Airway inflammation in asthma : from concept to the clinic

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Chapter

General introduction and aims of the studies

1. Definition of asthma

Asthma is a serious public health problem in countries throughout the world. It is one of the most common chronic diseases worldwide (1). The current Global Initiative for Asthma (GINA) guidelines provide the generally accepted definition for asthma:

Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation causes an associated increase in airway hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particular at night or in the early morning. These episodes are usually associated with widespread, but variable airflow obstruction that is often reversible either spontaneously or with treatment (1).

For most patients with asthma this means that they are having a life-long chronic disease. Asthma may affect children and adults of all ages, but in the majority of patients the first symptoms start at young age. The fundamental causes of asthma are still not known. Symptoms of breathlessness and wheezing occur often following exposure to allergens. 90% of the children with asthma are allergic to common airborne allergens such as house dust mite (HDM), animals, fungi or pollen. Inhaler therapy with bronchodilators may improve these symptoms, but in most patients continuous daily anti-inflammatory therapy is needed to control symptoms. Although major improvements in asthma medical treatment have taken place in the past decades, the disease may be severe and sometimes fatal and can still not be cured (1).

2. Burden of asthma

In children, the prevalence of asthma symptoms varies widely between countries from 1.6% to even 36.8% (2). However, when an objective measurement as airway hyperresponsiveness is also taken into account, the prevalence rates drop to 0-11.1% (1). Although fewer data are available for adults, similar prevalence rates have been described (3). The highest rates in Europe have been reported in the UK (2).

Despite the large variation in asthma occurrence between countries, the reported increase in prevalence of asthma during the eighties and nineties was very consistent word-wide (4). In the UK, the prevalence of asthma at age 16 increased from 3.8% in 1974 to 6.5% in 1986. In the same study, the prevalence of eczema and hay fever doubled in same period, suggesting that the increase in asthma was part of a general increase in atopic disease (5). Although improved diagnosis may be an explanation for this, it cannot explain the rise of asthma, since populations were studied with the same methods on many occasions several years apart. The reasons for the differences in prevalence and the recent increase are still poorly understood. There are now several reports suggesting that the tide has turned. Asthma prevalence seems to be decreasing or at least no longer increasing (6-10). A recent study in the Netherlands demonstrated that wheeze in Dutch children has decreased from 13.4% in 1989 to 9.1% in 2001 (11). Interestingly, the study

reported that the use of asthma medication has increased. Possibly, better asthma control may partly explain the concurrently decreasing trend in the prevalence of asthma.

Even with improvements in treatment, patients may still die of asthma. Although mortality data are unreliable in some countries, they may provide an indication of the burden of asthma. Recently, standardised mortality rates (SMR) were published for males (1.54 (1.10–2.09)) and for females (1.91 (1.44–2.49)) (12). Asthma morbidity and mortality seems to increase with socioeconomic deprivation and ethnicity (13).

Asthma may have considerable impact on physical, emotional, social and economic aspects of lives of patients (14). Despite the availability of effective therapies, asthma is not optimally controlled in many patients (15). Sleep disturbance is reported in one third of the children with asthma, whereas 60% report absence from school and activity restrictions (16). Disability adjusted life years (DALYs) is a measure of the burden of disease that assesses the years of healthy life lost due to disease or illness. The number of DALYs lost due to asthma worldwide has been estimated to be about 15 million/year. This makes asthma the 25th leading cause of DALYs lost worldwide in 2001 (17).

3. Risk factors for asthma

Asthma is a disease that may have multiple causes (1). The risk factors that might contribute to the development of asthma can be divided in two main factors: host factors and environmental exposure. These two factors may interact with each other both in the induction and subsequent expression of the disease (18) (Figure 1).

