



Contents lists available at ScienceDirect

Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/pharmthera

Associate editor: L. Murray

Patient stratification and the unmet need in asthma

Linda Swedin¹, Tiit Saarne¹, Maria Rehnberg, Pernilla Glader, Magdalena Niedzielska, Gustav Johansson, Petra Hazon, Matthew C. Catley^{*}

Respiratory, Inflammation and Autoimmunity iMED, Translational Biology, AstraZeneca R&D Gothenburg, Sweden

ARTICLE INFO

Keywords:

Asthma
Biomarkers
Endotype
Patient stratification
Phenotype
Asthma therapy

ABSTRACT

Asthma is often described as an inflammatory disease of the lungs and in most patients symptomatic treatment with bronchodilators or inhaled corticosteroids is sufficient to control disease. Unfortunately there are a proportion of patients who fail to achieve control despite treatment with the best current treatment. These severe asthma patients have been considered a homogeneous group of patients that represent the unmet therapeutic need in asthma. Many novel therapies have been tested in *unselected asthma* patients and the effects have often been disappointing, particularly for the highly specific monoclonal antibody-based drugs such as anti-IL-13 and anti-IL-5. More recently, it has become clear that asthma is a syndrome with many different disease drivers. Clinical trials of anti-IL-13 and anti-IL-5 have focused on biomarker-defined patient groups and these trials have driven the clinical progression of these drugs. Work on asthma phenotyping indicates that there is a group of asthma patients where T helper cell type 2 (Th2) cytokines and inflammation predominate and these type 2 high (T2-high) patients can be defined by biomarkers and response to therapies targeting this type of immunity, including anti-IL-5 and anti-IL-13. However, there is still a subset of T2-low patients that do not respond to these new therapies. This T2-low group will represent the new unmet medical need now that the T2-high-targeting therapies have made it to the market. This review will examine the current thinking on patient stratification in asthma and the identification of the T2-high subset. It will also look at the T2-low patients and examine what may be the drivers of disease in these patients.

© 2016 Elsevier Inc. All rights reserved.

1. Introduction

According to the Global Initiative for Asthma (GINA), asthma is defined in the following way:

“Asthma is a heterogeneous disease, usually characterized by chronic airway inflammation. It is defined by the history of respiratory

symptoms such as wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity, together with variable expiratory airflow limitation” (GINA, 2016).

This definition was changed in 2014 to include the statement that asthma is a heterogeneous disease. This change is in part driven by clinical experience from the monoclonal antibody trials for anti-interleukin (IL)-5, anti-IL-13 and anti-IL-4 receptor α (anti-IL-4R α), which demonstrated that these highly specific therapies are generally more efficacious in groups of patients selected on the bases of target mechanism or pathway activation.

Classically, asthma was thought of as a T helper cell type 2 (Th2)-driven disease and this has driven the development of therapies targeting this biology. In this review, we will use the emerging terminology of type 2 (T2) asthma, taking into consideration that other cells, such as group 2 innate lymphoid cells (ILC2s) may contribute to the immune responses involved (Barnes, 2001; McKenzie, 2014; Fahy, 2015). Importantly, recent work indicates that asthma has both a T2-high and a T2-low phenotype (Woodruff et al., 2009). The underlying biology and key pathological drivers in the T2-low patients are poorly understood meaning that there are few therapies being developed to treat these patients (Moore et al., 2010). This review will discuss current thinking on the pathology of asthma and the methods used to define stratified groups of patients. In addition, it will make the case for more work to understand the T2-low asthma group and define where the unmet

Abbreviations: ACOS, asthma and chronic obstructive pulmonary disease overlap syndrome; ACQ, Asthma Control Questionnaire; AHR, airway hyperresponsiveness; ASM, airway smooth muscle; BAL, bronchoalveolar lavage; BHR, bronchial hyperresponsiveness; CLCA1, chloride channel, calcium activated family member 1; COPD, chronic obstructive pulmonary disease; CRTh2, chemoattractant receptor-homologous molecule expressed on Th2 cells; cysLTs, cysteinyl leukotrienes; DCs, dendritic cells; DPP-4, dipeptidyl peptidase-4; FeNO, fractional exhaled nitric oxide; FEV1, Forced Expiratory Volume in 1 s; GINA, Global Initiative for Asthma; GM-CSF, granulocyte macrophage colony-stimulating factor; HRV, human rhinovirus; ICSs, inhaled corticosteroids; IFN, interferon; Ig, immunoglobulin; IL, interleukin; ILCs, innate lymphoid cells; LABA, long-acting beta-agonist; LTRA, leukotriene receptor antagonist; PEF, peak expiratory flow; PEFR, peak expiratory flow rate; PGD₂, prostaglandin D₂; POSTN, periostin, osteoblast specific factor; SARP, severe asthma research program; serpinB2, serine peptidase inhibitor, clade B, member2; STAT, signal transducer and activator of transcription factor; T2, type 2; Th2, T helper cell type 2; TLR, Toll-like receptor; TNF, tumor necrosis factor; TSLP, thymic stromal lymphopoietin.

^{*} Corresponding author at: AstraZeneca R&D Gothenburg, RIA, Translational Biology, Pepparedsleden 1, 431 83, Mölndal, Sweden. Tel.: +46 31 776 11 70, +46 709154838.

E-mail address: matthew.catley@astrazeneca.com (M.C. Catley).

¹ Shared first authorship.

medical need is likely to lie in light of the imminent approval of therapies targeting T2-high-associated pathways.

1.1. The molecular pathology of T Helper 2 cytokine high (T2-high) asthma

T2-high asthma is associated with allergic inflammation despite the fact that some eosinophilic patients with late-onset disease may be less allergic (Robinson et al., 1992; Peters, 2014). Allergens or other harmless stimuli activate the airway epithelium to release cytokines such as thymic stromal lymphopoietin (TSLP), IL-33 and IL-25 (Hammad & Lambrecht, 2015). Activation of the epithelium may occur through activation of protease activated receptor 2 (PAR2) by allergen-associated protease activity or the release of proteases from mast cells (Boitano et al., 2011). Tissue damage appears to be the key to production of IL-33 which is released by necrotic cells and acts as an alarmin (Luthi et al., 2009). There is evidence that TSLP is induced by oxidative stress and inflammatory cytokines such as tumor necrosis factor (TNF)- α and IL-1 β (Lee & Ziegler, 2007; Nakamura et al., 2008). TSLP, IL-33 and IL-25 drive the activation of a T2-immune response to allergens. In the theory of T cell-mediated responses, allergens are taken up by dendritic cells (DCs) that then migrate to the draining lymph nodes. TSLP causes the maturation of DCs that upregulate major histocompatibility (MHC) class II and OX40L and interact with naïve T cells in the draining lymph node (Liu, 2006; Wang & Liu, 2007). TSLP-activated DCs drive the differentiation of naïve T cells to inflammatory Th2 cells that produce high levels of IL-5, IL-13 and TNF- α but low levels of IL-10 (Soumelis et al., 2002; Liu, 2006). These T cells migrate back to the airways where they drive a T2-mediated inflammatory response. This model explains the activation of allergic inflammation by the adaptive immune system. However, mice lacking T and B cells will still develop eosinophilic airway inflammation to innocuous stimuli (Hammad & Lambrecht, 2015), indicating that there is an innate activation of T2 immunity. The characterization of innate lymphoid cells (ILCs) demonstrated how T2 responses could develop in the absence of Th2 cells (Hammad & Lambrecht, 2015). Broadly speaking, ILCs can be classed as ILC1, ILC2 or ILC3 depending on the cytokines that they produce (Spits et al., 2013) and transcription factor expression. ILC1s characteristically express the T-bet transcription factor and produce interferon (IFN)- γ and TNF- α ; ILC2s express the GATA3 transcription factor and produce the classical T2 cytokines IL-4, IL-5 and IL-13, whereas ILC3s express ROR γ t and produce IL-17A, IL-22 and granulocyte macrophage colony-stimulating factor (GM-CSF) (Spits et al., 2013; Artis & Spits, 2015). The biology of ILCs is still emerging and there may be many subtypes that fall between these groups. There is also literature to suggest that ILC phenotypes display a degree of plasticity, for example, IFN- γ producing ILC1 and IL-17 producing ILC3s can switch between these phenotypes depending on the cytokines in the environment (Vonarbourg et al., 2010; Bernink et al., 2013; Artis & Spits, 2015; Bernink et al., 2015). A full review of ILC biology is outside the scope of this review but there are a number of recent reviews on these cells (Artis & Spits, 2015; Sonnenberg & Artis, 2015). In the context of asthma, there has been considerable focus on the role of ILC2s since they are capable of producing the classical T2 cytokines that drive eosinophilic airway disease. ILC2s are activated and modulated by many of the epithelial-derived cytokines that are involved in the maturation of DCs in T2 responses including TSLP, IL-33 and IL-25 (Imai et al., 2013; Kim et al., 2013; Salimi et al., 2013; Artis & Spits, 2015). It is speculated that ILC2s may provide a first line of innate defense against infection of the airways prior to the induction of an adaptive immune response (Licona-Limon et al., 2013). Recently, it was demonstrated in vitro that virally induced IL-33 release from the bronchial epithelium may activate ILC2s and drive the production of T2 cytokines (Jackson et al., 2014). This provides a direct link between viral infection and T2 responses.

The link between Th2-mediated inflammation and asthma pathology is well founded and has now been clinically proven by the successful

trials of therapies that target aspects of Th2 biology, including anti-IL-4R α , anti-IL-13 (Barnes, 2008; Corren, 2013b; Wenzel et al., 2013) anti-IL-5 (Robinson et al., 1992; Sanderson, 1992; Corrigan et al., 1993; Carroll et al., 1996; Giermycz & Lindsay, 1999; Rothenberg & Hogan, 2006; Kouro & Takatsu, 2009), anti-immunoglobulin (Ig) E (Burrows et al., 1989; Sunyer et al., 1995; Hamelmann, 2007) and initial studies with anti-TSLP (Gauvreau et al., 2014b). However, to the surprise of many in the field of respiratory research, the initial results of anti-IL-13 and anti-IL-5 trials were disappointing. What changed the prospects for these drugs was the use of pre-selection of patients for clinical trials based on biomarkers that indicated active T2-mediated inflammation. The implications of this work are that there is a group of patients whose asthma is driven by T2 immunity and that there are severe asthma patients whose disease is independent of T2 immunity or low T2. These studies provide definitive clinical evidence that asthma is a heterogeneous disease. Considerable work has been performed to understand the different groups, phenotypes or endotypes of asthma patients and to define methods to identify patients in the clinic. This work is of vital importance to ensure that therapies are targeted to the appropriate patient groups.

2. Phenotyping asthma

2.1. Defining asthma: endotypes and phenotypes

The heterogeneous nature of asthma creates a problem for the development of therapies targeting specific disease mechanisms that may only be present in some patients; how do we identify the patients that are more likely to respond to a specific therapy? The process of classifying patients into different categories is often referred to as phenotyping. Phenotyping can be made at different levels from clinical manifestations, inflammatory cell counts in tissue and blood and down to molecular patterns in tissue and serum. However, it is not always clear how a specific phenotype may relate to the mechanisms that drive disease and recent literature highlights the need to define patients based on mechanistic endotypes (Anderson, 2008; Lotvall et al., 2011; Corren, 2013a). Here, we will briefly go through different ways of phenotyping and some of the phenotypes that have been suggested in asthma. There are many excellent reviews on phenotyping of asthma for those who want more detailed information on the subject (Wenzel, 2012; Chung & Adcock, 2015; Fajt & Wenzel, 2015; Chung, 2016).

2.1.1. Biased clinical and cellular phenotyping

Historically, asthma patients were categorized as having allergic (extrinsic) or non-allergic (intrinsic) asthma. Today, the terms T2-high and T2-low asthma (or sometimes still referred to as Th2 high and low) are more often used. Allergic asthma most often falls within the T2-high asthma but not all patients with T2-high asthma are allergic. T2 refers to the type 2 cytokines IL-4, IL-5, and IL-13, and the cells that produce them, including Th2 cells, ILC2s, mast cells, eosinophils, basophils, macrophages, and NKT cells (Chang et al., 2013; Karta et al., 2016). These cytokines are involved in eosinophil recruitment and antibody class switching to IgE, both which are traits of allergy. Eosinophil counts in both sputum and blood are often measured in asthma patients (Schleich et al., 2014; Zhang et al., 2014) and sometimes extrapolated to reflect T2-high asthma.

In addition to allergy and inflammatory cells, biased clinical phenotyping includes variables such as age at onset of disease, nasal symptoms and responsiveness to inhaled corticosteroids. Given these variables and others, the following phenotypes could be suggested based on interpretation from current literature (Wenzel, 2012), some of which will be described more in detail later on in this review:

T2-high asthma

1. Eosinophilic, early-onset, allergic, respond to inhaled corticosteroids (ICS)

2. Eosinophilic, late-onset, non-allergic, sinusitis, refractory to ICS
3. Exercised-induced asthma

T2-low asthma

4. Obesity-related, late-onset
5. Asthma and chronic obstructive pulmonary disease overlap syndrome (ACOS)/Neutrophilic, late-onset
6. Paucigranulocytic (no sputum eosinophilia or neutrophilia), smooth muscle-associated

2.1.2. Unbiased phenotyping

Unbiased analysis uses cluster analysis to evaluate how different parameters group together. The method is not completely unbiased since it is dependent on which parameters are used in the analysis and in some cases how many clusters have been pre-specified as part of the analysis. Here, we will review four studies on unbiased phenotyping of adult asthma (Haldar et al., 2008; Moore et al., 2010; Siroux et al., 2011; Moore et al., 2014). All four studies were based on clinical parameters and the Haldar study (Haldar et al., 2008) as well as the Moore study from 2014 (Moore et al., 2014) also included sputum cell data.

Haldar et al. mainly report on clusters from nonhierarchical analysis on two cohorts, one with mild/moderate asthma and one with more severe refractory asthma (Haldar et al., 2008). The different clusters are described in Table 1. Importantly, atopy was not a discriminator for patients ending up in one cluster or another; however, atopy prevalence differed between the clusters and is therefore included in the description of the phenotypes. In addition to this, Haldar et al. tested the clinical relevance of their clusters in a prospective study, showing that the outcome of corticosteroid titration, using eosinophilic inflammation as an indicator of response to therapy, differs between the clusters.

In 2010, Moore et al. used unsupervised hierarchical cluster analysis based on clinical parameters and identified five clusters within 726 subjects (of which 304 had severe asthma) in the severe asthma research program, SARP (Moore et al., 2010). The different clusters are described in Table 1. In 2014, the same group reported on a new clustering

analysis on 423 patients from the SARP cohort (Moore et al., 2014). This time four clusters were identified based on clinical and sputum inflammatory parameters (Table 1). In agreement with the Haldar study, atopy was not a discriminator of cluster affiliation but was equally spread throughout the four clusters.

Siroux et al. used the clustering model-based method latent class analysis (LCA) in two cohorts: the European Community Respiratory Health Survey (ECRHSII, $n = 1895$) and the follow-up of the Epidemiological study on the Genetics and Environment of Asthma (EGEA2, $n = 641$) (Siroux et al., 2011). The different clusters are described in Table 1. The clustering analysis in the study of Siroux et al. did not include sputum inflammatory cell counts, but blood cell counts in the EGEA2 cohort showed highest eosinophil count in cluster 1 and lowest in cluster 4 while neutrophil counts were highest in cluster 2 and lowest in cluster 3.

These clustering analysis studies differed from each other in several ways; they were based on cohorts with different inclusion/exclusion criteria, different variables were included into their respective analysis and different statistical methods were used in each study. Hence, the studies cannot end up with exactly the same clusters. However, there are still similarities between the clusters in these different studies and the clusters do, to some extent, confirm phenotypes described in studies based on more biased phenotyping. All of the above studies were cross sectional. Hence, from these studies it is impossible to make any conclusions regarding how robust these clusters would form if data from another time point, within the same cohort, would be analyzed. Newby et al. has published a study on the matter of phenotypic stability of clusters and repeatability of cluster membership based on longitudinal data from the British Society Severe Refractory Asthma Registry (Newby et al., 2014). The conclusion of the study was that the same clusters are likely to form when analysis is applied to different cohorts but that patients do tend to shift cluster membership when their medication is optimized (Newby et al., 2014).

2.1.3. Molecular phenotyping

A movement from phenotyping based on clinical parameters towards endotyping, based on variables relevant for defining patients based on the mechanistic drivers of disease, is strongly encouraged in the asthma field (Anderson, 2008; Lotvall et al., 2011; Corren, 2013a).

Table 1
Clusters from unbiased cluster analyses of asthma cohorts.

Study	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
Mild/moderate cohort Haldar et al., 2008	Eosinophilic Early-onset Atopic	Non-eosinophilic Obese Female	Inactive/mild asthma Few symptoms Low inflammation		
Refractory cohort Haldar et al., 2008	Eosinophilic Early-onset Atopic	Non-eosinophilic Obese	High symptom expression Low inflammation Early-onset	Eosinophilic Few symptoms High inflammation	
SARP cohort Moore et al., 2010	Early-onset Atopic Normal lung function Low use of medication	Early-onset Atopic Normal lung function Higher use of medication	Obesity related Late-onset Decreased lung function High symptom score High use of medication	Early-onset Atopic Severe asthma High use of medication	Late-onset Less atopic Severe asthma High use of medication
SARP cohort Moore et al., 2014	Low inflammation Early-onset Atopic Normal lung function Low use of medication	Eosinophilic or pauci-granulocytic Early-onset Atopic Slightly reduced lung function Higher use of medication	Neutrophilic or mixed granulocytic Obese Atopic Slightly reduced lung function High use of medication	Neutrophilic or mixed granulocytic Obese Atopic Severe asthma Markedly reduced lung function High use of medication	
ECRHSII cohort Siroux et al., 2011	Early-onset Allergic Active disease Treated	Late-onset Female Active disease Treated	Allergic Inactive/mild disease Untreated	Non-allergic Inactive/mild disease Untreated	
EGEA2 cohort Siroux et al., 2011	Early-onset Allergic Active disease Treated	Late-onset Active disease Treated	Early-onset Allergic Inactive/mild disease Untreated	Non-allergic Late-onset Inactive/mild disease Untreated	

Defining patients based on biomarkers that indicate activation of a specific molecular pathway, or molecular phenotyping, has recently generated a great deal of attention since it appears to be able to identify patients that will respond to anti-IL-13 therapy, as will be described in Section 3.2. Woodruff et al. have demonstrated that chloride channel, calcium activated family member 1 (CLCA1), periostin, osteoblast specific factor (POSTN) and serine peptidase inhibitor, clade B (ovalbumin), member2 (serpinB2) are up-regulated in the gene expression profiles of airway epithelial brushings from some asthmatic patients when compared to healthy subjects (Woodruff et al., 2007). Furthermore, the expression of these three genes was decreased by treatment with inhaled corticosteroids, and confirmatory data on epithelial cells in vitro showed that the gene expression increased by stimulation with IL-13 and decreased when corticosteroids were added to the cultures. Based on this, these genes were proposed as biomarkers of T2-driven (initially called Th2 high-driven) corticosteroid-responsive asthma (Woodruff et al., 2007). A follow-up study on the expression of the same three genes in bronchial brushings showed that it was possible to categorize asthma patients into T2-high and T2-low phenotypes based on a combination of the three genes. These genes served as good surrogate markers for T2-driven inflammation in the lungs, since patients belonging to the T2-high phenotype had higher expression of IL-5 and IL-13 in bronchial biopsies than did patients belonging to the T2-low phenotype. These phenotypes also showed to be clinically relevant since they differed from each other in clinical and cellular parameters such as airway hyperresponsiveness (AHR), blood and airway eosinophilia, responsiveness to corticosteroids and in terms of allergy/atopy and total IgE (Woodruff et al., 2009). Increased expression of *CLCA1* and *POSTN*, but not *serpinB2*, in patients with asthma compared to healthy subjects have also been shown in sputum cells (Peters et al., 2014). The same study reports that the gene expression of *IL4*, *IL5*, and *IL13*, as well as the eosinophil marker *eotaxin 3* (*CCL26*) and the mast cell markers *tryptase* and *CPA3*, differs between asthma patients and may be used to categorize patients into T2-high and T2-low asthma.

2.1.4. Biomarkers for T2-high asthma

As described above, T2 cytokines and eosinophilia seem to be important drivers of some of the asthma phenotypes and may predict responsiveness to corticosteroids and, as will be described later on in this review, responsiveness to anti-IL-13 and anti-IL-5 treatments. Finding an accurate biomarker for T2 inflammation and tissue eosinophilia is therefore important. Three of the top biomarker candidates for tissue eosinophilia are serum periostin, fractional exhaled nitric oxide (FeNO) and blood eosinophil counts (Zhang et al., 2014; Parulekar et al., 2016). Periostin is a protein produced by bronchial epithelial cells in response to IL-13 (Sidhu et al., 2010) that can be measured in serum (Jia et al., 2012). Nitric oxide is produced by the airway epithelium after upregulation of inducible nitric oxide synthase (iNOS) by pro-inflammatory cytokines (Pavord & Shaw, 2008). A study on patients with severe asthma and on high dose ICS showed low intra-individual changes in serum periostin levels over time and in these patients serum periostin proved to be a better single predictor of tissue eosinophilia than FeNO or blood eosinophil counts (Jia et al., 2012). However, the relevance of serum periostin as a predictor of airway eosinophilia was questioned in a recent study based on two asthma cohorts, one mild to moderate and one moderate to severe (Wagener et al., 2015). In this study, no correlation between serum periostin and sputum eosinophils was found in either cohort. The periostin levels in the Wagener study were based on a different assay than that used in the studies by Woodruff et al. and this may or may not explain some of the differences seen between the studies. In the study by Wagener et al., among blood eosinophil counts, FeNO and serum periostin, blood eosinophils proved to be the best predictor of sputum eosinophilia. In addition, smoking habits may affect serum periostin levels. In a study on patients with mild to severe asthma, non-smoking asthmatics showed higher serum periostin than smoking asthmatics while no difference was seen

between non-smoking asthmatics and healthy non-smokers or between mild/moderate and severe asthmatics (Thomson et al., 2015). Caution should be taken when interpreting different studies since there may be statistically significant associations between sputum eosinophils and biomarkers such as blood eosinophil counts and FeNO, although these markers may not always predict sputum eosinophilia, as concluded by Hastie et al. (Hastie et al., 2013). Apart from reflecting tissue eosinophilia, serum periostin has been suggested to reflect both severity and phenotype of asthma. When dividing asthmatics into groups with high and low serum periostin, Nagasaki et al. found that the high periostin group showed more frequent exacerbations and a more rapid decline in Forced Expiratory Volume in 1 s (FEV1) over time, suggesting that serum periostin reflects asthma severity (Nagasaki et al., 2014). Some researchers mean that serum periostin reflects a certain asthma phenotype rather than severity of disease. The high periostin phenotype has been described as patients with late age of disease onset, T2-signature in the means of high eosinophilia, rhinosinusitis and nasal polyps. IgE and atopy status were however not correlated to periostin (Matsusaka et al., 2015).

3. The targeting of IL-5 and IL-13 as case studies on the need for patient stratification in asthma

The two next sections will describe the need for patient stratification and how patients can be selected by molecular phenotyping in asthma. Using anti-IL-5 and anti-IL-13 as case studies, this section will demonstrate how specific therapies are generally more efficacious in groups of patients selected on the basis of target mechanism or pathway activation, thus molecular phenotyping should be applicable in drug discovery.

3.1. Anti-IL-5 and the role of patient phenotyping

Several studies directly link IL-5 to T2-high asthma pathophysiology (Robinson et al., 1992; Sanderson, 1992; Corrigan et al., 1993; Carroll et al., 1996; Giembycz & Lindsay, 1999; Rothenberg & Hogan, 2006; Kouro & Takatsu, 2009). The close association of eosinophils with T2-high asthma and its dependence on IL-5 (Shi et al., 1998) led to the development of therapies targeting IL-5. Two different types of anti-IL-5 antibodies have been progressed to the clinic. Mepolizumab and reslizumab bind to free circulating IL-5 whereas benralizumab targets the IL-5 receptor α (IL-5R- α). Thus all three antibodies prevent IL-5 from acting through its receptor but benralizumab has the additional effect of directly depleting eosinophils and other IL-5R α bearing cells through recruitment of effector function (Kolbeck et al., 2010).

Promising results in pre-clinical models showed that anti-IL-5 reduced circulating and bronchoalveolar lavage (BAL) fluid eosinophils and airflow obstruction following antigen challenge (Mauser et al., 1995; Foster et al., 1996; Shardonofsky et al., 1999; Hart et al., 2001). Given the encouraging pre-clinical data, these anti-IL-5 antibodies were trialed in human asthma, where the initial results were disappointing (Leckie et al., 2000). In the first clinical trial, a single intravenous dose of mepolizumab (SB-240563) was investigated in mild asthmatics. Mepolizumab was found to be effective in suppression of circulating eosinophils and reducing sputum eosinophil numbers, but without any effect on allergen-induced late asthmatic response or histamine-induced airway hyperreactivity (AHR) (Leckie et al., 2000). Several studies have continued evaluating the relevance of anti-IL-5 biologics in asthma as a therapy. Studies in mild to moderate asthmatics recognized mepolizumab as unsuccessful in improving end-point clinical symptoms (Leckie et al., 2000; Flood-Page et al., 2003; Flood-Page et al., 2007). Thus the efficacy of anti-IL-5 as a therapy in asthma was questioned. The fact that anti-IL-5 therapy seemed to be ineffective raised the question whether eosinophils are not the primary drivers of asthma pathology. However, Flood-Page et al. suggested that inhibition of IL-5 may reduce severe exacerbations and that exacerbations should

be studied as an end-point in the clinic, indicating that selection of patients may improve the outcome anti-IL-5 clinical trials (Flood-Page et al., 2007).

