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Dynamics of the allergic reaction in the airways and the skin

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DYNAMICS OF THE ALLERGIC REACTION IN THE AIRWAYS AND THE SKIN

Marjolein S. de Bruin-Weller

Dynamics of the allergic reaction in the airways and the skin

Stellingen behorend bij het proefschrift

Dynamics of the allergic reaction in the airways and the skin

1. Herhaalde allergeenprovocatie in de huid en luchtwegen kan zowel tot priming als tot uitdoving van de allergische reactie leiden. *Dit proefschrift*

2. Wanneer de allergische reactie in de huid gebruikt wordt als model voor de allergische reactie in de luchtwegen, moet de late reactie na herhaalde provocatie bestudeerd worden. *Dit proefschrift*

3. CD8+ T-lymfocyten spelen een rol bij het onderdrukken van de allergische reactie. *Dit proefschrift*

4. Wanneer de late reactie na allergeen provocatie in de luchtwegen bestudeerd wordt, moet de longfunctie minimaal 10 uur vervolgd worden. *Dit proefschrift*

5. Het beëindigen van de allergologie als zelfstandig specialisme kan leiden tot versnippering van het vakgebied.

6. Bij verrichten van fundamenteel wetenschappelijk onderzoek mag de relatie met de kliniek niet uit het oog verloren worden.

7. Bij het schrijven van een proefschrift is een belezen vader van grotere waarde dan de medline CD ROM.

8. Bij de behandeling van patienten met constitutioneel eczeem schiet het zorgaspect vaak tekort.

9. Een goede oppas is het halve werk.

10. Immunotherapy of allergic diseases: The future holds great promise and excitement (Nakagawa).

11 Naast het ziektebeeld zelf lijkt ook de interesse in de allergologie erfelijk bepaald.

12 Gezien het onmiskenbare kwaliteitsaspect van wetenschappelijk onderzoek, verdient het overweging wetenschappelijke productie (ook) in een perifeer ziekenhuis als budgetparameter aan te merken. (M.E.Vierhout, medisch contact jaargang 51, oktober 1996)

13 Kind en carrière kan!!

Marjolein de Bruin-Weller
Bilthoven, 14 mei 1997

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RIJKSUNIVERSITEIT GRONINGEN

Dynamics of the allergic reaction in the airways and the skin

Proefschrift

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Medische Wetenschappen
aan de Rijksuniversiteit Groningen
op gezag van de
Rector Magnificus, dr. F. van der Woude,
in het openbaar te verdedigen op
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des namiddags te 2.45 uur

door

Marjolein Saskia de Bruin-Weller

geboren op 12 januari 1961
te Groningen

Promotores: Prof. dr. J.G.R. de Monchy
Prof. dr. H.M. Jansen

Referenten: Dr. F. R. Weller
Dr. S. van der Baan

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Roeland en Digna

Promotiecommissie: Prof. dr. C.A.F.M. Bruijnzeel-Koomen
Prof. dr. H.C. Hoogsteden
Prof. dr .S.T. Durham

The investigations were performed in Astmacentrum Heideheuvel Hilversum, in close collaboration with the department of pulmonology of the Academic Medical Center, Amsterdam, the department of dermatology of the University Hospital Utrecht and the department of allergology of the University Hospital Groningen, The Netherlands.

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Abbreviations

AAC	Area Above the Curve
AR	Histamine-induced Airway Responsiveness
Baseline AR	Histamine-induced Airway Responsiveness before allergen challenge
BU	Biological Unit
Dpt	Dermatophagoides pteronissinus
ECP	Eosinophilic Cationic Protein
EAR	Early Asthmatic Reaction
FEV_1	Forced Expiratory Volume in 1 second
HDM	House dust mite
IVC	Inspiratory Vital Capacity
LAR	Late Asthmatic Reaction
NER	Nasal Early Reaction
NLR	Nasal Late Reaction
LNIT	Local Nasal Immunotherapy
Pa	Alveolar pressure
PC_{20} histamine	The concentration of histamine phosphate (mg/ml) that induces a 20% fall of FEV_1 value compared to baseline.
PD_{15} allergen	Dose of allergen (BU/ml) that induces a 15% fall of FEV_1 value compared to baseline.
RAC	Repeated Allergen Challenge.
RACn	Repeated Allergen Challenge in the nose
RACs	Repeated Allergen Challenge in the skin
RAC value nose	The difference between the size of the allergic reaction after challenge 3 and challenge 1, both expressed as mean % change in $Rn_{(insp)}$
RAC value skin	The difference between the size of the allergic reaction after challenge 3 and challenge 1, both expressed as reaction diameter
Raw	Airway resistance
$Rn_{(insp)}$	Inspiratory nasal resistance
SER	Skin Early Reaction
SLR	Skin Late Reaction
$\dot{V}$	Flow
VC	Vital Capacity

