the inhibitory region of factor H, a molecule directly targeting complement-activating surfaces, protected mice in both primary and secondary challenge models of AHR and lung inflammation. Thus, although endogenous factor H does play a role in limiting the development of AHR, strategies to deliver the complement-regulatory region of factor H specifically to the site of inflammation provide greater protection than that afforded by endogenous regulators. Such an agent may be an effective therapy for the treatment of asthma. The Journal of Immunology, 2012, 188: 661–667.

Asthma is the most common chronic respiratory disease in westernized countries. Because of the increasing prevalence, severity, and morbidity in asthma, particularly among certain ethnic groups, numerous studies have been carried out to address the underlying mechanisms of the disease and to identify potential therapeutic targets. To date, the most widely accepted mechanistic theory is that asthma is a Th2-type cell-mediated airway inflammatory disease (1). However, despite aggressive targeting of the Th2 pathway, only limited successful trials with Th2 modulators have been reported (2). Indeed, few new asthma drugs have been successfully introduced into the clinic, and corticosteroids remain the treatment of choice.

Several studies of asthma pathogenesis have focused on the role of the complement system in the induction of the allergic inflammatory response and propagation of the disease (3–11). Our previous studies have shown that mice treated with the mouse complement-inhibitory protein Crry-Ig developed decreased levels of airway hyperresponsiveness (AHR) and allergic airway inflammation than control mice (6). Subsequently, we demonstrated that mice genetically deficient in factor B, an essential molecule for the activation of the alternative pathway, were protected from the development of AHR and allergic airway inflammation (3). However, mice deficient in C4, a protein that is required for classical pathway activation, were not protected in this model. These studies suggested that complement was activated via the alternative pathway during the development of allergen-induced airway inflammation. However, the mechanisms by which the alternative pathway was activated have not yet been determined.

The alternative pathway regulatory protein factor H (fH) is the most important soluble regulator for the alternative pathway initiation and amplification system. fH is abundant in plasma and is capable through its N-terminal domain of binding to C3b, of accelerating the decay of the alternative pathway C3 convertase (C3bBb), and of acting as a cofactor for the factor I-mediated proteolytic cleavage and inactivation of C3b (12). As a result of these activities, fH regulates complement activation and amplification through the alternative pathway and is critical to the homeostatic regulation of the alternative pathway in the fluid phase (13). Moreover, several regions within the protein outside of the N-terminal alternative pathway regulatory domain mediate binding to ligands present on host tissues, consisting primarily of polyanions in combination with covalently fixed C3 activation fragments C3b and C3d, preventing autologous injury by the alternative pathway on self tissues (12).

One of the important characteristics of fH is its ability to limit inflammation in host tissues to which it binds while permitting complement activation on several types of pathogens that are not recognized by fH (14). The importance of the recognition of host by fH is evident in patients with mutations in the C-terminal regions of fH that bind to host ligands. These patients appear to regulate the
alternative pathway in the fluid phase, but lack of tissue binding predisposes individuals to certain diseases, including atypical hemolytic-uremic syndrome and other thrombotic microangiopathies (15).

Pathologic alternative pathway activation contributes to several inflammatory diseases even in the presence of fully functional membrane-bound regulatory proteins and fH, highlighting the limitations of one or more of these proteins in some tissues and disease states (3, 16–18). The central role of the alternative pathway in asthma models suggests that fH, a regulator specific to this pathway, has a limited ability to regulate the alternative pathway within the lung after allergen sensitization and challenge. A greater understanding of the mechanisms by which the alternative pathway is activated and by which endogenous fH is bypassed or overwhelmed may permit the development of effective new therapies.

The goals of the studies reported in this work were to examine the role of endogenous fH in the prevention or attenuation of experimental asthma, and to test strategies to overcome these limitations. To approach this, we used a recombinant protein that blocks the binding of fH to tissue surfaces, and another recombinant protein that links the alternative pathway regulatory region of fH to the iC3b/C3d binding region of complement receptor (CR)2. This latter protein was developed based upon the premise that the CR2 moiety would specifically direct the regulatory protein to sites of complement activation where iC3b/C3d was bound (19). These studies used primary and secondary OVA-induced allergic airway inflammation model of asthma. In the former, effects on development of lung allergic responses were assessed. In the secondary allergen challenge model, allergic airway inflammation was first established and allowed to resolve before secondary challenge. This model may be particularly relevant to the clinical setting, as patients typically have established disease at the time of treatment. Consequently, a complement-inhibitory agent that is effective at preventing and/or attenuating disease in this model could provide a promising addition to the therapeutic options for treating patients with severe asthma.