Indeed, by selecting patients based on persistent blood and sputum eosinophilia with a history of frequent exacerbations led to significant decrease in exacerbation frequencies and an increased asthma control with mepolizumab treatment (Haldar et al., 2009; Nair et al., 2009). In addition, a steroid-sparing effect was found (Nair et al., 2009). Similar reduction in exacerbations and decrease in blood eosinophilia was reflected in the DREAM study (Dose Ranging Efficacy and Safety with Mepolizumab in Severe Asthma) (Pavord et al., 2012). In line with the DREAM study, a phase III trial was conducted, which reported a reduction in exacerbation rates by 47% to 53%, depending on dose and administration route, together with an attenuation of blood eosinophil count (Ortega et al., 2014). These findings have been confirmed in a parallel phase III trial (Bel et al., 2014), however with a less pronounced reduction of exacerbation. A trial with another anti-IL-5 antibody, reslizumab, was in agreement with results of mepolizumab by showing a reduction in sputum eosinophils, but in addition an improvement in lung function parameters and asthma scores in patients with an eosinophilic asthma phenotype (Castro et al., 2011). In line with observations that mepolizumab treatment was successful in patients with blood and sputum eosinophilia, reslizumab showed greater improvements in asthma control in subjects with high baseline eosinophilia (≥ 400 cells/ μ L) (Bjermer et al., 2014), again highlighting the need for careful patient stratification. The fact that anti-IL-5 treatments are effective in reducing blood and BAL fluid eosinophils are clear outcomes from the clinical studies, whereas their effects on tissue eosinophils are more limited. Extended analysis of studies with mepolizumab indicated a disconnection between blood and tissue eosinophilia, with depletion of only 55% of lung tissue eosinophils and 52% of bone marrow eosinophils (Flood-Page et al., 2003).

A third anti-IL-5 biologic is under development, named benralizumab (MEDI-563). Unlike the two other anti-IL-5 biologics, benralizumab targets the IL-5R- α and is an afucosylated antibody which induces apoptosis by cellular toxicity mechanisms in its target cells (Kolbeck et al., 2010). Due to the different mode of action of benralizumab, this therapy could potentially have an increased efficacy in reducing eosinophils in comparison to other anti-IL-5 antibodies. In initial studies, benralizumab has proven to be safe without severe events and depletes circulating eosinophils to below detection limit within 24 h of dosing (Reed et al., 2008; Busse et al., 2010). Further characterization of the effects of benralizumab in the clinic has shown that this antibody can reduce exacerbations (Nowak et al., 2015), improve lung function and Asthma Control Questionnaire score, and like the other anti-IL-5 biologics, has greater efficacy in patients with high blood eosinophil levels (≥ 300 cells/ μ L) (Castro et al., 2014).

Lung tissue eosinophils appear to be relatively resistant to anti-IL-5 treatment and these cells may continue driving asthma symptoms locally in the lung. Since the IL-5 receptor has been found on lung tissue eosinophils from mild asthmatics (Reed et al., 2008; Kolbeck et al., 2010), it has been suggested that benralizumab also could deplete tissue eosinophils and therefore have an advantage over other anti-IL-5 therapies. In a recent study, Laviolette et al. investigated the efficacy of benralizumab in reducing airway eosinophils in asthmatic patients with elevated sputum eosinophil numbers (Laviolette et al., 2013). This study confirmed the reduction of eosinophils in sputum, bone marrow and blood. Benralizumab failed to show statistically significant decrease in tissue eosinophil numbers when individual cohorts were compared to placebo. This was likely due to a small sample size and significant variability. However, a reduction in eosinophil numbers (61% single dose, 96% multiple doses, change from baseline) in airway tissue could be observed when a post-hoc analysis was done combining data from the two cohorts (Laviolette et al., 2013).

The successes and failures of anti-IL-5 from pre-clinical models to progression into the clinic have extensively contributed to our

knowledge of the heterogeneity of asthma pathophysiology. One therapeutic approach is unlikely to offer clinical benefit to all patients with asthma and a careful assessment of the targeted pathway in patients included in the clinical studies is needed. As mentioned earlier, several promising biomarkers have been found in clinical studies and have shown to be able to predict an enhanced efficacy to the therapy and thereby improve asthma control in a subset of patients. Following the initial disappointing clinical results, the understanding that asthmatic patients with eosinophilic inflammation will have a benefit from an anti-IL-5 therapy, has been of great value and rescued these drugs from discontinuation. At present, mepolizumab (Nucala®) and reslizumab (CINQAIR®) are the first anti-IL-5 biologics approved by the U.S. Food and Drug Administration (FDA) for the maintenance treatment of adult asthma patients. Mepolizumab (Nucala®) and reslizumab (CINQAIR®) are approved as add-on maintenance treatments for patients with an eosinophilic phenotype who are uncontrolled despite receiving their current asthma medicines.

3.2. Anti-IL-13 and the role of patient phenotyping

IL-4 and IL-13 are implicated in many aspects of both T2 inflammation and structural changes of asthmatic airways (Barnes, 2008; Corren, 2013b). These two cytokines act through a heterodimeric receptor complex consisting of IL-4R α and either IL-13R α 1 for IL-13 or the common gamma chain (γ c) for IL-4 to deliver a biological response by activation of the downstream Signal transducer and activator of transcription factor 6 (STAT6) (LaPorte et al., 2008; Chiba et al., 2012). In subjects with asthma, IL-4 and IL-13 can be found in increased levels in serum, BAL fluid, sputum and airway mucosa (Walker et al., 1992; Wong et al., 2001; Berry et al., 2004; Saha et al., 2008) supporting a link between IL-4/IL-13 and asthma. Several pharmaceutical companies are focusing on targeting these two cytokines as a new therapy for asthma. Pre-clinical data from several experimental models of asthma supported progression of antibodies against the IL-4/IL-13 pathway into the clinic (Coyle et al., 1995; Corry et al., 1996).

Antibodies targeting IL-4 (pascolizumab, altrakincept; both neutralizing free circulating IL-4) and IL-13 (anrukinzumab, IMA-026, GSK679586; all neutralizing free circulating IL-13) that showed good safety and tolerability profiles have been progressed into clinical development, but these drugs have been discontinued (Borish et al., 1999; Hart et al., 2002; Steinke, 2004; Oh et al., 2010; Gauvreau et al., 2011; Akdis, 2012; Clinicaltrials.gov., 2012; De Boever et al., 2014). Tralokinumab and lebrikizumab are anti-IL-13 monoclonal antibodies, binding free IL-13, that are still in clinical development. Researchers have shown the effect of these drugs in subjects with moderate to severe asthma (Corren et al., 2011; Piper et al., 2013; Brightling et al., 2015; Hanania et al., 2015).

The biologic effects of IL-13 and IL-4 in allergic asthma are to some degree overlapping, and as they share a common receptor complex and signaling, it may be more effective to interfere with both cytokines. Therefore, an approach has been to target IL-4 and IL-13 as a dual IL-4/IL-13 antagonist. The first IL-4/IL-13 drug to enter the clinic was pitrakinra, a recombinant mutant form of human IL-4 protein that inhibits binding of IL-4 and IL-13 to IL-4R α (Wenzel et al., 2007). In clinical studies, treatment with pitrakinra reduced the allergen-induced decline in FEV₁ in patients with atopic asthma. It has been proposed that the response to pitrakinra could be influenced by a mutation in IL-4R α . Although, the overall frequency of asthma exacerbations was not significantly reduced in participants assigned to pitrakinra, a subanalysis of patients treated with pitrakinra with a specific nucleotide polymorphism in the IL-4R α gene showed significantly reduced asthma exacerbations, decreased nocturnal awakenings and activities limited by asthma after withdrawal of standard of care (Slager et al., 2012).

AMG 317 and dupilumab also target the IL-4R α but are fully human monoclonal antibodies. AMG 317 initially demonstrated a good safety profile and reduction of IgE levels (Corren et al., 2010). In this phase II

study, even though decreases in number and time to exacerbations were observed in patients with high doses of anti-IL-4 biologic, AMG 317 failed to show clinical efficacy on the primary endpoint of the Asthma Control Questionnaire (ACQ) symptom score. However, there was a patient population with high ACQ that appeared to benefit from the AMG317 treatment.

Indeed, several other studies point towards a concept of patient stratification, when it comes to anti-interleukin therapy. For example, data from a study with pitrakinra showed reduction of exacerbations in moderate to severe asthmatics with eosinophilic asthma and elevated FeNO, a highly selected patient population (Otulana et al., 2011). In a trial of anti-IL-13 using GSK679586 as therapy in severe asthma patients, no clinically meaningful improvement could be observed in asthma control or lung function over placebo, even though patients with detectable levels of serum IL-13 were analyzed separately. The authors speculated that this could be an effect of high dose ICS usage maximally suppressing IL-13 since only half of the patients under GSK679586 treatment had measurable levels of total serum IL-13. Another explanation given by the authors is that lung function or exacerbations in this patient population may not be driven by IL-13 (De Boever et al., 2014). Similar to the above described trial of GSK679586, a phase 2b study with tralokinumab treatment did not significantly reduce asthma exacerbation rates in subjects with severe uncontrolled asthma. However, results of a post-hoc subgroup analysis indicated an effect in a specific defined population in this particular severe asthma cohort. Patients with high baseline serum dipeptidyl peptidase-4 (DPP-4) or patients with high periostin showed additional improvements in prebronchodilator FEV₁ and asthma control. Patients with high periostin did also show significant improvements in asthma exacerbation rate. Future trials will investigate the potential of using DPP-4 and periostin as biomarkers of an activated IL-13 pathway (Brightling et al., 2015). In addition, lebrikizumab achieved best efficacy in reduction of exacerbations and improvements in lung function in patients with high periostin levels in the blood (Corren et al., 2011; Hanania et al., 2015). These studies suggest that periostin and blood eosinophils (≥ 240 cells/ μ L) may be good biomarkers for a successful anti-IL-13 approach. Patients with high periostin levels also displayed high levels of FeNO (≥ 21 ppb), another biomarker that has been discussed previously for asthma with a T2-high profile. As described earlier in Section 2.1.3, Woodruff et al. phenotyped human asthma and showed that there are two subpopulations of asthma defined by the degree of T2-inflammation (Woodruff et al., 2009). A T2-signature may be a useful tool to define responders to asthma therapy. In a study by Corren et al., 2011, patients with uncontrolled asthma were divided into high and low periostin subgroups. This study concluded that asthmatics with high periostin levels showed better efficacy to lebrikizumab when measuring FEV₁, FeNO and exacerbations. Interestingly, patients with low periostin levels treated with lebrikizumab showed a trend towards reduction of exacerbations compared to the placebo group, although not statistically significant. Thus, Corren et al. could show a benefit of anti-IL-13 therapy in high and low periostin groups, but with greater improvements in the high periostin group.

In a promising study conducted by Wenzel et al., 2013, dupilumab was given to patients with uncontrolled moderate to severe eosinophilic asthma. In this multicenter phase II study, treatment with dupilumab, as compared with placebo, resulted in fewer exacerbations when LABAs (long-acting beta-agonist) and ICS were withdrawn. In addition, dupilumab treatment resulted in improved lung function and asthma control as well as a significant reduction in T2 inflammatory markers, but did not affect changes in blood eosinophil counts. In line with previously described data, a combined anti-IL-4 and -IL-13 approach suggests a potential therapy in a specific subset of patients with poorly controlled eosinophilic asthma. Recent data demonstrating that an IL-4/IL-13 antagonist like dupilumab works both in asthma patients with high (≥ 300 cells/ μ L) and low baseline (< 300 cells/ μ L) eosinophil counts, which is promising for future therapy and may add to standard of care

for treatment of asthma (orally communicated by Wenzel S at ATS 2015). The reason why dupilumab also works in patients with low blood eosinophil counts may be that a cut-off of 300 cells/ μ L in baseline blood eosinophilia may be artificially high (Katz et al., 2014; Ortega et al., 2014). Data from studies with mepolizumab support a cut-off of 150 cells/ μ L in baseline blood eosinophilia. In this severe asthma population a cut-off of 150 cells/ μ L in baseline blood eosinophilia resulted in 72% reduction in asthma exacerbations whereas patients below that cut-off showed only 30% reduction in asthma exacerbations. The efficacy of mepolizumab was not different if the cut-off was set on absence or presence of sputum eosinophils of 3% or greater. That the number of blood eosinophils was a predictor of response as a treatment, whereas sputum eosinophils were not, was also seen in the DREAM study. Several studies highlight the value of an appropriate cut-off in baseline for blood eosinophilia when conducting asthma clinical trials. The use of ICS may already have reduced blood eosinophilia in severe asthma subjects and a cut-off of 300 cells/ μ L in baseline blood eosinophilia may be too high. Another explanation could be that eosinophils are not as directly linked to IL-13 as periostin and this would suggest that periostin is a better biomarker of an IL-13 responsive patient population. Patients with high periostin levels respond well to anti-IL-13 therapy independent of their blood eosinophilia. Thus, blood eosinophilia may not be the best biomarker for defining an IL-13 responsive endotype. It would be of interest to see periostin levels in relation to the level of response in the dupilumab studies since this would give an idea of the periostin level in patients with high or low eosinophils and could determine if periostin is a better predictor of clinical outcome for anti-IL4R α targeting therapies.

The data from anti-IL-4/IL-13 clinical trials indicate that there may be a stratified group of patients who would benefit from this type of therapy. Defining patients based on biomarkers and selecting patient populations with specific molecular pathways activated will identify patients that are likely responders to highly specific monoclonal antibody-based drugs such as anti-IL-13 therapy. Using a molecular phenotyping approach may identify potentially responsive patients and would allow clinical trials to investigate efficacy in this particular population.

4. Other T2-high targeting therapies

There are other therapies that have reached the market or are under development to treat T2-high asthma. This reflects the historical dogma that asthma is a T2-high driven eosinophilic disease of the lungs. This section will review some of these therapies that have passed clinical studies and reached the market (Section 4.1) as well as therapies still under development (Section 4.2).

4.1. Other therapies targeting T2-high patients currently on the market

4.1.1. Anti-IgE therapy to control asthma

Many asthma patients have atopy associated with elevated levels of allergen-specific IgE and there is a clear association between T2-high allergic asthma and IgE levels (Burrows et al., 1989; Sunyer et al., 1995; E, 2007). Therefore, targeting allergen-specific IgE represents an approach for treatment of asthma that may have therapeutic benefit. The process where IgE binds to its receptor on mast cells can be blocked by using monoclonal antibodies. Omalizumab (Xolair®) is the only anti-IgE biological drug currently approved for the treatment of asthma.

Omalizumab is a recombinant humanized monoclonal anti-IgE antibody which complexes with free IgE and disrupts the interaction between IgE and its receptor, resulting in a reduction of circulating serum uncomplexed IgE (Presta et al., 1993). Generally, subcutaneous administration of omalizumab reduces free serum IgE by approximately 90–95% (Milgrom et al., 1999; Milgrom et al., 2001) and this reduction is dose-dependent. This effect can be preserved with continued administration (Casale et al., 1997; Adelroth et al., 2000; Casale et al., 2001;

Milgrom et al., 2001). As a result of treatment with omalizumab, IgE receptors on immune cells are down-regulated and these cells become less responsive to antigen challenge (MacGlashan et al., 1997). These early findings demonstrate that reducing circulating IgE with omalizumab is a valuable approach for the treatment of allergic asthma and other allergic diseases. Early initial studies showed that omalizumab inhibits the early and late responses to inhaled allergens in patients with mild allergic asthma (Boulet et al., 1997; Fahy et al., 1997). Clinical efficacy of omalizumab has also been confirmed in long-term clinical studies (Busse et al., 2001; Soler et al., 2001; Buhl et al., 2002). In a large study with severe allergic asthmatics that were symptomatic despite moderate-to-high doses of ICSs, omalizumab improved asthma control and reduced the use of ICSs (Busse et al., 2001). Clinical improvement has been confirmed in several other studies alongside with significantly fewer asthma exacerbations (Milgrom et al., 1999; Holgate et al., 2001; Finn et al., 2003; Lanier et al., 2003). In addition to the clinical improvements, a combined analysis of results from 3 multicenter phase III clinical trials of omalizumab showed that hospitalization was reduced by up to 93% with an average reduction in duration of hospitalization of 63% (Corren et al., 2003). A French study, describing the effectiveness of omalizumab in patients treated in a real-life setting, strongly supports observations from clinical trials with similar clinical benefits from the reduction of free IgE (Molimard et al., 2008). These findings implicate that omalizumab may also be of value when discussing cost of care in patients with severe allergic asthma. Omalizumab has been developed in the clinic, using IgE as a biomarker for patients likely to respond to anti-IgE therapy. Allergic asthma patients with levels higher than 30 IU/mL of IgE are considered for omalizumab treatment. Recently, it has also been shown that asthmatic patients with higher levels of FeNO, peripheral blood eosinophils and periostin had a greater reduction of exacerbations in response to omalizumab indicating that they may be good biomarkers for the prediction of treatment response to omalizumab (Hanania et al., 2013). By targeting one of the underlying mechanisms of allergic disease, the use of omalizumab as an add-on therapy, will be of clinical relevance in patients with very poorly controlled severe allergic T2-high asthma.

4.1.2. Anti-leukotriene therapy to control asthma

Cysteinyl leukotrienes (cysLTs) C_4 , D_4 and E_4 are potent biological lipid mediators generated from arachidonic acid through the 5-lipoxygenase (5-LO) pathway, a mediator released upon mast cell activation (Henderson, 1994; Peters-Golden & Henderson, 2007; Okunishi & Peters-Golden, 2011). It has been known since the mid-twentieth century that these mediators mediate contraction of airway smooth muscle, a contractive activity distinct from that of histamine (Brocklehurst, 1960). Later it has been shown that leukotrienes play a central pathophysiological role in asthma mediating bronchoconstriction, mucus secretion, oedema and inflammation through activation of several receptors (Wenzel, 2003). Two classical cysLT receptors are characterized, cysLT₁ and cysLT₂ with different selectivity towards the cysLTs. cysLT₁ binds LTD₄ > LTC₄ or LTE₄ whereas cysLT₂ binds LTC₄ = LTD₄ > LTE₄ (Lynch et al., 1999; Heise et al., 2000). There are two classes of anti-leukotriene agents approved for clinical use: LT receptor antagonist (LTRA) targeting the cysLT₁ receptor and 5-LO inhibitor zileuton (ZyfloTM). cysLT₁ antagonists currently on the pharmaceutical market are zafirlukast (AccolateTM), pranlukast (UltairTM, OnonTM) and montelukast (SingulairTM), where montelukast is the most commonly used agent.

Anti-leukotriene agents have been extensively evaluated and a lot of information about clinical efficacy is available. Numerous studies have been conducted with the different agents, which have been shown to reduce bronchoconstrictor responses to various challenges including exercise, allergens, aspirin and methacholine (see reviews by Garcia-Marcos & Schuster, 2001 and Scott & Peters-Golden, 2013). In addition, studies have also shown that anti-leukotriene agents mediate anti-inflammatory effects by reduction of eosinophils in different human

body fluids from asthmatics (Kane et al., 1996; Liu et al., 1996; Calhoun et al., 1998; Nakamura et al., 1998; Reiss et al., 1998; Malmstrom et al., 1999; Pizzichini et al., 1999; Hasday et al., 2000). Other researchers have examined the bronchodilatory effect of these agents and a small increase in baseline FEV₁ could be observed for all of the above mentioned agents (Hui & Barnes, 1991; Kemp et al., 1999). However, an inter-individual variation in the response to cysLT receptor antagonists has been described to be up to 50% (Tanaka et al., 1999; Terashima et al., 2002; Szefer et al., 2005). There are few available comparative studies between leukotriene synthesis inhibitors and other receptor antagonists. One study compared zileuton to montelukast in chronic persistent asthmatics (Kubavat et al., 2013). Zileuton (2400 mg/day) or montelukast (10 mg/day) was given to mild to moderate patients with stable asthma for 12 weeks. Peak expiratory flow rate (PEFR) and asthma symptoms were assessed as the clinical outcomes. Both anti-leukotriene therapies improved PEFR and asthma symptoms, with better efficacy with zileuton treatment for both assessments.

cysLTs are involved in many asthma phenotypes and high urinary LTE₄ defines an endotype across phenotypes (Higashi et al., 2004; Rabinovitch et al., 2008; Giouleka et al., 2011; Thomson et al., 2014; Marmarinos et al., 2015). There are studies suggesting that an increase in cysLT levels measured as LTE₄ in urine correlates well to acute changes in asthma control. For example, an acute increase of urinary LTE₄ predicts a decrease in FEV₁ and increased salbutamol use (Rabinovitch et al., 2006). Urinary LTE₄ is also significantly increased in atopic and non-atopic asthmatic children during exacerbation (Marmarinos et al., 2015). cysLT levels persist in chronic asthma and are increased with asthma severity. In addition, studies have shown that increased levels of cysLTs are not adequately controlled by steroid treatment in asthma (Vachier et al., 2003; Mondino et al., 2004). Montelukast has been shown to be more effective in asthma subjects with a urine LTE₄ level of ≥ 200 pg/mg creatinine than in those with less than 200 pg/mg creatinine (Cai et al., 2007). Asthmatics with one doubling of baseline urine LTE₄ showed FEV₁ improvement in montelukast step-up therapy while no improvement was observed for LABA step-up therapy (Rabinovitch et al., 2014). These two studies indicate that urine LTE₄ levels are correlated with the response to anti-leukotriene treatment and that LTE₄ in urine is a good biomarker for selecting an anti-leukotriene therapy as treatment of asthmatics with an activated cysLT pathway.

Taken together, response to anti-leukotriene therapy seems to be on the level of patients' characteristics, again highlighting the importance of asthma phenotyping. Patients with signs of increased urinary LTE₄ levels, which demonstrates an activated cysLT pathway, is a phenotype likely to respond well to anti-leukotriene therapy even on top of steroids.

4.2. The most novel asthma therapies in development will target T2-high patients

There are many novel therapies that are under development for treatment of asthma and also these therapies will mainly focus on targeting T2-high asthma. This section will review some of these therapies.

4.2.1. CRTh2 receptor antagonists to control asthma

Another approach to control T2-high asthma has been to target the CRTh2 receptor (chemoattractant receptor-homologous molecule expressed on Th2 cells) also known as DP₂ receptor (Ly & Bacon, 2005; Pettipher & Whittaker, 2012; Norman, 2014). CRTh2 is a G-protein coupled receptor expressed on eosinophils, basophils as well as Th2 cells and the major ligand for CRTh2 is prostaglandin D₂ (PGD₂) (Nagata et al., 1999b; Hirai et al., 2001). PGD₂ is a pro-inflammatory mediator generated from arachidonic acid through the cyclooxygenase pathway. PGD₂ and its metabolite bind to and activate the CRTh2

receptor. Activation of the CRTh2 receptor leads to release of IL-4, IL-5 and IL-13 by Th2 cells (Nagata et al., 1999b; Tanaka et al., 2004; Xue et al., 2005) and thereby mediates chemotaxis of Th2 lymphocytes, eosinophils and basophils (Nagata et al., 1999a; Hirai et al., 2001; Monneret et al., 2001; Spik et al., 2005). There is growing evidence for a role of CRTh2 on ILC2 cells. CRTh2-positive ILC2s have been found to be present in lung tissue and CRTh2-positive ILC2s have also been discovered in nasal polyps from chronic rhinosinusitis patients (Mjosberg et al., 2011). Activation of ILC2 cells with PGD₂ causes cell migration and stimulates production of IL-4, IL-5 and IL-13 (Xue et al., 2014). Therefore, it is not unlikely that CRTh2-positive ILC2 cells contribute to the number of CRTh2-positive inflammatory cells and to the production of pro-inflammatory cytokines in asthmatic airways.

PGD₂ is released in large amounts during an allergic reaction (Lewis et al., 1982) and is accepted as a marker for mast cell activation. This ligand is found in high levels in BAL fluid from asthmatic patients (Liu et al., 1990; Crea et al., 1992; Balzar et al., 2011) and following acute antigen challenge (Murray et al., 1986; Wenzel et al., 1989; Nowak et al., 1993), and has also been found to be released during early and late phase asthmatic responses (O'Sullivan et al., 1998). Several CRTh2 antagonists have been progressed to clinical development with various efficacies, with limited efficacy in asthma (Barnes et al., 2012; Horak et al., 2012; Busse et al., 2013b; Singh et al., 2013; Snell et al., 2013; Straumann et al., 2013; Diamant et al., 2014; Krug et al., 2014; Pettipher et al., 2014; Berair et al., 2015). The mixed response to a CRTh2 therapy in asthmatic patients is likely an effect of the patient selection in these studies. Recently, when patients were selected based on their sputum eosinophil count or allergic status, beneficial effects on asthma symptoms like FEV₁, peak flow, quality of life and sputum eosinophil counts were achieved (Barnes et al., 2012; Singh et al., 2013; Diamant et al., 2014; Berair et al., 2015). It remains to be determined whether a CRTh2 antagonist approach is more effective in a selected patient population with high sputum eosinophilia. Future studies will give a better understanding of the potential for this approach.

4.2.2. Targeting TSLP in allergen-induced asthma

Another therapeutic candidate that may modify the T2-mediated pathway, but upstream of IL-4, IL-13 and IL-5 is TSLP. TSLP has recently been targeted with beneficial results in the first study on patients with allergen-induced asthma.

As mentioned in the introductory section 1.1., TSLP is a key mediator for initiating T2-responses in the development of allergic asthma. Alongside IL-33 and IL-25, which are also considered as important early inducers of allergic inflammation, TSLP is highly expressed by epithelial cells in barrier surfaces in the lung, gut and skin (Ziegler & Artis, 2010). High levels of TSLP mRNA have also been found in other human non-hematopoietic cells in the lung, such as smooth muscle cells and lung fibroblasts (Soumelis et al., 2002). The effect of TSLP has most widely been studied on DCs. TSLP signals through a complex of the IL-7 receptor α chain (IL-7R α) and the TSLP receptor chain (TSLPR) which are most highly co-expressed on CD11c⁺ DCs (Reche et al., 2001). Upon TSLP binding, the expression of OX40L is up-regulated on the surface of immature CD11c⁺ myeloid DCs (Arima et al., 2010). Subsequently, the DCs mature and migrate to the draining lymph nodes where they activate the differentiation of naïve CD4⁺ T cells into Th2 cells that migrate back to the tissue (Ito et al., 2005). The TSLP-induced OX40L binds to the OX40 receptor on the T cells, whereby they become inflammatory Th2 cells. There was recently an attempt to target the OX40L in patients with mild allergic asthma, but no effect was seen on allergen-induced early or late airway responses (Gauvreau et al., 2014a).