3.1. Host factors

One of the most important risk factors for asthma is heritability. If the mother and/or father have the disease, it is much more likely for the child to become allergic and asthmatic (18). Although multiple genes have been demonstrated to be related to this disorder, *the* asthma gene has not been identified yet and probably never will (19). The ADAM33 gene on chromosome 20p12 is such a gene that has been linked with asthma (20). It has been associated with asthma and bronchial hyperresponsiveness (21). Furthermore, polymorphisms of the ADAM33 are related to accelerated lung function decline (22). Atopy is another important host factor that predisposes individuals to develop this disease. It has been estimated that around one third of the asthma cases may be attributable to atopy (23). Finally, having (asymptomatic) airway hyperresponsiveness is an increased risk of becoming asthmatic (24).

3.2. Environmental exposures

In predisposed individuals, many environmental factors have been identified to increase the risk of developing asthma. An important determinant for the risk on asthma is exposure to house dust mite (25). An Australian study showed that in regions where HDM levels were high, more children were sensitised to HDM, and that subsequently

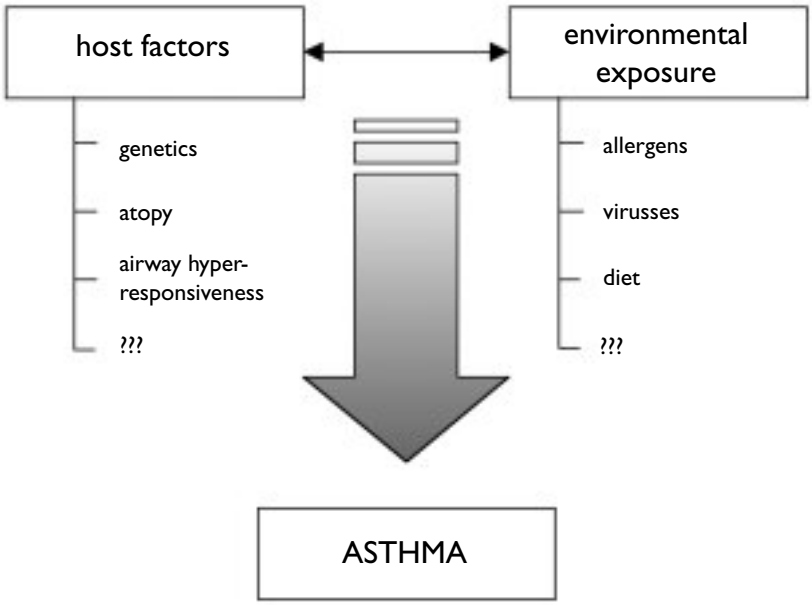

Figure 1. Causes of asthma.
A multi causal pathway may lead to the development of asthma. Several factors have been related with an increased risk of the disease.

these children had significantly more wheeze (26). The relationship with exposure to pets and asthma seems to be complex. Whereas dog ownership is not associated with sensitisation, the presence of a cat in the home may affect the risk on asthma (27). Exposure to cat dander may both increase as well as decrease the risk on sensitisation (27-30). It has been suggested that these conflicting results may be explained by the fact that the dose-response relationship with cat allergen is bell-shaped or that the timing of exposure (early or current pet ownership) is important or that high levels of other allergens influence sensitisation (31). Furthermore, these results have also been explained by the atopic status of the mother (32).

Respiratory syncytial virus (RSV) is a common cause of virus infection of the human respiratory tract during the first two years of life (33). It has been suggested that RSV infection may also predispose some children to the development of asthma (34). This is based on many observations that children who wheeze with RSV-induced bronchiolitis are more likely to develop into allergic asthmatics. In the Tucson Children's Respiratory study, one of the longest running respiratory cohort studies, RSV infection before the age of 3 was associated with an increased risk of wheezing by the age of 6 and 11. Remarkably, at the age of 13, RSV infection was no longer a risk factor for wheezing (35). It is still unknown whether RSV is a causal factor for asthma or targets children who are predisposed to asthma.
Growing up on a farm may be protective against the development of atopy (36-39).

However, exposures in the stables and farm in the first year of life seem to be crucial for this protective effect (40). It may also explain the absence of this association in New Zealand children (41). In contrast to central European farms where cattle are usually kept in stables built near the farmhouse, in New Zealand animals on large farm holdings stay outdoors throughout the year (42). The protective effect of living on a farm may result from elevated exposure to endotoxin, which is an intrinsic part of the outer membrane of gram-negative bacteria, since exposure to endotoxin is inversely related to the occurrence of asthma (43).

