**Artigo original**

# Asthma Pathogenesis
## Patogênese da Asma

*Michael C. Pynn, Catherine A. Thornton, Gwyneth A. Davies*[1]

## RESUMO

A asma resulta de uma interação complexa entre genes e meio ambiente que leva à tríade característica de obstrução variável do fluxo aéreo, hiper-responsividade e inflamação da via aérea.

Estímulos ambientais, como alérgenos e vírus, agindo em estágios críticos do desenvolvimento, promovem o início e a progressão da doença em pessoas geneticamente suscetíveis. O epitélio, ao interagir com estímulos ambientais e sinalizar para o mesênquima subjacente, direciona para o remodelamento da via aérea. Interações complexas entre subclasses de células T CD4+ efetoras, incluindo as tradicionais Th2 e as mais recentemente descobertas Th9 e Th17, células imunes e estruturais, desencadeiam inflamação e remodelamento da via aérea e são cruciais para a compreensão dos diversos fenótipos de asma. Novos fenótipos, como o de obesidade, podem ajudar a esclarecer mecanismos patogenéticos recentemente descritos na asma.

A patogênese de fenótipos distintos de asma está começando a ser descoberta; esse esclarecimento será crucial para a compreensão dessa doença complexa. O desenvolvimento de uma abordagem biológica e sistemática, integrando biologia molecular e características clínicas, poderá levar a definição de alvos terapêuticos, especialmente na asma grave, e também avançar em busca de um tratamento personalizado na asma.

**Descritores:** Asma/patologia; Asma/genética; Inflamação/patologia; Remodelação das vias aéreas; Interação gene-ambiente.

## ABSTRACT

Asthma arises from complex gene-environment interactions that drive the characteristic triad of variable airway obstruction, airway hyper-responsiveness, and airway inflammation.

Environmental stimuli (e.g., allergens and viruses), acting at critical stages of development, lead to the initiation and progression of disease in genetically susceptible individuals. The epithelium, by interacting with environmental stimuli and with the underlying mesenchyme, directs airway remodelling. Complex interactions between subsets of CD4+ effector T cells, including classical Th2 cells and the more recently discovered Th9 and Th17 cells, as well as immune and structural cells, drive airway inflammation and remodelling. Understanding such interactions is crucial to our understanding of the various asthma phenotypes. Descriptions of newer asthma phenotypes, such as the obesity phenotype, might clarify novel pathogenetic pathways in asthma.

The pathogenetic mechanisms of distinct asthma phenotypes are beginning to be unravelled; clarification will be crucial to our understanding of this complex disease. A systems biology approach integrating genetics, molecular biology, and clinical assessment is needed in order to develop targeted therapeutics, especially for patients with severe asthma, and advance toward tailored treatment of this disease.

**Keywords:** Asthma/pathology; Asthma/genetics; Inflammation/pathology; Airway remodeling; Gene-environment interaction.

1. College of Medicine, Swansea University, Swansea, United Kingdom.
The authors declare that they do not have any potential conflict of interest.

**Correspondence to:** Gwyneth A. Davies. College of Medicine. Grove Building. University of Wales Swansea, Singleton Park, Swansea, SA2 8PP, Wales, UK. Tel. 44 0 1792 513400, Fax: 44 0 1792 513430. E-mail: gwyneth.davies@gmail.com.

## INTRODUCTION

Asthma is a chronic inflammatory disease characterised by variable airway obstruction, inflammation, and airway hyper-responsiveness (AHR). The evolutionary origins of asthma are likely to lie in the advantage conferred by a vigorous Th2-cell mediated inflammatory response to parasites, which in asthma is directed against otherwise innocuous agents such as allergens (1). Complex gene-environment interactions underlie the pathogenesis of asthma, and genetic susceptibility interacts with particular environmental stimuli (e.g., allergens and viruses) at critical phases in early life.