Ying et al. presented the first human data supporting a role of TSLP in allergic asthma (Ying et al., 2005). Compared with normal controls, atopic asthmatics had increased numbers of TSLP mRNA-expressing cells in the epithelium and submucosa of bronchial biopsies (Ying et al., 2005). In addition, an elevated expression of TSLP was found in bronchial mucosa and BAL fluid in subjects with moderate/severe

asthma in comparison to controls with normal lung function (Ying et al., 2008). Importantly, genetic data, e.g. polymorphisms in the TSLP gene have associated TSLP with susceptibility to allergic asthma (Harada et al., 2011).

Subsequently, TSLP has been identified as a potential target in the treatment of allergen-induced asthma. In 2014, Gauvreau et al. reported the outcome of the first clinical trial for an anti-TSLP therapy (Gauvreau et al., 2014b). The study comprised of 31 patients with mild allergic asthma who were treated intravenously with three monthly doses of the humanized monoclonal anti-TSLP antibody AMG 157, which prevents the interaction of TSLP with TSLPR, or with placebo. Interestingly, both early and late asthmatic responses were reduced by the anti-TSLP treatment. Compared to placebo, the treatment with AMG 157 resulted in statistically significant reductions in allergen-induced bronchoconstriction and numbers of eosinophils in both blood and sputum. The numbers of adverse events were similar in the treatment and placebo groups, with no serious adverse events reported.

The results of the anti-TSLP trial support the hypothesis that TSLP is an important early mediator in allergic asthma and may be responsible for persistent airway inflammation. However, it remains to be seen whether patients with severe asthma could be treated with anti-TSLP and more studies are needed to prove the mechanism of action of the TSLPR blockade. Even though TSLP is considered to activate T2-immune responses, as described in the introduction, Gauvreau et al. could not demonstrate a statistically significant reduction in Th2/Th1 cell ratio from the anti-TSLP treatment in the first clinical trial (Gauvreau et al., 2014b). It will be interesting to see the effects of AMG 157/MEDI9929 from the ongoing Phase 2 study in patients with poorly controlled severe asthma (Clinicaltrials.gov., 2016).

4.2.3. Interferons in asthma

Respiratory virus infections are a major cause of exacerbations in asthmatics (see (Costa et al., 2014) and (Edwards et al., 2013) for comprehensive reviews) and there seems to be an impaired immune response to virus infections in some (Bufe et al., 2002; Wark et al., 2005; Contoli et al., 2006; Gehlhar et al., 2006; Sykes et al., 2012; Edwards et al., 2013) but not all asthmatics (DeMore et al., 2009; Lopez-Souza et al., 2009; Bochkov et al., 2010; Patel et al., 2014; Schwantes et al., 2014; Sykes et al., 2014). The impaired immune response appears to manifest itself as a decreased ability of both bronchial epithelial cells and immune cells to produce or release interferons. Further, it has been demonstrated that asthmatics have more severe and long-lasting rhinovirus-induced lower respiratory tract symptoms than healthy subjects (Corne et al., 2002), and there is a reduction in lung function after experimentally induced human rhinovirus (HRV) 16 infection in asthmatics (Message et al., 2008). It has been suggested that this is due to a reduced interferon production. This is supported by the observation that administration of exogenous IFN- β reduces virus replication in primary bronchial epithelial cells from asthmatics *ex vivo* (Wark et al., 2005; Cakebread et al., 2011; Becker et al., 2013), suggesting that IFN- β treatment may be beneficial to treat or prevent virus-induced exacerbations in asthmatic patients.

Recently, a positive phase 2 trial by Synairgen demonstrated that inhaled IFN- β treatment improves control of asthma symptoms (changes in ACQ-6 from pre-treatment baseline), as well as morning peak expiratory flow (PEF) in difficult-to-treat asthmatics (approximately 10–20% of all adult asthmatics) (Djukanovic et al., 2014). Among the IFN- β -treated patients, there was a steady improvement in morning PEF, while in the placebo-treated group there was an initial dip during the first week followed by an improvement. Fewer patients receiving IFN- β required additional treatment (e.g. corticosteroids and antibiotics; 1 of 22 patients) compared to the placebo-treated patients (5 of 25 patients). Further, the inhaled IFN- β was well tolerated.

Several signaling pathways have been implicated in the reduced interferon response in asthmatics. Toll-like receptor 3 (TLR3) and melanoma differentiation-associated gene 5 (MDA5) signaling, although not

expression, appear to be impaired in asthmatic epithelial cells in response to rhinovirus infection (Parsons et al., 2013). Another study reported lower baseline *TLR3* expression as well as impaired HRV-induced *TLR3*, retinoic acid-inducible gene 1 (*RIG-1*) and *MDA5* mRNA expression in bronchial epithelial cells from severe therapy-resistant atopic asthmatic children compared to non-atopic, non-asthmatic children (Edwards et al., 2013). Similarly, polyinosinic:polycytidylic acid (poly I:C)-induced induction of several interferon-related genes (*IRF3*, *IFNAR1*, *IFNB1*, *IFNGR1*, *IL28B*) are impaired in bronchial and/or nasal epithelial cells from asthmatics compared to healthy individuals (Wagener et al., 2014). In addition, an increased nuclear suppressor of cytokine signaling 1 (*SOCS1*) level in bronchial epithelial cells in asthmatics has been suggested to be the cause of suppressed HRV-induced interferons in asthmatics (Gielen et al., 2015). Conversely, another study reported that influenza A virus and respiratory syncytial virus induce very similar interferon responses in non-asthmatics and asthmatics (Patel et al., 2014).

There are also indications that respiratory viruses, especially rhinoviruses, are not only associated with exacerbations but also with the development of asthma, and that this may be T2-driven (see Rowe and Gill, 2015 for a comprehensive review). It has been reported that in asthma patients both IFN- γ and IL-10 baseline production by peripheral blood CD4⁺ T cells are negatively correlated with virus load and common cold symptoms (note small number of subjects). Further, IFN- γ production by BAL fluid CD4⁺ T cells was found to be negatively correlated with maximum fall in PEF after infection with HRV16, whereas BAL fluid CD4⁺ T cell production of the T2 cytokines IL-4, IL-5 and IL-13 were found to be associated with more severe lower respiratory symptom scores (Message et al., 2008). Taken together this indicates that a T1 signature correlates with less severe cold symptoms while a T2 signature correlates with more severe cold symptoms. This was further strengthened by the demonstration that the release of the T1 cytokines IFN- γ , IL-12 as well as IL-10 from BAL cells is induced by RV16 or LPS (vs control) in healthy individuals but not in asthmatics, while the T2 cytokines IL-5 and IL-13 were found to only be induced by phytohemagglutinin in BAL cells from asthmatics. Similarly, the phytohemagglutinin-induced IL-4 release was found to be higher in asthmatics than in healthy subjects (Message et al., 2008). Further evidence for the involvement of a T2 high signature in a deficient IFN- β response to respiratory viruses is the report that the T2 cytokines IL-4 and IL-13 impair the innate immune responses to HRV in respiratory epithelial cells by inhibition of *TLR3* expression and *IRF3* and *STAT1* activation (Contoli et al., 2015). Similarly, IL-4 mRNA expression and viral load was found to be higher in primary nasal epithelial cells from atopic rhinitis patients compared to non-atopic healthy subjects while *TLR3* expression was lower (Contoli et al., 2015). A deficient interferon response to HRV16 treatment of bronchial epithelial cells *ex vivo* was reported in children with asthma irrespective of their atopic status as well as in atopic children without asthma, further suggesting that an impaired immune response to viral infections may be T2-driven (Baraldo et al., 2012). Similarly, IFN- α secretion by plasmacytoid DCs induced by influenza A and B *ex vivo* was reduced in patients with allergic asthma and the secretion negatively correlated with serum IgE levels as well as plasmacytoid DC Fc ϵ R α expression (Gill et al., 2010). Gill and coworkers also demonstrated that IgE-crosslinking reduced the influenza-induced IFN- α production by plasmacytoid DCs, and that reciprocal inhibition of TLR7 and Fc ϵ R α expression occurs by stimulating either pathway, further supporting counter regulatory mechanisms between T2 responses and virus defense. This was supported by the observation that the exacerbations often observed in asthmatic children during the autumn were reduced during treatment with the anti-IgE drug omalizumab (Busse et al., 2011; Teach et al., 2015), and it can be speculated that similar reductions will be obtained with other treatments targeting T2-asthma, at least in some asthmatic subpopulations. Although recent publications suggest that only patients with the T2-high endotype have a reduced

type 1 interferon response to virus, there are currently no evidence suggesting that inhaled IFN- β would not be beneficial to the resolution of viral infections in both T1 and T2 asthmatics.

5. T2-low as the unmet need in asthma

5.1. Where are the treatments for T2-low asthma

Although asthma is often considered to be an eosinophilic disease responsive to corticosteroids, almost half of the mild-to-moderate asthmatics may be non-eosinophilic and unresponsive to current treatments (McGrath et al., 2012). In those patients, the drivers of asthma may not be related to eosinophils, serum periostin, FeNO and blood eosinophils which may or may not prove to be useful biomarkers to predict the outcome of new treatments for these patients.

Many of the T2-high-targeting therapies discussed above will come to the markets within the next few years. This will allow us to determine the long term effects of treating patients with these immunomodulatory therapies. They will also require novel treatment regimens for asthma which will probably involve biomarker-based selection of patients for treatment with specific drugs. This will be the beginning of personalized treatment for asthma patients. However, since all these therapies will target the T2-high patient population, the T2-low patients will be excluded from treatment with these drugs and there are currently few treatments in development for these patients. This means that T2-low patients will not benefit from novel therapies and will continue to suffer the symptoms of poorly controlled asthma. In order to develop novel therapies for these patients we will need to understand the key pathways that drive pathology and this will probably subdivide the group into smaller patient subsets. The next section of this review will describe what is known about the potential T2-low subgroups. However, at present there are no clearly defined T2-low endotypes, so the following section will examine the evidence for the existence of a number of phenotypes that have been suggested to fall into the T2-low patient group.

6. Proposed T2-low phenotypes and the evidence they exist

6.1. Obesity-related asthma

Recent literature suggests a link between obesity and asthma (Beckett et al., 2001; Gunnbjornsdottir et al., 2004; Chen et al., 2005; Beuther & Sutherland, 2007; Von Behren et al., 2009; Brumpton et al., 2013), although there have been contradictory findings (Stanley et al., 2005; Chinn et al., 2006; Barranco et al., 2011) leading to the possibility of asthma overdiagnosis based on dyspnea and wheezing that are more prevalent among obese individuals (Pakhale et al., 2010; Colak et al., 2016).

Obesity is not only a risk factor for asthma but it is also associated with poor asthma control, resistance to standard therapies and higher risk of hospitalization due to asthma exacerbations compared with lean asthmatics (Mosen et al., 2008; Taylor et al., 2008; Vortmann & Eisner, 2008; Dixon, 2012). At least two distinct phenotypes of asthma in obesity have been described so far that can be distinguished by age of onset and markers of T2 inflammation (Wenzel, 2012). Obese patients with early-onset asthma have more atopic disease, higher IgE, and greater bronchial hyperresponsiveness (BHR). In contrast, obese patients with late-onset asthma tend to have less atopy, less BHR and lower levels of T2 inflammation. It has been proposed that the T2-dependent early-onset asthma phenotype is complicated by coexisting obesity, whereas the late-onset asthma phenotype is likely to be a consequence of obesity-induced metabolic changes and the condition driven by distinct mechanisms (Holguin et al., 2011; Dixon, 2012; Sutherland et al., 2012; Fahy, 2015). The relationship between weight gain and asthma appears more clear in women than men (Seidell et al., 1986; Hancox et al., 2005), although some of the largest studies have not shown a sex difference (Beuther & Sutherland, 2007; Egan

et al., 2013). These results suggest that sex hormones may have a modulating effect on the expression of asthma in obesity (Sood, 2011; Ali & Ulrik, 2013). Gender effects on asthma could also be due to differences in perception and behavior between men and women (Zein & Erzurum, 2015). Interestingly, Moore et al. have identified a distinct asthma phenotype of mostly older obese women with late-onset non-atopic asthma, moderate reductions in FEV₁, and frequent use of oral corticosteroids to manage exacerbations (Moore et al., 2010).

The mechanisms underlying the obesity–asthma association remain largely unknown and have been discussed in detail elsewhere (Santamaria et al., 2012; Rasmussen & Hancox, 2014; Sideleva & Dixon, 2014; Sutherland, 2014; Leiria et al., 2015; Sivapalan et al., 2015). A brief overview is presented here. It is obvious that obesity significantly alters lung mechanics. Obese patients have reduced functional residual capacity (FRC) due to changes in the elastic properties of the chest wall (Naimark & Cherniack, 1960; Pelosi et al., 1998; Jones & Nzekwu, 2006). It has been proposed that the association between increasing body mass index (BMI) and a decrease in lung function is due to the fact that adiposity mainly compresses the chest and by that limits the expansion of the lungs (Nicolacakis et al., 2008; Thyagarajan et al., 2008). Obese humans also breathe spontaneously with lower tidal volume (Sampson & Grassino, 1983). Breathing at a lower lung volume can lead to AHR (Ding et al., 1987; Skloot et al., 1995). Recently, it was shown that late-onset non-allergic asthma pathophysiology may be linked to excessive collapsibility of the lung periphery (Al-Alwan et al., 2014).

To understand the effects of obesity particularly on late-onset non-allergic asthma, it is important to understand the perturbations in adipose tissue as well as the immune system that occur in obesity. It is now appreciated that obesity is a state of chronic low grade systemic inflammation affecting the expression of several inflammatory genes in the adipose tissue of obese versus lean mice or human beings (Xu et al., 2003; Shore, 2008; Ouchi et al., 2011). It is believed that this inflammation spills over locally as well as to sites distant to the adipose tissue (Shore, 2008).

Serum concentrations of C-reactive protein (CRP), TNF- α and IL-6 were significantly correlated with weight and BMI (Park et al., 2005). Williams et al. showed that TNF- α receptor 2 (TNFR2) signaling is required for the innate AHR that develops in obese mice. (Williams et al., 2013). Moreover, TNFR2 appeared to promote expression of IL-17A, endothelin, and neurotrophic tyrosine kinase, receptor, type 2 (TrkB). Recent research showed that primary alveolar macrophages derived from overweight/obese subjects had an enhanced response to lipopolysaccharide, particularly when they were primed *ex vivo* with leptin, resulting in increased levels of pro-inflammatory cytokines such as TNF- α (Lugogo et al., 2012). In addition, Periyalil et al. reported age- and sex-specific activation of macrophages from obese subjects (Periyalil et al., 2015). In a house dust mite model of AHR, mice that were on high-fat diet showed lower eosinophil and higher macrophage infiltration into the lungs and BAL fluid, which were less sensitive to dexamethasone regulation (Diaz et al., 2015). Kim et al. found that mice fed on a high-fat diet and *ob/ob* mice fed on a regular diet developed AHR dependent on innate immunity. They suggested that obesity-induced asthma is facilitated by inflammation mediated by the NLRP3-IL-1 β -macrophage axis and ILC3s (Kim et al., 2014).

Also neutrophils may contribute to the pathogenesis of asthma in obesity. It was shown that obese asthmatics, particularly woman, had more neutrophils in sputum and blood than non-obese patients (Telenga et al., 2012). Th17 inflammation has been strongly linked to neutrophilia but IL-17 was also reported to be involved in airway remodeling and steroid insensitivity (McKinley et al., 2008; Al-Ramli et al., 2009; Chesne et al., 2014). Together, these findings highlight the potential role of innate immunity in obesity-related asthma, although most studies in humans have failed to demonstrate an association between obesity and increased airway inflammation in asthma (Hancox et al., 2007; Todd et al., 2007; Sutherland et al., 2008; van Veen et al., 2008; Sideleva et al., 2012).

Mediators secreted by adipose tissue, collectively called adipokines, may be important in the pathogenesis of late-onset asthma in obesity (Ouchi et al., 2011). Sideleva et al. have shown that markers of adipose tissue metabolic inflammation are increased, particularly in visceral fat of obese late-onset, T2-low subjects (Sideleva et al., 2012). Aberrations in leptin and adiponectin signaling lead to obesity and metabolic dysfunction (Ouchi et al., 2011). Although currently inconclusive, human studies suggest that high-serum leptin and low-serum adiponectin levels predict asthma, independent of obesity, in selected population groups (Sood, 2010). On the other hand, increased adiponectin concentrations have been proposed to be associated with chronic obstructive pulmonary disease (COPD) pathogenesis (Chan et al., 2010). The role of adiponectin in asthma has recently been reviewed (Nigro et al., 2015). There is strong evidence that adiponectin acts as an anti-inflammatory mediator, however also pro-inflammatory actions have been reported (Esmaili et al., 2014). Much work remains to be done to understand the adiponectin biology and its contribution to many diseases, including obesity-associated asthma. Clearly, the experimental conditions chosen as well as the type and ratio of the different isoforms of adiponectin determine the outcome (Esmaili et al., 2014). For instance, low molecular-weight (LMW) adiponectin was associated with disease activity of rheumatoid arthritis (RA) (Li et al., 2015). Similarly, higher LMW adiponectin levels and a lower middle molecular weight (MMW)/total ratio may be associated with current asthma in both male and female subjects and a low total adiponectin level may lead to airway obstruction, especially in women (Hayashikawa et al., 2015). Interestingly, Azambuja et al. proposed that adiponectin does not have protective effects in asthma and may in fact exacerbate the disease (de Lima Azambuja et al., 2015).

Leptin appears to be involved in lung development in mice (Huang et al., 2008), may have stimulatory effects on the proliferation of cells of tracheal epithelial cells (Tsuchiya et al., 1999) and has pro-fibrotic effects (Jain et al., 2011). Further, leptin has been shown to act as a survival cytokine for human eosinophils (Suzukawa et al., 2011) and activated neutrophils in terms of chemotaxis and production of reactive oxygen species (Montecucco et al., 2006), enhance leukotriene synthesis by alveolar macrophages (Mancuso et al., 2004), and inhibit the function of regulatory T cells (De Rosa et al., 2007). These findings suggest that leptin actions may partly explain the obesity and asthma relationship.

A high fat diet may worsen airway inflammation and reduce bronchodilator responsiveness (Wood et al., 2011). A recent study suggested that increasing anti-oxidants in the diet may improve asthma control (Wood et al., 2012). Obesity is associated with increased oxidative stress and obese subjects have higher levels of oxidative stress biomarkers compared with their lean counterparts (Keaney et al., 2003; Steffes et al., 2006). Oxidative stress is likely to modulate asthma severity and alter response to medications rather than be a causative agent. Lifestyle changes are one of the important factors that have an effect on the epigenome. Interestingly, peripheral blood mononuclear cells derived from pre-adolescent obese asthmatic children had distinct patterns of DNA methylation compared with those observed in control group (Rastogi et al., 2013). Differentially methylated molecules were associated with pathways linked to T cell signaling and macrophage activation.

Further studies are necessary to identify the mechanisms underlying the distinct asthma phenotypes present in obese populations. It is clear that the glucocorticoid treatment does not suffice for achieving good asthma management in obese patients (Dixon, 2012; Sutherland, 2014). Studies in bariatric surgery suggest significant improvements in asthma control through weight loss (Dixon et al., 2011; Boulet et al., 2012; van Huisstede et al., 2015) but given the expense and risks associated with this intervention, it is unlikely to be an effective strategy available for all patients with obesity-associated asthma. It seems that 5–10% weight loss is required to improve asthma control in obese asthmatics (Ma et al., 2015) but weight regain is a common problem. Therefore, establishing well-characterized markers for each

phenotype will facilitate the correct diagnosis and will help to develop effective treatment of obese-related asthma.

Nevertheless, dietary intervention also represents a possible approach that could also safely contribute to the prevention of allergic diseases and obesity. Several classes of dietary components of parental diet may influence asthma and allergies through effects on the neonatal immune system and lung development (Calvani et al., 2006; Devereux, 2006; Devereux et al., 2007; Willers et al., 2007; Julia et al., 2015).

6.2. Smoking-associated asthma

It has been speculated that there is a smoking-associated neutrophilic phenotype in asthma. However, the literature is not consistent and the evidence for a well-defined smoking phenotype is sparse. It is possible that a smaller smoking-associated patient population is lost among a more variable smoking asthmatic population.

The evidence for a neutrophilic phenotype in asthma is still debated and the failure of neutrophil targeting approaches suggests that neutrophils may not be a driver of disease. Corticosteroids are widely used in the treatment of asthma and they potentially induce neutrophilia and possibly act as a modulator of the disease (Thomson et al., 2004; Wenzel, 2012). Interestingly, smoking asthmatics are generally considered corticosteroid-insensitive indicating that there may be unique drivers of disease in smoking asthmatics (Pedersen et al., 1996; Chalmers et al., 2001; Chaudhuri et al., 2003). Tobacco smoke in itself contains many different chemicals that may play a role in negatively affecting the lung and possibly causing inflammation, as previously reviewed (Thomson et al., 2004). In addition, smoking has further been shown to have severe effects on the airways and can lead to the development of several diseases such as lung cancer and COPD.

Asthmatics demonstrate almost the same level of smoking as in healthy individuals, despite their impaired lung function (Wakefield et al., 1995; Cerveri et al., 2012). Smoking asthmatics have a more steep decline in FEV₁ than do non-smoking asthmatics, and airway obstruction is worse and more prevalent (Lange et al., 1998; Aanerud et al., 2015). Besides affecting the lung function, smoking has been associated with worse asthma symptoms, more severe asthma attacks, a higher number of near-fatal or fatal asthma attacks, and a worse health status (Ulrik & Frederiksen, 1995; Sippel et al., 1999; Siroux et al., 2000; Mitchell et al., 2002). However, it is unclear whether smoking is driving a unique and stable asthma phenotype or just modulating and exacerbating an already existing condition.

The question whether smoking has a causative role for asthma has been addressed in several studies, some showing an association with onset of asthma (Flodin et al., 1995; Plaschke et al., 2000; Rasmussen et al., 2000), whereas others do not (Vesterinen et al., 1988; Troisi et al., 1995; Siroux et al., 2000). The association seems to be stronger in females than in males (Chen et al., 1999; Langhammer et al., 2000). Several studies have shown a correlation between children to smoking parents and a higher risk of developing asthma later in life (Strachan & Cook, 1998; Skorge et al., 2005; Burke et al., 2012). However, more studies are needed to confirm whether smoking is a cause of asthma or solely a risk factor. It is conceivable that a form of asthma directly caused by smoking may form a unique endotype. However, the complex mixture of compounds in tobacco smoke means it could be modulating normal airway physiology through multiple pathways.

The inflammation in smokers and asthmatic smokers has been described previously, but in many cases, the data seem to be conflicting. In most studies, the peripheral inflammation is characterized by an increase of eosinophils in healthy smokers (Halonen et al., 1982; Taylor et al., 1985; Jensen et al., 1998) whereas this relationship seems to be reversed in asthmatics, where smokers have reduced eosinophils in the blood compared to asthmatic non-smokers (Sunyer et al., 2003). In the lung, smoking has been shown to cause an inflammation that is characterized by infiltration of T cells, macrophages, mast cells and neutrophils to different compartments of the lung (Kuschnier

et al., 1996; Jeffery, 1998; Roth et al., 1998; Di Stefano et al., 2001; Saetta et al., 2001; Broekema et al., 2009). As for induced sputum, the eosinophils are reduced in asthmatic smokers with elevated neutrophils, as compared to asthmatic non-smokers (Chalmers et al., 2001) but there are also studies showing conflicting results (Broekema et al., 2009). It is clear that the cellular changes due to smoking and asthma are variable and possibly not stable as discussed by Polosa and Thomson, (2013), and there is a need for better designed studies that can properly characterize the asthmatic smoking population in a reliable and controlled way.

Some studies have taken atopy into account when stratifying the patients and most of them have studied the onset of asthma in relation to smoking history and atopy, as well as AHR and its association to smoking and atopy. One study found that smoking was predictive for onset of asthma in adult atopic patients where atopy was defined as presence of allergic rhinitis (10 year follow-up study) (Polosa et al., 2008). In contrast to this, another longitudinal study showed a strong association with onset of asthma in smoking non-atopics (Plaschke et al., 2000), suggesting that there may be atopic and non-atopic smoking related asthma.

In a recent study by Aanerud et al. (2015), the relationship between airway obstruction and smoking was studied in a large cohort of patients. Smokers with asthma had the greatest prevalence of airway obstruction (defined as pre-bronchodilator FEV₁/FVC (forced vital capacity) < 0.7 and FEV₁ < 80% predicted), and when adjusting for atopy, the association was strongest in smoking non-atopic asthmatics (Aanerud et al., 2015). Sunyer et al. (1997) also found that BHR is associated with smoking in non-atopic patients but not in atopic. In another recent large study by Perret et al. (2013), the opposite relationship of atopy and airway obstruction was found, however, the definition of certain parameters differed to that of Aanerud et al. (2015).

There seems to be some evidence that atopy could be protective for the adverse effects of smoking, or perhaps non-atopic asthmatics are more sensitive to the damaging effects of cigarette smoke. It is, however, known that atopic asthmatics have a more rapid decline in lung function than do non-atopic subjects (Weiss, 2000). It has been speculated that atopic asthmatics have a more severe lung inflammation than do non-atopic asthmatics, and that the inflammation is masking the effects of cigarette smoke on the lung which therefore appears less affected.