Diet is known to have a large effect on risk of many different diseases. Also relations between diet and the risk on asthma have been found. Consumption of fruit and vegetables, in particularly vitamin C, has been associated with a decreased prevalence of wheeze and asthma and may lead to a better lung function (44-46). In addition the consumption of full cream milk and butter has been suggested to reduce the risk of asthma (47).

4. Primary prevention of asthma

In view of the high prevalence of asthma and the fact that asthma cannot be cured, primary prevention must be considered (48). Several randomised controlled trials (RCTs) have attempted to reduce the risk on asthma. Studies investigating the effect of house dust mite avoidance by mattress covers have shown disappointing results (49-51). On the other hand, RCTs using multiple interventions have shown more effect. In the Isle of Wight Prevention Study, the risk on asthma and allergic sensitization was significantly reduced after an intervention on HDM avoidance combined with food restrictions (52). Moreover, these results were still significant after 8 years of follow-up (53). Similar results have been obtained in the Canadian Asthma Primary Prevention study after two and seven years of follow-up (54;55). The intervention in this study included reduced exposure to indoor allergens, avoidance of environmental tobacco smoke (ETS), encouragement of breast-feeding, and delayed introduction of other foods during the first 12 months of life.

Interesting data for asthma prevention come from intervention studies using probiotics (56). The term probiotics is referring to living or inactivated organisms that may exert beneficial effects on health when ingested. The most commonly used probiotics are lactobacilli and bifidobacteria. The proposed rationale for using probiotics against allergies and asthma is based on the relationship between the composition of intestinal flora and the presence of allergies. The gut of infants born in poor areas of developing countries, where allergy prevalence is low, is colonized earlier by lactobacilli compared to the gut of infants born in developed countries (57). A prospective study demonstrated that allergic children in Estonia and Sweden were less often colonized with lactobacilli, as compared with non-allergic children (58). Finish and Swedish studies also showed that differences in the neonatal gut microflora precede the development of

atopy, suggesting a crucial role of the balance of intestinal bacteria for the maturation of human immunity (59;60). The effect of probiotics on the development of asthma and allergies has been tested in clinical studies. Kalliomäki and coworkers have shown that *Lactobacillus* GG given prenatally to mothers and postnatally to their infants reduces the frequency of atopic eczema in a randomized placebo-controlled trial (61). Furthermore, the preventive effect of probiotics was persistent even at 4 years of age (62). Moreover, even in infants, who manifested atopic eczema, probiotics may counteract the allergic inflammation and thereby prevent further allergic disease (63). However, these studies have not yet been able to demonstrate positive effects of probiotics on the development of asthma.

Asthma is a multi-causal disease. As described above, several risk factors have been associated with the development of asthma. Nevertheless, the disease is complex and up to now can neither be cured nor be prevented. More understanding of the underlying pathology might lead to better knowledge and thereby treatment of asthma.

5. Airway inflammation

Inflammation of the airways is the main pathological characteristic of asthma. The inflammatory process can be separated into acute inflammation, chronic inflammation and airway remodelling. These pathologic mechanisms may lead to specific and overlapping clinical consequences for patients with asthma for example bronchoconstriction (acute), exacerbations (chronic), and persistent airflow obstruction (remodelling) (64). However, the link between these inflammatory features and clinical expression of the disease is often weak (Figure 2).