In individuals between 5 and 25 years of age, the main predictor of asthma is atopy, which is defined as a genetic predisposition toward the development of IgE-mediated immediate hypersensitivity reactions against common environmental antigens (allergens). However, asthma is not synonymous with atopy, and the population attributable fraction for aeroallergen sensitisation in adults is estimated at 30% (2). In fact, in late-onset, "intrinsic" asthma, certain types of occupational asthma (e.g., isocyanate-induced asthma), and non-allergic asthma in children, Th2 inflammation can occur without elevating circulating levels of IgE (3). Viral infections are major factors in asthma exacerbations, and emerging evidence suggests that such infections play a role in the origins of this heterogenous disease, including the programming of dendritic cells to direct Th2 responses (4,5).

The pathogenesis of airway obstruction in asthma is characterised by the following:

• airway hyper-responsiveness (AHR), resulting in excessive smooth-muscle contraction in response to environmental stimuli

• airway inflammation, involving mast cells, eosinophils, and Th2 cells

Once asthma has become established, the airway remodelling process begins, as evidenced by goblet cell hyperplasia, reticular basement membrane thickening, and smooth-muscle hypertrophy (Figure 1).

### Airway Inflammation

It has been postulated that epithelial dysfunction renders the airways susceptible to damage inflicted by viruses, allergens, pollutants, and other insults (6,7). Bidirectional interaction between epithelial and dendritic cells is pivotal to the development of immunological tolerance or inflammation resulting from exposure of the airways to antigens (8). The sub-populations of effector T cells thus generated propagate the inflammatory response in the airways of asthma sufferers. Subsets of CD4+ effector T cells are defined by the

arsenal of cytokines they secrete, which reflect the signature expression of transcription factor profiles.

The Th1/Th2 paradigm has underpinned research into the contribution of T cells to airway inflammation for many years. Consequently, the roles that IL-4, IL-5, and IL-13 play in atopy and asthma are well documented: IL-4 promotes allergic sensitisation and IgE production; IL-5 influences the differentiation, maturation, and survival of eosinophils; and IL-13 mediates mucus production, remodelling, and AHR. However, a more extensive array of effector T-cell subsets has emerged (9).

A third major subset of CD4+ effector T cells is that composed of Th17 cells, which play roles in host defence and auto-immunity. The characteristic Th17 cytokines are IL-17A and IL-17F, and Th17 cells primarily influence neutrophil recruitment and activation (10). Interest in Th9 cells, characterised by IL-9 secretion, is also growing. Although IL-9 is considered a Th2 cytokine, a distinct population of IL-9-producing Th9 cells can arise in chronic diseases. The contribution of Th9 cells to chronic airway inflammation has only begun to be elucidated (11,12). The emergent hypothesis is that CD4+ effector T-cell subpopulations differentially contribute to asthma phenotypes. For example, Th17 cells might be more involved in neutrophilic rather than eosinophilic asthma (10).

Other key T-cell subsets postulated to play a role in airway inflammation are regulatory T (Treg) cells, including the naturally occurring CD4+CD25+FoxP3+ subset and induced adaptive CD4+ Treg cells (13,14). In
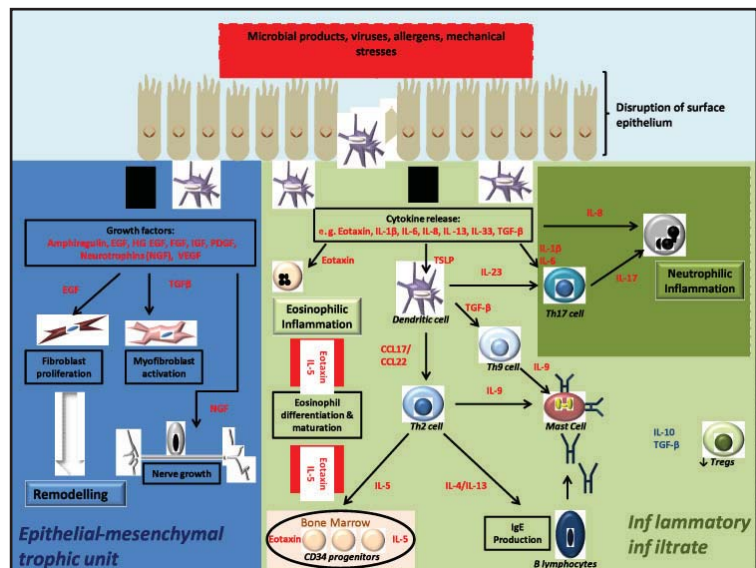