Materials and Methods

Animals

Female C57BL/6 (wild-type) mice from 6–8 wk of age were obtained from Harlan (Indianapolis, IN). The animals were maintained on an OVA-free diet. Experiments were conducted under a protocol approved by the Institutional Animal Care and Use Committee of National Jewish Health.

Sensitization and airway challenge

In the primary allergen challenge protocol, mice were sensitized by i.p. injection of 20 μg OVA (Fisher Scientific, Pittsburgh, PA) or short ragweed (Rw; Greer Laboratories, Lenoir, NC) emulsified in 2.0 mg alum (Alum-Muject; Pierce, Rockford, IL) in a total volume of 100 μl on days 1 and 14. Mice were challenged via the airways with OVA or Rw (1% in saline) for 20 min on days 28–30 using an ultrasonic nebulizer (model NE-07; Omron Healthcare, Vernon Hills, IL) (OVA/OVA or Rw/Rw). The control mice were sensitized with PBS, followed by OVA or Rw challenge in the same way (PBS/OVA or PBS/Rw). In the secondary allergen challenge protocol, mice were sensitized with 10 μg OVA with alum on days 1 and 7, followed by 0.2% OVA challenge on days 14–16 (primary allergen challenge). Fourteen days after the last primary allergen challenge, during which time mice were not treated, mice were challenged again with a single, provocative aerosolized dose of 1% OVA for 20 min (secondary allergen challenge) (OVA/OVA/OVA). As controls, mice were not sensitized, but received primary and secondary allergen challenge (PBS/OVA/OVA), or were sensitized and primary challenged, but were not exposed to secondary challenge (OVA/OVA/PBS). In all groups, assays were carried out 48 h after the last allergen challenge.

Preparation and administration of complement inhibitors

CR2-Cry and CR2-IH were prepared as previously described (19, 20). In the primary model, sensitized mice were treated with 250 μg CR2-Cry or CR2-IH by i.p. injection on days 28–30, 2 h prior to each airway challenge with OVA. In the secondary allergen challenge protocol, mice received a treatment on days 32 and 33, 2 h prior to and 24 h after secondary allergen challenge. PBS or CR2 alone (125 μg/mouse) were administered as controls.

To block complement inhibition by endogenous fH on host cells, a recombinant fragment of mouse fH, encompassing the 19th and 20th short consensus repeats (SCRs) of the protein (rH 19–20), was generated. Briefly, a cDNA fragment containing murine fH SCRs 19–20 was cloned into pPlc expression vector pPICZ A (Invitrogen, Carlsbad, CA) upstream from the Saccharomyces cerevisiae factor secretion sequence. The protein was expressed and deglycosylated by incubation with endoglycosidase H (New England Biolabs, Ipswich, MA). rH 19–20 was purified by cation exchange chromatography, dialyzed against PBS, filtered sterilized, and stored at −80˚C until use. A recombinant protein derived from SCRs 3–5 of the human iH protein (referred to as rH 3–5) was generated in a similar fashion and used as a negative control, because this protein should not interfere with the binding of fH to cell surfaces. rH 19–20 or the control molecule rH 3–5 was administered at a dose of 150 μg i.p. 2 h prior to the provocative allergen challenge in the secondary challenge model. Control groups received PBS as vehicle or the mice were treated with rH 19–20 prior to the saline challenge instead of the OVA challenge.

Determination of airway responsiveness

Airway responsiveness was measured as the change in lung resistance (RL) after exposure to increased concentrations of aerosolized methacholine (MCh; Sigma-Aldrich). Mice were anesthetized, tracheostomized, and mechanically ventilated, and lung function was assessed, as described previously (21). RL was continuously monitored for up to 3 min after aerosolized MCh exposure. Maximum values of RL were taken to express changes in airway function. Baseline RL in saline (saline) for RL were not significantly different among the groups.