5.1. Acute inflammation

After inhalation of allergens, the acute allergic reaction is initiated when an allergen interacts with IgE that is bound to mast cells and basophils (65). These high-affinity IgE receptor (FcϵRI) bearing cells release following activation preformed mediators, membrane-derived lipids, cytokines and chemokines (66). The release of the pro-inflammatory mediators such as histamine induces bronchoconstriction, mucus secretion and vasodilatation (64). The narrowing of the airway lumen is further increased via the induced microvascular leakage and edema (67). Antigen-presenting cells such as dendritic cells are also crucial in the allergic reaction. Dendritic cells can take up allergen and following the presentation of allergen to T cells, induce proliferation of naïve T cells (68). In the “late-phase” reaction inflammatory cells, such as eosinophils, CD4+ cells, basophils, neutrophils and macrophages are recruited and activated (64). The activation of T cells leads to the release of T helper cell, type 2 (Th2)-like cytokines that include IL-4, IL-5, IL-9, IL-13 (69).

The recruitment of cells into the airway wall is dependent on cytokines such as IL-5. IL-5 plays an important role in the mobilisation of eosinophils from the bone marrow (70).

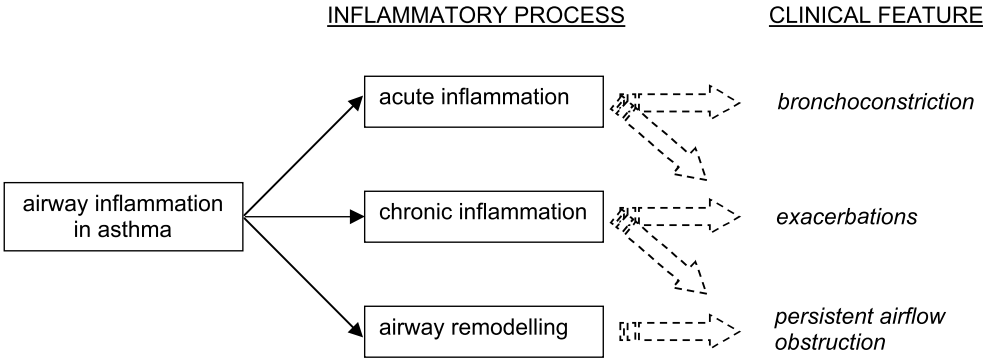

Figure 2. Airway inflammation in asthma.
Examples of clinical features that may result from a typical inflammatory process. These features are overlapping.

The Koch postulates of causation might be useful to confirm the central role of IL-5 in asthma. Indeed, elevated levels of IL-5 have been demonstrated in patients with asthma and have been related with severity (71). Second, administration of IL-5 to animals and humans has been associated with increased numbers of eosinophils, however its effects on airway hyperresponsiveness are unclear (72;73). This has led to the following questions: **Would exogenous IL-5 lead to airway inflammation in asthma? Is the route of IL-5 production crucial to its effect on the airways in patients with asthma? (Chapter 2)**

The fundamental importance of IgE in the pathogenesis of asthma has been clearly identified (74). Burrows *et al.* were the first who showed a strong association between serum IgE levels and self-reported asthma (75). Since then many epidemiological studies have demonstrated the relationship between asthma and IgE (65;76). Furthermore, high levels of circulating IgE have been shown to correlate with the risk of emergency room admissions in patients with asthma (77). Like other antibodies, IgE has two identical light and heavy chains. IgE has a very short half-life (<1 day) and is present in very low concentration in the circulation (78). Cross-linking of IgE molecules on high-affinity IgE receptors triggers the release of mediators by mast cells and basophils (79). Therefore, these cells are highly sensitive to allergens even when the concentration of IgE is very low. IgE production requires at least two distinct signals. The first signal is IL-4 (produced by T-cells, mast cells, basophils and eosinophils) and IL-13 (produced in addition by natural killer cells). The second signal is delivered by the interaction of CD40L on T cells with CD40 on B cells. The combination of these signals causes class switching to IgE and B cell proliferation (80). Recently, the first monoclonal antibody against IgE has been approved by Food and Drug Administration (FDA) in the US for the treatment of patients with severe allergic asthma. This antibody recognizes IgE at the same site as the high-affinity receptor for IgE (FcϵRI), but does not provoke histamine release from IgE-sensitized mast cells (81).
The acute symptoms of breathlessness and wheezing, which occur following exposure to

allergens are the clear clinical features of this acute inflammatory process. This has led to the following question: **Is treatment with anti-IgE improving both the clinical features and the inflammatory process in patients with asthma? (Chapter 7)**