Figure 1 - Schematic representation of the epithelial-mesenchymal trophic unit and inflammatory cells that play important roles in the pathogenesis of asthma.

HB-EGF: heparin-binding EGF; CCL: chemokine (C-C motif) ligand

Note: The epithelium releases a number of growth factors important to the coordination of airway remodelling. The cytokines released promote migration and activation of various inflammatory cells. Th2 cells are pivotal to orchestrating eosinophilic inflammation and IgE production; Th9 cells might play a role in local IL-9 generation. IL-17 (produced by Th17 cells) and IL-8 could play roles in the development of neutrophilic disease. The numbers of Treg cells, which promote immunological tolerance, are decreased, as is their functionality, in asthma sufferers.

individuals without asthma, Treg cells promote immunological tolerance to aeroallergens. Changes in the number, phenotype, or function of Treg cells, including those present in the lungs, have been described in upper and lower airway disease (13,14). Early life events seem to be critical in programming immunoregulatory pathways that underpin immune homeostasis in the airways and other tissues (15,16). Immunoregulatory invariant natural killer T cells, which are reactive to CD1d-presented glycolipids, are also of interest. Such cells are found in human airways where they are relatively rare, but numbers are similar between mild/moderately severe asthma, COPD and controls (17). Additional T-cells subsets implicated in airways inflammation and asthma include CD8+ T cells and gamma-delta T cells (9).

Effector CD4+ T-cell subsets mediate their effects via the release of cytokines that then modulate, either directly or indirectly, the activity of other cell types—including eosinophils, neutrophils, and mast cells, all of which in turn augment the inflammatory response. High numbers of eosinophils are observed in the airways of many asthma patients. However, non-eosinophilic asthma occurs across a range of asthma severity, and there is particular interest in the role of neutrophils in severe asthma. The release of bioactive molecules such as histamines and leukotrienes from degranulated mast cells contributes to airway inflammation and the clinical symptoms of asthma.

The prime function of a barrier is to discriminate between relatively innocuous environmental antigens (including allergens) and infectious pathogens. Various stimuli drive cytokine production at barriers and this creates a local cytokine milieu that further informs the innate immune response and educates the adaptive immune response. Pattern recognition receptors, including Toll-like receptors (TLRs), nucleotide-binding oligomerisation domain-like receptors, and retinoic acid-inducible gene I-like receptors, are critical to that process. The repertoire of pattern recognition receptor expression differs by cell type, is developmentally programmed, and is further influenced by genetic variation and the local microenvironment. Dendritic cells are exquisitely placed, both physically and functionally, to bridge innate and adaptive immunity: they are in intimate contact with the epithelium where they can interact with environmental cues that shape the repertoire of cytokines, chemokines, and co-stimulatory molecules, which they express during antigen-specific priming of the immune response. This determines the nature of the allergen-specific CD4+ effector T-cell response generated.

Epithelium-derived cytokines and chemokines orchestrate the recruitment and activity of multiple cell types. Epithelium-derived eotaxin and IL-8 direct the recruitment of eosinophils and neutrophils, respectively, to the airways. Currently, there is much focus on IL-25, IL-33,