Cytokines, C3a, and pH levels in bronchoalveolar lavage fluid

Immediately after assessment of AHR, lungs were lavaged via the tracheal tube with HBSS (1 × 1 ml, 37˚C). The number of leukocytes was counted (Coulter Counter; Coulter, Hialeah, FL), and differential cell counts were performed by counting at least 200 cells on cytospun preparations in a blinded manner (Cytospin 3; Shandon, Runcorn, Cheshire, U.K.). Slides were stained with Wright–Giemsa and differentiated by standard hematoxylin and eosin procedures.

Cytokine levels in the bronchoalveolar lavage (BAL) fluid were measured by ELISA, as previously described (21). Briefly, measurements of IFN-γ, IL-4, and IL-5 were performed using OptEIA sets (BD Pharmingen, San Diego, CA). IL-13 measurements were performed using an ELISA kit (Quantikine M; R&D Systems, Minneapolis, MN). All followed the manufacturers’ protocols. The limits of detection were 1.5 pg/ml for IL-13, 4 pg/ml for IL-4 and IL-5, and 10 pg/ml for IFN-γ.

The levels of C3a in BAL fluid were measured by ELISA as a marker of complement activation. ELISA was performed using a mAb to mouse C3a as the capture Ab and a biotinylated mAb to C3a for detection (BD Biosciences, Franklin Lakes, NJ), according to the manufacturer’s instructions. The samples were collected into 10 mM EDTA to prevent complement activation ex vivo, and they were stored at −80˚C until the assay was performed. pH was measured in the BAL fluid by Western blot analysis. Equal volumes of BAL fluid (20 μl) were separated by SDS-PAGE and transferred to nitrocellulose. fH was detected using a mAb to mouse fH (Santa Cruz Biotechnology, Santa Cruz, CA).

Histological studies

After obtaining BAL fluid, the right lung was inflated with 1 ml 10% formalin through the trachea, and fixed in formalin by immersion. Tissue blocks of lung tissue from four to five mice in each group were cut from around the main bronchi and embedded in paraffin blocks; two to three tissue sections (5 μm) per mouse were then affixed to microscope slides and deparaffinized. The slides were then stained with H&E or periodic acid-Schiff (PAS). The slide images were captured using a microscope (BX40; Olympus America, Melville, NY) equipped with a digital camera (Q-color 3; Olympus America). Goblet cell metaplasia was quantified as the number of pixels on the computer converted from PAS areas along the airway epithelium. The quantification was performed using NIH Image J software (version 1.38), available at http://rsbweb.nih.gov/ij/. Four different microscopic slide in four to six samples from each group of mice were examined in a blinded manner.

Statistical analysis

All results were expressed as the mean ± SEM. ANOVA and the Tukey-Kramer multiple-means comparison test were used for comparisons be-
Results

Endogenous fH limits airway inflammation after allergen challenge

Previous work demonstrated that alternative pathway complement activation occurs within the alveolar and/or bronchial surfaces during the development of AHR (3). The inability of endogenous fH to prevent this complement activation could be due to inadequate access of the protein to this location or insufficient ability to regulate complement activation on the activating surface. To determine whether fH is present in the airways, we performed Western blot analysis on BAL fluid from mice subjected to sensitization and challenge and then secondary allergen challenge or mice that were not sensitized and then exposed to primary and secondary allergen challenge (Fig. 1A). fH was detected in the BAL fluid of sensitized and challenged mice, but not in unsensitized mice. Thus, fH is present in the airways of mice that developed allergen-induced airway inflammation.

To determine whether fH is functionally protective in the airways of mice with established allergen-induced airway inflammation, a competitive antagonist of the C-terminal cell surface binding region of fH (rH 19–20; 150 μg) (22) was administered to the mice i.p., 2 h prior to the provocative (secondary) OVA challenge. C3a levels were higher in the BAL fluid of secondary challenged mice treated with rH 19–20 compared with those that did not receive the antagonist (Fig. 1B). rH 19–20 had no effect in mice that did not receive secondary allergen challenge. Treatment with rH 19–20 was also associated with an enhancement of AHR (Fig. 1C) and significantly more eosinophils in the BAL fluid compared with vehicle- or control protein-treated mice (Fig. 1D). Cytokine analysis revealed that both IL-5 and IL-13 levels in BAL fluid were increased following administration of rH 19–20 (Fig. 1E); the levels of IFN-γ were not altered (data not shown). Histological analysis also demonstrated increased cellular infiltration into the submucosal areas of the airways and enhanced goblet cell metaplasia following rH treatment compared with control protein treatment (Fig. 1F). This development of enhanced lung allergic responses that developed when endogenous fH was blocked confirmed its capacity to limit the magnitude of complement activation, and

