

EXPERT OPINION

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Eculizumab for treatment of asthma

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Introduction: Asthma is an inflammatory disease, which can be exacerbated by stimuli such as viral infections and exposure to allergens. Asthma continues to be a profound public health problem due to asthma exacerbation in a low proportion of patients in need of more effective medications.

Areas covered: The C5 complement pathway has been proposed as a new target for the treatment of asthma, supported by clinical observations of increased C5a levels in asthmatic airways, constitutive expression of C5 receptors on bronchial epithelium and smooth muscle cells, and preclinical studies in mice demonstrating inhibition of C5 cleavage reduced established airway inflammation and improves lung function. Eculizumab is a monoclonal antibody, which binds to the complement protein C5, thereby preventing the formation of C5a and C5b-9. The research discussed in this review describes development of eculizumab for the treatment of paroxysmal nocturnal hemoglobinuria and the efficacy of eculizumab on allergen-induced asthmatic responses in a placebo-controlled study.

Expert opinion: In an allergen-challenge model of asthma, there was a significant period effect with eculizumab, with inhibition of the late asthmatic response in subjects who received placebo treatment first. Although this study provides some evidence that eculizumab may be effective to attenuate allergen-induced responses, the role of C5 in asthma remains to be clarified.

Keywords: allergen challenge, allergic asthma, C5 antibody, late asthmatic response

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1. Introduction

1.1 Allergic asthma

Asthma is an inflammatory disease, which is characterized by variable airflow obstruction, airway hyperresponsiveness (AHR) and airway inflammation. Symptoms can be triggered by exercise, viral infections, exposure to allergens and/or inhaled chemical irritants [1]. Exposure to allergens, the most extensively studied mechanism, is a significant cause of asthma development [2] and has been linked to asthma severity [3]. Sensitivity to allergens such as house dust mite, cat, dog and mold spores is associated with an increased risk of asthma [3]. Thus the immunological response to allergens is a significant focus of asthma research.

Links have been made between the physiological responses after allergen challenge and cellular mechanisms. A correlation of airway inflammatory cells and physiological responses (forced expiratory flow rate and AHR) has been observed in asthmatics post-allergen challenge [4]. Increased circulating IgE and AHR predict the presence of the early asthmatic response [5], whereas the influx of eosinophils into the airway correlates with the late asthmatic response and AHR [5]. While early studies have formed the framework for studying the cellular mechanisms involved in the pathogenesis of allergic asthma, the cellular targets have evolved over the years.

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Box 1. Drug summary.

Drug Name (Generic)	Eculizumab
Phase (for indication under discussion)	Phase II
Indication (specific to discussion)	Asthma
Pharmacology description/mechanism of action	Humanized monoclonal antibody to C5 (C5 blockade)
Route of Administration	Intravenous infusion
Pivotal trial(s) – please name and describe	Allergen challenge, proof of concept

1.2 Complement pathway

The complement system is an enzyme cascade activated by recognition of foreign substances and thereby protects the body against pathogens. Despite the well-documented protective role, the complement system has also been implicated in several inflammatory diseases, including sepsis [6], multiple sclerosis [7] and asthma [8].

The complement system includes numerous soluble and membrane-bound receptors along with associated plasma proteins and glycoproteins [9], and occurs through three possible pathways: the classical, the alternative and the lectin pathway, to produce several complement proteins; C5, the focus of this review and the target for eculizumab (Box 1), is cleaved into terminal complement proteins, C5a and C5b-9, which contribute to the pathogenesis of asthma (Figure 1).

1.3 The C5 complement pathway and allergic asthma

Clinical observations suggest that C5 plays a significant role in the development of allergies and asthma. In a Japanese population, a single nucleotide polymorphism in the gene encoding for the C5 protein in both adults and children is associated with protection against bronchial asthma [10]. Further evidence for asthma risk associated with the C5 gene came from genome-wide screening studies that identified links between asthma-related traits to both the C5 and C5a receptor chromosomal regions [10,11].

Mediator measurements in airway samples from asthmatic patients also support the view that the complement pathway is active in allergic asthma. When compared with healthy controls, asthmatic subjects have increased levels of C5a in bronchoalveolar lavage (BAL) fluid [12]. In a challenge model, the levels of C5a increase in BAL fluid 24 h after segmental allergen challenge in subjects with asthma [13] and correlate with eosinophils in BAL [13]. This relationship is also demonstrated in the whole lung allergen inhalation model, where increased levels of C5a measured in sputum of allergic asthmatic subjects 24 h post allergen correlate positively with sputum eosinophil numbers [14]. Clinically, the increased nasal symptoms following nasal allergen challenges are associated with increased levels of C5a in nasal lavage fluid in subjects with allergic rhinitis [15], and the severity of clinical symptoms in ragweed-allergic patients studied during a pollen season

positively correlates with the generation of complement activation products measured in serum [16]. These observational studies support further investigation of C5a and its role in allergic asthma.

