Allergic asthma: Environmental factors challenging the immune system
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General introduction
INTRODUCTION

Asthma

Asthma is a chronic inflammatory disorder of the airways that is associated with airway hyperresponsiveness and variable airflow obstruction leading to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in the early morning. Symptom-free periods are interrupted by periods of acute worsening of asthma (exacerbations), often caused by viral infections and/or increased exposure to allergens. In research, the diagnosis of allergic asthma is based on objective measures, including (1) lung function (reversibility in forced expiratory volume in 1 second (FEV1), variability in peak expiratory flow), airway hyperresponsiveness (narrowing of the airways in response to triggers that have little or no effect in healthy individuals), (2) indirect measures of airway inflammation (nitric oxide in exhaled air, eosinophils and neutrophils and their soluble activation products (eosinophil cationic protein (ECP) and myeloperoxidase (MPO)) in induced sputum) and (3) responsiveness to allergens (the presence of positive skin-prick tests, levels of specific-immunoglobulin (Ig)E in serum, or the clinical or cellular response (in vivo and ex vivo) to common environmental allergens).

Although allergic sensitization in early childhood, through the induction of allergen-specific T helper (Th)2-type inflammation, is the major risk factor for children to become asthmatic, only 50% of adults who have asthma are atopic. Wheezing-associated infections in the first 3 years of life with respiratory syncytial virus or with rhinovirus, which is also the main cause of asthma exacerbations in adults, are associated with the development of asthma. Recent data have shown that also structural cells, such as airway epithelial cells and dendritic cells play an important role in the initiation and exacerbations of asthma, probably due to epigenetic changes induced by environmental factors.

Allergy and asthma

Allergy is defined as a hypersensitivity reaction initiated by immunological mechanisms that can be antibody- or cell-mediated. In the majority of cases the antibody responsible for an allergic reaction is IgE. Atopic individuals are genetically pre-disposed to become IgE-sensitized to common environmental allergens, to which everyone is exposed, but to which the majority does not produce a prolonged IgE-antibody response. Atopy, or more specifically, sensitization to indoor allergens is strongly associated with the development of asthma. For house dust mite (HDM), for example, the prevalence of sensitization and the development of asthma appears to be directly correlated with exposure. Although the association between allergy and asthma is well established, the mechanisms responsible for the allergen-induced Th2-type inflammation and the initiation of asthma remain poorly understood.
Once sensitized, the antigens interact, upon inhalation of allergen, with allergen-specific IgE receptors, which are bound to the cell membrane of mast cells that are present in increased numbers in the lungs of sensitized subjects. After cross-linking the high-affinity IgE receptors, mast cells immediately start to release mediators (histamine, cysteinyl leukotrienes and prostaglandin D2) that induce typical allergic responses such as vasodilatation and smooth muscle contraction. This results in an acute bronchoconstriction (early asthmatic response, EAR), which is maximal 15-30 minutes after exposure and resolves in about 2 hours. At the same time many chemokines and cytokines, including the T helper (Th)-2 cytokines interleukin (IL)-4, IL-5 and IL-13, are released both locally and systemically. Whereas IL-4 and IL-13 are important for IgE secretion, IL-5 plays a pivotal role in the recruitment, activation and survival of eosinophils in the airways. These activated eosinophils subsequently release inflammatory mediators in the airways, such as eosinophil cationic protein (ECP), leukotrienes and major basic protein (MBP) that damage airway epithelial cells and thus promote the development of a second episode of bronchoconstriction (LAR), which occurs 3-8 hours after inhalation of allergen, and an increased airway hyperresponsiveness (AHR), which may last for up to several days or weeks.

**Environmental risk factors for the development of allergy and asthma**

Over the last century, the prevalence of allergic diseases has increased markedly, especially in industrialized countries. Although genetic factors mostly govern susceptibility to allergic diseases, the increase occurred within too short a time frame to be explained by genetic changes alone. In 1989, Strachan proposed the hygiene hypothesis, which stated that improved hygiene and better infection control reduced microbial exposure in early life and led to an insufficient microbial-induced Th1-immune response and thus an imbalance in the Th1/Th2 balance in favor of the pro-allergic Th2 response. Since, the hygiene hypothesis has been revised many times and comprises nowadays a complex interplay between innate and adaptive immune responses of the host, characteristics of symbiotic bacteria and infectious agents during early childhood, and the level and variety of environmental pollution and allergens.

Interestingly, the prevalence of asthma is reduced in children raised in a rural setting, which may be linked to the presence of higher levels of endotoxin in these environments. Endotoxin or lipopolysaccharide (LPS) is a cell wall component of Gram-negative bacteria, which is ubiquitous in our living environment. Several murine and human studies have demonstrated that the dose of endotoxin, present during allergen sensitization or allergen challenge was critical for the outcome of the immune response. Exposure to high doses of endotoxin during sensitization was associated with the
prevention of asthma and allergy, whereas low doses of endotoxin acted like an adjuvant and stimulated a Th2-mediated immune response\textsuperscript{26,27}. In established asthma, however, high doses of endotoxin are able to induce severe asthma exacerbations\textsuperscript{28}.