and thymic stromal lymphopoietin (TSLP), all of which have been implicated in allergic airway disease (and anti-helminth immune responses) but are not alone in demonstrating such properties (18). Models of airway over-expression are typically used to reveal their role but immunohistochemical and other analyses demonstrate the relevance of IL-25, IL-33, and TSLP to airway inflammation in humans. It has been shown that IL-25 plays a role in regulating IL-9 expression by CD4+ T cells (19), and IL-33 is a chromatin binding nuclear cytokine of the IL-1 family, implicated in classical Th2 cell- and mast cell-mediated asthma and anaphylaxis (20). However, IL-33 is an alarmin released by necrotic cells to recruit and activate immune cells, i.e., to signal damage and amplify the innate immune response (21). Given the recent identification of neutrophil-derived enzymes as critical to the generation of mature bioactive IL-33 (22), neutrophil infiltrates might amplify IL-33 bioavailability in the airways. For its part, TSLP can promote Th2 cytokine-associated inflammation, modulate activity of granulocyte populations, limit the expression of dendritic cell-derived proinflammatory cytokines, and promote Treg responses (23). The recently identified natural helper cells have also been shown to play a role in Th2-dependent immune response. Natural helper cells are non-B, non-T innate effector cells that are activated via IL-25 or IL-33 to promote Th2 cytokine responses and might represent an ancient evolutionary conserved pathway (24,25).

Neuro-effector mechanisms have also been implicated in airway inflammation, with loss of pre-ganglionic, auto-regulatory muscarinic 2 receptors observed after exposure to allergens and viruses. These receptors normally limit the release of the neurotransmitter acetylcholine, which causes bronchoconstriction. Non-adrenergic, non-cholinergic systems might also play a role in airway bronchoconstriction, with substance P and tachykinins causing bronchoconstriction via the natural killer cell receptors 1 and 2, respectively (18).

### The Epithelium and Remodelling

There is compelling evidence to support a fundamental role for the airway epithelium and the underlying mesenchyme (the epithelial-mesenchymal trophic unit) in the pathogenesis of asthma. It has been suggested that genetically susceptible individuals have impaired epithelial barrier function with disrupted tight junctions and defective anti-oxidant and innate immune defences (7,26). The epithelium is therefore vulnerable to viral infection in early life, conditioning immature dendritic cells to drive a Th2 "allergic" phenotype. A dysfunctional epithelium is susceptible to allergen sensitisation, and further environmental insults (e.g., exposure to viruses or pollutants), acting in concert with a susceptible genotype during the critical stages of immune system development, are critical to the development and persistence of asthma. In asthma sufferers, bronchial epithelial cells are more

susceptible to rhinovirus infection due to reduced IFN-β production and defences are restored by exogenous IFN-β (27). Susceptibility to recurrent exacerbations is associated with an O-secretor mucin glycan

phenotype (28). The concept of epithelial dysfunction provides an explanation for certain aetiological factors in asthma (Table 1) and of exacerbation in response to environmental insults such as exposure to pollutants.

Table 1 - Summary of the main environmental factors (exposures) implicated in the aetiology of asthma.

| Factor | Example(s) | Study type | Findings |
|---|---|---|---|
| Allergens | House dust mite | Prospective | Sensitisation increases asthma risk |
| | | | Early childhood exposure increases asthma risk |
| | | | Minimal threshold level of allergen exposure |
| | Cockroach allergens | Case control | Increased sensitisation in asthmatics |
| | Animal allergens: cat/dog | Prospective cohort | Exposure decreases sensitisation to other aeroallergens |
| | | | No protective effect on asthma |
| Pollutants | Nitrogen dioxide (NO$_2$) | Cross-sectional, prospective | High NO$_2$ - increased asthma prevalence |
| | | | Indoor gas stove use associated with asthma symptoms |
| | | | Proximity to roads/elevated NO$_2$ - increased asthma risk |
| | Diesel exhaust particles | Mechanistic | Diesel exhaust particles promote dendritic cell maturation |
| | | | Diesel exhaust particles cause airway epithelial activation and pro-inflammatory cytokine release |
| Viral infections | | Prospective cohort | High number of viral infections in infancy - reduced risk of asthma and atopy |
| Smoking | Active smoking | Prospective cohort | Smoking increases risk of asthma development |
| | Second hand smoking | Cross-sectional | Pre-natal maternal smoking - increased asthma risk |
| | | | Post-natal maternal smoking - increased asthma risk |
| | | | Adult passive smoking - increased physician-diagnosed asthma |
| Medication use | Antibiotic use in childhood | Meta-analysis of prospective and retrospective studies | Antibiotic use in first year of life - increased asthma risk |
| | Hormone replacement therapy (HRT) | Cross-sectional, prospective | HRT use - increased asthma incidence |
| Obesity | | Prospective | Dose-dependent association between body mass index and asthma risk |
| | | | Weight loss studies improve disease control |
| Early menarche | | Cross-sectional, longitudinal | Early menarche - higher asthma risk |
| Peri-natal | Maternal diet | Prospective cohort | ↑ Maternal vitamin E - ↓ childhood asthma risk |
| | | | ↓ Maternal vitamin D - ↑ childhood asthma risk |
| | Prematurity | Retrospective meta-analyses | Prematurity - higher asthma risk |
| | Breastfeeding | Prospective cohort | Breastfeeding for 3-6 months - reduced wheeze |