5.2. Chronic inflammation

All cells of the airways, including eosinophils, T cells, mast cells, macrophages, and epithelial cells are involved in the chronic inflammation of asthma (82). Although eosinophilic inflammation plays a central role in the disease, it is not specific to asthma (83). Moreover, a sub-type of non-eosinophilic asthma has been described (84). The number of bronchial eosinophils has been associated with the severity of asthma (85). Eosinophils are potentially harmful in asthma through the release of highly toxic products (MBP, ECP, EDN and oxygen free radicals). The role of IL-5 in the observed eosinophilia in asthma is important: IL-5 regulates the development and differentiation of eosinophils, stimulates the release of eosinophils from the bone marrow into the peripheral circulation and is involved in the activation and survival of eosinophils (86). Interestingly, a study investigating the role of anti-IL-5 challenged the central place of eosinophils in asthma. In this study, a monoclonal antibody against IL-5 reduced the levels of eosinophils in blood and sputum of patients with asthma. However, it had no effect on early and late allergen response or airway hyperresponsiveness (87). Therefore, it may be questionable whether eosinophils and airway hyperresponsiveness are causally related in asthma.

T cells are likely to play a role in controlling the chronic inflammation through the release of Th2-cytokines (88). The majority of T cells are CD4+ cells, whereas CD8+ cells are less frequently identified, even during exacerbations of asthma (89). The frequency of cytokine producing CD4+ and CD8+ cells is similar and is increased as compared with normals (90;91). Interestingly, following glucocorticoid withdrawal, eosinophils are elevated in all patients, whereas increases in airway T cells (both CD4+ and CD8+) were found in only those who developed an exacerbation (92).

There is growing interest for the role of CD8+ T-cells in asthma. A cross-sectional relationship between CD8+ T-cells and the outcome of asthma has been observed in patients with fatal asthma (93). Furthermore, increased cytokine production of sputum CD8+ T-cells has been shown to be related with disease severity in patients with asthma (93). Antigen specific CD8+ T-cells in the lungs demonstrate a high cytotoxic activity and proliferate rapidly (94). These specific effector/memory T cells can be rapidly activated by antigens, like allergens and viruses (95). In the host response to virus infections, CD8+ T-cells may initiate eosinophil recruitment (95). Indeed, in mouse models CD8+ T-cells appear to be essential for the influx of eosinophils into the lung and the development of airway hyperresponsiveness during respiratory virus infections (96). Furthermore, CD8+ T-cells are required for the development of airway hyperresponsiveness following allergic sensitization (97) leading to increased inflammation (98;99). Although an important role for CD8+ T-cells in asthma seems likely, the association of CD8+ T-cells with asthma severity has only been demonstrated in

cross-sectional studies. This has led to the following question: **Are CD8 cells related to the outcome of asthma in a longitudinal study? (Chapter 5)**

5.3. Airway wall remodelling

The structural changes in the airways of asthmatics, which are referred to as airway wall remodelling, include thickening of the reticular basement membrane, increased airway smooth muscle, epithelial shedding, altered deposition of extracellular matrix (ECM) proteins. Since this process begins early in the development of asthma, remodelling may occur in parallel or could even be required for the development of chronic inflammation (100).

The basement membrane of the surface epithelium is composed of the basal lamina and lamina reticularis. Thickening of the latter is an early characteristic feature of patients with asthma (101). Epithelial reticular basement membrane thickening has been demonstrated in children with asthma, although in symptomatic infants with reversible airflow obstruction, it could not be found (102). In adults, following treatment with inhaled steroids, the wall thickness was reduced, however it remained elevated as compared to controls (103). Interestingly, a thicker airway wall as assessed by computed tomography (CT) appears to be related to reduced airway hyperresponsiveness (104). This suggests that the increased airway wall thickness, as observed in asthma, is protective for airway hyperresponsiveness. In some (105;106), but not all studies (107), the thickness of the sub-epithelial reticular layer was inversely associated with the level of lung function in asthma. The clinical features of airway remodeling may be the irreversible airway obstruction and decline in lung function, which is observed in patients with asthma. However, until now there are no prospective studies, which have shown a relationship between the thickness of the sub-epithelial reticular layer and lung function decline in patients with asthma. This has led to the following question: **Is the thickness of the sub-epithelial reticular layer related to the outcome of asthma in a prospective follow-up study? (Chapter 5)**