Voorwoord

Op 15 maart 1990 zette ik mijn eerste schreden op het terrein van Heideheuvel. Er moest onderzoek gestart worden. Op dat moment waren de enigste ingrediënten een protocol en een bureau in de koffiekamer van het laboratorium. Dit laatste lijkt misschien wat ongebruikelijk, maar is misschien wel één van de belangrijke aspecten geweest voor het slagen van mijn onderzoek binnen Heideheuvel. Zonder duidelijke plek binnen de organisatie leek mijn positie als eerste arts-onderzoeker op Heideheuvel aanvankelijk vrij eenzaam. Ik ben dan ook heel dankbaar voor de steun en gezelligheid dit ik heb ervaren op de afdeling laboratorium en longfunktie. Alice, Aty, Christien, Anita, Irma, Margreet, Astrid, Lucie, Jeanette ,Wil en Else, mede dankzij jullie hulp is dit proefschrift tot stand gekomen. Ik denk met veel plezier terug aan het traditionele kerstontbijt en aan onze gezellige personeelsuitjes.
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Chapter 1

General introduction

Definitions and epidemiology

1.1 Allergy

Allergy can be defined as a clinically relevant, harmful immunological reaction, directed towards heterologeous material, leading to a more or less reversible injury of the host. The pathogenesis of allergy is not restricted to IgE-binding, but involves various immunological mechanisms. Apart from these immunological mechanisms, the clinical manifestations of allergy are also determined by the nature of the exposed allergen and the target organ sensitivity to mediators produced by the immunological processes. The prevalence of allergy in random populations, generally defined as the presence of positive skin tests to one or more allergens, ranges from 30 to almost 50 % (1-4). The prevalence of specific IgE to aeroallergens in the Dutch general population is 32% (5).

Atopy is defined as an hereditary predisposition to the development of specific IgE antibody after exposure to even small amounts of allergen. Studies on genetics of atopy and asthma has gained much interest; several regions of interest on the human genome have emerged in the last five years, namely the β chain of the high affinity IgE receptor on chromosome 11q13 (6), T-cell receptor α on chromosome 14 (7), the HLA complex on chromosome 6p (8) and the cytokine cluster on chromosome 5q (9-12). In addition to genetical factors, environmental factors are also crucial for the expression of the phenotype.

Although allergic reactions can involve any organ system of the body, in this thesis only patients with asthma and/or rhinitis were studied. In these studies the "healthy" skin is used as a model to study allergic processes in the airways. The pathogenesis of atopic skin disorders is not the primary subject of the studies.

1.2 Asthma

Traditionally asthma is regarded as a disease of acute bronchoconstriction, resulting in variable airflow limitation over 24 hours and from day tot day. The major respiratory symptoms of asthma are paroxysms of dyspnoea and wheezing which may vary from mild and almost undetectable to severe and unremitting (13). Bronchial asthma can be clinically defined as reversible airway obstruction, pathophysiologically as airway hyperresponsiveness and histopathologically as an inflammatory response of the airways (14). Asthma affects about 10% of the population and there is a strong evidence that the prevalence and severity of asthma are rising (15). In a recent study in a Dutch general population the prevalence of current asthma (doctor's diagnose) was 3.1%, while the prevalence of patients who frequently experienced wheezing with dyspnoea without cold was 9% (5).