Sustained epithelial injury leads to disordered communication with the underlying mesenchyme, thereby triggering airway remodelling (29). The inhibition of epithelial repair results in the release of growth factors, including TGF-β2, which activate subepithelial fibroblasts to form myofibroblasts and promote mucous metaplasia. Myofibroblasts deposit extracellular matrix, thickening the epithelial *lamina reticularis*, and secrete mitogens causing smooth-muscle hypertrophy. Remodelling, including angiogenesis, can occur

in childhood asthma, even before a clinical diagnosis of asthma has been made (30-32).

Airway remodelling represents a crucial part of AHR in established asthma and is a major cause of fixed airflow obstruction and declining lung function in more severe asthma. Repeated airway exposure to environmental insults promotes a milieu of persistent inflammation and remodelling associated with progressive disease. Targeting therapeutics toward enhancing epithelial barrier function, as well as exploring novel

anti-inflammatory targets, presents an opportunity to advance asthma treatment.

## NEW INSIGHTS – ASTHMA PHENOTYPES AND "ENDOTYPES"

The heterogeneity of asthma is evidenced by the identification of distinct phenotypes, including the early-onset "extrinsic" allergic phenotype, the late-onset "intrinsic" eosinophilic phenotype, and the late-onset, non-eosinophilic female version of the obesity phenotype (33). Despite sharing the common defining feature of variable airflow obstruction, the underlying pathophysiology is likely to differ. Recently, there has been a move toward describing asthma "endotypes", subtypes defined by distinct pathophysiological mechanisms. Several asthma endotypes have been described, including aspirin-sensitive asthma, allergic bronchopulmonary aspergillosis, allergic adult asthma, predictive indices of asthma in childhood, late-onset eosinophilic asthma, and asthma in cross-country skiers (34).

## SEVERE ASTHMA

Severe asthma encompasses the early-onset, eosinophilic, neutrophilic, and obesity phenotypes (35). Neutrophilic asthma with no evidence of eosinophilic inflammation is often seen in individuals with severe disease on high-dose steroids. Neutrophilic asthma shows features of innate immune activation within the airways, including upregulation of TLR2 and TLR4 and soluble CD14, as well as enhanced expression of proinflammatory cytokines such as IL-1β and IL-8 (36). This asthma phenotype might also involve systemic changes in innate immune function with upregulation of genes promoting neutrophil survival observed in peripheral blood (37). Upregulation of TNF-α within the airways has also been implicated and identified as a potential therapeutic target (38). Although early studies in steroid-resistant disease, using the anti-TNF-α drug etanercept, have produced promising results (39), those results have not been replicated in larger multi-centre studies (40). Adaptive immune mechanisms, including neutrophil inflammation promoted by Th17 cells, have also been implicated (10).