Smoking has been shown to increase the decline in lung function in healthy subjects and since asthmatics already have lower lung function than non-asthmatics, the argument for asthmatics to stop smoking is even stronger. Several studies have assessed smoking cessation, most of them showing positive results on lung function and lung inflammation. An early study by Fennerty et al. showed that seven days after smoking cessation, there was a change in AHR and PEF (Fennerty et al., 1987). More recent studies report that asthmatics who quit smoking have a better quality of life, they use less asthma medication (inhaled bronchodilators and corticosteroids), have a better asthma control, and show improvement in AHR and lung function (Tonnesen et al., 2005; Chaudhuri et al., 2006; Piccillo et al., 2008). This was only true for the patients who quit smoking and not for the patients who relapsed (Tonnesen et al., 2005). Some patients also reported a worsening of asthma symptoms after smoking cessation (Hillerdahl & Rylander, 1984; Fennerty et al., 1987). Another study examined BAL fluid from smokers and non-smokers 1, 3, 6, 9 and 15 months after smoking cessation and found that the cellular composition and levels of inflammatory cells in the BAL fluid was back to the levels of non-smoking subjects 9 months after smoking cessation (Skold et al., 1996).

All these studies show a benefit of smoking cessation for asthmatics, however, the asthma still persists. The difficulty to quit smoking also affects the success rate and many asthmatics who try to quit fail (Eisner et al., 2000), and often they also lack the understanding of the risk of smoking and how it can worsen their asthma (Wakefield et al., 1995; Silverman et al., 2003).

Smoking asthmatics are hard to treat, since they have a reduced response to inhaled and oral corticosteroid treatment and several studies show that smoking asthmatics are steroid insensitive (Pedersen et al., 1996; Chalmers et al., 2001; Chaudhuri et al., 2003). Mechanisms for steroid insensitivity have been discussed and it remains unclear exactly how to successfully treat smoking asthmatics (Thomson et al., 2004). Therapeutics targeting neutrophils have been addressed in asthma since cigarette smoke can trigger IL-8 production, working as a chemotactic factor for neutrophils (Keatings et al., 1996), but both a C-X-X chemokine receptor type 2 (CXCR2) antagonist and IL-17-related targets have failed to show any efficacy (Nair et al., 2012; Busse et al., 2013a). It should however be noted that smokers are often excluded from studies and clinical trials and it is possible that with a different patient selection the outcome of these studies would have been different. Clearly, there is a big need for new therapies that can relieve smoking asthmatics. However, a clear understanding of the smoking asthma phenotype is needed in order to develop these therapies that will have to be efficacious in patients who have ceased smoking.

6.3. Asthma and COPD overlap syndrome

In recent years, it has become clear that many asthma patients share symptoms and features of both asthma and COPD. GINA has published guidelines for asthma and COPD overlap syndrome (ACOS) and the definition of it (GINA, 2015) but there are currently several other definitions in use, as covered in a review by Papaiwannou et al. (2014). In the GINA document, there are several criteria that should be fulfilled for a diagnosis of ACOS. Two of the most important ones are that patients should have post-bronchodilator $FEV_1/FVC < 0.7$, which is also required for diagnosis of COPD, and they should be highly reversible and have a post-bronchodilator $FEV_1 > 12\%$ and 400 mL from baseline, which is most probable for asthmatics (GINA, 2015). Physicians also take age, pattern of symptoms, lung function, family history, time course and chest x-ray results into account when making a diagnosis (GINA, 2015).

Since ACOS has characteristics of both asthma and COPD and is a relatively newly described syndrome, there has most likely been a certain degree of misdiagnosis in the past. Several studies including smoking asthmatics are, according to the present guidelines, most likely studying not only smoking asthmatics but also ACOS patients.

The cellular infiltration in the lung of a COPD patient differs from that of an asthmatic. A COPD patient has more neutrophils, monocytes and $CD8^+$ T cells than does an asthmatic patient who usually has more $CD4^+$ T cells and eosinophils (Barnes et al., 2003; Fabbri et al., 2003; Louie et al., 2013). An ACOS patient would lay somewhere in between, with a mixed inflammation in the lung (Louie et al., 2013). This has some implications for the treatment strategies applied to these patients as they are usually more sensitive to inhaled corticosteroids than COPD patients, which is the first-line treatment in combination with LABA and LAMA (long-acting muscarinic antagonist) (Louie et al., 2013; GINA, 2015). It is however clear that the current treatment strategies are not sufficient, as ACOS patients have more frequent exacerbations than asthma and COPD patients, poor quality of life (according to health questionnaires), high mortality and a steep decline in lung function (Gibson & Simpson, 2009; Hardin et al., 2011; Kauppi et al., 2011).

Christenson et al. (2015) recently published a paper on genetic signatures in COPD and asthma. They found that one hundred T2-associated genes up-regulated in asthma, among them the IL-13-inducible genes *POSTN*, *CLCA1*, and *SERPINE2*, were also expressed in a subgroup of COPD patients, identifying a T2 profile in both diseases. This could be therapeutically important as it is known that COPD patients respond poorly to inhaled corticosteroids, but there might be a subpopulation of patients that would benefit from such treatments. It also opens up for T2-related pharmacological intervention such as the anti-IL-5R α monoclonal antibody benralizumab that affects eosinophils. It has gone through phase IIb studies for asthma and phase IIa

studies for COPD, but could only meet the primary endpoint for asthma (Brightling et al., 2014; Castro et al., 2014). A subgroup analysis of the COPD patients with high eosinophil counts in the phase IIa study showed that some patients seemed to benefit from the treatment (Brightling et al., 2014). Perhaps similar findings would be made in an ACOS patient cohort.

This is an emerging area in biology but it is still unclear if a syndrome like ACOS really exists. Most studies agree that more research is needed to form proper guidelines and to assess underlying mechanisms responsible for ACOS (GINA, 2015).

6.4. Paucigranulocytic asthma

About 40% of asthmatic patients have normal levels of both eosinophils and neutrophils and can be classified as paucigranulocytic asthmatics (Schleich et al., 2013). Paucigranulocytic asthmatics are a group of patients that on average have low levels of serum-IgE and T2 cytokines, in contrast to the T2-high phenotype (Manise et al., 2013).

Even so, paucigranulocytic asthmatics show responsiveness to corticosteroid treatment which is difficult to understand since the levels of inflammatory cytokines and sputum cells are similar to healthy subjects (Cowan et al., 2010). One explanation to why paucigranulocytic asthmatics have a benefit of ICS may be that they appear as pseudo paucigranulocytic, suggesting that ICS treatment decreases their eosinophil levels and hides the true eosinophilic asthma phenotype (Cowan et al., 2010). The possibility of paucigranulocytic asthma being a true phenotype has been discussed and it is important to stratify these patients to true or pseudo paucigranulocytic asthmatics, since the stability of this phenotype has been questioned (Simpson et al., 2006; Cowan et al., 2010; Schleich et al., 2013). This is also evident as the variability of eosinophils and levels of cytokines is significantly larger in the paucigranulocytic group than in healthy subject even though the average is the same in both groups (Manise et al., 2013).

Cowan et al. investigated if withdrawal of ICS would change the asthma phenotype in subjects with stable persistent asthma (Cowan et al., 2010). This study showed that after complete steroid withdrawal for 28 days or until patients lost control of their asthma, whichever occurred first, 63 (67%) out of 94 patients had eosinophilic asthma, 29 patients (31%) were classified as paucigranulocytic and two patients (2%) had a mixed phenotype. The same patients were then treated again with fluticasone daily for more than 28 days to investigate a possible change of phenotype upon steroid treatment. After fluticasone treatment, 32 (51%) of the original 63 eosinophilic asthma patients remained having an eosinophilic phenotype, while 22 (35%) became paucigranulocytic, two (3%) got a mixed and two (3%) a neutrophilic phenotype. Of the original paucigranulocytic patients, 21 (72%) stayed within this classification, while four (14%) showed signs of eosinophilic asthma and three (10%) switched to a neutrophilic phenotype. As a conclusion, this study demonstrated that 46% of all patients on ICS treatment with stable persistent asthma fell into a paucigranulocytic asthma phenotype but more than 50% of these patients had been classified as eosinophilic when ICS was withdrawn, i.e. pseudo paucigranulocytic asthma patients. Therefore, classifying patients as paucigranulocytic in a population treated with ICS may lead to a high number of false positives. Moreover, what the proportion of pseudo paucigranulocytic asthmatics vs paucigranulocytic asthmatics will be in a severe asthmatic group is unknown and cannot be investigated with the same method, as withdrawal of ICS would then put the patient at risk.

In a small long-term reproducibility study by Simpson et al. (2006), 86% of non-eosinophilic asthmatics remained in the same asthma phenotype five years after the initial examination. However, non-eosinophilic asthma is defined using a cut-off level in blood eosinophil count and cannot be defined as a pure paucigranulocytic phenotype, since it also includes a neutrophilic asthma phenotype. Also, one issue in this study is the use of ICS and its influence on asthma phenotype. A non-eosinophilic asthma patient could in fact have eosinophilic

asthma that has responded to ICS, and therefore falls into a pseudo non-eosinophilic phenotype.

Paucigranulocytic asthma is still a poorly investigated phenotype of asthma, even though it is suggested that a large proportion of asthmatics are phenotyped as being paucigranulocytic (Simpson et al., 2006; Cowan et al., 2010; Schleich et al., 2013). There is a need for further investigations of the stability of this particular phenotype in long-term reproducibility studies with a focus on understanding the underlying mechanism of airway contraction. Paucigranulocytic asthmatics have classical asthma symptoms like hyperresponsiveness to methacholine and low FEV₁ which is reversible to bronchodilators. Similar to T2-high asthmatics, the patients with a paucigranulocytic phenotype experience on average approximately one exacerbation per patient a year (Manise et al., 2013).

In T2-high asthmatics, airway smooth muscle (ASM) contractility can be explained by the inflammatory milieu and T2-driven mediators (Hakonarson et al., 1999; Tliba et al., 2003; Saha et al., 2008; Manson, 2015). In a non-inflammatory milieu, the underlying mechanism for AHR in asthma is more difficult to understand. Either intrinsic or extrinsic factors can cause smooth muscle cells to contract (Ma et al., 2002; Saha et al., 2008). An intrinsic factor could be a genetic association that may increase AHR in these patients. It may also be speculated that epigenetic factors could affect the proliferation and reactivity in ASM cells, possibly through long-lasting inflammation which has been refracted and resolved. Extrinsic factors that affect ASM may be an excess or lack of paracrine factors. It has been revealed that insulin increases the contractility of smooth muscle cells in vitro, which has further been validated by a demonstrated negative association between type one diabetes and asthma (Stene & Nafstad, 2001). Endocrine factors can also lead to contraction indirectly through the nervous system, for example serum leptin levels correlate with asthma severity (Tsaroucha et al., 2013). ASM contraction can also be induced by activation of airway autonomic nerves through the action of acetylcholine (Canning et al., 2012). Contraction caused by a spasmogen such as acetylcholine can be resolved by non-cholinergic neurotransmitters, as reviewed by Canning (2006). Dysregulation of any of these functions might change the contractility response of the ASM cell and may be involved in the pathogenesis of at least some subtypes of T2-low asthma. Further studies are needed to address what is driving the contraction in a paucigranulocytic environment.

7. Potential T2-low cytokines in asthma

In this review we have defined T2-high patients that have a disease with demonstrated links to the classical Th2 cytokines IL-4, IL-5 and IL-13. A number of non-Th2 cytokines have been implicated in asthma and it is possible that these cytokines could drive distinct T2-low endotypes of asthma. In the following section, we have reviewed the evidence that IL-17, TNF- α and IL-6 may have a role in T2-low asthma.

7.1. IL-17

Th17 cell biology has been implicated in several autoimmune diseases, as reviewed (Yang et al., 2014). Th17 cells are the main producer of IL-17A and IL-17F, which have been observed at high levels in severe asthmatics both in sputum, lung tissue and BAL fluid (Molet et al., 2001; Barczyk et al., 2003; Chakir et al., 2003; Al-Ramli et al., 2009). Other cell types can produce IL-17, such as innate lymphoid cells and NK T cells. The rationale for the involvement of Th17 biology, and more specifically IL-17 in asthma, is that many lung epithelial cells and smooth muscle cells respond to IL-17 with the production of pro-inflammatory cytokines like IL-6, IL-1, TNF- α , GM-CSF and IL-8. These cytokines will promote neutrophil maturation and recruitment as well as induce acute-phase reactants, thereby increasing inflammation (Nembrini et al., 2009). This inflammation falls outside typical T2 inflammation and it is possible that IL-17 biology plays a disease driving

role in T2 low patients. In most studies with asthmatics, IL-17 cannot be linked to neutrophilia in the lung (Chakir et al., 2003; Doe et al., 2010). However, in one study, a correlation was seen between neutrophil counts and IL-17 levels in sputum (Bullens et al., 2006). In another recent study, a Th17 asthma phenotype was associated with a high eosinophil count, both in blood and bronchial biopsies (Choy et al., 2015).

Even though the evidence for IL-17 involvement in severe asthma seems to be present, clinical trials targeting IL-17 have failed for asthma as an indication. Brodalumab, a human anti-IL-17RA blocking monoclonal antibody has recently undergone phase IIa clinical trials for the treatment of moderate to severe asthma, but no treatment effect was observed, perhaps a different patient stratification/inclusion criteria may have been more successful (Busse et al., 2013a). After dividing the patients into subgroups, there was a significant improvement in the high reversibility group, but further studies are needed to confirm this finding (Busse et al., 2013a). The effect observed might also have been due to antagonism of IL-25 (IL-17E) which has been shown to induce T2-related cytokines and inflammatory responses, as IL-25 shares receptor complex with IL-17A (Gaffen, 2009). Another monoclonal antibody, secukinumab, was also tried for asthma in a clinical trial (Clinicaltrials.gov., 2015) but no data has been reported. Patient selection might also be a reason for a potential lack of efficacy since patients with high blood eosinophils (<400 eosinophils/ μ L) were excluded from the secukinumab trial, which according to Choy et al. might benefit from such treatment (Choy et al., 2015). Choy et al. suggest that an IL-17 gene signature can be seen in asthma and it is separate from the T2-high patient group. Their data suggests the presence of three groups, T2-high, Th17-high and T2-low/Th17-low. In order to validate this finding, there would need to be a clinical trial of anti-IL-17 treatment in the Th17-high group. However, at present the only way of selecting these patients is by bronchoscopy and gene signature analysis. Blood biomarkers of the Th17-high group would facilitate the development of an anti-IL-17 treatment in asthma.

The presence of IL-17 is apparent in asthmatics but IL-17-targeting therapies do not seem to be efficacious. Perhaps IL-17 is a result of, and not a driver of inflammation in asthma. More studies are needed to elucidate the exact role of IL-17 in asthma.

7.2. TNF- α

TNF- α is a proinflammatory cytokine that can be released by many different cell types, including structural cells. Dysregulation of TNF- α can lead to increased inflammation and TNF- α inhibitors have had great progress in the treatment of autoimmune diseases such as RA (Feldmann & Maini, 2003). Given the characteristics of TNF- α , one could hypothesize that inhibiting it could be efficacious in asthma. It has been shown that TNF- α is elevated in BAL fluid of symptomatic asthma patients (Broide et al., 1992; Cembrzynska-Nowak et al., 1993), in sputum of asthmatics and in patients with a history of childhood asthma (Obase et al., 2001). Asthmatic patients and healthy subjects who inhaled TNF- α experienced increased BHR and increased numbers of inflammatory cells in sputum (Thomas et al., 1995; Thomas & Heywood, 2002).

TNF- α inhibitors have been tried in asthma but the results have not been promising, despite the proinflammatory profile of TNF- α (Howarth et al., 2005; Berry et al., 2006; Erin et al., 2006; Morjaria et al., 2008; Wenzel et al., 2009). Three TNF- α inhibitors have been tested in clinical trials so far: infliximab, etanercept and golimumab. In two studies of etanercept, patients showed upregulation of TNF- α in BAL fluid, TNF- α gene expression as well as TNF- α expressing immunoreactive cells, and showed improved post-bronchodilator FEV₁ and asthma symptoms (Howarth et al., 2005; Berry et al., 2006). Another study showed decrease in systemic inflammatory parameters like CRP and improved asthma control (Morjaria et al., 2008). Infliximab was tested on moderate asthmatics and a decrease in exacerbations was

observed (Erin et al., 2006). A larger study was also performed with golimumab on uncontrolled, severe persistent asthmatics with no beneficial effects in the overall population. However, subgroup analysis appeared to demonstrate a potentially significant benefit in highly reversible patients, patients with a history of sinusitis and patients with disease onset after 12 years of age. The trial was terminated due to serious adverse events including infections and malignancies (Wenzel et al., 2009).

In summary, TNF- α clinical trials have been performed and initially demonstrated some improvement of symptoms but unfortunately the safety issues reported make it highly unlikely that an anti-TNF will ever be used to treat asthma.

7.3. IL-6

The production of IL-6 is induced in response to infection, tissue damage or exercise and functions as a warning signal that activates host defense mechanisms. The cytokine is produced by a large number of inflammatory cells such as macrophages, dendritic cells, mast cells, neutrophils, B cells and some CD4 effector cells (Rincon & Irvin, 2012) as well as endothelial cells, fibroblasts and epithelial cells (Tanaka et al., 2014).

In classical IL-6 signaling, IL-6 binds to the membrane-bound IL-6 receptor (IL-6R), which is homodimerised with the membrane-bound signal transducer glycoprotein gp130 (thoroughly reviewed by Hunter and Jones (2015)). Classical IL-6 signaling occurs only in cells that express the IL-6R subunit of the receptor complex, that are hepatocytes, megakaryocytes and some leukocytes (Hunter & Jones, 2015). Cells that do not express the membrane-bound IL-6R can use an alternative IL-6 signaling pathway, known as trans-signaling. Here, IL-6 associates with a soluble form of the receptor (sIL-6R), and the IL-6/sIL-6R complex then binds gp130, which is expressed on all human cells, whereby trans-signaling can occur in any cell (Hunter & Jones, 2015). Even though classical IL-6 signaling may be associated with chronic inflammatory and autoimmune diseases, the disease association has recently grown stronger to IL-6 trans-signaling (Scheller et al., 2014). It is hypothesized that trans-signaling could control critical situations by serving as a quick boost of IL-6-dependent responses while under some yet undefined conditions the IL-6 trans-signaling leads to uncontrollable inflammation.

IL-6 is established as a critical target in a number of inflammatory diseases as well as some cancers. Nevertheless, this cytokine plays different roles in different diseases and while it may be effective to block IL-6 in many conditions, it may have to be targeted differently in other disease states, as recently reviewed by Hunter and Jones (2015). As a result, a large number of therapies are under pre-clinical or clinical development targeting IL-6, IL-6R, gp130, IL-6 trans-signaling or signaling downstream of the IL-6R. The monoclonal antibody tocilizumab (ACTEMRA® or RoActemra®) that targets both membrane-bound and soluble IL-6R is approved for treatment of RA worldwide as well as juvenile idiopathic arthritis in several countries and is undergoing clinical trials in a few other inflammatory conditions (Tanaka et al., 2014).

Several human studies have related both IL-6 and the sIL-6R to asthma. Primary bronchial epithelial cells from asthmatics have been shown to produce increased amounts of IL-6 compared to cells from non-asthmatics (Marini et al., 1992; Kicic et al., 2006). Increased levels of IL-6 in induced sputum have been found in mild to moderate asthmatics (Neveu et al., 2010), severe asthmatics (Morjaria et al., 2011) and, at the time of exacerbation, in patients with moderate to severe asthma (Chu et al., 2015). Interestingly, Neveu et al., 2010 found a correlation between increased IL-6 and IL-13 whereas Chu et al. (2015) demonstrated that sputum IL-6 was associated with a mixed eosinophilic/neutrophilic asthmatic airway inflammation. Acute exacerbation of asthma has further been associated with an elevated level of IL-6 in BAL fluid (Tillie-Leblond et al., 1999).

Yokoyama et al. (1997) found increased serum levels of sIL-6R in asthmatic patients compared to control subjects. Interestingly, here the sIL-6R levels were significantly increased during an asthma exacerbation. However, no correlation was found to bronchial hyperreactivity or serum-IgE. Increased levels of sIL-6R have also been found in BAL fluid from asthmatic patients compared to controls (Doganci et al., 2005). The concentration was further significantly increased after segmental allergen challenge. Recently, a study measured both IL-6 and sIL-6R in induced sputum of patients with mild to moderate asthma (Ullah et al., 2015). Only a weak correlation was found between IL-6 and sIL-6R, but two groups with neutrophilic or mixed eosinophilic/neutrophilic asthma were found to have both high IL-6 and high sIL-6R in sputum.

In addition, genetic studies have shown a strong link between IL-6 signaling and asthma. A large genome-wide association study of nearly 58,000 subjects identified the *IL6R* gene as an asthma risk gene (Ferreira et al., 2011). At least two coding changes in the *IL6R* gene that promote IL-6 trans-signaling have been associated with increased levels of sIL-6R in serum and with reduced lung function in asthma (Galicia et al., 2004; Hawkins et al., 2012; Revez et al., 2013). One of these gene variants was found at higher frequency in patients with severe asthma (Hawkins et al., 2012) and the authors proposed it as a potential genetic marker of asthma severity.

There is strong evidence showing an association between IL-6 and asthma. Even though it is not a key cytokine in most subsets of asthma, it may be important to identify the subset with the strongest link to increased IL-6 signaling. Whether it is a subset of neutrophilic asthmatics, a group of severe asthmatics with a specific variant of the *IL6R* gene or some other subset of patients with increased IL-6 that does not respond to other treatments, tocilizumab could be a potential therapy to investigate.

8. Summary

It is clear that a number of novel asthma therapies are now close to the market and these will provide new options for the treatment of severe asthmatics. Some of these therapies have taken a significant time to demonstrate efficacy in clinical asthma. The difficulties of developing therapies such as anti-IL-5 and anti-IL-13 are related to the heterogenic nature of asthma and both therapies have had to adopt a biomarker-based strategy to identify responder patients. Work on these therapies has led to the understanding that asthma can be divided into two broad populations of T2-high patients and T2-low patients. Asthma has classically been thought of as a T2-high disease and most therapies in development target this pathology. The T2-high patients seem to form a relatively large population where the key pathological drivers are well characterized. Review of the literature makes it clear that there is a very limited understanding of T2-low pathology and there are no treatments on the market or in development for these patients. Therefore, once the T2-high-targeting therapies are on the market, patients with T2-low asthma will remain poorly treated. This review looked at some of the suggested T2-low patient populations but with the exception of obese-associated asthma it is clear there is limited understanding of the phenotype of these patients or the pathological drivers in these T2-low patients. Without clinical characterization of the T2-low patients it is impossible to identify potential pathological drivers of the disease and develop novel therapies to target this pathology. Therefore, future research should focus on identifying T2-low patients, understanding the heterogeneity of T2-low asthma and identifying the key pathological drivers. This will allow us to develop targeted therapies to treat what will become the unmet clinical need in asthma once the T2-targeting therapies are approved.

Studies have been initiated to understand which patients will be suitable for novel T2-high asthma treatments and these studies will also identify T2-low patients (Heaney et al., 2016). The problem then is to determine the phenotypes and endotypes of T2-low asthma and

this is not a trivial task. Identification of T2-high asthma was based on knowledge that T2 immunity played an important role in asthma. Unfortunately, T2-low asthma does not have such an obvious driver and is probably not a single endotype. Identification of pathological drivers in T2-low asthma will require sampling of the lung and analysis in a similar manner to the way Woodruff et al. identified T2-high asthma. The weakness of this approach is that it is based on epithelial brushings and it is possible that T2-low asthma is not driven through altered epithelial cell biology, meaning that future studies may have to look at alternative cell types, such as airway smooth muscle cells or macrophages.

This review considers the T2-high group as a relatively homogeneous group of patients defined by responsiveness to therapies that target T2 biology and the presence of T2-associated biomarkers, such as eosinophils, periostin or IgE. This indicates that the biological drivers of disease are closely related to the classical Th2 cytokines IL-4, IL-5 and IL-13. The development of multiple therapies targeting different aspects of T2 biology will determine if there is a way to differentiate between patients responsive to anti-IL-5, anti-IL-13, anti-IL-4R α or anti-TSLP. Of the patient groups discussed in this review, it is only the periostin-defined anti-IL-13-responsive population that represents a true pathway-defined endotype. It could be argued that IL-5 biology is so closely linked to eosinophils that the patients with persistent eosinophils, despite ICS treatment, also represents an endotype. Only clinical experience with these therapies will determine if there are multiple T2 endotypes and how they will respond to different T2 therapies. It is possible that there will be multiple T2 sub-endotypes requiring different treatment regimens. T2 biology and eosinophilic inflammation is also present in a large proportion of patients that are successfully controlled by current standard of care therapy and these patients will not require expensive biological drugs to control their disease. There is a possibility that non-compliant T2-high patients will be treated with biologics unnecessarily, and biomarkers may be required to differentiate between steroid-responsive T2-high, steroid-refractory T2-high and non-compliant patients. A recently published protocol indicated that this may be achieved using composite T2 biomarkers linked to a simple algorithm (Heaney et al., 2016). Through most of this review, we have mainly discussed single biomarker-defined patient populations, but recently it has become clear that there is considerable effort being focused on using composite biomarkers to define endotypes, and this approach may be required to fully endotype both T2-high and T2-low asthma (Nagasaki et al., 2014).

It is worth noting that although novel therapies will be developed to help control asthma, none of the therapies under development will cure asthma or completely eliminate the risk of exacerbations. Therefore, there is still a need to develop a next generation of asthma therapies for both T2-high and T2-low asthma. These therapies should be aimed to provide remission and possibly drug-free remission of disease. These treatments would truly revolutionize the treatment of asthma.

Conflicts of interest

1. LS, TS, MR, PG, MN, GJ, PH and MC are full time employees of AstraZeneca.
2. LS, TS, MR, PG, MN, GJ, PH and MC own shares in AstraZeneca.
3. MC owns shares in UCB Pharma.

References

- Aanerud, M., Carsin, A. E., Sunyer, J., Dratva, J., Gislason, T., Jarvis, D., et al. (2015). Interaction between asthma and smoking increases the risk of adult airway obstruction. *Eur Respir J* 45, 635–643.
- Adelroth, E., Rak, S., Hahtela, T., Aasand, G., Rosenhall, L., Zetterstrom, O., et al. (2000). Recombinant humanized mab-e25, an anti-ige mab, in birch pollen-induced seasonal allergic rhinitis. *J Allergy Clin Immunol* 106, 253–259.
- Akdis, C. A. (2012). Therapies for allergic inflammation: refining strategies to induce tolerance. *Nat Med* 18, 736–749.