![Image](http://www.jimmunol.org/)

**FIGURE 1.** Endogenous fH limits complement activation and the development of AHR after allergen challenge. A, Western blot analysis was performed to determine whether fH was present in BAL fluid. fH was detected in BAL fluid from mice sensitized and challenged with OVA and then exposed to a single provocative (secondary) allergen challenge (OVA/OVA/OVA), and the levels were significantly higher than in BAL fluid from nonsensitized, but primary and secondary challenged mice (PBS/OVA/OVA) (*p < 0.05, n = 3 for each group). B, C3a levels in the BAL fluid were measured by ELISA (n = 8 per group). Levels of C3a were significantly increased in sensitized and primary and secondary challenged mice (OVA/OVA/OVA) compared with those that were not sensitized (PBS/OVA/OVA) (*p < 0.05). When the cell surface-binding activity of endogenous fH was blocked with rH 19–20, even greater levels of C3a were generated after sensitization and primary and secondary challenge (p < 0.05 for OVA/OVA/OVA rH 19–20 versus OVA/OVA/OVA PBS, **p < 0.001 versus PBS/OVA/OVA). C3a levels were not significantly affected in the rH 19–20 group, which was sensitized and exposed to primary, but not secondary challenge (OVA/OVA/PBS rH 19–20). C, Changes in RL 48 h after secondary allergen challenge. OVA-sensitized and secondary challenged mice were treated with vehicle (OVA/OVA/OVA PBS), rH 19–20 (OVA/OVA/OVA rH 19–20), or the control peptide rH 3–5 (OVA/OVA/OVA rH 3–5), and compared with the groups treated with rH 19–20, but secondary challenged with PBS (OVA/OVA/PBS rH 19–20). Effects of the treatments on cellular composition (D) and IL-5 and IL-13 levels (E) in BAL fluid obtained 48 h after the last allergen challenge were examined. Values are expressed as mean ± SEM (n = 8). *p < 0.05 compared with OVA/OVA/OVA PBS and OVA/OVA/OVA rH 3–5, #p < 0.01 versus PBS/OVA/OVA PBS. **p < 0.001 compared with OVA/OVA/OVA PBS. D, Representative histological sections are shown stained with H&E (a, b) and PAS (c–e). Effect of the treatments on goblet cell metaplasia 48 h after secondary allergen challenge was quantified in PAS-stained sections and expressed per mm basement membrane. *p < 0.05 compared with OVA/OVA/OVA rH 3–5. Eo, eosinophils; Ly, lymphocytes; Mac, macrophages; Nt, neutrophils.
AHR and inflammation as a result of allergen sensitization and challenge.

**Targeted delivery of fH to sites of complement activation protects mice from the development of AHR**

These results also indicate that although endogenous fH limits the development of AHR and airway inflammation, the protection afforded by fH is incomplete. Given that fH is present within the airways (Fig. 1A), we hypothesized that it fails to fully prevent complement activation and the development of lung allergic responses at this location due to insufficient levels or a deficiency in its interaction with the activating surfaces. To test this hypothesis, we employed a chimeric protein that links the iC3b/C3d binding region of CR2 to the complement-regulatory region of fH (19). This fusion protein with a high affinity for iC3b/C3d would target fH-protective functions specifically to the airways where complement is activated, increasing the local complement-activating surface concentration of the complement-regulatory region of fH. In parallel, we also tested another targeted complement inhibitor, CR2-Crry, that prevents activation of both the classical and the alternative pathways on tissue surfaces (20).