1.4 Mechanisms of C5a in allergic asthma

The C5 protein is produced in normal human serum in a dose-dependent manner in the presence of house dust mite extract [17], and one of the additional pathways proposed for activation of the complement cascade, relevant to allergic disease, is through proteolytic cleavage of C5 to C5a by proteases found in allergen extracts [18]. Studies examining the role of the complement protein C5a in asthma have described it as a mediator of inflammation. C5a receptors are constitutively expressed on bronchial epithelium and smooth muscle cells in the human lung [19], providing a pathway for signaling in response to C5a in airways.

C5a was originally described as a classical anaphylatoxin because it induced the secretion of histamine from mast cells [20], but it is also recognized as a potent chemoattractant of neutrophils, eosinophils [21,22] and macrophages [22]. Furthermore, C5a is a pleiotropic molecule that can modulate the activity of all cells of the myeloid lineage (Figure 2) [23-25], including IgG-mediated histamine release from basophils [26], release of MMP-9 from human neutrophils and cysteinyl-leukotrienes from human lung fragments [27,28], and thus enriches the pro-inflammatory milieu in affected tissue.

Intratracheal administration of anaphylatoxin C5a in rats potentiates antigen-induced late-phase responses and the accompanying inflammation in the bronchial submucosal tissue; these responses were mediated in part through the prolonged production of cysteinyl-leukotrienes [29]. Addition of an antibody to the C5a receptor inhibits cell activation, including the release of histamine from basophils [26], suggesting that blockade of C5a signaling may effectively suppress various pro-inflammatory cellular responses to C5a.

1.5 Effects of C5 blockade in animal models of asthma

Studies in animal models demonstrate that inhibition of C5 cleavage can reduce established inflammation of the airways and improve lower airway function. Various complement inhibitors evoke markedly reduced AHR and airway inflammation in rodent models of asthma [30,31]. In particular, studies targeting the C5 system of mice post-antigen sensitization demonstrate suppression of the allergic response to challenges with house dust mite, OVA and *Aspergillus fumigatus* [27,32]. Findings from a comprehensive study by Peng *et al.*, which blocked C5 following antigen sensitization of mice suggest that C5 significantly contributes i) to the development of airway responses and inflammation during an initial allergen provocation challenge, ii) to AHR in mice with established airway inflammation and iii) to the inflammatory mediators produced by neutrophils and eosinophils [27]. Additional studies by Köhl *et al.* confirm and extend these findings,

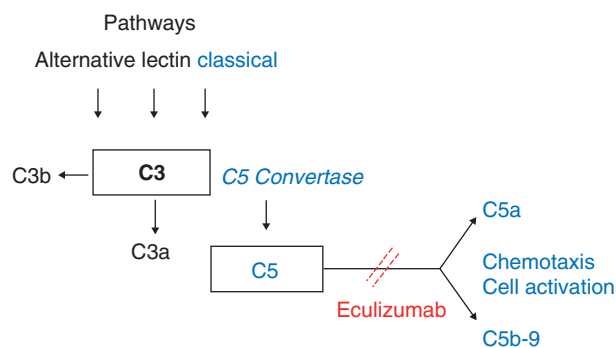


Figure 1. Antigen-induced activation of the complement cascade through the classical pathway leads to accumulation and activation of inflammatory cells in the asthmatic airways.

demonstrating firstly that C5a receptor blockade during established inflammation indeed suppresses cardinal features of asthma, whereas C5a receptor blockade upon first exposure to inhaled allergen promotes Th2 sensitization through induction of Th2-polarized immune responses, airway inflammation and AHR [33]. Summarization of these data suggests that C5a is dominant during allergen sensitization by protecting against the development of Th2 responses, but by contrast C5a drives a Th2 response in those already sensitized. As such, the efficacy of C5 blockade for treatment of allergic disease may very well depend on the temporal implementation of treatment. Since patients with allergic asthma are already sensitized to allergens, one would hypothesize that targeting C5a as a treatment for established asthma would be beneficial.

Different models of experimental allergic asthma suggest that the small complement fragments of C3 and the anaphylatoxin C3a promote proallergic effector functions during the allergic effector phase and regulate the development of Th2 immunity during allergen sensitization [34,32]. Comparisons of C3 and C5 activity are reviewed in depth by Zhang *et al.* [35].

2. Overview of the market

2.1 Currently available therapies – the unmet needs

Corticosteroids, β -agonists and leukotriene inhibitors are most frequently used for treatment of asthma [36]. Inhaled corticosteroids (ICS) have been the gold standard anti-inflammatory treatment for asthma for several decades. Clinical trials have confirmed that ICS significantly reduce the frequency of asthma exacerbations [37]; however, the benefit of ICS treatment occurs primarily in patients with eosinophilic airway inflammation [38]. Some asthmatic patients respond poorly to ICS [39,40], especially those with predominantly neutrophilic airway inflammation [41]. The safety of long-acting β -agonists (LABA) in asthmatic patients has been carefully tracked following a publication that demonstrated increased asthma-related deaths in patients with salmeterol added to

their regular therapy [42]. A large meta-analysis has concluded that salmeterol monotherapy is associated with increased mortality [43], yet when combining LABA with ICS is safe and more effective than ICS alone [43]. Leukotriene inhibitors have fewer side effects than corticosteroids [36]; however, they are less effective than inhaled corticosteroids alone [44] and less effective in combination with ICS than LABA [45].