6. Asthma management

The GINA guidelines propose a six-part asthma management program (1). The *first* part involves patient education. In this way, patients will be able to achieve control of asthma by adjusting medication according to a management plan.

In the *second* part, the assessment and monitoring of asthma severity is based on symptoms and lung function (1). In order to quantify asthma symptoms, several symptom scores and quality of life questionnaires have been developed (108;109). The relationship between symptoms and other clinical measures is poor. Interestingly, a factor analysis has shown that this is primarily due to the fact that asthma health status has 4 distinct components: 1: asthma-specific quality of life, 2: airway calibre, 3: daytime symptoms and daytime $ß_2$-agonist use and 4: night-time symptoms and night-time $ß_2$-

agonist use (110). Measurements of lung function are recommended and overcome the problems of poor perception or under-reporting of symptoms by patients. Lung function measurements, in particularly, the degree of reversibility to bronchodilators such as $ß_2$-agonists, give insight in airflow limitation, whereas measuring the variability of lung function provides an assessment of airway hyperresponsiveness (1).

Peak expiratory flow (PEF) meters are useful for regular home monitoring of lung function. PEF is mostly being recorded two times a day. The measurements can be analysed and summarised in different ways: PEF-level (mean daily PEF, expressed as a percentage of predicted or of personal best) and PEF-variability (amplitude as percentage of mean value) (111) for the diagnosis of asthma, although its usage may be limited due to poor sensitivity of the measurement (112-114). It has been suggested that PEF-variability may provide additional information when used in conjunction with other clinical parameters for asthma patients, who are already on treatment (115). Indeed, the effectiveness of treatment appeared to be associated with the level of PEF-variablity (116). However, PEF-variability may fail to detect an asthma exacerbation (117).Therefore, the minimum morning PEF over 1 week, expressed as a percentage of recent best (Min%Max) has been suggested as an alternative, simpler index of PEF-variability (118;119). The are few studies, which have shown contribution of using PEF-variability to classify asthma control. This has led to the following question: **What is the value of including PEF-variability in addition to symptoms and $ß_2$-agonist use, in predicting the development of poor asthma control? (Chapter 6)**

The *third* part of asthma management involves avoidance of exposure to risk factors. The effectiveness of the reduction of allergen levels was shown in studies, which are performed at high altitude where allergens levels are low (120;121). Although the use of impermeable mattress cover seems to be ineffective, several other measures can be taken (122;123).

Pharmacological treatment (part *four* of the asthma management program) is needed in most patients. Treatment of asthma aims to reverse and prevent symptoms and airflow limitation (1). Medication can be divided into controllers and relievers. Controller medications (inhaled glucocorticosteroids, long-acting $ß_2$-agonists, and leukotriene modifiers) have to be used daily for long-term to maintain control of persistent asthma. At this moment, glucocorticosteroids are considered to be the most effective controller medications (1). In RCTs, inhaled steroids have shown to improve airway hyperresponsiveness and sputum eosinophils even in patients with mild asthma (124;125). Furthermore, in studies using bronchial biopsies, reduction in the thickness of the sub-epithelial reticular layer and a decrease in mast cells and eosinophils could be demonstrated (126;127). Reliever medications (short-acting $ß_2$-agonists and anticholinergics) are needed to act quickly in reducing bronchoconstriction.

Despite optimal treatment, exacerbations of asthma may occur. Part *five* of the asthma management plan concerns the managing of exacerbations. Exacerbations are often

related to a viral infection (128). An increase in symptoms usually precedes the worsening in lung function (129). Therefore, symptoms may be a sensitive marker for the early onset of an exacerbation. The severity of an exacerbation is difficult to define. Therapies include repetitive step-wise administration of short-acting $ß_2$-agonists, systemic glucocorticosteroids or even oxygen supplementation (1).