- Al-Alwan, A., Bates, J. H., Chapman, D. G., Kaminsky, D. A., DeSarno, M. J., Irvin, C. G., et al. (2014). The nonallergic asthma of obesity. A matter of distal lung compliance. *Am J Respir Crit Care Med* 189, 1494–1502.
- Ali, Z., & Ulrik, C. S. (2013). Obesity and asthma: a coincidence or a causal relationship? A systematic review. *Respir Med* 107, 1287–1300.
- Al-Ramli, W., Prefontaine, D., Chouiali, F., Martin, J. G., Olivenstein, R., Lemiere, C., et al. (2009). T(h)17-associated cytokines (il-17a and il-17f) in severe asthma. *J Allergy Clin Immunol* 123, 1185–1187.
- Anderson, G. P. (2008). Endotyping asthma: new insights into key pathogenic mechanisms in a complex, heterogeneous disease. *Lancet* 372, 1107–1119.
- Arima, K., Watanabe, N., Hanabuchi, S., Chang, M., Sun, S. C., & Liu, Y. J. (2010). Distinct signal codes generate dendritic cell functional plasticity. *Sci Signal* 3, ra4.
- Artis, D., & Spits, H. (2015). The biology of innate lymphoid cells. *Nature* 517, 293–301.
- Balzar, S., Fajt, M. L., Comhair, S. A., Erzurum, S. C., Bleeker, E., Busse, W. W., et al. (2011). Mast cell phenotype, location, and activation in severe asthma. Data from the severe asthma research program. *Am J Respir Crit Care Med* 183, 299–309.
- Baraldo, S., Contoli, M., Bazzan, E., Turato, G., Padovani, A., Marku, B., et al. (2012). Deficient antiviral immune responses in childhood: distinct roles of atopy and asthma. *J Allergy Clin Immunol* 130, 1307–1314.
- Barczyk, A., Pierzchala, W., & Sozanska, E. (2003). Interleukin-17 in sputum correlates with airway hyperresponsiveness to methacholine. *Respir Med* 97, 726–733.
- Barnes, P. J. (2001). Th2 cytokines and asthma: an introduction. *Respir Res* 2, 64–65.
- Barnes, P. J. (2008). The cytokine network in asthma and chronic obstructive pulmonary disease. *J Clin Invest* 118, 3546–3556.
- Barnes, N., Pavord, I., Chuchalin, A., Bell, J., Hunter, M., Lewis, T., et al. (2012). A randomized, double-blind, placebo-controlled study of the CRTH2 antagonist OC000459 in moderate persistent asthma. *Clin Exp Allergy* 42, 38–48.
- Barnes, P. J., Shapiro, S. D., & Pauwels, R. A. (2003). Chronic obstructive pulmonary disease: molecular and cellular mechanisms. *Eur Respir J* 22, 672–688.
- Barranco, P., Garcia-Rio, F., Olivares, J., Lopez-Carrasco, V., Alvarez-Sala, R., & Quirce, S. (2011). Asthma diagnosis is not associated with obesity in a population of adults from Madrid. *J Investig Allergol Clin Immunol* 21, 540–545.
- Becker, T. M., Durrani, S. R., Bochkov, Y. A., Devries, M. K., Rajamanickam, V., & Jackson, D. J. (2013). Effect of exogenous interferons on rhinovirus replication and airway inflammatory responses. *Ann Allergy Asthma Immunol* 111, 397–401.
- Beckett, W. S., Jacobs, D. R., Jr., Yu, X., Iribarren, C., & Williams, O. D. (2001). Asthma is associated with weight gain in females but not males, independent of physical activity. *Am J Respir Crit Care Med* 164, 2045–2050.
- Bel, E. H., Wenzel, S. E., Thompson, P. J., Prazma, C. M., Keene, O. N., Yancey, S. W., et al. (2014). Oral glucocorticoid-sparing effect of mepolizumab in eosinophilic asthma. *N Engl J Med* 371, 1189–1197.
- Berair, R. G. S., Singapur, A., Hartley, R., Laurencin, M., Bacher, G., Holzhauser, B., ... Brightling, C. E. (2015). Effect of qaw039, an oral prostaglandin D2 receptor (DP2/CRTH2) antagonist, upon sputum and bronchial eosinophilic inflammation and clinical outcomes in treatment-resistant asthma: a phase 2a randomized placebo-controlled trial. *American Thoracic Society: ATS Conference* (pp. A6361).
- Bernink, J. H., Krabbendam, L., Germar, K., de Jong, E., Gronke, K., Kofoed-Nielsen, M., et al. (2015). Interleukin-12 and -23 control plasticity of cd127(+) group 1 and group 3 innate lymphoid cells in the intestinal lamina propria. *Immunity* 43, 146–160.
- Bernink, J. H., Peters, C. P., Munneke, M., te Velde, A. A., Meijer, S. L., Weijer, K., et al. (2013). Human type 1 innate lymphoid cells accumulate in inflamed mucosal tissues. *Nat Immunol* 14, 221–229.
- Berry, M. A., Hargadon, B., Shelley, M., Parker, D., Shaw, D. E., Green, R. H., et al. (2006). Evidence of a role of tumor necrosis factor alpha in refractory asthma. *N Engl J Med* 354, 697–708.
- Berry, M. A., Parker, D., Neale, N., Woodman, L., Morgan, A., Monk, P., et al. (2004). Sputum and bronchial submucosal il-13 expression in asthma and eosinophilic bronchitis. *J Allergy Clin Immunol* 114, 1106–1109.
- Beuther, D. A., & Sutherland, E. R. (2007). Overweight, obesity, and incident asthma: a meta-analysis of prospective epidemiologic studies. *Am J Respir Crit Care Med* 175, 661–666.
- Bjermer, L., Lemiere, C., Maspero, J., Ciesielska, M., O'Brien, C., & Zangrilli, J. (2014). A randomized phase 3 study of the efficacy and safety of reslizumab in subjects with asthma with elevated eosinophils. *Eur Respir J* 44(Suppl. 58), 299.
- Bochkov, Y. A., Hanson, K. M., Keles, S., Brockman-Schneider, R. A., Jarjour, N. N., & Gern, J. E. (2010). Rhinovirus-induced modulation of gene expression in bronchial epithelial cells from subjects with asthma. *Mucosal Immunol* 3, 69–80.
- Boitano, S., Flynn, A. N., Sherwood, C. L., Schulz, S. M., Hoffman, J., Gruzina, I., et al. (2011). Alternaria alternata serine proteases induce lung inflammation and airway epithelial cell activation via par2. *Am J Physiol Lung Cell Mol Physiol* 300, L605–L614.
- Borish, L. C., Nelson, H. S., Lanz, M. J., Claussen, L., Whitmore, J. B., Agosti, J. M., et al. (1999). Interleukin-4 receptor in moderate atopic asthma. A phase I/II randomized, placebo-controlled trial. *Am J Respir Crit Care Med* 160, 1816–1823.
- Boulet, L. P., Chapman, K. R., Cote, J., Kalra, S., Bhagat, R., Swystun, V. A., et al. (1997). Inhibitory effects of an anti-ige antibody e25 on allergen-induced early asthmatic response. *Am J Respir Crit Care Med* 155, 1835–1840.
- Boulet, L. P., Turcotte, H., Martin, J., & Poirier, P. (2012). Effect of bariatric surgery on airway response and lung function in obese subjects with asthma. *Respir Med* 106, 651–660.
- Brightling, C. E., Bleeker, E. R., Panettieri, R. A., Jr., Bafadhel, M., She, D., Ward, C. K., et al. (2014). Benralizumab for chronic obstructive pulmonary disease and sputum eosinophilia: a randomised, double-blind, placebo-controlled, phase 2a study. *Lancet Respir Med* 2, 891–901.
- Brightling, C. E., Chaney, P., Leigh, R., O'Byrne, P. M., Korn, S., She, D., et al. (2015). Efficacy and safety of tralokinumab in patients with severe uncontrolled asthma: a randomised, double-blind, placebo-controlled, phase 2b trial. *Lancet Respir Med* 3, 692–701.

- Brocklehurst, W. E. (1960). The release of histamine and formation of a slow-reacting substance (srs-a) during anaphylactic shock. *J Physiol* 151, 416–435.
- Broekema, M., ten Hacken, N. H., Volbeda, F., Lodewijk, M. E., Hylkema, M. N., Postma, D. S., et al. (2009). Airway epithelial changes in smokers but not in ex-smokers with asthma. *Am J Respir Crit Care Med* 180, 1170–1178.
- Broide, D. H., Lotz, M., Cuomo, A. J., Coburn, D. A., Federman, E. C., & Wasserman, S. I. (1992). Cytokines in symptomatic asthma airways. *J Allergy Clin Immunol* 89, 958–967.
- Brumpton, B., Langhammer, A., Romundstad, P., Chen, Y., & Mai, X. M. (2013). General and abdominal obesity and incident asthma in adults: the Hunt study. *Eur Respir J* 41, 323–329.
- Bufe, A., Gehlhar, K., Grage-Griebenow, E., & Ernst, M. (2002). Atopic phenotype in children is associated with decreased virus-induced interferon- α release. *Int Arch Allergy Immunol* 127, 82–88.
- Buhl, R., Soler, M., Matz, J., Townley, R., O'Brien, J., Noga, O., et al. (2002). Omalizumab provides long-term control in patients with moderate-to-severe allergic asthma. *Eur Respir J* 20, 73–78.
- Bullens, D. M., Truyen, E., Coteur, L., Dilissen, E., Hellings, P. W., Dupont, L. J., et al. (2006). IL-17 mRNA in sputum of asthmatic patients: linking T cell driven inflammation and granulocytic influx? *Respir Res* 7, 135.
- Burke, H., Leonardi-Bee, J., Hashim, A., Pine-Abata, H., Chen, Y., Cook, D. G., et al. (2012). Prenatal and passive smoke exposure and incidence of asthma and wheeze: systematic review and meta-analysis. *Pediatrics* 129, 735–744.
- Burrows, B., Martinez, F. D., Halonen, M., Barbee, R. A., & Cline, M. G. (1989). Association of asthma with serum IgE levels and skin-test reactivity to allergens. *N Engl J Med* 320, 271–277.
- Busse, W., Corren, J., Lanier, B. Q., McAlary, M., Fowler-Taylor, A., Cioppa, G. D., et al. (2001). Omalizumab, anti-IgE recombinant humanized monoclonal antibody, for the treatment of severe allergic asthma. *J Allergy Clin Immunol* 108, 184–190.
- Busse, W. W., Holgate, S., Kerwin, E., Chon, Y., Feng, J., Lin, J., et al. (2013a). Randomized, double-blind, placebo-controlled study of brodalumab, a human anti-IL-17 receptor monoclonal antibody, in moderate to severe asthma. *Am J Respir Crit Care Med* 188, 1294–1302.
- Busse, W. W., Katial, R., Gossage, D., Sari, S., Wang, B., Kolbeck, R., et al. (2010). Safety profile, pharmacokinetics, and biologic activity of medi-563, an anti-IL-5 receptor α antibody, in a phase I study of subjects with mild asthma. *J Allergy Clin Immunol* 125(1237–1244), e1232.
- Busse, W. W., Morgan, W. J., Gergen, P. J., Mitchell, H. E., Gern, J. E., Liu, A. H., et al. (2011). Randomized trial of omalizumab (anti-IgE) for asthma in inner-city children. *N Engl J Med* 364, 1005–1015.
- Busse, W. W., Wenzel, S. E., Meltzer, E. O., Kerwin, E. M., Liu, M. C., Zhang, N., et al. (2013b). Safety and efficacy of the prostaglandin D2 receptor antagonist AMG 853 in asthmatic patients. *J Allergy Clin Immunol* 131, 339–345.
- Cai, C., Yang, J., Hu, S., Zhou, M., & Guo, W. (2007). Relationship between urinary cysteinyl leukotriene e4 levels and clinical response to antileukotriene treatment in patients with asthma. *Lung* 185, 105–112.
- Cakebread, J. A., Xu, Y., Grainge, C., Kehagia, V., Howarth, P. H., Holgate, S. T., et al. (2011). Exogenous IFN- β has antiviral and anti-inflammatory properties in primary bronchial epithelial cells from asthmatic subjects exposed to rhinovirus. *J Allergy Clin Immunol* 127(1148–1154), e1149.
- Calhoun, W. J., Lavins, B. J., Minkwitz, M. C., Evans, R., Gleich, G. J., & Cohn, J. (1998). Effect of zafirlukast (accolate) on cellular mediators of inflammation: bronchoalveolar lavage fluid findings after segmental antigen challenge. *Am J Respir Crit Care Med* 157, 1381–1389.
- Calvani, M., Alessandri, C., Sopo, S. M., Panetta, V., Pingitore, G., Tripodi, S., et al. (2006). Consumption of fish, butter and margarine during pregnancy and development of allergic sensitizations in the offspring: role of maternal atopy. *Pediatr Allergy Immunol* 17, 94–102.
- Canning, B. J. (2006). Reflex regulation of airway smooth muscle tone. *J Appl Physiol* (1985) 101, 971–985.
- Canning, B. J., Woo, A., & Mazzone, S. B. (2012). Neuronal modulation of airway and vascular tone and their influence on nonspecific airways responsiveness in asthma. *J Allergy (Cairo)* 2012, 108149.
- Carroll, N., Carello, S., Cooke, C., & James, A. (1996). Airway structure and inflammatory cells in fatal attacks of asthma. *Eur Respir J* 9, 709–715.
- Casale, T. B., Bernstein, I. L., Busse, W. W., LaForce, C. F., Tinkelman, D. G., Stoltz, R. R., et al. (1997). Use of an anti-IgE humanized monoclonal antibody in ragweed-induced allergic rhinitis. *J Allergy Clin Immunol* 100, 110–121.
- Casale, T. B., Condemni, J., LaForce, C., Nayak, A., Rowe, M., Watrous, M., et al. (2001). Effect of omalizumab on symptoms of seasonal allergic rhinitis: a randomized controlled trial. *JAMA* 286, 2956–2967.
- Castro, M., Mathur, S., Hargreave, F., Boulet, L. P., Xie, F., Young, J., et al. (2011). Reslizumab for poorly controlled, eosinophilic asthma: a randomized, placebo-controlled study. *Am J Respir Crit Care Med* 184, 1125–1132.
- Castro, M., Wenzel, S. E., Bleeker, E. R., Pizzichini, E., Kuna, P., Busse, W. W., et al. (2014). Benralizumab, an anti-interleukin 5 receptor α monoclonal antibody, versus placebo for uncontrolled eosinophilic asthma: a phase 2b randomised dose-ranging study. *Lancet Respir Med* 2, 879–890.
- Cembrzynska-Nowak, M., Szklarz, E., Inglot, A. D., & Teodorczyk-Injeyan, J. A. (1993). Elevated release of tumor necrosis factor- α and interferon- γ by bronchoalveolar leukocytes from patients with bronchial asthma. *Am Rev Respir Dis* 147, 291–295.
- Cerveri, I., Cazzoletti, L., Corsico, A. G., Marcon, A., Niniano, R., Grosso, A., et al. (2012). The impact of cigarette smoking on asthma: a population-based international cohort study. *Int Arch Allergy Immunol* 158, 175–183.
- Chakir, J., Shannou, J., Molet, S., Fukakusa, M., Elias, J., Laviolette, M., et al. (2003). Airway remodeling-associated mediators in moderate to severe asthma: effect of steroids on
- tgf- β , IL-11, IL-17, and type I and type III collagen expression. *J Allergy Clin Immunol* 111, 1293–1298.
- Chalmers, G. W., MacLeod, K. J., Thomson, L., Little, S. A., McSharry, C., & Thomson, N. C. (2001). Smoking and airway inflammation in patients with mild asthma. *Chest* 120, 1917–1922.
- Chan, K. H., Yeung, S. C., Yao, T. J., Ip, M. S., Cheung, A. H., Chan-Yeung, M. M., et al. (2010). Elevated plasma adiponectin levels in patients with chronic obstructive pulmonary disease. *Int J Tuberc Lung Dis* 14, 1193–1200.
- Chang, Y. J., DeKruyff, R. H., & Umetsu, D. T. (2013). The role of type 2 innate lymphoid cells in asthma. *J Leukoc Biol* 94, 933–940.
- Chaudhuri, R., Livingston, E., McMahon, A. D., Lafferty, J., Fraser, I., Spears, M., et al. (2006). Effects of smoking cessation on lung function and airway inflammation in smokers with asthma. *Am J Respir Crit Care Med* 174, 127–133.
- Chaudhuri, R., Livingston, E., McMahon, A. D., Thomson, L., Borland, W., & Thomson, N. C. (2003). Cigarette smoking impairs the therapeutic response to oral corticosteroids in chronic asthma. *Am J Respir Crit Care Med* 168, 1308–1311.
- Chen, Y., Dales, R., Krewski, D., & Breithaupt, K. (1999). Increased effects of smoking and obesity on asthma among female Canadians: the National Population Health Survey, 1994–1995. *Am J Epidemiol* 150, 255–262.
- Chen, Y., Rennie, D., Cormier, Y., & Dosman, J. (2005). Sex specificity of asthma associated with objectively measured body mass index and waist circumference: the Humboldt study. *Chest* 128, 3048–3054.
- Chesne, J., Braza, F., Mahay, G., Brouard, S., Aronica, M., & Magnan, A. (2014). IL-17 in severe asthma. Where do we stand? *Am J Respir Crit Care Med* 190, 1094–1101.
- Chiba, Y., Goto, K., & Misawa, M. (2012). Interleukin-13-induced activation of signal transducer and activator of transcription 6 is mediated by an activation of janus kinase 1 in cultured human bronchial smooth muscle cells. *Pharmacol Rep* 64, 454–458.
- Chinn, S., Downs, S. H., Anto, J. M., Gerbase, M. W., Leynaert, B., de Marco, R., et al. (2006). Incidence of asthma and net change in symptoms in relation to changes in obesity. *Eur Respir J* 28, 763–771.
- Choy, D. F., Hart, K. M., Borthwick, L. A., Shikotra, A., Nagarkar, D. R., Siddiqui, S., et al. (2015). Th2 and Th17 inflammatory pathways are reciprocally regulated in asthma. *Sci Transl Med* 7, 301ra129.
- Christenson, S. A., Steiling, K., van den Berge, M., Hijazi, K., Hiemstra, P. S., Postma, D. S., et al. (2015). Asthma–COPD overlap. Clinical relevance of genomic signatures of type 2 inflammation in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 191, 758–766.
- Chu, D. K., Al-Garawi, A., Llop-Guevara, A., Pillai, R. A., Radford, K., Shen, P., et al. (2015). Therapeutic potential of anti-IL-6 therapies for granulocytic airway inflammation in asthma. *Allergy Asthma Clin Immunol* 11, 14.
- Chung, K. F. (2016). Asthma phenotyping: a necessity for improved therapeutic precision and new targeted therapies. *J Intern Med* 279, 192–204.
- Chung, K. F., & Adcock, I. M. (2015). Clinical phenotypes of asthma should link up with disease mechanisms. *Curr Opin Allergy Clin Immunol* 15, 56–62.
- Clinicaltrials.gov (2012). Pilot study in patients with symptomatic steroid-naïve asthma. <http://clinicaltrials.gov/show/NCT00024544>
- Clinicaltrials.gov (2015). Safety, tolerability, and efficacy of ain457 in patients with uncontrolled asthma. <http://clinicaltrials.gov/ct2/show/NCT01478360>
- Clinicaltrials.gov (2016). A phase 2 randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of medi9929 (amg 157) in adult subjects with inadequately controlled, severe asthma. <https://clinicaltrials.gov/ct2/show/NCT02054130>
- Colak, Y., Afzal, S., Lange, P., & Nordestgaard, B. G. (2016). Obese individuals experience wheezing without asthma but not asthma without wheezing: a Mendelian randomisation study of 85 437 adults from the Copenhagen general population study. *Thorax* 71, 247–254.
- Contoli, M., Ito, K., Padovani, A., Poletti, D., Marku, B., Edwards, M. R., et al. (2015). Th2 cytokines impair innate immune responses to rhinovirus in respiratory epithelial cells. *Allergy* 70, 910–920.
- Contoli, M., Message, S. D., Laza-Stanca, V., Edwards, M. R., Wark, P. A., Bartlett, N. W., et al. (2006). Role of deficient type III interferon- λ production in asthma exacerbations. *Nat Med* 12, 1023–1026.
- Corne, J. M., Marshall, C., Smith, S., Schreiber, J., Sanderson, G., Holgate, S. T., et al. (2002). Frequency, severity, and duration of rhinovirus infections in asthmatic and non-asthmatic individuals: a longitudinal cohort study. *Lancet* 359, 831–834.
- Corren, J. (2013a). Asthma phenotypes and endotypes: an evolving paradigm for classification. *Discov Med* 15, 243–249.
- Corren, J. (2013b). Role of interleukin-13 in asthma. *Curr Allergy Asthma Rep* 13, 415–420.
- Corren, J., Busse, W., Meltzer, E. O., Mansfield, L., Bensch, G., Fahrenholz, J., et al. (2010). A randomized, controlled, phase 2 study of amg 317, an IL-4/ α antagonist, in patients with asthma. *Am J Respir Crit Care Med* 181, 788–796.
- Corren, J., Casale, T., Deniz, Y., & Ashby, M. (2003). Omalizumab, a recombinant humanized anti-IgE antibody, reduces asthma-related emergency room visits and hospitalizations in patients with allergic asthma. *J Allergy Clin Immunol* 111, 87–90.
- Corren, J., Lemanske, R. F., Hanania, N. A., Korenblat, P. E., Parsey, M. V., Aron, J. R., et al. (2011). Lebrikizumab treatment in adults with asthma. *N Engl J Med* 365, 1088–1098.
- Corrigan, C. J., Haczku, A., Gemou-Engesaeth, V., Doi, S., Kikuchi, Y., Takatsu, K., et al. (1993). CD4 T-lymphocyte activation in asthma is accompanied by increased serum concentrations of interleukin-5. Effect of glucocorticoid therapy. *Am Rev Respir Dis* 147, 540–547.
- Corry, D. B., Folkesson, H. G., Warnock, M. L., Erle, D. J., Matthay, M. A., Wiener-Kronish, J. P., et al. (1996). Interleukin 4, but not interleukin 5 or eosinophils, is required in a murine model of acute airway hyperreactivity. *J Exp Med* 183, 109–117.
- Costa, L. D., Costa, P. S., & Camargos, P. A. (2014). Exacerbation of asthma and airway infection: is the virus the villain? *J Pediatr (Rio J)* 90, 542–555.
- Cowan, D. C., Cowan, J. O., Palmay, R., Williamson, A., & Taylor, D. R. (2010). Effects of steroid therapy on inflammatory cell subtypes in asthma. *Thorax* 65, 384–390.