Currently, available asthma therapies are effective for the majority of patients; however, asthma continues to be a profound public health problem due to exacerbation of asthma in a small population of steroid-resistant patients who are not effectively controlled with inhaled or oral corticosteroid treatment. New classes of therapeutic agents developed for moderate-to-severe asthmatic patients have been met with limited success, in part due to the complexity of the pathogenesis and lack of complete understanding of key mechanisms of the disease.

2.2 New compounds for treatment of asthma

Pharmaceutical companies continue to test new compounds to fill the unmet need in asthma treatment. Omalizumab is a humanized anti-IgE monoclonal antibody approved for use in for patients with severe uncontrolled allergic asthma [46]. This drug targets circulating IgE and prevents cross-linking of IgE receptors on cell surfaces. Implementation of this therapeutic approach has fueled clinical development of small molecules and biological therapies that target very specific components of the immune response.

Anti-cytokine approaches for treatment of asthma include monoclonal antibodies that block interleukin (IL)-5 or the receptor for IL-5. The IL-5 signaling pathway is critical for the terminal differentiation of eosinophils and thus the development of eosinophilic airway inflammation. Although blocking IL-5 has not been clinically beneficial in a broad population of asthmatics [47], it has proved to be effective in patients with refractory eosinophilic asthma by reducing exacerbations, improving quality of life and allowing doses of oral steroids to be reduced [48,49]. Antisense therapy with ASM8, which also targets eosinophils by blocking signaling by IL-5, IL-3 and granulocyte macrophage colony-stimulating factor (GM-CSF), significantly inhibits the allergen-induced late asthmatic response, AHR and airway levels of mature and immature eosinophils in subjects with mild allergic asthma [50]. Thus, investigational drugs aimed at interfering with the differentiation and function of eosinophils, and probably also basophils, have shown promising results in clinical trials of carefully selected patients. Following extensive evidence supporting a major role for the IL-13 pathway in the pathobiology of asthma, several small-molecule inhibitors and monoclonal antibodies have been developed to block the signaling of IL-4 and IL-13. IMA-638, a monoclonal antibody to IL-13, and pitrakinra, an interleukin-4 variant, were both shown to reduce the allergen-induced late-phase response [51,52] in subjects with mild asthma. AMG-317, a monoclonal antibody to the IL-4R α , improved the number and time to exacerbation in moderate-to-severe asthma [53]. These anti-cytokine approaches