## ASTHMA IN THE OBESE – A NEW ASTHMA PHENOTYPE?

The increasingly prevalent obesity phenotype of severe asthma is characterised by the absence of eosinophilic inflammation, a predominance of females, and late onset (35). Corticosteroid therapy is poorly effective in this group (41,42), which shows a dramatic improvement with weight loss measures, including bariatric surgery (43). Neutrophilic inflammation might explain the relative corticosteroid resistance observed (44). The underlying pathophysiology of the obesity phenotype is currently unknown, although several mechanisms have been suggested (Figure 2). Obesity enhances systemic inflammation through the release of pro-inflammatory cytokines (45), by increasing oxidative stress (46), or via adipocyte-derived hormones (adipokines), which have immunomodulatory effects (47). Although there is much interest in the impact that these mechanisms have on the airways, studies of the topic have produced inconsistent results. Further studies examining the interactions among obesity, asthma, and innate immune function are warranted.

The elucidation of asthma phenotypes and their pathogenesis is crucial to our understanding of this complex disease. A systems biology approach integrating genetics, molecular biology, and clinical assessment is needed in order to develop targeted therapeutics, especially for patients with severe asthma.
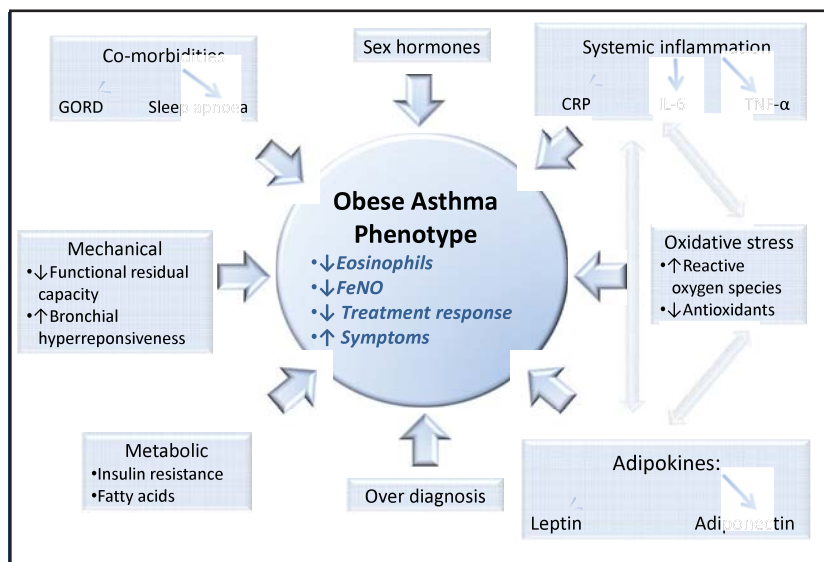


Figure 2 - Diagram illustrating the mechanisms that might link obesity and asthma.

GORD: gastro-oesophageal reflux disease; CRP: C-reactive protein.

Note: Some of these factors could interact with each other: adipokines are known to have many immunomodulatory effects and might promote reactive oxygen species generation; metabolic factors such as insulin resistance might play a role in systemic inflammation; and fatty acids might moderate inflammation via Toll-like receptor signalling.

## REFERÊNCIAS

1. Hopkin J. Immune and genetic aspects of asthma, allergy and parasitic worm infections: evolutionary links. Parasite Immunol. 2009;31(5):267-73.
2. Jaakkola MS, Ieromnimon A, Jaakkola JJ. Are atopy and specific IgE to mites and molds important for adult asthma? J Allergy Clin Immunol. 2006;117(3):642-8.
3. Mapp CE, Boschetto P, Miotto D, De Rosa E. Asthma induced by isocyanates: a model of IgE-independent asthma. Acta Biomed 2005;76(Suppl):15–19.
4. Walton RP, Johnston SL. Role of respiratory viral

infections in the development of atopic conditions. Curr Opin Allergy Clin Immunol. 2008;8(2):150-3. Review.

5.  Strickland, D. H., J. W. Upham, et al. Epithelial-dendritic cell interactions in allergic disorders. Current Opinion in Immunology 2010;22(6): 789-794.