- Coyle, A. J., Le Gros, G., Bertrand, C., Tsuyuki, S., Heusser, C. H., Kopf, M., et al. (1995). Interleukin-4 is required for the induction of lung Th2 mucosal immunity. *Am J Respir Cell Mol Biol* 13, 54–59.
- Crea, A. E., Nakhosteen, J. A., & Lee, T. H. (1992). Mediator concentrations in bronchoalveolar lavage fluid of patients with mild asymptomatic bronchial asthma. *Eur Respir J* 5, 190–195.
- De Boever, E. H., Ashman, C., Cahn, A. P., Locantore, N. W., Overend, P., Pouliquen, I. J., et al. (2014). Efficacy and safety of an anti-IL-13 mab in patients with severe asthma: a randomized trial. *J Allergy Clin Immunol* 133, 989–996.
- de Lima Azambuja, R., da Costa Santos Azambuja, L. S., Costa, C., & Rufino, R. (2015). Adiponectin in asthma and obesity: protective agent or risk factor for more severe disease? *Lung* 193, 749–755.
- De Rosa, V., Procaccini, C., Cali, G., Pirozzi, G., Fontana, S., Zappacosta, S., et al. (2007). A key role of leptin in the control of regulatory t cell proliferation. *Immunity* 26, 241–255.
- DeMore, J. P., Weisshaar, E. H., Vrtis, R. F., Swenson, C. A., Evans, M. D., Morin, A., et al. (2009). Similar colds in subjects with allergic asthma and nonatopic subjects after inoculation with rhinovirus-16. *J Allergy Clin Immunol* 124, 245–252 (252 e241–243).
- Devereux, G. (2006). The increase in the prevalence of asthma and allergy: food for thought. *Nat Rev Immunol* 6, 869–874.
- Devereux, G., Litonjua, A. A., Turner, S. W., Craig, L. C., McNeill, G., Martindale, S., et al. (2007). Maternal vitamin D intake during pregnancy and early childhood wheezing. *Am J Clin Nutr* 85, 853–859.
- Di Stefano, A., Capelli, A., Lusuadi, M., Caramori, G., Balbo, P., Ioli, F., et al. (2001). Decreased T lymphocyte infiltration in bronchial biopsies of subjects with severe chronic obstructive pulmonary disease. *Clin Exp Allergy* 31, 893–902.
- Diamant, Z., Sidharta, P. N., Singh, D., O'Connor, B. J., Zuiker, R., Leaker, B. R., et al. (2014). Setipiprant, a selective CRTH2 antagonist, reduces allergen-induced airway responses in allergic asthmatics. *Clin Exp Allergy* 44, 1044–1052.
- Diaz, J., Warren, L., Helfner, L., Xue, X., Chatterjee, P. K., Gupta, M., et al. (2015). Obesity shifts house dust mite-induced airway cellular infiltration from eosinophils to macrophages: effects of glucocorticoid treatment. *Immunol Res* 63, 197–208.
- Ding, D. J., Martin, J. G., & Macklem, P. T. (1987). Effects of lung volume on maximal methacholine-induced bronchoconstriction in normal humans. *J Appl Physiol* (1985) 62, 1324–1330.
- Dixon, A. (2012). The treatment of asthma in obesity. *Expert Rev Respir Med* 6, 331–340.
- Dixon, A. E., Pratley, R. E., Forgione, P. M., Kaminsky, D. A., Whittaker-Leclair, L. A., Griffes, L. A., et al. (2011). Effects of obesity and bariatric surgery on airway hyperresponsiveness, asthma control, and inflammation. *J Allergy Clin Immunol* 128(508–515), e501–e502.
- Djukanovic, R., Harrison, T., Johnston, S. L., Gabbay, F., Wark, P., Thomson, N. C., et al. (2014). The effect of inhaled ifn-beta on worsening of asthma symptoms caused by viral infections. A randomized trial. *Am J Respir Crit Care Med* 190, 145–154.
- Doe, C., Bafadhel, M., Siddiqui, S., Desai, D., Mistry, V., Rugman, P., et al. (2010). Expression of the t helper 17-associated cytokines IL-17a and IL-17f in asthma and copd. *Chest* 138, 1140–1147.
- Doganci, A., Eigenbrod, T., Krug, N., De Sanctis, G. T., Hausding, M., Erpenbeck, V. J., et al. (2005). The IL-6 α chain controls lung CD4+CD25+ Treg development and function during allergic airway inflammation in vivo. *J Clin Invest* 115, 313–325.
- Edwards, M. R., Regamey, N., Varelle, M., Kieninger, E., Gupta, A., Shoemark, A., et al. (2013). Impaired innate interferon induction in severe therapy resistant atopic asthmatic children. *Mucosal Immunol* 6, 797–806.
- Egan, K. B., Ettinger, A. S., & Bracken, M. B. (2013). Childhood body mass index and subsequent physician-diagnosed asthma: a systematic review and meta-analysis of prospective cohort studies. *BMC Pediatr* 13, 121.
- Eisner, M. D., Yelin, E. H., Katz, P. P., Shiboski, S. C., Henke, J., & Blanc, P. D. (2000). Predictors of cigarette smoking and smoking cessation among adults with asthma. *Am J Public Health* 90, 1307–1311.
- Erin, E. M., Leaker, B. R., Nicholson, G. C., Tan, A. J., Green, L. M., Neighbour, H., et al. (2006). The effects of a monoclonal antibody directed against tumor necrosis factor- α in asthma. *Am J Respir Crit Care Med* 174, 753–762.
- Esmaili, S., Xu, A., & George, J. (2014). The multifaceted and controversial immunometabolic actions of adiponectin. *Trends Endocrinol Metab* 25, 444–451.
- Fabbri, L. M., Romagnoli, M., Corbetta, L., Casoni, G., Busljetic, K., Turato, G., et al. (2003). Differences in airway inflammation in patients with fixed airflow obstruction due to asthma or chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 167, 418–424.
- Fahy, J. V. (2015). Type 2 inflammation in asthma—present in most, absent in many. *Nat Rev Immunol* 15, 57–65.
- Fahy, J. V., Fleming, H. E., Wong, H. H., Liu, J. T., Su, J. Q., Reimann, J., et al. (1997). The effect of an anti-IgE monoclonal antibody on the early- and late-phase responses to allergen inhalation in asthmatic subjects. *Am J Respir Crit Care Med* 155, 1828–1834.
- Fajit, M. L., & Wenzel, S. E. (2015). Asthma phenotypes and the use of biologic medications in asthma and allergic disease: the next steps toward personalized care. *J Allergy Clin Immunol* 135, 299–310 quiz 311.
- Feldmann, M., & Maini, R. N. (2003). Lasker clinical medical research award. Tnf defined as a therapeutic target for rheumatoid arthritis and other autoimmune diseases. *Nat Med* 9, 1245–1250.
- Fennerty, A. G., Banks, J., Ebdon, P., & Bevan, C. (1987). The effect of cigarette withdrawal on asthmatics who smoke. *Eur J Respir Dis* 71, 395–399.
- Ferreira, M. A., Matheson, M. C., Duffy, D. L., Marks, G. B., Hui, J., Le Souef, P., et al. (2011). Identification of IL6r and chromosome 11q13.5 as risk loci for asthma. *Lancet* 378, 1006–1014.
- Finn, A., Gross, G., van Bavel, J., Lee, T., Windom, H., Everhard, F., et al. (2003). Omalizumab improves asthma-related quality of life in patients with severe allergic asthma. *J Allergy Clin Immunol* 111, 278–284.
- Flodin, U., Jonsson, P., Ziegler, J., & Axelsson, O. (1995). An epidemiologic study of bronchial asthma and smoking. *Epidemiology* 6, 503–505.
- Flood-Page, P. T., Menzies-Gow, A. N., Kay, A. B., & Robinson, D. S. (2003). Eosinophil's role remains uncertain as anti-interleukin-5 only partially depletes numbers in asthmatic airway. *Am J Respir Crit Care Med* 167, 199–204.
- Flood-Page, P., Swenson, C., Faiferman, I., Matthews, J., Williams, M., Brannick, L., et al. (2007). A study to evaluate safety and efficacy of mepolizumab in patients with moderate persistent asthma. *Am J Respir Crit Care Med* 176, 1062–1071.
- Foster, P. S., Hogan, S. P., Ramsay, A. J., Matthaei, K. I., & Young, I. G. (1996). Interleukin 5 deficiency abolishes eosinophilia, airways hyperreactivity, and lung damage in a mouse asthma model. *J Exp Med* 183, 195–201.
- Gaffen, S. L. (2009). Structure and signalling in the IL-17 receptor family. *Nat Rev Immunol* 9, 556–567.
- Galicija, J. C., Tai, H., Komatsu, Y., Shimada, Y., Akazawa, K., & Yoshie, H. (2004). Polymorphisms in the IL-6 receptor (IL-6r) gene: strong evidence that serum levels of soluble IL-6r are genetically influenced. *Genes Immun* 5, 513–516.
- Garcia-Marcos, L., & Schuster, A. (2001). Antileukotrienes in asthma: present situation. *Expert Opin Pharmacother* 2, 441–466.
- Gauvreau, G. M., Boulet, L. P., Cockcroft, D. W., FitzGerald, J. M., Mayers, I., Carlsten, C., et al. (2014a). OX40 blockade and allergen-induced airway responses in subjects with mild asthma. *Clin Exp Allergy* 44, 29–37.
- Gauvreau, G. M., O'Byrne, P. M., Boulet, L. P., Wang, Y., Cockcroft, D., Bigler, J., et al. (2014b). Effects of an anti-TSLP antibody on allergen-induced asthmatic responses. *N Engl J Med* 370, 2102–2110.
- Gauvreau, G. M., Boulet, L. P., Cockcroft, D. W., Fitzgerald, J. M., Carlsten, C., Davis, B. E., et al. (2011). Effects of interleukin-13 blockade on allergen-induced airway responses in mild atopic asthma. *Am J Respir Crit Care Med* 183, 1007–1014.
- Gehlhar, K., Bilitewski, C., Reinitz-Rademacher, K., Rohde, G., & Bufer, A. (2006). Impaired virus-induced interferon- α 2 release in adult asthmatic patients. *Clin Exp Allergy* 36, 331–337.
- Gibson, P. G., & Simpson, J. L. (2009). The overlap syndrome of asthma and COPD: what are its features and how important is it? *Thorax* 64, 728–735.
- Gielen, V., Sykes, A., Zhu, J., Chan, B., MacIntyre, J., Regamey, N., et al. (2015). Increased nuclear suppressor of cytokine signaling 1 in asthmatic bronchial epithelium suppresses rhinovirus induction of innate interferons. *J Allergy Clin Immunol* 136(177–188), e111.
- Giembycz, M. A., & Lindsay, M. A. (1999). Pharmacology of the eosinophil. *Pharmacol Rev* 51, 213–340.
- Gill, M. A., Bajwa, G., George, T. A., Dong, C. C., Dougherty, I. I., Jiang, N., et al. (2010). Counterregulation between the Fc epsilon RI pathway and antiviral responses in human plasmacytoid dendritic cells. *J Immunol* 184, 5999–6006.
- GINA (2015). Initiative for Asthma: Asthma, COPD and Asthma-COPD Overlap Syndrome (ACOS). <http://ginasthma.org/asthma-copd-and-asthma-copd-overlap-syndrome-acos/>.
- GINA (2016). Global Initiative for Asthma: Global Strategy for Asthma Management and Prevention. <http://ginasthma.org/2016-gina-report-global-strategy-for-asthma-management-and-prevention/>.
- Giouleka, P., Papatheodorou, G., Lyberopoulos, P., Karakatsani, A., Alchanatis, M., Roussos, C., et al. (2011). Body mass index is associated with leukotriene inflammation in asthmatics. *Eur J Clin Invest* 41, 30–38.
- Gunnbjornsdottir, M. I., Omenaas, E., Gislason, T., Norrman, E., Olin, A. C., Jogi, R., et al. (2004). Obesity and nocturnal gastro-oesophageal reflux are related to onset of asthma and respiratory symptoms. *Eur Respir J* 24, 116–121.
- Hakonarson, H., Maskeri, N., Carter, C., & Grunstein, M. M. (1999). Regulation of Th1- and Th2-type cytokine expression and action in atopic asthmatic sensitized airway smooth muscle. *J Clin Invest* 103, 1077–1087.
- Halder, P., Brightling, C. E., Hargadon, B., Gupta, S., Monteiro, W., Sousa, A., et al. (2009). Mepolizumab and exacerbations of refractory eosinophilic asthma. *N Engl J Med* 360, 973–984.
- Halder, P., Pavord, I. D., Shaw, D. E., Berry, M. A., Thomas, M., Brightling, C. E., et al. (2008). Cluster analysis and clinical asthma phenotypes. *Am J Respir Crit Care Med* 178, 218–224.
- Halonen, M., Barbee, R. A., Lebowitz, M. D., & Burrows, B. (1982). An epidemiologic study of interrelationships of total serum immunoglobulin e, allergy skin-test reactivity, and eosinophilia. *J Allergy Clin Immunol* 69, 221–228.
- Hamelmann, E. (2007). The rationale for treating allergic asthma with anti-IgE. *Eur Respir Rev* 16, 61–66.
- Hammad, H., & Lambrecht, B. N. (2015). Barrier epithelial cells and the control of type 2 immunity. *Immunity* 43, 29–40.
- Hanania, N. A., Noonan, M., Corren, J., Korenblat, P., Zheng, Y., Fischer, S. K., et al. (2015). Lebrikizumab in moderate-to-severe asthma: pooled data from two randomised placebo-controlled studies. *Thorax* 70, 748–756.
- Hanania, N. A., Wenzel, S., Rosen, K., Hsieh, H. J., Mosesova, S., Choy, D. F., et al. (2013). Exploring the effects of omalizumab in allergic asthma: an analysis of biomarkers in the extra study. *Am J Respir Crit Care Med* 187, 804–811.
- Hancox, R. J., Milne, B. J., Poulton, R., Taylor, D. R., Greene, J. M., McLachlan, C. R., et al. (2005). Sex differences in the relation between body mass index and asthma and atopy in a birth cohort. *Am J Respir Crit Care Med* 171, 440–445.
- Hancox, R. J., Poulton, R., Greene, J. M., Filsell, S., McLachlan, C. R., Rasmussen, F., et al. (2007). Systemic inflammation and lung function in young adults. *Thorax* 62, 1064–1068.
- Harada, M., Hirota, T., Jodo, A. I., Hitomi, Y., Sakashita, M., Tsunoda, T., et al. (2011). Thymic stromal lymphopoietin gene promoter polymorphisms are associated with susceptibility to bronchial asthma. *Am J Respir Cell Mol Biol* 44, 787–793.
- Hardin, M., Silverman, E. K., Barr, R. G., Hansel, N. N., Schroeder, J. D., Make, B. J., et al. (2011). The clinical features of the overlap between COPD and asthma. *Respir Res* 12, 127.
- Hart, T. K., Blackburn, M. N., Brigham-Burke, M., Dede, K., Al-Mahdi, N., Zia-Amirhosseini, P., et al. (2002). Preclinical efficacy and safety of pascolizumab (sb 240683): a

- humanized anti-interleukin-4 antibody with therapeutic potential in asthma. *Clin Exp Immunol* 130, 93–100.
- Hart, T. K., Cook, R. M., Zia-Amirhosseini, P., Minthorn, E., Sellers, T. S., Maleeff, B. E., et al. (2001). Preclinical efficacy and safety of mepolizumab (sb-240563), a humanized monoclonal antibody to IL-5, in cynomolgus monkeys. *J Allergy Clin Immunol* 108, 250–257.
- Hasday, J. D., Meltzer, S. S., Moore, W. C., Wisniewski, P., Hebel, J. R., Lanni, C., et al. (2000). Anti-inflammatory effects of zileuton in a subpopulation of allergic asthmatics. *Am J Respir Crit Care Med* 161, 1229–1236.
- Hastie, A. T., Moore, W. C., Li, H., Rector, B. M., Ortega, V. E., Pascual, R. M., et al. (2013). Biomarker surrogates do not accurately predict sputum eosinophil and neutrophil percentages in asthmatic subjects. *J Allergy Clin Immunol* 132, 72–80.
- Hawkins, G. A., Robinson, M. B., Hastie, A. T., Li, X., Li, H., Moore, W. C., et al. (2012). The IL6r variation asp(358)ala is a potential modifier of lung function in subjects with asthma. *J Allergy Clin Immunol* 130(510–515), e511.
- Hayashikawa, Y., Iwata, M., Inomata, M., Kawagishi, Y., Tokui, K., Taka, C., et al. (2015). Association of serum adiponectin with asthma and pulmonary function in the Japanese population. *Endocr J* 62, 695–709.
- Heaney, L. G., Djukanovic, R., Woodcock, A., Walker, S., Matthews, J. G., Pavord, I. D., et al. (2016). Research in progress: medical research council United Kingdom refractory asthma stratification programme (RASP-UK). *Thorax* 71, 187–189.
- Heise, C. E., O'Dowd, B. F., Figueroa, D. J., Sawyer, N., Nguyen, T., Im, D. S., et al. (2000). Characterization of the human cysteinyl leukotriene 2 receptor. *J Biol Chem* 275, 30531–30536.
- Henderson, W. R., Jr. (1994). The role of leukotrienes in inflammation. *Ann Intern Med* 121, 684–697.
- Higashi, N., Taniguchi, M., Mita, H., Kawagishi, Y., Ishii, T., Higashi, A., et al. (2004). Clinical features of asthmatic patients with increased urinary leukotriene e4 excretion (hyperleukotrienuria): involvement of chronic hyperplastic rhinosinusitis with nasal polyposis. *J Allergy Clin Immunol* 113, 277–283.
- Hillerdahl, G., & Rylander, R. (1984). Asthma and cessation of smoking. *Clin Allergy* 14, 45–47.
- Hirai, H., Tanaka, K., Yoshie, O., Ogawa, K., Kenmotsu, K., Takamori, Y., et al. (2001). Prostaglandin D2 selectively induces chemotaxis in T helper type 2 cells, eosinophils, and basophils via seven-transmembrane receptor CRTH2. *J Exp Med* 193, 255–261.
- Holgate, S., Bousquet, J., Wenzel, S., Fox, H., Liu, J., & Castellsague, J. (2001). Efficacy of omalizumab, an anti-immunoglobulin e antibody, in patients with allergic asthma at high risk of serious asthma-related morbidity and mortality. *Curr Med Res Opin* 17, 233–240.
- Holguin, F., Bleecker, E. R., Busse, W. W., Calhoun, W. J., Castro, M., Erzurum, S. C., et al. (2011). Obesity and asthma: an association modified by age of asthma onset. *J Allergy Clin Immunol* 127(1486–1493), e1482.
- Horak, F., Ziegelmayer, P., Ziegelmayer, R., Lemell, P., Collins, L. P., Hunter, M. G., et al. (2012). The CRTH2 antagonist OC000459 reduces nasal and ocular symptoms in allergic subjects exposed to grass pollen, a randomised, placebo-controlled, double-blind trial. *Allergy* 67, 1572–1579.
- Howarth, P. H., Babu, K. S., Arshad, H. S., Lau, L., Buckley, M., McConnell, W., et al. (2005). Tumour necrosis factor (tnfalpa) as a novel therapeutic target in symptomatic corticosteroid dependent asthma. *Thorax* 60, 1012–1018.
- Huang, K., Rabold, R., Abston, E., Schofield, B., Misra, V., Galdzicka, E., et al. (2008). Effects of leptin deficiency on postnatal lung development in mice. *J Appl Physiol* (1985) 105, 249–259.
- Hui, K. P., & Barnes, N. C. (1991). Lung function improvement in asthma with a cysteinyl-leukotriene receptor antagonist. *Lancet* 337, 1062–1063.
- Hunter, C. A., & Jones, S. A. (2015). IL-6 as a keystone cytokine in health and disease. *Nat Immunol* 16, 448–457.
- Imai, Y., Yasuda, K., Sakaguchi, Y., Haneda, T., Mizutani, H., Yoshimoto, T., et al. (2013). Skin-specific expression of IL-33 activates group 2 innate lymphoid cells and elicits atopic dermatitis-like inflammation in mice. *Proc Natl Acad Sci USA* 110, 13921–13926.
- Ito, T., Wang, Y. H., Duramad, O., Hori, T., Delespess, G. J., Watanabe, N., et al. (2005). TSLP-activated dendritic cells induce an inflammatory T helper type 2 cell response through OX40 ligand. *J Exp Med* 202, 1213–1223.
- Jackson, D. J., Makrinioti, H., Rana, B. M., Shamji, B. W., Trujillo-Torralbo, M. B., Footitt, J., et al. (2014). IL-33-dependent type 2 inflammation during rhinovirus-induced asthma exacerbations in vivo. *Am J Respir Crit Care Med* 190, 1373–1382.
- Jain, M., Budinger, G. R., Lo, A., Ulrich, D., Rivera, S. E., Ghosh, A. K., et al. (2011). Leptin promotes fibroproliferative acute respiratory distress syndrome by inhibiting peroxisome proliferator-activated receptor-gamma. *Am J Respir Crit Care Med* 183, 1490–1498.
- Jeffery, P. K. (1998). Structural and inflammatory changes in COPD: a comparison with asthma. *Thorax* 53, 129–136.
- Jensen, E. J., Pedersen, B., Narvestadt, E., & Dahl, R. (1998). Blood eosinophil and monocyte counts are related to smoking and lung function. *Respir Med* 92, 63–69.
- Jia, G., Erickson, R. W., Choy, D. F., Mosesova, S., Wu, L. C., Solberg, O. D., et al. (2012). Periostin is a systemic biomarker of eosinophilic airway inflammation in asthmatic patients. *J Allergy Clin Immunol* 130(647–654), e610.
- Jones, R. L., & Nzekwu, M. M. (2006). The effects of body mass index on lung volumes. *Chest* 130, 827–833.
- Julia, V., Macia, L., & Dombrowicz, D. (2015). The impact of diet on asthma and allergic diseases. *Nat Rev Immunol* 15, 308–322.
- Kane, G. C., Pollice, M., Kim, C. J., Cohn, J., Dworski, R. T., Murray, J. J., et al. (1996). A controlled trial of the effect of the 5-lipoxygenase inhibitor, zileuton, on lung inflammation produced by segmental antigen challenge in human beings. *J Allergy Clin Immunol* 97, 646–654.
- Karta, M. R., Broide, D. H., & Doherty, T. A. (2016). Insights into group 2 innate lymphoid cells in human airway disease. *Curr Allergy Asthma Rep* 16, 8.
- Katz, L. E., Gleich, G. J., Hartley, B. F., Yancey, S. W., & Ortega, H. G. (2014). Blood eosinophil count is a useful biomarker to identify patients with severe eosinophilic asthma. *Ann Am Thorac Soc* 11, 531–536.
- Kauppi, P., Kupiainen, H., Lindqvist, A., Tammilehto, L., Kilpelainen, M., Kinnula, V. L., et al. (2011). Overlap syndrome of asthma and COPD predicts low quality of life. *J Asthma* 48, 279–285.
- Keaney, J. F., Jr., Larson, M. G., Vasan, R. S., Wilson, P. W., Lipinska, I., Corey, D., et al. (2003). Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham study. *Arterioscler Thromb Vasc Biol* 23, 434–439.
- Keatings, V. M., Collins, P. D., Scott, D. M., & Barnes, P. J. (1996). Differences in interleukin-8 and tumor necrosis factor-alpha in induced sputum from patients with chronic obstructive pulmonary disease or asthma. *Am J Respir Crit Care Med* 153, 530–534.
- Kemp, J. P., Korenblat, P. E., Scherger, J. E., & Minkwitz, M. (1999). Zafirlukast in clinical practice: results of the Accolate Clinical Experience and Pharmacoeconomics Trial (ACCEPT) in patients with asthma. *J Fam Pract* 48, 425–432.
- Kicic, A., Sutanto, E. N., Stevens, P. T., Knight, D. A., & Stick, S. M. (2006). Intrinsic biochemical and functional differences in bronchial epithelial cells of children with asthma. *Am J Respir Crit Care Med* 174, 1110–1118.
- Kim, H. Y., Lee, H. J., Chang, Y. J., Pichavant, M., Shore, S. A., Fitzgerald, K. A., et al. (2014). Interleukin-17-producing innate lymphoid cells and the NLRP3 inflammasome facilitate obesity-associated airway hyperactivity. *Nat Med* 20, 54–61.
- Kim, B. S., Siracusa, M. C., Saenz, S. A., Noti, M., Monticelli, L. A., Sonnenberg, G. F., et al. (2013). TSLP elicits IL-33-independent innate lymphoid cell responses to promote skin inflammation. *Sci Transl Med* 5, 170ra116.
- Kolbeck, R., Kozhich, A., Koike, M., Peng, L., Andersson, C. K., Damschroder, M. M., et al. (2010). Medi-563, a humanized anti-IL-5 receptor alpha mab with enhanced antibody-dependent cell-mediated cytotoxicity function. *J Allergy Clin Immunol* 125, 1344–1353, e1342.
- Kouro, T., & Takatsu, K. (2009). IL-5- and eosinophil-mediated inflammation: from discovery to therapy. *Int Immunol* 21, 1303–1309.
- Krug, N., Gupta, A., Badorrek, P., Koenen, R., Mueller, M., Pivovarov, A., et al. (2014). Efficacy of the oral chemoattractant receptor homologous molecule on Th2 cells antagonist bi 671800 in patients with seasonal allergic rhinitis. *J Allergy Clin Immunol* 133, 414–419.
- Kubavat, A. H., Khippal, N., Tak, S., Rijhwani, P., Bhargava, S., Patel, T., et al. (2013). A randomized, comparative, multicentric clinical trial to assess the efficacy and safety of zileuton extended-release tablets with montelukast sodium tablets in patients suffering from chronic persistent asthma. *Am J Ther* 20, 154–162.
- Kuschner, W. G., D'Alessandro, A., Wong, H., & Blanc, P. D. (1996). Dose-dependent cigarette smoking-related inflammatory responses in healthy adults. *Eur Respir J* 9, 1989–1994.
- Lange, P., Parner, J., Vestbo, J., Schnohr, P., & Jensen, G. (1998). A 15-year follow-up study of ventilatory function in adults with asthma. *N Engl J Med* 339, 1194–1200.
- Langhammer, A., Johnsen, R., Holmen, J., Gulsvik, A., & Bjerner, L. (2000). Cigarette smoking gives more respiratory symptoms among women than among men. The Nord-Trøndelag Health Study (HUNT). *J Epidemiol Community Health* 54, 917–922.
- Lanier, B. Q., Corren, J., Lumry, W., Liu, J., Fowler-Taylor, A., & Gupta, N. (2003). Omalizumab is effective in the long-term control of severe allergic asthma. *Ann Allergy Asthma Immunol* 91, 154–159.
- LaPorte, S. L., Juo, Z. S., Vacklavikova, J., Cof, L. A., Qi, X., Heller, N. M., et al. (2008). Molecular and structural basis of cytokine receptor pleiotropy in the interleukin-4/13 system. *Cell* 132, 259–272.
- Laviolette, M., Gossage, D. L., Gauvreau, G., Leigh, R., Olivenstein, R., Katial, R., et al. (2013). Effects of benralizumab on airway eosinophils in asthmatic patients with sputum eosinophilia. *J Allergy Clin Immunol* 132, 1086–1096, e1085.
- Leckie, M. J., ten Brinke, A., Khan, J., Diamant, Z., O'Connor, B. J., Walls, C. M., et al. (2000). Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 356, 2144–2148.
- Lee, H. C., & Ziegler, S. F. (2007). Inducible expression of the proallergic cytokine thymic stromal lymphopoietin in airway epithelial cells is controlled by nfkappab. *Proc Natl Acad Sci USA* 104, 914–919.
- Leiria, L. O., Martins, M. A., & Saad, M. J. (2015). Obesity and asthma: beyond T(h)2 inflammation. *Metabolism* 64, 172–181.
- Lewis, R. A., Soter, N. A., Diamond, P. T., Austen, K. F., Oates, J. A., & Roberts, L. J., 2nd. (1982). Prostaglandin D2 generation after activation of rat and human mast cells with anti-ige. *J Immunol* 129, 1627–1631.
- Li, P., Yang, L., Ma, C., Liu, B., Zhang, X., Ding, R., et al. (2015). Low-molecular-weight adiponectin is more closely associated with disease activity of rheumatoid arthritis than other adiponectin multimeric forms. *Clin Rheumatol* 34, 1025–1030.
- Licona-Limon, P., Kim, L. K., Palm, N. W., & Flavell, R. A. (2013). Th2, allergy and group 2 innate lymphoid cells. *Nat Immunol* 14, 536–542.
- Liu, Y. J. (2006). Thymic stromal lymphopoietin: master switch for allergic inflammation. *J Exp Med* 203, 269–273.
- Liu, M. C., Bleecker, E. R., Lichtenstein, L. M., Kagey-Sobotka, A., Niv, Y., McLemore, T. L., et al. (1990). Evidence for elevated levels of histamine, prostaglandin D2, and other bronchoconstricting prostaglandins in the airways of subjects with mild asthma. *Am Rev Respir Dis* 142, 126–132.
- Liu, M. C., Dube, L. M., & Lancaster, J. (1996). Acute and chronic effects of a 5-lipoxygenase inhibitor in asthma: a 6-month randomized multicenter trial. Zileuton study group. *J Allergy Clin Immunol* 98, 859–871.
- Lopez-Souza, N., Favoreto, S., Wong, H., Ward, T., Yagi, S., Schnurr, D., et al. (2009). In vitro susceptibility to rhinovirus infection is greater for bronchial than for nasal airway epithelial cells in human subjects. *J Allergy Clin Immunol* 123, 1384–1390, e1382.
- Lotvall, J., Akdis, C. A., Bacharier, L. B., Bjerner, L., Casale, T. B., Custovic, A., et al. (2011). Asthma endotypes: a new approach to classification of disease entities within the asthma syndrome. *J Allergy Clin Immunol* 127, 355–360.
- Louie, S., Zeki, A., Schivo, M., Chan, A. L., Yoneda, K. Y., Avdalovic, M., et al. (2013). The asthma-chronic obstructive pulmonary disease overlap syndrome: pharmacotherapeutic considerations. *Expert Rev Clin Pharmacol* 6, 197–219.