Finally, provision of regular follow-up care is needed in order to ensure that asthma control is maintained. Even in patients who are in clinical remission of asthma airway inflammation may still be present and treatment with inhaled steroids can be effective (130-132).

6.1. Limitations of asthma management

The assessment and monitoring of asthma control in the current guidelines is based on symptoms and lung function. Although this approach leads to good control of asthma in many patients, there is still room for improvement. As mentioned before, the burden of asthma is high. Large surveys have shown that the level of asthma control falls short of the goals for asthma management (15;133). Furthermore, asthma prognosis can be poor. Long-term follow-up has shown that patients with asthma have an accelerated decline in lung function (FEV_1) as compared to controls (134-136). Moreover, in adult patients with severe asthma, irreversible airway obstruction is common (137).

6.2. Improving asthma management

Several approaches are being undertaken to increase asthma control. First, asthma management may be improved by optimizing the use of current available treatment. Poor compliance to asthma medication has been repeatedly reported (138). Monitoring medication adherence might improve compliance and thereby asthma control (139). Interestingly, the internet appears to be a useful tool to reach this goal (140;141). It has also been hypothesized that patients do not perceive the need for daily therapy and therefore are not taking their medication. Based on this hypothesis, the efficacy of as-needed corticosteroids has recently been investigated in mild intermittent asthma (142).

Second, new drugs for asthma are being developed. These therapies include anti-inflammatory drugs (such as phosphodiesterase 4 (PDE4) inhibitors (143)) or "anti-allergic" drugs directed against specific components of allergic inflammation (such as omalizumab). Omalizumab, which is a humanized monoclonal antibody against IgE, is the first monoclonal antibody drug developed for the treatment of moderate-to-severe asthmatics to receive approval by the FDA in the US. The first clinical studies with intravenous anti-IgE have shown that both early (EAR) and late (LAR) asthmatic response to inhaled allergen are attenuated in patients with asthma (144;145). A recent double-blind, placebo-controlled study confirmed the effectiveness of anti-IgE treatment in inadequately controlled severe persistent asthma by showing a reduction in exacerbation rate and emergency room visits and an improvement in quality of life and morning peak expiratory flow rate (146). Although anti-IgE treatment has been shown to reduce IgE positive cells in bronchial mucosa of patients with asthma, a direct

association with its clinical effect has not been made (147). This has led to the following question: **Can the clinical effect of anti-IgE be explained by a reduction in allergen-induced airway inflammation? (Chapter 7).**

Third, the measurement of non-invasive (more direct) markers of inflammation might be beneficial for asthma management. The use of airway hyperresponsiveness (AHR), inflammatory markers in sputum and exhaled nitric oxide (NO) in the management of asthma will be discussed in the next paragraph.

7. Monitoring inflammation

Airway hyperresponsiveness can be defined as an increase in sensitivity to a wide variety of airway narrowing stimuli (148). The degree of AHR is related to asthma severity and airway inflammation (149;150). Furthermore, repeated measurements of AHR seem to reflect the changes in asthma control in response to treatment (151). Interestingly, asthma management based on reducing AHR on top of improving symptoms and lung function leads to more effective asthma control (152). Compared with conventional management, the AHR strategy resulted in fewer exacerbations, improved FEV_1 and a greater reduction in thickness of the sub-epithelial reticular layer.