6.  Xiao, C., S. M. Puddicombe, et al. Defective epithelial barrier function in asthma. Journal of Allergy and Clinical Immunology. 2011;128(3):549-556.

7.  Holgate ST. The sentinel role of the airway epithelium in asthma pathogenesis. Immunol Rev. 2011;242(1):205-19.

8.  Lambrecht BN, Hammad H. The role of dendritic and epithelial cells as master regulators of allergic airway inflammation. Lancet. 2010 Sep 4;376(9743):835-43.

9.  Lloyd, C. M. and E. M. Hessel. Functions of T cells in asthma: more than just TH2 cells. Nat Rev Immunol. 2010;10(12):838-848.

10. Cosmi, L., F. Liotta, et al. Th17 cells: new players in asthma pathogenesis. Allergy 2011;66(8): 989-998.

11. Soroosh, P. and T. A. Doherty. Th9 and allergic disease. Immunology 2009;127(4): 450-458.

12. Stassen, M., E. Schmitt, et al. From interleukin-9 to T helper 9 cells. Annals of the New York Academy of Sciences 2011;1247(1): 56-68.

13. Lloyd, C. M. and C. M. Hawrylowicz. Regulatory T Cells in Asthma. Immunity 2009;31(3): 438-449.

14. Robinson, D. S. Regulatory T cells and asthma. Clinical & Experimental Allergy 2009;39(9): 1314-1323.

15. Smith, M., M. R. Tourigny, et al. Children with egg allergy have evidence of reduced neonatal CD4+CD25+CD127lo/- regulatory T cell function. Journal of Allergy and Clinical Immunology 2008;121(6): 1460-1466.

16. Strickland, D. H. and P. G. Holt. T regulatory cells in childhood asthma. Trends in Immunology 2011;32(9): 420-427.

17. Vijayanand, P., G. g. Seumois, et al. Invariant Natural Killer T Cells in Asthma and Chronic Obstructive Pulmonary Disease. New England Journal of Medicine 2007;356(14): 1410-1422.

18. Finkelman, F. D., S. P. Hogan, et al. Importance of Cytokines in Murine Allergic Airway Disease and Human Asthma. The Journal of Immunology 2010;184(4): 1663-1674.

19. Angkasekwinai, P., S. H. Chang, et al. Regulation of IL-9 expression by IL-25 signaling. Nat Immunol 2010;11(3): 250-256.

20. Liew, F. Y., N. I. Pitman, et al. Disease-associated functions of IL-33: the new kid in the IL-1 family. Nat Rev Immunol 2010;10(2): 103-110.

21. Moussion, C., N. Ortega, et al. The IL-1-Like Cytokine IL-33 Is Constitutively Expressed in the Nucleus of Endothelial Cells and Epithelial Cells In Vivo: A Novel 'Alarmin'? PLoS ONE 2008;3(10): e3331.

22. Lefrancais, E., S. Roga, et al. IL-33 is processed into mature bioactive forms by neutrophil elastase and cathepsin G. Proceedings of the National Academy of Sciences 2012;109(5): 1673-1678.

23. Ziegler, S. F. and D. Artis. Sensing the outside world: TSLP regulates barrier immunity. Nat Immunol 2010;11(4): 289-293.

24. Neill, D. R., S. H. Wong, et al. Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. Nature 2010;464(7293): 1367-1370.

25. Saenz, S. A., M. Noti, et al. Innate immune cell populations function as initiators and effectors in Th2 cytokine responses. Trends in Immunology 2010;31(11): 407-413.

26. Message SD, Johnston SL. Host defense function of the airway epithelium in health and disease: clinical background. J Leukoc Biol. 2004 Jan;75(1):5-17.

27. Cakebread JA, Xu Y, Grainge C et al. Exogenous IFN-β has antiviral and anti-inflammatory properties in primary bronchial epithelial cells from asthmatic subjects exposed to rhinovirus. J Allergy Clin Immunol. 2011;127(5):1148-54.