- Lugogo, N. L., Hollingsworth, J. W., Howell, D. L., Que, L. G., Francisco, D., Church, T. D., et al. (2012). Alveolar macrophages from overweight/obese subjects with asthma demonstrate a proinflammatory phenotype. *Am J Respir Crit Care Med* 186, 404–411.
- Luthi, A. U., Cullen, S. P., McNeela, E. A., Duriez, P. J., Afonina, I. S., Sheridan, C., et al. (2009). Suppression of interleukin-33 bioactivity through proteolysis by apoptotic caspases. *Immunity* 31, 84–98.
- Ly, T. W., & Bacon, K. B. (2005). Small-molecule CRTH2 antagonists for the treatment of allergic inflammation: an overview. *Expert Opin Investig Drugs* 14, 769–773.
- Lynch, K. R., O'Neill, G. P., Liu, Q., Im, D. S., Sawyer, N., Metters, K. M., et al. (1999). Characterization of the human cysteinyl leukotriene cyslt1 receptor. *Nature* 399, 789–793.
- Ma, X., Cheng, Z., Kong, H., Wang, Y., Unruh, H., Stephens, N. L., et al. (2002). Changes in biophysical and biochemical properties of single bronchial smooth muscle cells from asthmatic subjects. *Am J Physiol Lung Cell Mol Physiol* 283, L1181–L1189.
- Ma, J., Strub, P., Xiao, L., Lavori, P. W., Camargo, C. A., Jr., Wilson, S. R., et al. (2015). Behavioral weight loss and physical activity intervention in obese adults with asthma. A randomized trial. *Ann Am Thorac Soc* 12, 1–11.
- MacGlashan, D. W., Jr., Bochner, B. S., Adelman, D. C., Jardieu, P. M., Togias, A., McKenzie-White, J., et al. (1997). Down-regulation of fε(epsilon)ri expression on human basophils during in vivo treatment of atopic patients with anti-IgE antibody. *J Immunol* 158, 1438–1445.
- Malmstrom, K., Rodriguez-Gomez, G., Guerra, J., Villaran, C., Pineiro, A., Wei, L. X., et al. (1999). Oral montelukast, inhaled beclomethasone, and placebo for chronic asthma. A randomized, controlled trial. Montelukast/beclomethasone study group. *Ann Intern Med* 130, 487–495.
- Mancuso, P., Canetti, C., Gottschalk, A., Tithof, P. K., & Peters-Golden, M. (2004). Leptin augments alveolar macrophage leukotriene synthesis by increasing phospholipase activity and enhancing group IVC IPLA2 (CPLA2gamma) protein expression. *Am J Physiol Lung Cell Mol Physiol* 287, L497–L502.
- Manise, M., Holtappels, G., Van Crombruggen, K., Schleich, F., Bachert, C., & Louis, R. (2013). Sputum IgE and cytokines in asthma: relationship with sputum cellular profile. *PLoS One* 8, e58388.
- Manson, M. (2015). *Airway smooth muscle as a target in asthma: new insights into bronchorelaxation and hyperreactivity*. Stockholm, Sweden: Karolinska Institutet.
- Marini, M., Vittori, E., Hollemberg, J., & Mattoli, S. (1992). Expression of the potent inflammatory cytokines, granulocyte-macrophage-colony-stimulating factor and interleukin-6 and interleukin-8, in bronchial epithelial cells of patients with asthma. *J Allergy Clin Immunol* 89, 1001–1009.
- Marmarinos, A., Saxon-Papageorgiou, P., Cassimos, D., Manoussakis, E., Tsentidis, C., Doxara, A., et al. (2015). Urinary leukotriene e4 levels in atopic and non-atopic preschool children with recurrent episodic (viral) wheezing: a potential marker? *J Asthma* 52, 554–559.
- Matsusaka, M., Kabata, H., Fukunaga, K., Suzuki, Y., Masaki, K., Mochimaru, T., et al. (2015). Phenotype of asthma related with high serum periostin levels. *Allergol Int* 64, 175–180.
- Mausner, P. J., Pittman, A. M., Fernandez, X., Foran, S. K., Adams, G. K., 3rd, Kreutner, W., et al. (1995). Effects of an antibody to interleukin-5 in a monkey model of asthma. *Am J Respir Crit Care Med* 152, 467–472.
- McGrath, K. W., Icitovic, N., Boushey, H. A., Lazarus, S. C., Sutherland, E. R., Chinchilli, V. M., et al. (2012). A large subgroup of mild-to-moderate asthma is persistently noneosinophilic. *Am J Respir Crit Care Med* 185, 612–619.
- McKenzie, A. N. (2014). Type-2 innate lymphoid cells in asthma and allergy. *Ann Am Thorac Soc* 11(Suppl. 5), S263–S270.
- McKinley, L., Alcorn, J. F., Peterson, A., Dupont, R. B., Kapadia, S., Logar, A., et al. (2008). Th17 cells mediate steroid-resistant airway inflammation and airway hyperresponsiveness in mice. *J Immunol* 181, 4089–4097.
- Message, S. D., Laza-Stanca, V., Mallia, P., Parker, H. L., Zhu, J., Kebadze, T., et al. (2008). Rhinovirus-induced lower respiratory illness is increased in asthma and related to virus load and th1/2 cytokine and il-10 production. *Proc Natl Acad Sci U S A* 105, 13562–13567.
- Milgrom, H., Berger, W., Nayak, A., Gupta, N., Pollard, S., McAlary, M., et al. (2001). Treatment of childhood asthma with anti-immunoglobulin e antibody (omalizumab). *Pediatrics* 108, E36.
- Milgrom, H., Fick, R. B., Jr., Su, J. Q., Reimann, J. D., Bush, R. K., Watrous, M. L., et al. (1999). Treatment of allergic asthma with monoclonal anti-ige antibody. Rhumab-e25 study group. *N Engl J Med* 341, 1966–1973.
- Mitchell, I., Tough, S. C., Semple, L. K., Green, F. H., & Hessel, P. A. (2002). Near-fatal asthma: a population-based study of risk factors. *Chest* 121, 1407–1413.
- Mjosberg, J. M., Trifari, S., Crellin, N. K., Peters, C. P., van Drunen, C. M., Piet, B., et al. (2011). Human IL-25- and IL-33-responsive type 2 innate lymphoid cells are defined by expression of CRTH2 and CD161. *Nat Immunol* 12, 1055–1062.
- Molet, S., Hamid, Q., Davoine, F., Nutku, E., Taha, R., Page, N., et al. (2001). IL-17 is increased in asthmatic airways and induces human bronchial fibroblasts to produce cytokines. *J Allergy Clin Immunol* 108, 430–438.
- Molimard, M., de Blay, F., Didier, A., & Le Gros, V. (2008). Effectiveness of omalizumab (xolair) in the first patients treated in real-life practice in france. *Respir Med* 102, 71–76.
- Mondino, C., Ciabattini, G., Koch, P., Pistelli, R., Trove, A., Barnes, P. J., et al. (2004). Effects of inhaled corticosteroids on exhaled leukotrienes and prostanoids in asthmatic children. *J Allergy Clin Immunol* 114, 761–767.
- Monneret, G., Gravel, S., Diamond, M., Rokach, J., & Powell, W. S. (2001). Prostaglandin D2 is a potent chemoattractant for human eosinophils that acts via a novel dp receptor. *Blood* 98, 1942–1948.
- Montecucco, F., Bianchi, G., Gnerre, P., Bertolotto, M., Dallegri, F., & Ottonello, L. (2006). Induction of neutrophil chemotaxis by leptin: crucial role for p38 and SRC kinases. *Ann N Y Acad Sci* 1069, 463–471.
- Moore, W. C., Hastie, A. T., Li, X., Li, H., Busse, W. W., Jarjour, N. N., et al. (2014). Sputum neutrophil counts are associated with more severe asthma phenotypes using cluster analysis. *J Allergy Clin Immunol* 133, 1557–1563, e1555.
- Moore, W. C., Meyers, D. A., Wenzel, S. E., Teague, W. G., Li, H., Li, X., et al. (2010). Identification of asthma phenotypes using cluster analysis in the severe asthma research program. *Am J Respir Crit Care Med* 181, 315–323.
- Morjaria, J. B., Babu, K. S., Vijayanand, P., Chauhan, A. J., Davies, D. E., & Holgate, S. T. (2011). Sputum il-6 concentrations in severe asthma and its relationship with fev1. *Thorax* 66, 537.
- Morjaria, J. B., Chauhan, A. J., Babu, K. S., Polosa, R., Davies, D. E., & Holgate, S. T. (2008). The role of a soluble TNFalpha receptor fusion protein (etanercept) in corticosteroid refractory asthma: a double blind, randomised, placebo controlled trial. *Thorax* 63, 584–591.
- Mosen, D. M., Schatz, M., Magid, D. J., & Camargo, C. A., Jr. (2008). The relationship between obesity and asthma severity and control in adults. *J Allergy Clin Immunol* 122, 507–511, e506.
- Murray, J. J., Tonnel, A. B., Brash, A. R., Roberts, L. J., 2nd, Gosset, P., Workman, R., et al. (1986). Release of prostaglandin D2 into human airways during acute antigen challenge. *N Engl J Med* 315, 800–804.
- Nagasaki, T., Matsumoto, H., Kanemitsu, Y., Izuhara, K., Tohda, Y., Horiguchi, T., et al. (2014). Using exhaled nitric oxide and serum periostin as a composite marker to identify severe/steroid-insensitive asthma. *Am J Respir Crit Care Med* 190, 1449–1452.
- Nagata, K., Hirai, H., Tanaka, K., Ogawa, K., Aso, T., Sugamura, K., et al. (1999a). CRTH2, an orphan receptor of t-helper-2-cells, is expressed on basophils and eosinophils and responds to mast cell-derived factor(s). *FEBS Lett* 459, 195–199.
- Nagata, K., Tanaka, K., Ogawa, K., Kemmotsu, K., Imai, T., Yoshie, O., et al. (1999b). Selective expression of a novel surface molecule by human Th2 cells in vivo. *J Immunol* 162, 1278–1286.
- Naimark, A., & Cherniack, R. M. (1960). Compliance of the respiratory system and its components in health and obesity. *J Appl Physiol* 15, 377–382.
- Nair, P., Gaga, M., Zervas, E., Alagha, K., Hargreave, F. E., O'Byrne, P. M., et al. (2012). Safety and efficacy of a CXCR2 antagonist in patients with severe asthma and sputum neutrophils: a randomized, placebo-controlled clinical trial. *Clin Exp Allergy* 42, 1097–1103.
- Nair, P., Pizzichini, M. M., Kjarsgaard, M., Inman, M. D., Efthimiadis, A., Pizzichini, E., et al. (2009). Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. *N Engl J Med* 360, 985–993.
- Nakamura, Y., Hoshino, M., Sim, J. J., Ishii, K., Hosaka, K., & Sakamoto, T. (1998). Effect of the leukotriene receptor antagonist pranlukast on cellular infiltration in the bronchial mucosa of patients with asthma. *Thorax* 53, 835–841.
- Nakamura, Y., Miyata, M., Ohba, T., Ando, T., Hatsushika, K., Suenaga, F., et al. (2008). Cigarette smoke extract induces thymic stromal lymphopoietin expression, leading to T(h)2-type immune responses and airway inflammation. *J Allergy Clin Immunol* 122, 1208–1214.
- Nembrini, C., Marsland, B. J., & Kopf, M. (2009). IL-17-producing t cells in lung immunity and inflammation. *J Allergy Clin Immunol* 123, 986–994 quiz 995–986.
- Neveu, W. A., Allard, J. L., Raymond, D. M., Bourassa, L. M., Burns, S. M., Bunn, J. Y., et al. (2010). Elevation of IL-6 in the allergic asthmatic airway is independent of inflammation but associates with loss of central airway function. *Respir Res* 11, 28.
- Newby, C., Heaney, L. G., Menzies-Gow, A., Niven, R. M., Mansur, A., Bucknall, C., et al. (2014). Statistical cluster analysis of the British Thoracic Society Severe Refractory Asthma Registry: clinical outcomes and phenotype stability. *PLoS One* 9, e102987.
- Nicolacakis, K., Skowronski, M. E., Coreno, A. J., West, E., Nader, N. Z., Smith, R. L., et al. (2008). Observations on the physiological interactions between obesity and asthma. *J Appl Physiol* (1985) 105, 1533–1541.
- Nigro, E., Daniele, A., Scudiero, O., Ludovica Monaco, M., Roviezzo, F., D'Agostino, B., et al. (2015). Adiponectin in asthma: implications for phenotyping. *Curr Protein Pept Sci* 16, 182–187.
- Norman, P. (2014). Update on the status of DP2 receptor antagonists; from proof of concept through clinical failures to promising new drugs. *Expert Opin Investig Drugs* 23, 55–66.
- Nowak, D., Grimminger, F., Jorres, R., Oldigs, M., Rabe, K. F., Seeger, W., et al. (1993). Increased LTb4 metabolites and PGD2 in BAL fluid after methacholine challenge in asthmatic subjects. *Eur Respir J* 6, 405–412.
- Nowak, R. M., Parker, J. M., Silverman, R. A., Rowe, B. H., Smithline, H., Khan, F., et al. (2015). A randomized trial of benralizumab, an antiinterleukin 5 receptor alpha monoclonal antibody, after acute asthma. *Am J Emerg Med* 33, 14–20.
- Obase, Y., Shimoda, T., Mitsuta, K., Matsuo, N., Matsuse, H., & Kohno, S. (2001). Correlation between airway hyperresponsiveness and airway inflammation in a young adult population: Eosinophil, ECP, and cytokine levels in induced sputum. *Ann Allergy Asthma Immunol* 86, 304–310.
- Oh, C. K., Geba, G. P., & Molino, N. (2010). Investigational therapeutics targeting the IL-4/IL-13/st-6 pathway for the treatment of asthma. *Eur Respir Rev* 19, 46–54.
- Okunishi, K., & Peters-Golden, M. (2011). Leukotrienes and airway inflammation. *Biochim Biophys Acta* 1810, 1096–1102.
- Ortega, H. G., Liu, M. C., Pavord, I. D., Brusselle, G. G., FitzGerald, J. M., Chetta, A., et al. (2014). Mepolizumab treatment in patients with severe eosinophilic asthma. *N Engl J Med* 371, 1198–1207.
- O'Sullivan, S., Dahlen, S. E., Larsson, K., Larsson, B. M., Malmberg, P., Kumlin, M., et al. (1998). Exposure of healthy volunteers to swine house dust increases formation of leukotrienes, prostaglandin D2, and bronchial responsiveness to methacholine. *Thorax* 53, 1041–1046.
- Otulana, B. A., Wenzel, S. E., Ind, P. W., Bowden, A., Puthukkeril, S., Tomkinson, A., ... Yen, Y. (2011). A phase 2b study of inhaled pitrakinra, an IL-4/IL-13 antagonist, successfully identified responder subpopulations of patients with uncontrolled asthma. *Am J Respir Crit Care Med* 183, A6179.
- Ouchi, N., Parker, J. L., Lugus, J. J., & Walsh, K. (2011). Adipokines in inflammation and metabolic disease. *Nat Rev Immunol* 11, 85–97.
- Pakhale, S., Doucette, S., Vandemheen, K., Boulet, L. P., McIvor, R. A., Fitzgerald, J. M., et al. (2010). A comparison of obese and nonobese people with asthma: exploring an asthma-obesity interaction. *Chest* 137, 1316–1323.

- Papaiwannou, A., Zarogoulidis, P., Porpodis, K., Spyrtas, D., Kioumis, I., Pitsiou, G., et al. (2014). Asthma–chronic obstructive pulmonary disease overlap syndrome (ACOS): current literature review. *J Thorac Dis* 6(Suppl. 1), S146–S151.
- Park, H. S., Park, J. Y., & Yu, R. (2005). Relationship of obesity and visceral adiposity with serum concentrations of CRP, TNF- α and IL-6. *Diabetes Res Clin Pract* 69, 29–35.
- Parsons, K. S., Hsu, A. C., & Wark, P. A. B. (2013). TLR3 and MDA5 signalling, although not expression, is impaired in asthmatic epithelial cells in response to rhinovirus infection. *Clin Exp Allergy* 44, 91–101.
- Parulekar, A. D., Diamant, Z., & Hanania, N. A. (2016). Role of T2 inflammation biomarkers in severe asthma. *Curr Opin Pulm Med* 22, 59–68.
- Patel, D. A., You, Y., Huang, G., Byers, D. E., Kim, H. J., Agapov, E., et al. (2014). Interferon response and respiratory virus control are preserved in bronchial epithelial cells in asthma. *J Allergy Clin Immunol* 134, 1402–1412, e1407.
- Pavord, I. D., & Shaw, D. (2008). The use of exhaled nitric oxide in the management of asthma. *J Asthma* 45, 523–531.
- Pavord, I. D., Korn, S., Howarth, P., Bleecker, E. R., Buhl, R., Keene, O. N., et al. (2012). Mepolizumab for severe eosinophilic asthma (dream): a multicentre, double-blind, placebo-controlled trial. *Lancet* 380, 651–659.
- Pedersen, B., Dahl, R., Karlstrom, R., Peterson, C. G., & Venge, P. (1996). Eosinophil and neutrophil activity in asthma in a one-year trial with inhaled budesonide. The impact of smoking. *Am J Respir Crit Care Med* 153, 1519–1529.
- Pelosi, P., Croci, M., Ravagnan, I., Tredici, S., Pedoto, A., Lissoni, A., et al. (1998). The effects of body mass on lung volumes, respiratory mechanics, and gas exchange during general anesthesia. *Anesth Analg* 87, 654–660.
- Periyalil, H. A., Wood, L. G., Scott, H. A., Jensen, M. E., & Gibson, P. G. (2015). Macrophage activation, age and sex effects of immunometabolism in obese asthma. *Eur Respir J* 45, 388–395.
- Perret, J. L., Dharmage, S. C., Matheson, M. C., Johns, D. P., Gurrin, L. C., Burgess, J. A., et al. (2013). The interplay between the effects of lifetime asthma, smoking, and atopy on fixed airflow obstruction in middle age. *Am J Respir Crit Care Med* 187, 42–48.
- Peters, S. P. (2014). Asthma phenotypes: nonallergic (intrinsic) asthma. *J Allergy Clin Immunol Pract* 2, 650–652.
- Peters, M. C., Mekonnen, Z. K., Yuan, S., Bhakta, N. R., Woodruff, P. G., & Fahy, J. V. (2014). Measures of gene expression in sputum cells can identify Th2-high and Th2-low subtypes of asthma. *J Allergy Clin Immunol* 133, 388–394.
- Peters-Golden, M., & Henderson, W. R., Jr. (2007). Leukotrienes. *N Engl J Med* 357, 1841–1854.
- Pettipher, R., & Whittaker, M. (2012). Update on the development of antagonists of chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2). From lead optimization to clinical proof-of-concept in asthma and allergic rhinitis. *J Med Chem* 55, 2915–2931.
- Pettipher, R., Hunter, M. G., Perkins, C. M., Collins, L. P., Lewis, T., Baillet, M., et al. (2014). Heightened response of eosinophilic asthmatic patients to the CRTH2 antagonist OC000459. *Allergy* 69, 1223–1232.
- Piccolo, G., Caponnetto, P., Barton, S., Russo, C., Origlio, A., Bonaccorsi, A., et al. (2008). Changes in airway hyperresponsiveness following smoking cessation: comparisons between MCH and AMP. *Respir Med* 102, 256–265.
- Piper, E., Brightling, C., Niven, R., Oh, C., Faggioni, R., Poon, K., et al. (2013). A phase II placebo-controlled study of tralokinumab in moderate-to-severe asthma. *Eur Respir J* 41, 330–338.
- Pizzichini, E., Leff, J. A., Reiss, T. F., Hendeles, L., Boulet, L. P., Wei, L. X., et al. (1999). Montelukast reduces airway eosinophilic inflammation in asthma: a randomized, controlled trial. *Eur Respir J* 14, 12–18.
- Plaschke, P. P., Janson, C., Norrman, E., Bjornsson, E., Ellbjär, S., & Jarvholm, B. (2000). Onset and remission of allergic rhinitis and asthma and the relationship with atopic sensitization and smoking. *Am J Respir Crit Care Med* 162, 920–924.
- Polosa, R., & Thomson, N. C. (2013). Smoking and asthma: dangerous liaisons. *Eur Respir J* 41, 716–726.
- Polosa, R., Knoke, J. D., Russo, C., Piccillo, G., Caponnetto, P., Sarva, M., et al. (2008). Cigarette smoking is associated with a greater risk of incident asthma in allergic rhinitis. *J Allergy Clin Immunol* 121, 1428–1434.
- Presta, L. G., Lahr, S. J., Shields, R. L., Porter, J. P., Gorman, C. M., Fendly, B. M., et al. (1993). Humanization of an antibody directed against IgE. *J Immunol* 151, 2623–2632.
- Rabinovitch, N., Mauger, D. T., Reisdorph, N., Covar, R., Malka, J., Lemanske, R. F., Jr., et al. (2014). Predictors of asthma control and lung function responsiveness to step 3 therapy in children with uncontrolled asthma. *J Allergy Clin Immunol* 133, 350–356.
- Rabinovitch, N., Strand, M., Stuhlman, K., & Gelfand, E. W. (2008). Exposure to tobacco smoke increases leukotriene E4-related albuterol usage and response to montelukast. *J Allergy Clin Immunol* 121, 1365–1371.
- Rabinovitch, N., Zhang, L., & Gelfand, E. W. (2006). Urine leukotriene E4 levels are associated with decreased pulmonary function in children with persistent airway obstruction. *J Allergy Clin Immunol* 118, 635–640.
- Rasmussen, F., & Hancox, R. J. (2014). Mechanisms of obesity in asthma. *Curr Opin Allergy Clin Immunol* 14, 35–43.
- Rasmussen, F., Siersted, H. C., Lambrechtsen, J., Hansen, H. S., & Hansen, N. C. (2000). Impact of airway liability, atopy, and tobacco smoking on the development of asthma-like symptoms in asymptomatic teenagers. *Chest* 117, 1330–1335.
- Rastogi, D., Suzuki, M., & Greally, J. M. (2013). Differential epigenome-wide DNA methylation patterns in childhood obesity-associated asthma. *Sci Rep* 3, 2164.
- Reche, P. A., Soumelis, V., Gorman, D. M., Clifford, T., Liu, M., Travis, M., et al. (2001). Human thymic stromal lymphopoietin preferentially stimulates myeloid cells. *J Immunol* 167, 336–343.
- Reed, J., Kolbeck, R., Molino, N. A., Kozhich, A., Humbles, A. A., Erjefalt, J. S., ... White, B. (2008). Med-563 a humanised anti-IL-5ra antibody with enhanced effector function, induces reversible blood eosinopenia in mild asthmatics. *J Allergy Clin Immunol* 121, s47.
- Reiss, T. F., Chervinsky, P., Dockhorn, R. J., Shingo, S., Seidenberg, B., & Edwards, T. B. (1998). Montelukast, a once-daily leukotriene receptor antagonist, in the treatment of chronic asthma: a multicenter, randomized, double-blind trial. Montelukast clinical research study group. *Arch Intern Med* 158, 1213–1220.
- Revez, J. A., Bain, L., Chapman, B., Powell, J. E., Jansen, R., Duffy, D. L., et al. (2013). A new regulatory variant in the interleukin-6 receptor gene associates with asthma risk. *Genes Immun* 14, 441–446.
- Rincon, M., & Irvin, C. G. (2012). Role of IL-6 in asthma and other inflammatory pulmonary diseases. *Int J Biol Sci* 8, 1281–1290.
- Robinson, D. S., Hamid, Q., Ying, S., Tsicopoulos, A., Barkans, J., Bentley, A. M., et al. (1992). Predominant Th2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 326, 298–304.
- Roth, M. D., Arora, A., Barsky, S. H., Kleerup, E. C., Simmons, M., & Tashkin, D. P. (1998). Airway inflammation in young marijuana and tobacco smokers. *Am J Respir Crit Care Med* 157, 928–937.
- Rothenberg, M. E., & Hogan, S. P. (2006). The eosinophil. *Annu Rev Immunol* 24, 147–174.
- Rowe, R. K., & Gill, M. A. (2015). Asthma: the interplay between viral infections and allergic diseases. *Immunol Allergy Clin North Am* 35, 115–127.
- Saetta, M., Turato, G., Maestrelli, P., Mapp, C. E., & Fabbri, L. M. (2001). Cellular and structural bases of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 163, 1304–1309.
- Saha, S. K., Berry, M. A., Parker, D., Siddiqui, S., Morgan, A., May, R., et al. (2008). Increased sputum and bronchial biopsy IL-13 expression in severe asthma. *J Allergy Clin Immunol* 121, 685–691.
- Salimi, M., Barlow, J. L., Saunders, S. P., Xue, L., Gutowska-Owsiak, D., Wang, X., et al. (2013). A role for IL-25 and IL-33-driven type-2 innate lymphoid cells in atopic dermatitis. *J Exp Med* 210, 2939–2950.
- Sampson, M. G., & Grassino, A. E. (1983). Load compensation in obese patients during quiet tidal breathing. *J Appl Physiol Respir Environ Exerc Physiol* 55, 1269–1276.
- Sanderson, C. J. (1992). Interleukin-5, eosinophils, and disease. *Blood* 79, 3101–3109.
- Santamaria, F., Montella, S., & Pietrobello, A. (2012). Obesity and pulmonary disease: unanswered questions. *Obes Rev* 13, 822–833.
- Scheller, J., Garbers, C., & Rose-John, S. (2014). Interleukin-6: from basic biology to selective blockade of pro-inflammatory activities. *Semin Immunol* 26, 2–12.
- Schleich, F. N., Chevremont, A., Paulus, V., Henket, M., Manise, M., Seidel, L., et al. (2014). Importance of concomitant local and systemic eosinophilia in uncontrolled asthma. *Eur Respir J* 44, 97–108.
- Schleich, F. N., Manise, M., Sele, J., Henket, M., Seidel, L., & Louis, R. (2013). Distribution of sputum cellular phenotype in a large asthma cohort: predicting factors for eosinophilic vs neutrophilic inflammation. *BMC Pulm Med* 13, 11.
- Schwantes, E. A., Manthei, D. M., Denlinger, L. C., Evans, M. D., Gern, J. E., Jarjour, N. N., et al. (2014). Interferon gene expression in sputum cells correlates with the asthma index score during virus-induced exacerbations. *Clin Exp Allergy* 44, 813–821.
- Scott, J. P., & Peters-Golden, M. (2013). Antileukotriene agents for the treatment of lung disease. *Am J Respir Crit Care Med* 188, 538–544.
- Seidell, J. C., de Groot, L. C., van Sonsbeek, J. L., Deurenberg, P., & Hautvast, J. G. (1986). Associations of moderate and severe overweight with self-reported illness and medical care in Dutch adults. *Am J Public Health* 76, 264–269.
- Shardonofsky, F. R., Venzor, J., 3rd, Barrios, R., Leong, K. P., & Huston, D. P. (1999). Therapeutic efficacy of an anti-IL-5 monoclonal antibody delivered into the respiratory tract in a murine model of asthma. *J Allergy Clin Immunol* 104, 215–221.
- Shi, H. Z., Xiao, C. Q., Zhong, D., Qin, S. M., Liu, Y., Liang, G. R., et al. (1998). Effect of inhaled interleukin-5 on airway hyperreactivity and eosinophilia in asthmatics. *Am J Respir Crit Care Med* 157, 204–209.
- Shore, S. A. (2008). Obesity and asthma: possible mechanisms. *J Allergy Clin Immunol* 121, 1087–1093 (quiz 1094–1085).
- Sideleva, O., & Dixon, A. E. (2014). The many faces of asthma in obesity. *J Cell Biochem* 115, 421–426.
- Sideleva, O., Suratt, B. T., Black, K. E., Tharp, W. G., Pratley, R. E., Forgione, P., et al. (2012). Obesity and asthma: an inflammatory disease of adipose tissue not the airway. *Am J Respir Crit Care Med* 186, 598–605.
- Sidhu, S. S., Yuan, S., Innes, A. L., Kerr, S., Woodruff, P. G., Hou, L., et al. (2010). Roles of epithelial cell-derived periostin in TGF- β activation, collagen production, and collagen gel elasticity in asthma. *Proc Natl Acad Sci U S A* 107, 14170–14175.
- Silverman, R. A., Boudreaux, E. D., Woodruff, P. G., Clark, S., & Camargo, C. A., Jr. (2003). Cigarette smoking among asthmatic adults presenting to 64 emergency departments. *Chest* 123, 1472–1479.
- Simpson, J. L., Scott, R., Boyle, M. J., & Gibson, P. G. (2006). Inflammatory subtypes in asthma: assessment and identification using induced sputum. *Respirology* 11, 54–61.
- Singh, D., Cadden, P., Hunter, M., Pearce Collins, L., Perkins, M., Pettipher, R., et al. (2013). Inhibition of the asthmatic allergen challenge response by the CRTH2 antagonist OC000459. *Eur Respir J* 41, 46–52.
- Sippel, J. M., Pedula, K. L., Vollmer, W. M., Buist, A. S., & Osborne, M. L. (1999). Associations of smoking with hospital-based care and quality of life in patients with obstructive airway disease. *Chest* 115, 691–696.
- Siroux, V., Basagana, X., Boudier, A., Pin, I., Garcia-Aymerich, J., Vesin, A., et al. (2011). Identifying adult asthma phenotypes using a clustering approach. *Eur Respir J* 38, 310–317.
- Siroux, V., Pin, I., Oryszczyn, M. P., Le Moual, N., & Kauffmann, F. (2000). Relationships of active smoking to asthma and asthma severity in the egea study. Epidemiological study on the genetics and environment of asthma. *Eur Respir J* 15, 470–477.
- Sivapalan, P., Diamant, Z., & Ulrik, C. S. (2015). Obesity and asthma: current knowledge and future needs. *Curr Opin Pulm Med* 21, 80–85.
- Skloot, G., Permut, S., & Togias, A. (1995). Airway hyperresponsiveness in asthma: a problem of limited smooth muscle relaxation with inspiration. *J Clin Invest* 96, 2393–2403.