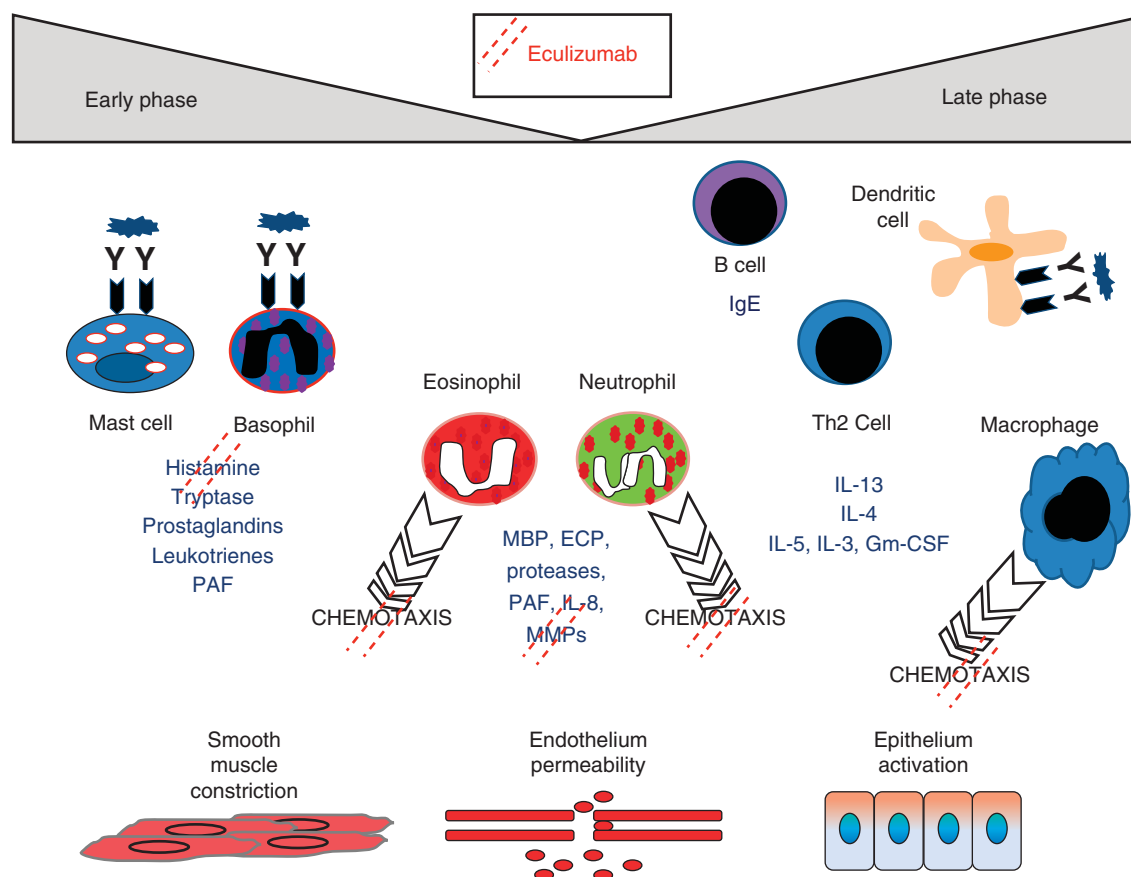


Figure 2. Cross-linking of high-affinity IgE receptors on mast cells, basophils and dendritic cells by antigen leads to activation of airway inflammatory cells. The development of allergen-induced early- and late-phase bronchoconstriction through activation of C5 receptor-bearing inflammatory cells can be modulated by eculizumab.

are still in clinical development and, therefore, not approved at this time for treatment of asthma.

2.3 Introduction to the compound

A limited number of compounds have been developed to interfere with the complement protein C5 pathway in humans. Eculizumab is the first approved drug that specifically targets complement activation. Eculizumab is a monoclonal antibody that specifically binds to the complement protein C5, preventing its cleavage and thereby arresting the complement cascade by preventing the formation of the terminal complement proteins, C5a and C5b-9. Eculizumab was developed for treatment of paroxysmal nocturnal hemoglobinuria (PNH), which is a disorder of the hemopoietic stem cells, known for its triad of clinical manifestations: intravascular hemolysis, thrombosis and reduced hematopoiesis [54].

2.4 Chemistry, pharmacokinetics and pharmacodynamics

Eculizumab is an IgG kappa immunoglobulin with a hybrid of IgG2 and IgG4 Fc portion. As such, it behaves only as a blocking antibody that prevents the cleavage and thus

activation of C5, with no other downstream activity [55]. Eculizumab specifically binds to the complement protein C5 with high affinity and remains bound to C5 until the complex is removed from the circulation. The drug information label shows that eculizumab has a half-life of 272 – 291 h and volume of distribution of 6.1 – 7.7 liters, depending on the population of patients studied. In PNH, eculizumab restores terminal complement regulation in the blood [56]. Complement complex 5b-9a is also known to induce potent biological responses including tissue injury, chemotaxis, inflammation and anaphylaxis [20-23]. Through blocking these inflammatory processes, eculizumab was tested in asthmatic subjects for effects on the allergen-induced late-phase response [57].

2.5 Clinical efficacy

In the first placebo-controlled study using a small group of patients with active PNH [56], eculizumab treatment significantly prevented intravascular hemolysis, stabilized hemoglobin levels and reduced the need for blood transfusions. A subsequent open-label, non-placebo-controlled Phase III clinical safety and efficacy study evaluated eculizumab in a

broader PNH patient population [58] and confirmed that eculizumab is very effective in the treatment of intravascular hemolysis and all its sequelae. Excitingly, eculizumab has revolutionized the therapeutic approach to hemolytic PNH [59].

In 2007, eculizumab received accelerated approval by the US Food and Drug Administration and the European Medicines Agency (EMA) for the treatment of patients with PNH to reduce hemolysis. More recently, the eculizumab was studied in an open-label, 12-week Phase II registration study in 29 Japanese patients [60] and in a 24-week study in six Korean patients [61], both studies demonstrated that eculizumab is effective, safe and well tolerated in Asian populations of patients with PNH. The approval of eculizumab as a first-in-class complement inhibitor for the treatment of PNH has validated the concept of complement inhibition as a therapy and provided rationale for investigation of other indications in which complement plays a role, such as macular degeneration [62,63], rheumatoid arthritis [64], atypical hemolytic uremic syndrome [65] and asthma [57].