Table 1: Classification of the severity of astma (16)

CLINICAL FEATURES BEFORE TREATMENT	DIALY MEDICATION REQUIRED TO MAINTAIN CONTROL
Intermittent	**Intermittent**
Intermittent symptoms < week Brief exacerbations (from a few hours to a few days) Nighttime asthma symptoms < 2 times a month Asymptomatic and normal lungfunction between exacerbations Peak flow of FEV_1 value ≥ 80% predicted; variability< 20%	Intermittent reliever medication taken as needed only; inhaled short acting β_2-agonist Intensity of treatment depends on severity of exacerbation: oral corticosteroids may be required
Mild persistent	**Mild persistent**
Symptoms ≥ 1 time a week, but < 1 time a day Exacerbations may affect activity and sleep Nighttime asthma symptoms > 2 times a month Peak flow of FEV_1 value ≥ 80% predicted; variability 20-30%	One daily controller medication; possibly add a long-acting bronchodilator to anti-inflammatory medication (especially for nighttime symptoms)
Moderate persistent	**Moderate persistent**
Symptoms daily Exacerbations may affect activity and sleep Nighttime asthma symptoms > 2 time a week Peak flow of FEV_1 value > 60% < 80% predicted; variability >30%	One daily controller medication; inhaled corticosteroids and long-acting bronchodilator (especially for nighttime symptoms)
Severe persistent	**Severe persistent**
Continuous symptoms Frequent exacerbations Frequent nighttime asthma symptoms Physical activities limited by asthma symptoms Peak flow of FEV_1 value ≤ 60%; variability > 30%	Multiple daily controller medications; high dose inhaled corticosteroids, long-acting bronchodilator, and oral corticosteroids long term.

Clinically, asthma is characterised by episodic wheeze, which varies considerable within short period of time and is reversible either spontaneously or with treatment. Asthma can be divided in allergic and non-allergic asthma. Based on symptoms and the level of airflow limitation, the severity of asthma can be subdivided into intermittent, mild persistent, moderate persistent and severe persistent (16)(Table 1). In our studies only patients with mild to moderate allergic asthma were studied. The severity of the symptoms is also determined by the degree of bronchial responsiveness.

Bronchial responsiveness is the term used to describe the tendency of the airways to bronchoconstrict to both specific stimuli, such as allergens in sensitised individuals, and to non-specific stimuli, such as methacholine or histamine. Increases in non-specific bronchial responsiveness can occur during seasonal pollen exposure (17, 18) and reductions in reactivity can be induced by prolonged allergen avoidance (19). Loss of epithelial cells, infiltration of inflammatory cells, especially eosinophils, and increased deposition of subepithelial collagen play major roles in the severity of asthma and non-specific bronchial responsiveness (20). The prevalence of bronchial responsiveness in the Dutch population is about 15% (5).

1.3 Rhinitis

Rhinitis is characterised by the symptoms of nasal itch, sneezing, rhinorrhoea, obstruction to nasal airflow and in some instances, loss of sense of smell (21). Rhinitis can be classified as allergic, infectious or caused by other factors. In allergic rhinitis symptoms can be triggered from allergic and non-allergic stimuli. Apart from nasal symptoms, patients with allergic rhinitis often suffer from headache and sinusitis due to impaired sinus drainage, disturbance of sleep and day time cognitive performance. The cumulative prevalence rate of allergic rhinitis is approximately 5-15% in the population of Western Europe (22) and epidemiological studies have identified an increase in the prevalence of allergic rhinitis over the last 20-30 years (21, 23). In a recent study the prevalence of patients with symptoms of allergic rhinitis in the Dutch population was 32% (5).

Nasal hyperresponsiveness can be described as a clinical feature, characterised by occurrence of symptoms on exposure to daily life stimuli, such as cold air, hot spicy food, dust and fumes. As in the lower airways nasal hyperreactivity can be measured in the laboratory by challenge testing, however only a few studies investigated the relationship between clinical hyperresponsiveness and the laboratory model (24). More than 80% of the patients with perennial rhinitis find that these non-specific

stimuli provoke symptoms (25). This information suggests that rhinitis is characterised by nasal hyperreactivity, however a clinical distinction between patients and healthy controls is not easy to make, because healthy subjects also react to challenge with non-specific stimuli such as cold air (26). This is contrast to the lower airways, where inhalation of cold dry air during exercise results in bronchoconstriction in patients and not in healthy controls.