- Skold, C. M., Blaschke, E., & Eklund, A. (1996). Transient increases in albumin and hyaluronan in bronchoalveolar lavage fluid after quitting smoking: Possible signs of reparative mechanisms. *Respir Med* 90, 523–529.
- Skorge, T. D., Eagan, T. M., Eide, G. E., Gulsvik, A., & Bakke, P. S. (2005). The adult incidence of asthma and respiratory symptoms by passive smoking in uterus or in childhood. *Am J Respir Crit Care Med* 172, 61–66.
- Slager, R. E., Otulana, B. A., Hawkins, G. A., Yen, Y. P., Peters, S. P., Wenzel, S. E., et al. (2012). IL-4 receptor polymorphisms predict reduction in asthma exacerbations during response to an anti-IL-4 receptor alpha antagonist. *J Allergy Clin Immunol* 130, 516–522, e514.
- Snell, N., Foster, M., & Vestbo, J. (2013). Efficacy and safety of AZD1981, a CRTH2 receptor antagonist, in patients with moderate to severe COPD. *Respir Med* 107, 1722–1730.
- Soler, M., Matz, J., Townley, R., Buhl, R., O'Brien, J., Fox, H., et al. (2001). The anti-IgE antibody omalizumab reduces exacerbations and steroid requirement in allergic asthmatics. *Eur Respir J* 18, 254–261.
- Sonnenberg, G. F., & Artis, D. (2015). Innate lymphoid cells in the initiation, regulation and resolution of inflammation. *Nat Med* 21, 698–708.
- Sood, A. (2010). Obesity, adipokines, and lung disease. *J Appl Physiol* (1985) 108, 744–753.
- Sood, A. (2011). Sex differences: implications for the obesity–asthma association. *Exerc Sport Sci Rev* 39, 48–56.
- Soumelis, V., Reche, P. A., Kanzler, H., Yuan, W., Edward, G., Homey, B., et al. (2002). Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing tslp. *Nat Immunol* 3, 673–680.
- Spik, I., Brenuchon, C., Angeli, V., Staumont, D., Fleury, S., Capron, M., et al. (2005). Activation of the prostaglandin D2 receptor DP2/CRTH2 increases allergic inflammation in mouse. *J Immunol* 174, 3703–3708.
- Spits, H., Artis, D., Colonna, M., Diefenbach, A., Di Santo, J. P., Eberl, G., et al. (2013). Innate lymphoid cells—a proposal for uniform nomenclature. *Nat Rev Immunol* 13, 145–149.
- Stanley, A. H., Demissie, K., & Rhoads, G. G. (2005). Asthma development with obesity exposure: observations from the cohort of the national health and nutrition evaluation survey epidemiologic follow-up study (nhefs). *J Asthma* 42, 97–99.
- Steffes, M. W., Gross, M. D., Lee, D. H., Schreiner, P. J., & Jacobs, D. R., Jr. (2006). Adiponectin, visceral fat, oxidative stress, and early macrovascular disease: the coronary artery risk development in young adults study. *Obesity (Silver Spring)* 14, 319–326.
- Steinke, J. W. (2004). Anti-interleukin-4 therapy. *Immunol Allergy Clin North Am* 24, 599–614 (vi).
- Stene, L. C., & Nafstad, P. (2001). Relation between occurrence of type 1 diabetes and asthma. *Lancet* 357, 607–608.
- Strachan, D. P., & Cook, D. G. (1998). Health effects of passive smoking. 6. Parental smoking and childhood asthma: longitudinal and case-control studies. *Thorax* 53, 204–212.
- Straumann, A., Hoesli, S., Bussmann, C., Stuck, M., Perkins, M., Collins, L. P., et al. (2013). Anti-eosinophil activity and clinical efficacy of the CRTH2 antagonist OC000459 in eosinophilic esophagitis. *Allergy* 68, 375–385.
- Sunyer, J., Anto, J. M., Kogevinas, M., Soriano, J. B., Tobias, A., & Munoz, A. (1997). Smoking and bronchial responsiveness in nonatopic and atopic young adults. Spanish group of the European study of asthma. *Thorax* 52, 235–238.
- Sunyer, J., Anto, J. M., Sabria, J., Roca, J., Morell, F., Rodriguez-Roisin, R., et al. (1995). Relationship between serum IgE and airway responsiveness in adults with asthma. *J Allergy Clin Immunol* 95, 699–706.
- Sunyer, J., Springer, G., Jamieson, B., Conover, C., Detels, R., Rinaldo, C., et al. (2003). Effects of asthma on cell components in peripheral blood among smokers and non-smokers. *Clin Exp Allergy* 33, 1500–1505.
- Sutherland, E. R. (2014). Linking obesity and asthma. *Ann N Y Acad Sci* 1311, 31–41.
- Sutherland, T. J., Cowan, J. O., Young, S., Goulding, A., Grant, A. M., Williamson, A., et al. (2008). The association between obesity and asthma: interactions between systemic and airway inflammation. *Am J Respir Crit Care Med* 178, 469–475.
- Sutherland, E. R., Goleva, E., King, T. S., Lehman, E., Stevens, A. D., Jackson, L. P., et al. (2012). Cluster analysis of obesity and asthma phenotypes. *PLoS One* 7, e36631.
- Suzukawa, M., Nagase, H., Ogahara, I., Han, K., Tashimo, H., Shibui, A., et al. (2011). Leptin enhances survival and induces migration, degranulation, and cytokine synthesis of human basophils. *J Immunol* 186, 5254–5260.
- Sykes, A., Edwards, M. R., Macintyre, J., del Rosario, A., Bakhsoliani, E., Trujillo-Torralbo, M.B., et al. (2012). Rhinovirus 16-induced IFN- α and IFN- β are deficient in bronchoalveolar lavage cells in asthmatic patients. *J Allergy Clin Immunol* 129(1506–1514), e1506.
- Sykes, A., Macintyre, J., Edwards, M. R., Del Rosario, A., Haas, J., Gielen, V., et al. (2014). Rhinovirus-induced interferon production is not deficient in well controlled asthma. *Thorax* 69, 240–246.
- Szefer, S. J., Phillips, B. R., Martinez, F. D., Chinchilli, V. M., Lemanske, R. F., Strunk, R. C., et al. (2005). Characterization of within-subject responses to fluticasone and montelukast in childhood asthma. *J Allergy Clin Immunol* 115, 233–242.
- Tanaka, K., Hirai, H., Takano, S., Nakamura, M., & Nagata, K. (2004). Effects of prostaglandin D2 on helper T cell functions. *Biochem Biophys Res Commun* 316, 1009–1014.
- Tanaka, T., Narazaki, M., & Kishimoto, T. (2014). IL-6 in inflammation, immunity, and disease. *Cold Spring Harb Perspect Biol* 6, a016295.
- Tanaka, H., Saito, T., Kurokawa, K., Teramoto, S., Miyazaki, N., Kaneko, S., et al. (1999). Leukotriene (LT)-receptor antagonist is more effective in asthmatic patients with a low baseline ratio of urinary LTE4 to 2,3-dinor-6-keto-prostaglandin (PG)F1 α . *Allergy* 54, 489–494.
- Taylor, R. G., Gross, E., Joyce, H., Holland, F., & Pride, N. B. (1985). Smoking, allergy, and the differential white blood cell count. *Thorax* 40, 17–22.
- Taylor, B., Mannino, D., Brown, C., Crocker, D., Twum-Baah, N., & Holguin, F. (2008). Body mass index and asthma severity in the National Asthma Survey. *Thorax* 63, 14–20.
- Teach, S. J., Gill, M. A., Togias, A., Sorkness, C. A., Arbes, S. J., Jr., Calatroni, A., et al. (2015). Preseasonal treatment with either omalizumab or an inhaled corticosteroid boost to prevent fall asthma exacerbations. *J Allergy Clin Immunol*.
- Telenga, E. D., Tideman, S. W., Kerstjens, H. A., Hacken, N. H., Timens, W., Postma, D. S., et al. (2012). Obesity in asthma: more neutrophilic inflammation as a possible explanation for a reduced treatment response. *Allergy* 67, 1060–1068.
- Terashima, T., Amakawa, K., Matsumaru, A., & Yamaguchi, K. (2002). Correlation between cysteinyl leukotriene release from leukocytes and clinical response to a leukotriene inhibitor. *Chest* 122, 1566–1570.
- Thomas, P. S., & Heywood, G. (2002). Effects of inhaled tumour necrosis factor alpha in subjects with mild asthma. *Thorax* 57, 774–778.
- Thomas, P. S., Yates, D. H., & Barnes, P. J. (1995). Tumor necrosis factor- α increases airway responsiveness and sputum neutrophilia in normal human subjects. *Am J Respir Crit Care Med* 152, 76–80.
- Thomson, N. C., Chaudhuri, R., & Livingston, E. (2004). Asthma and cigarette smoking. *Eur Respir J* 24, 822–833.
- Thomson, N. C., Chaudhuri, R., Spears, M., Haughney, J., & McSharry, C. (2015). Serum periostin in smokers and never smokers with asthma. *Respir Med*.
- Thomson, N. C., Chaudhuri, R., Spears, M., Messow, C. M., Jelinsky, S., Miele, G., et al. (2014). Arachidonic acid metabolites and enzyme transcripts in asthma are altered by cigarette smoking. *Allergy* 69, 527–536.
- Thyagarajan, B., Jacobs, D. R., Jr., Apostol, G. G., Smith, L. J., Jensen, R. L., Crapo, R. O., et al. (2008). Longitudinal association of body mass index with lung function: The cardia study. *Respir Res* 9, 31.
- Tillie-Leblond, I., Pugin, J., Marquette, C. H., Lamblin, C., Saulnier, F., Brichet, A., et al. (1999). Balance between proinflammatory cytokines and their inhibitors in bronchial lavage from patients with status asthmaticus. *Am J Respir Crit Care Med* 159, 487–494.
- Tliba, O., Deshpande, D., Chen, H., Van Besien, C., Kannan, M., Panettieri, R. A., Jr., et al. (2003). IL-13 enhances agonist-evoked calcium signals and contractile responses in airway smooth muscle. *Br J Pharmacol* 140, 1159–1162.
- Todd, D. C., Armstrong, S., D'Silva, L., Allen, C. J., Hargreave, F. E., & Parameswaran, K. (2007). Effect of obesity on airway inflammation: a cross-sectional analysis of body mass index and sputum cell counts. *Clin Exp Allergy* 37, 1049–1054.
- Tonnesen, P., Pisinger, C., Hvidberg, S., Wennike, P., Bremann, L., Westin, A., et al. (2005). Effects of smoking cessation and reduction in asthmatics. *Nicotine Tob Res* 7, 139–148.
- Troisi, R. J., Speizer, F. E., Rosner, B., Trichopoulos, D., & Willett, W. C. (1995). Cigarette smoking and incidence of chronic bronchitis and asthma in women. *Chest* 108, 1557–1561.
- Tsaroucha, A., Daniil, Z., Malli, F., Georgoulas, P., Minas, M., Kostikas, K., et al. (2013). Leptin, adiponectin, and ghrelin levels in female patients with asthma during stable and exacerbation periods. *J Asthma* 50, 188–197.
- Tsuchiya, T., Shimizu, H., Horie, T., & Mori, M. (1999). Expression of leptin receptor in lung: leptin as a growth factor. *Eur J Pharmacol* 365, 273–279.
- Ullah, M. A., Revez, J. A., Loh, Z., Simpson, J., Zhang, V., Bain, L., et al. (2015). Allergen-induced IL-6 trans-signaling activates gamma delta T cells to promote type 2 and type 17 airway inflammation. *J Allergy Clin Immunol* 136, 1065–1073.
- Ulrik, C. S., & Frederiksen, J. (1995). Mortality and markers of risk of asthma death among 1,075 outpatients with asthma. *Chest* 108, 10–15.
- Vachier, I., Kumlin, M., Dahlen, S. E., Bousquet, J., Godard, P., & Chanez, P. (2003). High levels of urinary leukotriene E4 excretion in steroid treated patients with severe asthma. *Respir Med* 97, 1225–1229.
- van Huisstede, A., Rudolphus, A., Castro Cabezas, M., Biter, L. U., van de Geijn, G. J., Taube, C., et al. (2015). Effect of bariatric surgery on asthma control, lung function and bronchial and systemic inflammation in morbidly obese subjects with asthma. *Thorax* 70, 659–667.
- van Veen, I. H., Ten Brinke, A., Sterk, P. J., Rabe, K. F., & Bel, E. H. (2008). Airway inflammation in obese and nonobese patients with difficult-to-treat asthma. *Allergy* 63, 570–574.
- Vesterinen, E., Kaprio, J., & Koskenvuo, M. (1988). Prospective study of asthma in relation to smoking habits among 14,729 adults. *Thorax* 43, 534–539.
- Von Behren, J., Lipsett, M., Horn-Ross, P. L., Delfino, R. J., Gilliland, F., McConnell, R., et al. (2009). Obesity, waist size and prevalence of current asthma in the California Teachers Study Cohort. *Thorax* 64, 889–893.
- Vonarbourg, C., Mortha, A., Bui, V. L., Hernandez, P. P., Kiss, E. A., Hoyler, T., et al. (2010). Regulated expression of nuclear receptor rorgammat confers distinct functional fates to nk cell receptor-expressing rorgammat(+) innate lymphocytes. *Immunity* 33, 736–751.
- Vortmann, M., & Eisner, M. D. (2008). Bmi and health status among adults with asthma. *Obesity (Silver Spring)* 16, 146–152.
- Wagener, A. H., de Nijs, S. B., Lutter, R., Sousa, A. R., Weersink, E. J., Bel, E. H., et al. (2015). External validation of blood eosinophils, Fe(NO) and serum periostin as surrogates for sputum eosinophils in asthma. *Thorax* 70, 115–120.
- Wagener, A. H., Zwinderman, A. H., Luiten, S., Fokkens, W. J., Bel, E. H., Sterk, P. J., et al. (2014). DSRNA-induced changes in gene expression profiles of primary nasal and bronchial epithelial cells from patients with asthma, rhinitis and controls. *Respir Res* 15, 9.
- Wakefield, M., Ruffin, R., Campbell, D., Roberts, L., & Wilson, D. (1995). Smoking-related beliefs and behaviour among adults with asthma in a representative population sample. *Aust N Z J Med* 25, 12–17.
- Walker, C., Bode, E., Boer, L., Hansel, T. T., Blaser, K., & Virchow, J. C., Jr. (1992). Allergic and nonallergic asthmatics have distinct patterns of ϵ -cell activation and cytokine production in peripheral blood and bronchoalveolar lavage. *Am Rev Respir Dis* 146, 109–115.
- Wang, Y. H., & Liu, Y. J. (2007). OX40–OX40L interactions: a promising therapeutic target for allergic diseases? *J Clin Invest* 117, 3655–3657.
- Wark, P. A., Johnston, S. L., Buchieri, F., Powell, R., Puddicombe, S., Laza-Stanca, V., et al. (2005). Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. *J Exp Med* 201, 937–947.

- Weiss, S. T. (2000). Atopy as a risk factor for chronic obstructive pulmonary disease: epidemiological evidence. *Am J Respir Crit Care Med* 162, S134–S136.
- Wenzel, S. E. (2003). The role of leukotrienes in asthma. *Prostaglandins Leukot Essent Fatty Acids* 69, 145–155.
- Wenzel, S. E. (2012). Asthma phenotypes: the evolution from clinical to molecular approaches. *Nat Med* 18, 716–725.
- Wenzel, S. E., Barnes, P. J., Bleecker, E. R., Bousquet, J., Busse, W., Dahlen, S. E., et al. (2009). A randomized, double-blind, placebo-controlled study of tumor necrosis factor- α blockade in severe persistent asthma. *Am J Respir Crit Care Med* 179, 549–558.
- Wenzel, S., Ford, L., Pearlman, D., Spector, S., Sher, L., Skobieranda, F., et al. (2013). Dupilumab in persistent asthma with elevated eosinophil levels. *N Engl J Med* 368, 2455–2466.
- Wenzel, S. E., Westcott, J. Y., Smith, H. R., & Larsen, G. L. (1989). Spectrum of prostanoid release after bronchoalveolar allergen challenge in atopic asthmatics and in control groups. An alteration in the ratio of bronchoconstrictive to bronchoprotective mediators. *Am Rev Respir Dis* 139, 450–457.
- Wenzel, S., Wilbraham, D., Fuller, R., Getz, E. B., & Longphre, M. (2007). Effect of an interleukin-4 variant on late phase asthmatic response to allergen challenge in asthmatic patients: results of two phase 2a studies. *Lancet* 370, 1422–1431.
- Willers, S. M., Devereux, G., Craig, L. C., McNeill, G., Wijga, A. H., Abou El-Magd, W., et al. (2007). Maternal food consumption during pregnancy and asthma, respiratory and atopic symptoms in 5-year-old children. *Thorax* 62, 773–779.
- Williams, A. S., Chen, L., Kasahara, D. I., Si, H., Wurmbbrand, A. P., & Shore, S. A. (2013). Obesity and airway responsiveness: role of TNFR2. *Pulm Pharmacol Ther* 26, 444–454.
- Wong, C. K., Ho, C. Y., Ko, F. W., Chan, C. H., Ho, A. S., Hui, D. S., et al. (2001). Proinflammatory cytokines (IL-17, IL-6, IL-18 and IL-12) and Th cytokines (IFN- γ , IL-4, IL-10 and IL-13) in patients with allergic asthma. *Clin Exp Immunol* 125, 177–183.
- Wood, L. G., Garg, M. L., & Gibson, P. G. (2011). A high-fat challenge increases airway inflammation and impairs bronchodilator recovery in asthma. *J Allergy Clin Immunol* 127, 1133–1140.
- Wood, L. G., Garg, M. L., Smart, J. M., Scott, H. A., Barker, D., & Gibson, P. G. (2012). Manipulating antioxidant intake in asthma: a randomized controlled trial. *Am J Clin Nutr* 96, 534–543.
- Woodruff, P. G., Boushey, H. A., Dolganov, G. M., Barker, C. S., Yang, Y. H., Donnelly, S., et al. (2007). Genome-wide profiling identifies epithelial cell genes associated with asthma and with treatment response to corticosteroids. *Proc Natl Acad Sci U S A* 104, 15858–15863.
- Woodruff, P. G., Modrek, B., Choy, D. F., Jia, G., Abbas, A. R., Ellwanger, A., et al. (2009). T-helper type 2-driven inflammation defines major subphenotypes of asthma. *Am J Respir Crit Care Med* 180, 388–395.
- Xu, H., Barnes, G. T., Yang, Q., Tan, G., Yang, D., Chou, C. J., et al. (2003). Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest* 112, 1821–1830.
- Xue, L., Gyles, S. L., Wettestad, F. R., Gazi, L., Townsend, E., Hunter, M. G., et al. (2005). Prostaglandin D2 causes preferential induction of proinflammatory Th2 cytokine production through an action on chemoattractant receptor-like molecule expressed on Th2 cells. *J Immunol* 175, 6531–6536.
- Xue, L., Salimi, M., Panse, I., Mjosberg, J. M., McKenzie, A. N., Spits, H., et al. (2014). Prostaglandin D2 activates group 2 innate lymphoid cells through chemoattractant receptor-homologous molecule expressed on Th2 cells. *J Allergy Clin Immunol* 133, 1184–1194.
- Yang, J., Sundrud, M. S., Skepner, J., & Yamagata, T. (2014). Targeting Th17 cells in autoimmune diseases. *Trends Pharmacol Sci* 35, 493–500.
- Ying, S., O'Connor, B., Ratoff, J., Meng, Q., Fang, C., Cousins, D., et al. (2008). Expression and cellular provenance of thymic stromal lymphopoietin and chemokines in patients with severe asthma and chronic obstructive pulmonary disease. *J Immunol* 181, 2790–2798.
- Ying, S., O'Connor, B., Ratoff, J., Meng, Q., Mallett, K., Cousins, D., et al. (2005). Thymic stromal lymphopoietin expression is increased in asthmatic airways and correlates with expression of Th2-attracting chemokines and disease severity. *J Immunol* 174, 8183–8190.
- Yokoyama, A., Kohno, N., Sakai, K., Kondo, K., Hirasawa, Y., & Hiwada, K. (1997). Circulating levels of soluble interleukin-6 receptor in patients with bronchial asthma. *Am J Respir Crit Care Med* 156, 1688–1691.
- Zein, J. G., & Erzurum, S. C. (2015). Asthma is different in women. *Curr Allergy Asthma Rep* 15, 28.
- Zhang, X. Y., Simpson, J. L., Powell, H., Yang, I. A., Upham, J. W., Reynolds, P. N., et al. (2014). Full blood count parameters for the detection of asthma inflammatory phenotypes. *Clin Exp Allergy* 44, 1137–1145.
- Ziegler, S. F., & Artis, D. (2010). Sensing the outside world: TSLP regulates barrier immunity. *Nat Immunol* 11, 289–293.