Presently, targeting the complement protein C5 pathway in patients with asthma has been conducted in only two clinical trials. Patients receiving an oral dose of a C5aR antagonist (NGD-2000-1) did not show any improvement in lung function, as outlined in a review by Monk *et al.* [66]. However, in a separate study of allergic asthma provoked by allergen inhalation, a single infusion of eculizumab showed promising effects on the late-phase asthmatic response [57]. In this study, 19 subjects demonstrating allergen-induced bronchoconstriction were recruited in placebo-controlled crossover study to evaluate the effects of eculizumab on the late-phase asthmatic response. Eculizumab was administered as a single infusion 24 h before the allergen challenge, with a minimum 4-week washout between treatment periods. A 4-week washout of drug from the circulation was thought to be sufficient based on the established half-life of eculizumab of approximately 11 days. The two to three half-lives of loss of activity during the washout period would reduce the concentration of eculizumab below the level required to inhibit C5. To this end, normal circulating complement activity was demonstrated before crossing over into the second treatment arm. The findings, however, demonstrated a significant period effect, which invalidated the primary analysis. A post hoc analysis demonstrated significant attenuation of the late-phase asthmatic response by eculizumab in group 1 subjects who received placebo in the first treatment arm and eculizumab in the second treatment arm. Attenuation of the late response was not observed in group 2 subjects who received eculizumab in the first treatment arm and placebo in the second treatment arm [57]. This pattern could be explained if the eculizumab administered to group 2 in the first treatment arm was effective and still active in the airways during the placebo arm of the study, resulting in no difference between active drug and placebo treatment. As such, it is tempting to speculate that eculizumab was not effectively washed out, and this warrants further investigation in clinical trial with a more appropriate

study design. To date, no additional studies of eculizumab in asthma have been reported.

2.6 Safety and tolerability

The safety database for eculizumab in 2007 included 196 adult patients with PNH. Significant findings included the development of human antihuman antibody responses in three patients and serious meningococcal infections in three patients [67]. Increased susceptibility to infection with encapsulated bacteria such as *Neisseria meningitidis* may be a potential adverse effect since eculizumab inhibits late components of complement activation (C5b-9) and thus the formation of membrane attack complex, as well as inhibition of neutrophil and monocyte function. As such, it was recommended that patients receiving eculizumab undergo meningococcal vaccination at least 2 weeks prior to receiving the first treatment, with revaccination according to medical guidelines. More recently, eculizumab has been shown to be safe and well tolerated in Asian populations of patients with PNH [60,61] and mild allergic asthma [57].

2.7 Regulatory affairs

Eculizumab, marketed as Soliris®, is approved in the US, European Union, Japan and other countries as the first and only treatment for patients with PNH. Alexion reported approximately 50% increase in total revenues from net product sales of eculizumab over the last year reflecting steady addition of new patients with PNH. In addition to PNH, eculizumab has very recently been approved by the US Food and Drug Administration as the first and only treatment indicated for patients with atypical hemolytic uremic syndrome (aHUS) [65]. This approval together with optimism of a favorable decision by the European Committee for Medicinal Products for Human Use to include the treatment of pediatric and adult patients with aHUS in the European Union, it is not surprising that this breakthrough approach in complement inhibition has received some of the pharmaceutical industry's highest honors.

3. Conclusion

Due to the high prevalence of allergic asthma, the immunological response to allergens is a significant focus of asthma research. Clinical observations have suggested that the complement protein C5 plays a significant role in the development of allergies and asthma. Furthermore, the terminal complement protein, C5a, is recognized as a mediator of inflammation in asthma, supported by studies in animal models of asthma and by cellular and airway responses to C5a blockade. Despite the efficacy of current therapies in the majority of asthmatic patients, asthma control in moderate to severe asthmatic patients has been met with limited success. Targeting the complement C5 pathway in patients with asthma has been conducted in very limited number of clinical trials; one of the trials demonstrates attenuation of the

allergen-induced late asthmatic response in one group of subjects treated with eculizumab; however, the results are difficult to interpret with certainty due to a significant period effect observed during the trial. Eculizumab has a favorable safety profile, making it attractive for further evaluation in asthma.

4. Expert opinion

Eculizumab is a humanized antibody to C5, which has been evaluated in only one clinical trial of allergic asthmatic subjects. The results from this trial demonstrate a clear period effect, with efficacy of eculizumab in the group of subjects receiving placebo in the first treatment period, and no effect in the group of subjects receiving eculizumab in the first treatment period of the crossover design study. The results of this trial are not definitive; however, one interpretation for the observed period effect supports the notion that the biological effect of eculizumab is still present in the airways 4 weeks after dosing. If this is the case, and eculizumab is indeed effective, this therapy could provide a substantially longer duration of action than the best of the current asthma therapies, offering clear advantages over daily dosing, which is vulnerable to poor patient compliance. Whether or not this interpretation is correct will require additional trials with suitable study designs. While early studies in mouse models were controversial as to whether C5 blockade would improve or worsen asthma, these limited studies in humans have given no reason to believe the latter.

This research holds the potential for a new approach to treating asthma. Admittedly, asthma is a very complex disease for which a pathway critical to all asthma phenotypes has not yet been identified. The complement protein C5 is a unique target, and thus it is conceivable that C5 blockade by eculizumab could be effective in the population of patients not well controlled with inhaled steroids or that it may be complementary to therapies that are in use. Eculizumab is currently formulated for intravenous injection for treatment of the systemic diseases PNH and aHUS. Targeted delivery of eculizumab to the airways using an inhaled formulation may be interesting to evaluate.