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Chapter 2

The allergic reaction

2.1 Physiological response to allergen challenge

2.1.1 Lower airways

Experimental allergen challenge in allergen-sensitive patients often results in an early asthmatic reaction. This early reaction starts within minutes after allergen challenge, reaching its maximum after 15-20 minutes and usually resolves after 1-2 hours. The severity of the early reaction is dependent on the level of specific IgE, the degree of bronchial hyperreactivity and the amount of allergen delivered(1, 2). In 50 to 80% of the individuals tested the early response is followed by a late asthmatic response, usually starting 3-4 hours after allergen challenge and reaching a maximum response after 7-12 hours. In many cases the lungfunction is still depressed 24 hours after challenge. Although the intensity of the airway obstruction, developed during the late response is more prolonged and usually more severe than during the early response, the patient's perception of breathlessness during the late response is less compared to the early response (3).

A useful parameter for the registration of allergic reactions after experimental allergen challenge is the FEV_1 value. To get more information on the level of bronchoconstriction additional parameters, such as vital capacity (VC) and FEV_1/VC, can be taken into account. In patients with spirometer-induced asthma, or in patients who have technical problems in performing FEV_1/VC manoeuvres, it is also possible to use the airway resistance as follow up parameter (4, 5). However effort-depending tests , such as FEV_1, are more reproducible than the effort-independent tests, such as airway resistance in the registration of allergen-induced airway obstruction(6). Technical and methodological aspects of experimental bronchial challenge are described in chapter 5.

The occurrence of a late phase reaction after experimental allergen challenge is dependent on various factors, such as circadian rhythm and viral respiratory tract infection (7-9). Mohiuddin et al (8) investigated the circadian basis of the late asthmatic response. When performing allergen challenge tests in the evening, 9 of the 10 patients studied experienced a late phase response, while 4 out of 10 patients showed late phase responses after challenge in the morning. In addition the maximal decrease in FEV_1 value during the late phase response after the evening challenge was significantly higher compared to the morning challenge. The higher frequency of late phase responses and the more severe late phase responses after evening challenges can possibly be explained by a higher number of neutrophils, eosinophils and lymphocytes in the bronchoalveolar lavage fluid at night (10, 11). The eosinophil has also been shown to have a circadian rhythm in the blood (12), which is highest in

the late evening. Circadian rhythms to histamine, cortisol, cyclic-AMP and catecholamines correlate with circadian rhythms in lungfunction (13, 14). Decreased endogenous corticosteroids has been identified as an important factor in the development of the late phase response (15, 16). In patients who were challenged in the evening decreased endogenous corticosteroids may play an important role in the development of the plate phase response at that time. In addition an increase in bronchial responsiveness has also been demonstrated at night in patients with asthma (11, 17, 18).

The occurrence of a late phase bronchial response after allergen challenge seems to have a clinical relevance. When comparing groups of patients with single (only early response) or dual response (early and late response), in the latter group usually a more severe type of disease is found. Using serial pulmonary function tests, Machado et al (19) found that the changes in pulmonary function occurring during dual responses were widespread, also involving the small airways. Theses changes resemble those seen in clinically more severe asthmatic patients. Results from a study of Metzger et al (20), who used helium-oxygen-mixture flow-volume loop measurements after experimental allergen challenge, suggest that during the early response especially large airways are narrowed, while the small airways are narrowed during the late response.

The relationship between late asthmatic reactions and bronchial responsiveness has received considerable interest in recent years. Late phase reactions can enhance bronchial reactivity (21-25) and may therefore contribute to the chronic morbidity seen in asthmatic patients. The severity of the late asthmatic response is significantly correlated with the degree of allergen-induced bronchial reactivity (23, 24). On the other hand it is not possible to predict the occurrence of a late asthmatic response from baseline bronchial responsiveness alone in individual patients (24, 26). Crimi et al (27) were able to determine a predictive index of late bronchial responses to housedustmite, which incorporated values for methacholine sensitivity, allergen-specific IgE in serum and baseline FEV_1.

In conclusion, experimental allergen challenge in the lower airways frequently results in dual responses. The fall in FEV_1 value is a sensitive method to measure early and late allergic reactions. The early fall in FEV_1 value is mainly caused by narrowing of large airways , while during late responses narrowing of small airways is the major cause of obstruction.