CR2-fH or CR2-Crry was administered to sensitized mice 2 h prior to each of the primary OVA challenges. Unlike mice that received vehicle (PBS) alone or control protein (CR2), treatment with CR2-fH or CR2-Crry significantly prevented the development of AHR and eosinophil infiltration into the airways after allergen challenge (Fig. 2A, 2B). In addition, treatment with CR2-fH or CR2-Crry significantly reduced production of BAL Th2-type cytokines, IL-5, and IL-13. Conversely, the levels of IFN-γ were increased (Fig. 2C). Histological analyses revealed that cellular infiltration into the airway submucosal areas and goblet cell metaplasia along the airways was also reduced in mice that had received CR2-fH or CR2-Crry (Fig. 2D, 2E).

To determine whether the effects of CR2-fH were demonstrable with another allergen besides OVA, CR2-fH was also administered to mice sensitized and challenged with Rw, the most common allergen in humans. Similar to the findings in the OVA-induced allergen challenge model, CR2-fH prevented the development of

**FIGURE 2.** Targeted delivery of fH to sites of complement activation protects mice from AHR after allergen challenge. The effects of targeted complement-inhibitory molecules were determined in OVA-sensitized and (primary) -challenged mice. Mice were treated with vehicle (OVA/OVA PBS), the control protein CR2 (OVA/OVA CR2), CR2-Crry (OVA/OVA CR2-Crry), or CR2-fH (OVA/OVA CR2-fH) and compared with OVA challenge-only group (PBS/OVA). Forty-eight hours after the last OVA challenge, changes in RL were obtained in response to increasing concentrations of inhaled MCh (A). Effects of the treatments on cellular composition (B) and cytokine levels (C) in BAL fluid obtained 48 h after the last allergen challenge were evaluated. Values are expressed as mean ± SEM (n = 8). *p < 0.05 compared with OVA/OVA PBS, **p < 0.05 compared with PBS/OVA. Representative histological sections are shown stained with H&E (D) and PAS (E). a, PBS/OVA; b, OVA/OVA PBS; c, OVA/OVA CR2; d, OVA/OVA CR2-Crry; and e, OVA/OVA CR2-fH. Quantitative analysis of goblet cell metaplasia 48 h after the last allergen challenge (f). PAS-stained sections were expressed per mm basement membrane. Effect of CR2-fH treatment in Rw-sensitized and -challenged mice (Rw/Rw) on RL changes to inhaled MCh (G). Control mice were sham sensitized, followed by Rw challenge (PBS/Rw). *p < 0.05 compared with OVA/OVA or Rw/Rw PBS and OVA/OVA or Rw/Rw CR2, **p < 0.05 compared with PBS/OVA or PBS/Rw. Eo, eosinophils; Ly, lymphocytes; Mac, macrophages; Nt, neutrophils.
AHR and eosinophil inflammation in Rw-sensitized and -challenged mice (Fig. 2F, 2G).

These results directly demonstrated that fH that is targeted to the tissues with sufficient concentration and with the proper localization can reduce the development of AHR and airway inflammation. The limitations of endogenous fH thus appear to be due to the nature of its interaction with the activating surfaces in this model. Furthermore, a recombinant form of fH that is targeted to sites of complement activation was as efficacious as a complement inhibitor (CR2-Crry) that blocked both the classical and alternative pathways.

**Effects of CR2-Crry and CR2-fH on airway responses following secondary allergen challenge**

To determine the effects of CR2-fH and CR2-Crry on established allergic airway disease, mice were systemically sensitized and challenged with OVA, rested, then exposed to a single, provocative aerosolized OVA challenge (secondary allergen challenge). Following secondary allergen challenge, mice developed AHR, demonstrated more eosinophils in the airways, and had increased levels of Th2-type cytokines in the BAL fluid. When mice were treated with CR2-fH or CR2-Crry just prior to the provocative allergen challenge, significant decreases in AHR (Fig. 3A), fewer airway eosinophils (Fig. 3B), and lower levels of IL-5 and IL-13 in the BAL fluid (Fig. 3C) were detected. Also, as shown in the primary allergen challenge model, levels of IFN-γ were increased in mice that received either CR2-fH or CR2-Crry. In parallel, histological analyses revealed that cellular infiltration into the airway submucosal areas and goblet cell metaplasia along the airways were also reduced in mice treated with CR2-fH or CR2-Crry compared with vehicle-treated mice (Fig. 3D–F).