At this time it is unclear whether eculizumab will continue in clinical development for treatment of asthma. Any future studies will require careful consideration of prolonged biological effects in tissue despite evidence of wash-out from the circulation and normalization of complement activity. An investigation of this nature could readily be accomplished using a parallel-group study design and proof-of-concept asthma model. Although there is some evidence that eculizumab may be effective to attenuate allergen-induced responses, the role of C5 in asthma remains to be clarified.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

Bibliography

Papers of special note have been highlighted as either of interest (●) or of considerable interest (●●) to readers.

- Public Health Agency of Canada. Life and Breath: Respiratory Disease in Canada. Public Health Agency of Canada; Ottawa: 2007
- Sporik R, Holgate ST, Platts-Mills TA, Cogswell JJ. Exposure to house-dust mite allergen (Der p I) and the development of asthma in childhood. A prospective study. *N Engl J Med* 1990;323:502-7
- Nelson HS. The importance of allergens in the development of asthma and the persistence of symptoms. *J Allergy Clin Immunol* 2000;105:S628-32
- Gauvreau GM, Watson RM, O'Byrne PM. Kinetics of allergen-induced airway eosinophilic cytokine production and airway inflammation. *Am J Respir Crit Care Med* 1999;160:640-7
- Cockcroft DW, Hargreave FE, O'Byrne PM, Boulet LP. Understanding allergic asthma from allergen inhalation tests. *Can Respir J* 2007;14:414-18
- Ward PA. The dark side of C5a in sepsis. *Nat Rev Immunol* 2004;4:133-42
- Ffrench-Constant C. Pathogenesis of multiple sclerosis. *Lancet* 1994;343:271-5
- Hawlish H, Wills-Karp M, Karp CL, Kohl J. The anaphylatoxins bridge innate and adaptive immune responses in allergic asthma. *Mol Immunol* 2004;41:123-31
- Guo RF, Ward PA. Role of C5a in inflammatory responses. *Annu Rev Immunol* 2005;23:821-52
- Hasegawa K, Tamari M, Shao C, et al. Variations in the C3, C3a receptor, and C5 genes affect susceptibility to bronchial asthma. *Hum Genet* 2004;115:295-301
- The Collaborative Study on the Genetics of Asthma (CSGA). A genome-wide search for asthma susceptibility loci in ethnically diverse populations. *Nat Genet* 1997;15:389-92
- Teran LM, Campos MG, Begishvilli BT, et al. Identification of neutrophil chemotactic factors in bronchoalveolar lavage fluid of asthmatic patients. *Clin Exp Allergy* 1997;27:396-405
- Krug N, Tschernig T, Erpenbeck VJ, et al. Complement factors C3a and C5a are increased in bronchoalveolar lavage fluid after segmental allergen provocation in subjects with asthma. *Am J Respir Crit Care Med* 2001;164:1841-3
- Strinich TX, Wang Y, Watson RM, et al. Levels of C5a increase in sputum following allergen challenge. *Am J Respir Crit Care Med* 2007;175:A835
- Andersson M, Michel L, Llull JB, Pipkorn U. Complement activation on the nasal mucosal surface—a feature of the immediate allergic reaction in the nose. *Allergy* 1994;49:242-5
- Goncz Z, Varga L, Hidvegi T, et al. The severity of clinical symptoms in ragweed-allergic patients is related to the extent of ragweed-induced complement activation in their sera. *Allergy* 1997;52:1110-14
- Nagata S, Glovsky MM. Activation of human serum complement with allergens. I. Generation of C3a, C4a, and C5a and induction of human neutrophil aggregation. *J Allergy Clin Immunol* 1987;80:24-32
- Maruo K, Akaike T, Ono T, et al. Generation of anaphylatoxins through proteolytic processing of C3 and C5 by house dust mite protease. *J Allergy Clin Immunol* 1997;100:253-60
- Drouin SM, Kildsgaard J, Haviland J, et al. Expression of the complement anaphylatoxin C3a and C5a receptors on bronchial epithelial and smooth muscle cells in models of sepsis and asthma. *J Immunol* 2001;166:2025-32
- Johnson AR, Hugli TE, Muller-Eberhard HJ. Release of histamine from rat mast cells by the complement peptides C3a and C5a. *Immunology* 1975;28(6):1067-80
- Snyderman R, Phillips J, Mergenhagen SE. Polymorphonuclear Leukocyte Chemotactic activity in rabbit serum and guinea pig serum treated with immune complexes: evidence for C5a as the major chemotactic factor. *Infect Immun* 1970;1:521-5
- Morita E, Schroder JM, Christophers E. Differential sensitivities of purified human eosinophils and neutrophils to defined chemotaxins. *Scand J Immunol* 1989;29(6):709-16
- Becker EL. The relationship of the chemotactic behavior of the complement-derived factors, C3a, C5a, and C567, and a bacterial chemotactic factor to their ability to activate the proesterase 1 of rabbit polymorphonuclear leukocytes. *J Exp Med* 1972;135:376-87
- Hook WA, Siraganian RP, Wahl SM. Complement-induced histamine release from human basophils. I. Generation of activity in human serum. *J Immunol* 1975;114:1185-90
- McCarthy K, Henson PM. Induction of lysosomal enzyme secretion by alveolar macrophages in response to the purified complement fragments C5a and C5a des-arg. *J Immunol* 1979;123:2511-17
- Kikuchi Y, Kaplan AP. A role for C5a in augmenting IgG-dependent histamine release from basophils in chronic urticaria. *J Allergy Clin Immunol* 2002;109:114-18
- Peng T, Hao L, Madri JA, et al. Role of C5 in the development of airway inflammation, airway hyperresponsiveness, and ongoing airway response. *J Clin Invest* 2005;115:1590-600
- **C5 was shown to contribute to initiation of airway inflammation, maintenance of airway hyperresponsiveness and ongoing airway responses to allergen provocation.**
- McKinley L, Kim J, Bolgos GL, et al. Allergens induce enhanced bronchoconstriction and leukotriene production in C5 deficient mice. *Respir Res* 2006;7:129
- Kodani M, Sakata N, Takano Y, et al. Intratracheal administration of anaphylatoxin C5a potentiates antigen-induced pulmonary reactions through the prolonged production of cysteinyl-leukotrienes. *Immunopharmacology* 2000;49(3):263-74
- Abe M, Shibata K, Akatsu H, et al. Contribution of anaphylatoxin C5a to late airway responses after repeated exposure of antigen to allergic rats. *J Immunol* 2001;167:4651-60
- Lukacs NW, Glovsky MM, Ward PA. Complement-dependent immune complex-induced bronchial inflammation and hyperreactivity. *Am J Physiol Lung Cell Mol Physiol* 2001;280:L512-18
- Baelder R, Fuchs B, Bausch W, et al. Pharmacological targeting of