The methods for sputum induction and processing have been recently standardized (153;154). The number of eosinophils in sputum is associated with asthma severity (155). Furthermore, sputum eosinophils already increase before the onset of an exacerbation (156). Following inhaled steroid treatment eosinophils in sputum significantly decrease (157). Asthma management based on minimising eosinophils in sputum has also been investigated. Patients in the “sputum management group” had fewer exacerbations than the patients who received the current standard treatment (158). Other inflammatory markers in sputum for monitoring airway inflammation in patients with asthma have been less thoroughly examined. Microvascular leakage, which is also an important characteristic of airways inflammation in asthma (67), has not often been measured to monitor inflammation in asthma. This has led to the following question: **Can induced sputum be applied to measure microvascular leakage in patients with asthma? (Chapter 4).**

Measurement of markers in exhaled air is non-invasive. In addition, exhaled NO has also been proposed as a marker for disease severity in asthma (159;160). Patients with asthma have increased levels of exhaled NO as compared to normals (161). Inhaled steroids reduce the levels of exhaled NO in patients with asthma (162) in a dose-dependent way (163). Moreover, increases in exhaled NO are associated with a worsening in asthma control (164). It was recently demonstrated that patients with asthma can be successfully treated based on the levels of exhaled NO (165;166).

One or more of the above-described non-invasive markers of inflammation will probably be implemented in future asthma guidelines. However, a comparative analysis is required before any of these markers can be recommended in the monitoring of asthma therapy. This has led to the following question: **What are the treatment-induced changes in airway hyperresponsiveness, sputum eosinophils and exhaled NO in one comparative study? (Chapter 3).**

8. Aims of the studies

In summary, this thesis addresses three different aspects of airway inflammation in asthma: role of inflammatory mediators in airway inflammation, monitoring of airway inflammation and asthma management of airway inflammation. The above-mentioned questions have been addressed in six studies relating to airway inflammation and asthma management in asthma.

Role of inflammatory mediators in airway inflammation
Chapter 2. Would exogenous IL-5 lead to airway inflammation in asthma? Is the route of IL-5 production crucial to its effect on the airways in patients with asthma? In this chapter the effects of IL-5 administered intravenously or by inhalation to patients with mild asthma was investigated on eosinophil counts in blood and in sputum, and on airway hyperresponsiveness.

Monitoring of airway inflammation
Chapter 3. What are the corticosteroid-induced changes in airway hyperresponsiveness, sputum eosinophils and exhaled NO in a comparative study? Twenty-five patients with asthma were treated for 4 weeks with inhaled steroids or placebo. Before, during and after treatment airway hyperresponsiveness, sputum eosinophils and exhaled NO were measured.

Chapter 4. Can induced sputum be applied to measure microvascular leakage in patients with asthma? This chapter examines the levels of albumin, fibrinogen, ceruloplasmin and alpha-2-macroglobulin as markers of leakage in induced sputum before and after a substance P challenge in patients with asthma. Inhaled NKA was used as a control challenge in this randomised, placebo-controlled, crossover study.

Chapter 5. Are CD8+ T cells related to the outcome of asthma in a follow-up study? Is the thickness of the sub-epithelial reticular layer related to the outcome of asthma in a longitudinal study? In this chapter, we aimed to investigate the prognostic significance of airway inflammation and remodelling on the decline in lung function in asthma. In 32 patients with asthma the relationship between bronchial eosinophils, CD8+ T cell, the thickness of the sub-epithelial layer, and the annual decline in lung function after 7½ years of follow-up was determined.

Asthma management

Chapter 6. What is the value of including PEF-variability in addition to symptoms and $ß_2$-agonist use, in predicting the development of poor asthma control? In a prospective study, we examined in 75 patients with asthma the value of including PEF-variability in addition to symptoms and $ß_2$-agonist use, in predicting the development of poor asthma control.

Chapter 7. Can the clinical effect of anti-IgE be explained by a reduction in allergen-induced airway inflammation? In a randomized, double-blind, placebo-controlled study, the effect of anti-IgE on allergen-induced airway inflammation in bronchial biopsies and on the expression FcεRI receptors and IgE+ cells was investigated in 25 patients with asthma. Furthermore, the effect of anti-IgE treatment on peak flow, airway hyperresponsiveness and sputum was determined.

Summary and conclusion

Chapter 8. A summary of the main results of the different studies is given in this chapter. In addition, implications of these findings are discussed.

Chapter 9. In this chapter, a summary of this thesis is given in Dutch.

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