**Discussion**

A large body of data from animal models and asthmatic patients demonstrates that the complement system is activated during the development of AHR (3, 4, 7–11, 23–30). In support of a specific role for activation of the alternative pathway in the full development of altered airway function, we previously showed that therapies that specifically block this pathway prevent the development of experimental asthma (3). The mechanisms by which the alternative pathway is activated in this model have not been identified. In particular, it was unclear why fH, an effective endogenous inhibitor of the alternative pathway, did not prevent alternative pathway activation during the development of asthma.

![FIGURE 3](http://www.jimmunol.org/) Targeted delivery of complement-inhibitory proteins to sites of complement activation protects mice from AHR in a secondary allergen challenge model. The effects of complement-inhibitory molecules in established allergic airway inflammation were examined. As described in Materials and Methods, OV A-sensitized and (secondary) -challenged mice were treated with vehicle (OVA/OVA/OVA PBS), the control protein CR2 (OVA/OVA/OVA CR2), CR2-Crry (OVA/OVA/OVA CR2-Crry), or CR2-fH (OVA/OVA/OVA CR2-fH) and compared with the nonsensitized, but challenged group (PBS/OVA). Forty-eight hours after the last OVA challenge, changes in RL to MCh were examined (A). The effects of the treatments on cellular composition (B) and cytokine levels (C) in BAL fluid obtained 48 h after the last allergen challenge were evaluated. Values are expressed as mean ± SEM (n = 8). *p < 0.05 compared with OVA/OVA/OVA PBS, †p < 0.05 compared with PBS/OVA/OVA. Representative histological sections were shown stained with H&E (D) and PAS (E). a, PBS/OVA/OVA; b, OVA/OVA/OVA PBS; c, OVA/OVA/OVA CR2; d, OVA/OVA/OVA CR2-Crry; and e, OVA/OVA/OVA CR2-fH. Quantitative analysis of goblet cell metaplasia 48 h after the last allergen challenge (F). PAS-stained sections were expressed per mm basement membrane. Significant differences, *p < 0.05 compared with OVA/OVA/OVA PBS and OVA/OVA/OVA CR2, †p < 0.05 compared with PBS/OVA/OVA. Eo, eosinophils; Ly, lymphocytes; Mac, macrophages; Nt, neutrophils.
We hypothesized that the effectiveness of endogenous fH was limited, at least in part, by the nature of its interaction with activating surfaces during the development of AHR and inflammation. In the current study, we addressed this question directly by employing agents that could either enhance or block the interaction of fH with tissue surfaces.

The results demonstrated that endogenous fH did limit alternative complement pathway-mediated injury during the development of AHR, but that this protection was incomplete. We showed that fH gained access to the airways during the development of AHR, and was present in significant quantities in the BAL fluid following provocative challenge of previously sensitized and challenged mice. When the interaction of fH with tissue surfaces was blocked with rH 19–20 in mice that had established allergic airway disease, the levels of C3a in BAL fluid were significantly higher than in vehicle-treated mice, demonstrating that fH does limit complement activation on activating surfaces in the airways during the development of AHR. rH 19–20 does not block fluid-phase fH function (22), therefore, enhanced C3a generation in its presence suggests that the relevant effects are due to surface activation. Mice treated with rH 19–20 also developed a greater degree of AHR, increased infiltration of the airways with eosinophils, goblet cell metaplasia along the airways, and higher levels of IL-5 and IL-13 in the BAL fluid. Treatment with rH 19–20 without allergen challenge did not have an effect on airway responses, however, indicating that fH is not functionally necessary to control airway function or inflammation in the absence of the allergic insult.