- anaphylatoxin receptors during the effector phase of allergic asthma suppresses airway hyperresponsiveness and airway inflammation. *J Immunol* 2005;174:783-9
33. Kohl J, Baelder R, Lewkowich IP, et al. A regulatory role for the C5a anaphylatoxin in type 2 immunity in asthma. *J Clin Invest* 2006;116:783-96
 - **This study demonstrates a dual role of C5a; targeting the C5 pathway prior to initial allergen sensitization in murine models of allergic asthma results in enhancement of allergen-induced responses whereas targeting the C5 pathway in sensitized mice results in suppressed airway inflammation and airway hyperresponsiveness.**
34. Drouin SM, Sinha M, Sfyroera G, et al. A protective role for the fifth complement component (c5) in allergic airway disease. *Am J Respir Crit Care Med* 2006;173:852-7
35. Zhang X, Kohl J. A complex role for complement in allergic asthma. *Expert Rev Clin Immunol* 2010;6(2):269-77
36. Zdanowicz MM. Pharmacotherapy of asthma. *Am J Pharm Educ* 2007;71:98
37. Desai D, Siddiqui S, Brightling C. Can inhaled corticosteroids prevent asthma exacerbations? *Curr Opin Pulm Med* 2011;17:16-22
38. Green RH, Brightling CE, Woltmann G, et al. Analysis of induced sputum in adults with asthma: identification of subgroup with isolated sputum neutrophilia and poor response to inhaled corticosteroids. *Thorax* 2002;57:875-9
39. Strek ME. Difficult asthma. *Proc Am Thorac Soc* 2006;3:116-23
40. Leung DY, Spahn JD, Szeffler SJ. Steroid-unresponsive asthma. *Semin Respir Crit Care Med* 2002;23:387-98
41. Berry M, Morgan A, Shaw DE, et al. Pathological features and inhaled corticosteroid response of eosinophilic and noneosinophilic asthma. *Thorax* 2007;62:1043-9
42. Nelson HS, Weiss ST, Bleecker ER, et al. The salmeterol multicenter asthma research trial: a comparison of usual pharmacotherapy for asthma or usual pharmacotherapy plus salmeterol. *Chest* 2006;129:15-26
43. Weatherall M, Wijesinghe M, Perrin K, et al. Meta-analysis of the risk of mortality with salmeterol and the effect of concomitant inhaled corticosteroid therapy. *Thorax* 2010;65:39-43
44. Busse W, Raphael GD, Galant S, et al. Low-dose fluticasone propionate compared with montelukast for first-line treatment of persistent asthma: a randomized clinical trial. *J Allergy Clin Immunol* 2001;107:461-8
45. Nelson HS, Busse WW, Kerwin E, et al. Fluticasone propionate/salmeterol combination provides more effective asthma control than low-dose inhaled corticosteroid plus montelukast. *J Allergy Clin Immunol* 2000;106:1088-95
46. Walker S, Monteil M, Phelan K, et al. Anti-IgE for chronic asthma in adults and children. *Cochrane Database Syst Rev* 2006(2):CD003559
47. Flood-Page P, Swenson C, Faierman I, et al. A study to evaluate safety and efficacy of mepolizumab in patients with moderate persistent asthma. *Am J Respir Crit Care Med* 2007;176:1062-71
48. Haldar P, Brightling CE, Hargadon B, et al. Mepolizumab and exacerbations of refractory eosinophilic asthma. *N Engl J Med* 2009;360:973-84
49. Nair P, Pizzichini MM, Kjarsgaard M, et al. Mepolizumab for prednisone dependent asthma with sputum eosinophilia. *N Engl J Med* 2009;360:985-93
50. Imaoka H, Campbell H, Babirad I, et al. TPI ASM8 reduces eosinophil progenitors in sputum after allergen challenge. *Clin Exp Allergy* 2011;41:1740-6
51. Gauvreau GM, Boulet LP, Cockcroft DW, et al. The effects of IL-13 blockade on allergen-induced airway responses in mild atopic asthma. *Am J Respir Crit Care Med* 2011;183(8):1007-14