**Therapy**

Because many asthma patients respond to multiple factors in the environment, complete avoidance of these factors is usually impractical and very limiting to the patient. Thus medications to maintain asthma control have an important role because patients are often less responsive to environmental allergens and viruses when their asthma is well controlled\textsuperscript{1}. Current standard treatment is based mainly on suppression of airway inflammation with (inhaled) corticosteroids and relief of symptoms with bronchodilators. Nevertheless, these therapies only reduce symptoms and do not consistently alter the inflammatory processes in the airways of patients with asthma\textsuperscript{29}. The ‘hygiene hypothesis’ of asthma, has led to the suggestion that strategies to prevent allergic sensitization should focus on redirecting the immune response in early life toward a Th1, non-allergic response or on modulating T regulatory cells\textsuperscript{30}, but research in this area has just started and still requires further investigation.

**SCOPE OF THIS THESIS**

The role of environmental factors such as allergens, endotoxin and microbes in the development and maintenance of allergic asthma has been an area of great interest ever since the ‘hygiene hypothesis’ was proposed. Although it is well known that allergy is strongly associated with asthma, the specific mechanisms underlying this process are still not fully understood. With the studies presented in this thesis I aimed to elucidate some aspects of these mechanisms.

Allergic sensitization to common environmental allergens often occurs at early childhood, which makes it difficult to study the allergen-specific and nonspecific cellular responses during the development of allergy. Laboratory animal allergy, however, can be found in up to 30% of exposed workers and appears to be very similar to sensitization to common environmental allergens with regard to symptoms and immunologic mechanisms\textsuperscript{31,32}. Because these allergies usually develop within 2-3 years of exposure\textsuperscript{33,34} and the moment of first exposure is known, laboratory animal allergy provides an attractive model to investigate changes in cellular responses during the development of an allergy longitudinally. In chapter 2 we describe the dynamics in cytokine responses during the development of allergic sensitization to rats in a nested case-control setting. In a cohort of starting laboratory animal workers, allergen-specific and non-specific cytokine responses,
measured *ex-vivo* in blood samples from incident animal workers, who developed rat-specific sensitization during 2 years of follow-up, were compared to the cytokine responses in blood samples from control animal workers.

Besides exposure to allergens, also other environmental factors have been shown to be positively or negatively related to the development of allergy and asthma\textsuperscript{33,35}. Considering the observation that the dose of endotoxin present during allergen sensitization or allergen challenge is critical for the outcome of the immune response, we investigated in chapter 3 the cytokine response of peripheral blood mononuclear cells (PBMC) from allergic and non-allergic subjects to HDM to a very low dose of LPS (10 pg/ml) that is comparable with the daily indoor exposure to endotoxin and the endogenous endotoxin contamination in our HDM extract.

Experimental bronchial allergen challenge followed by sputum induction of bronchoalveolar lavage is often performed to provide insight into the cellular mechanisms of airway inflammatory processes caused by inhaled allergens\textsuperscript{36}. Although several studies concerning the safety and reproducibility of bronchial allergen challenge show that the airway-related symptoms of the early and late asthmatic response are transient and return to baseline within 2 weeks, little is known yet about any longer-lasting systemic effects of the challenge. In chapter 4 the allergen-specific B- and T-cell responses were investigated in blood samples from house dust mite (HDM)-allergic asthmatics that were collected 5 weeks after exposure to (high dose) HDM during a single bronchial challenge and compared with those in blood samples that were collected before the challenge.

The activated eosinophils that are recruited to the airways of sensitized asthmatics after allergen challenge have been proposed to play a role in local activation of coagulation in the airways\textsuperscript{37,38}. As coagulation and inflammation often act in parallel, the activation of coagulation within the airways of asthmatic patients is considered to aggravate local inflammation. To investigate the role of local coagulation activation on inflammatory processes and *vice versa* in the airways of allergic asthmatics, we compared, in chapter 5, the activation of coagulation in the bronchoalveolar space and the acute effect of a segmental allergen challenge hereon in asthmatic patients with the activation in healthy controls.

In search for new therapies to prevent and/or treat allergic diseases, modulation of the intestinal microbiota by the intake of probiotic bacteria has been proposed to alter, directly or indirectly, the immune response to allergens toward a non-allergic Th1 response. Although several clinical trials have successfully shown the preventive potential of probiotics on the development of allergic disease in young children, the few studies with probiotics in older populations with established allergic diseases have shown conflicting results\textsuperscript{39}. In chapter 6 we describe the results of a double-blind placebo-
controlled clinical intervention study, investigating the effect of supplementing synbiotics, a specific combination of prebiotic oligosaccharides and a probiotic *Bifidobacterium breve* strain, on the allergic responses in adults with established allergic asthma. In this study a bronchial allergen challenge was performed at study entry and after a 4 week treatment period to determine whether supplementing synbiotics would have a positive effect on allergen-induced bronchial inflammation, lung function and immunological parameters, as was previously observed in a mouse model.

The analysis of induced sputum is an important tool in the diagnosis of pulmonary disease and in pulmonary research to investigate the airway inflammatory cells and related mediators. Since the sputum plugs are coughed up from the airways and collected into a tube, certain contamination with saliva cannot be avoided. In chapter 7 we propose a new method to correct whole sputum data for saliva contamination, which improves the validity of cellular and molecular biomarkers, determined in whole induced sputum of asthma and COPD patients.

Finally, in chapter 8, the results of previously described studies are summarized and the implications for further research are discussed.

REFERENCES