To test whether improved targeting of fH to activating surfaces could overcome the limitations of endogenous fH, we employed a recombinant complement inhibitor that incorporates the complement-regulatory region of fH, but also contains the binding region of CR2 for tissue-fixed iC3b/C3d. In this way, the inhibitory potential of fH was modulated through direct targeting to C3 activation fragments. CR2-fH has previously been shown to provide alternative pathway-specific local control of complement activation both in vitro and in vivo (19). Functionally, treatment with this agent during primary allergen challenge significantly reduced the development of AHR, the number of eosinophils in BAL fluid, and goblet cell metaplasia in the airways. Treatment with CR2-fH also decreased the levels of the Th2 cytokines IL-5 and IL-13 in BAL fluid. Conversely, in mice treated with CR2-fH, levels of the Th1 cytokine IFN-γ were increased. Similar inhibitory effects of CR2-fH treatment on the development of AHR and eosinophilic airway inflammation were observed in RW-sensitized and -challenged mice. The overall degree of protection in mice treated with CR2-fH was equivalent to that obtained with another targeted complement inhibitor, CR2-Cry, which can control both the classical and alternative pathways. This suggests that fH, when targeted to sites of C3 cleavage, can prevent complement-mediated injury induced by allergen sensitization and challenge as effectively as a broader spectrum inhibitor that blocks both the classical and the alternative pathways.

Given the effectiveness of CR2-fH in preventing the development of AHR and airway inflammation in the primary challenge model, we also sought to determine whether this agent can be used to suppress established allergic airway inflammation induced by a single, provocative allergen challenge in mice that were previously sensitized and challenged. Similar to the findings in the primary allergen challenge model, when treated with CR2-fH at the time of provocative allergen challenge in mice with established allergic airway disease, the degree of airway responsiveness to inhaled MCh, the number of eosinophils, and the levels of Th2-type cytokines in BAL fluid were significantly decreased, whereas IFN-γ levels in BAL fluid were increased. As with the primary model, CR2-fH was equally effective as CR2-Cry at controlling these outcomes. These results demonstrated that the CR2-fH was effective at controlling allergen-induced exacerbation of lung allergic responses.

These findings highlight three important points: 1) the limitations of endogenous fH to completely prevent alternative complement pathway-mediated injury in the primary and secondary challenge models were primarily due to deficiencies in the delivery of the molecule to the site where it can function most effectively; 2) targeted complement inhibitors can effectively prevent the full spectrum of allergen-induced changes in AHR, airway inflammation, and Th2 cytokine production; and 3) therapeutic complement inhibitors can ameliorate injury both in the primary and secondary models of AHR. The iC3b/C3d binding region of CR2 has been successfully used to develop several targeted complement inhibitors (19, 20, 31, 32). These targeted inhibitors appear to work at lower concentrations than untargeted complement inhibitors and may pose a lower risk of infection (22). The efficacy of these agents in both the primary and secondary models of asthma was particularly informative, establishing the role of the alternative pathway of complement activation in asthma and the potential for therapeutic intervention.

Mutations in fH have been associated with the development of several diseases, including age-related macular degeneration, atypical hemolytic uremic syndrome, and membranoproliferative glomerulonephritis type 2 (15). Even in the presence of fully functional fH, however, uncontrolled alternative pathway activation contributes to several different diseases, including asthma (33). The inability of endogenous fH to limit complement activation on the disparate tissues involved in these diseases may be due to the nature of the interaction of fH with the activating surfaces in these diseases. Endogenous fH contains C3b binding regions, but protection of a given surface by fH is also believed to depend upon fH interacting with other surface molecules, such as sialic acid (12, 14). Thus, the relative degree of activation of the alternative pathway on a particular surface appears to be a function of the interaction between fH and the repertoire of molecules expressed by that surface. It is also possible that the failure of endogenous fH to fully prevent alternative pathway activation in these models was due to an insufficient concentration of fH within the airways. Nevertheless, the systemic administration of CR2-fH provided even greater protection from alternative pathway-mediated injury than that provided by the endogenous fH.

We describe the effectiveness of two CR2-targeted complement inhibitors in preventing the development of AHR and airway inflammation in both primary and secondary models of experimental asthma. The targeted delivery of fH was as effective in these models as the targeted delivery of Cry, indicating that the complement-regulatory region of endogenous fH was capable of preventing complement-mediated injury in this disease when directed to sites of complement activation. CR2-fH may have fewer side effects than CR2-Cry because it does not block the classical pathway of complement. Nevertheless, the efficacy of both CR2-fH and CR2-Cry in these models identified the general effectiveness of using CR2 to target complement inhibitors to sites of complement activation. Future approaches could include the targeted delivery of complement-regulatory proteins to specific molecular targets expressed within the lung (34). Given some of the limitations and concerns with current therapies, targeted complement-inhibitory therapies hold promise in the treatment of severe asthma.
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Disclosures
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