52. Wenzel S, Wilbraham D, Fuller R, et al. Effect of an interleukin-4 variant on late phase asthmatic response to allergen challenge in asthmatic patients: results of two phase 2a studies. *Lancet* 2007;370:1422-31
53. Corren J, Busse W, Meltzer EO, et al. A randomized, controlled, phase 2 study of AMG 317, an IL-4Ralpha antagonist, in patients with asthma. *Am J Respir Crit Care Med* 2010;181:788-96
54. Hillmen P, Young NS, Schubert J, et al. The complement inhibitor eculizumab in paroxysmal nocturnal hemoglobinuria. *N Engl J Med* 2006;355(12):1233-43
 - **A double-blind, randomized, placebo-controlled, multicenter, Phase III trial showing safety and efficacy of eculizumab in patients with paroxysmal nocturnal hemoglobinuria.**
55. Rother RP, Rollins SA, Mojcik CF, et al. Discovery and development of the complement inhibitor eculizumab for the treatment of paroxysmal nocturnal hemoglobinuria. *Nat Biotechnol* 2007;25:1256-64
56. Hillmen P, Lewis SM, Bessler M, et al. Natural history of paroxysmal nocturnal hemoglobinuria. *N Engl J Med* 1995;333(19):1253-8
57. Gauvreau GM, Boulet LP, Serverino B, et al. The effect of C5 inhibition by eculizumab on allergen-induced asthmatic responses in patients. *Allergy* 2009;64:1-98
 - **The only published report of eculizumab in asthma, demonstrating inhibition of the allergen-induced late-phase response in allergic subjects receiving placebo first and eculizumab second, in a crossover study. There was no difference between eculizumab and placebo in subjects receiving eculizumab first and placebo second.**
58. Brodsky RA, Young NS, Antoniolli E, et al. Multicenter phase 3 study of the complement inhibitor eculizumab for the treatment of patients with paroxysmal nocturnal hemoglobinuria. *Blood* 2007;111(4):1840-7
59. Risitano AM, Notaro R, Luzzatto L, et al. Paroxysmal nocturnal hemoglobinuria-hemolysis before and after eculizumab. *N Engl J Med* 2010;363(23):2270-2
60. Kanakura Y, Ohyashiki K, Shichishima T, et al. Safety and efficacy of the terminal complement inhibitor eculizumab in Japanese patients with paroxysmal nocturnal hemoglobinuria: the AEGIS clinical trial. *Int J Hematol* 2011;93(1):36-46
61. Kim JS, Lee JW, Kim BK, et al. The use of the complement inhibitor eculizumab (Soliris®) for treating Korean patients with paroxysmal nocturnal hemoglobinuria. *Korean J Hematol* 2010;45(4):269-74

62. Baas DC, Ho L, Ennis S, et al. The complement component 5 gene and age-related macular degeneration. *Ophthalmology* 2010;117(3):500-11
63. Lockington D, Imrie F, Gillen J, et al. Visual improvement in established central retinal vein occlusion with long-standing macular edema following systemic eculizumab treatment. *Can J Ophthalmol* 2010;45(6):649
64. Wang Y, Rollins SA, Madri JA, Matis LA. Anti-C5 monoclonal antibody therapy prevents collagen-induced arthritis and ameliorates established disease. *Proc Natl Acad Sci USA* 1995;92(19):8955-9
65. Lapeyraque AL, Fremeaux-Bacchi V, Robitaille P. Efficacy of eculizumab in a patient with factor-H-associated atypical haemolytic uremic syndrome. *Pediatr Nephrol* 2011;26(4):621-4
66. Monk PN, Scola AM, Madala P, Fairlie DP. Function, structure and therapeutic potential of complement C5a receptors. *Br J Pharmacol* 2007;152:429-48
67. Dmytrijuk A, Robie-Suh K, Cohen MH, et al. FDA report: eculizumab (Soliris) for the treatment of patients with paroxysmal nocturnal hemoglobinuria. *Oncologist* 2008;13(9):993-1000

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