28. Innes AL, McGrath KW, Dougherty RH et al. The H antigen at epithelial surfaces is associated with susceptibility to asthma exacerbation. Am J Respir Crit Care Med. 2011;183(2):189-94.

29. Holgate ST, Davies DE, Lackie PM, et al. Epithelial-mesenchymal interactions in the pathogenesis of asthma. J Allergy Clin Immunol. 2000 Feb;105(2 Pt 1):193-204. Review.

30. Baena-Cagnani C, Rossi GA, Canonica GW Airway remodelling in children: when does it start? Curr Opin Allergy Clin Immunol. 2007 Apr;7(2):196-200..

31. Barbato A, Turato G, Baraldo S, et al. Epithelial damage and angiogenesis in the airways of children with asthma. Am J Respir Crit Care Med. 2006;174(9):975-81.

32. Malmström K, Pelkonen AS, Malmberg LP, et al. Lung function, airway remodelling and inflammation in symptomatic infants: outcome at 3 years. Thorax. 2011;66(2):157-62.

33. Haldar P, Pavord D, Shaw DE, Berry MA, et al. Cluster analysis and clinical asthma phenotypes. American Journal of Respiratory and Critical Care Medicine, 2008;178:218-224.

34. Lotvall J, Akdis CA, Bacharier LB, Bjermer L, et al. Asthma endotypes: A new approach to classification of disease entities within the asthma syndrome. Journal of Allergy and Clinical Immunology, 2011;127: 355-360.

35. Wenzel S, Severe asthma: from characteristics to phenotypes to endotypes. Clinical and Experimental Allergy, 2012 Jan 18. doi: 10.1111/j.1365-2222.2011.03929.x.

36. Simpson JL, Grissell TV, Douwes J et al. Innate immune activation in neutrophilic asthma and bronchiectasis. Thorax 2007;(62):211-218.

37. Baines KJ, Simpson JL, Bowden NA et al. Differential gene expression and cytokine production from neutrophils in asthma phenotypes. European Respiratory Journal, 2010;35: 522-531.

38. Howarth PH, Babu KS, Arshad HS et al. Tumour necrosis factor (TNFalpha) as a novel therapeutic target in symptomatic corticosteroid dependent asthma. Thorax, 2005;60(12): 1012-1018.

39. Morjaria JB, Chauhan AJ, Babu KS, et al. The role of a soluble TNFalpha receptor fushion protein (etanercept) in corticosteroid refractory asthma: a double blind randomised placebo-controlled trial. Thorax, 2008;63:584-591.

40. Holgate ST, Noonan M, Chanez P et al. Efficacy and safety of etanercept in moderate-to-severe asthma: a randomised controlled trial. European Respiratory Journal, 2011; 37(6):1352-1359.

41. Boulet LP, Franssen E Influence of obesity on response to fluticasone with or without salmeterol in moderate

asthma. Respiratory Medicine, 2007;101: 2240-2247.

42. Peters-Golden M, Swern A, Bird SS et al. Influence of body mass index on the response to asthma controller agents. European Respiratory Journal, 2006;27:495-503.

43. Dixon AE, Prately RE, Forgione PM et al. Effects of obesity and bariatric surgery on airway hyperresponsiveness, asthma control, and inflammation. Journal of Allergy and Clinical Immunology, 2011;128(3):508-515.

44. Scott HA, Gibson PG, Garg ML et al. Airway inflammation is augmented by obesity and fatty acids in asthma. European Respiratory Journal, 2011. Sep;38(3):594-602.

45. Yudkin JS, Stehouwer CD, Emeis JJ et al. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? Arterioscler Thromb Vasc Biol, 1999;19: 972-978.

46. Kearney JF, Larson MG, Vasan RS et al. Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham study. Arteriorsclerosis, Thrombosis and Vacular Biology, 2003;23: 434-439.

47. La Cava A, Maltrese G. The weight of leptin on immunity. Nature Reviews Immunology, 2004;4: 371-379.