2.1.2 Upper airways

Allergen challenge in the sensitised nose results in an early reactions within minutes after challenge. These early reactions can easily be detected by measuring clinical symptoms (sneezing, blockage and rhinorrhoea) or by measurements on nasal patency, such as rhinomanometry and peak inspiratory nasal flow (28-31). A number of studies report on a reoccurrence of symptoms after some hours (32-35) and a few studies have documented a late phase response using rhinomanometry (36, 37). The reproducibility and the consistency of the late nasal response has not been convincingly documented. In contrast to the early response, the changes in nasal patency during nasal late response are rather subtle. The influence of various factors, such as nasal cycle, posture and unphysiological stimulation by nasal instruments, can disturb the registration of the nasal late response (38, 39). The large variation in reported numbers of late phase nasal responses after allergen challenge (3% to 75 %) depends on differences in challenge procedures, investigation techniques and different positivity cut-offs (40). In general the reported number of nasal late reactions, based on inflammatory changes in the nasal secretions, is higher than the number of late reactions based on physiological methods. It seems that an influx of inflammatory cells in the nose during the late phase period is common in all patients, but these changes are not always detectable with physiological methods. Furthermore, no clear criteria have been developed for the definition of the late nasal response, in contrast to the late phase bronchial reaction. A new sensitive, non-invasive method for the measurement of nasal resistance after allergen challenge is described in chapter 6 and criteria for early and late nasal responses are defined.

The interrelationship between the nasal late phase response and allergen-induced nasal responsiveness to non-specific stimuli is not clarified (41). This may be due to the fact that the late nasal response is not so easy to document and also has a much more varied response pattern than the similar response in the lower airways (41). Klementsson et al (42) demonstrated an increased responsiveness to methacholine in the nose 30 minutes after nasal allergen challenge.

In conclusion, the large variety in methods, used for the documentation of allergic responses in the nose, results in a large variation in reported numbers of late phase reactions. The subtle changes in nasal patency during late phase responses can only be detected with very sensitive methods.

2.2 Immunohistology of the allergic reaction in the airways

2.2.1 Introduction

Allergic inflammation is caused by effector cells, such as mast cells, eosinophils, basophils and neutrophils and is controlled by immunological mechanisms. In the following chapter cells that are involved in the manifestation and control of allergic inflammation are described. In the chapter 2.4 the major differences between allergic reactions in nose and bronchi will be described.

2.2.2 Resident cells

Mast cells

Mast cells are abundant in skin, nose and lung. A separation of human mast cells into two subtypes can be made on the basis of their neutral protease composition and the associated ultrastructure of their secretory granules (43, 44). The predominant mast cell present in the lung mucosa contains tryptase with little, if any, chymase and has been designated MC_T. In the skin mast cells contain both proteases and has been designated MC_{TC}. Skin mast cells differ from the lung in producing prostaglandin D_2, but little or no leukotriene C_4. Nasal mucosa contain substantial numbers of both mast cells, although MC_{TC} cells usually predominate.

Mast cells have been traditionally associated with allergic type I reactions, mediated via surface bound IgE (45). In both atopic asthma and rhinitis there is a epithelial accumulation of these cells and there is ultrastructural evidence of degranulation on electron microscopy (46, 47). Experimental allergen challenge in bronchi, nose and skin leads to the production of several mast cell dependent mediators, such as histamine, prostaglandin D_2, leukotrienes C_4, D_4 and E_4 and tryptase (48, 49). The concurrent finding of histamine, prostaglandin D_2 and tryptase suggests a mast cell source for these mediators. More recently it has become evident that human mast cells generate and store preformed cytokines such as IL-3, IL-4, IL-5, IL-6, GM-CSF and $TNF\alpha$ (50, 51).

The effect of histamine on asthmatic airways and nasal passages have been well described. In the lower airways histamine induces bronchoconstriction, acting both directly on smooth muscle and indirectly via the vagus nerve. In the nose insufflation with histamine results in itching, sneezing, rhinorhoea and short-lasting nasal blockage.

In conclusion, through the release of various mediators, mast cells are important effector cells in the early phase reaction. In addition, mast cells play a role in initiating inflammatory responses by the release of cytokines.

Antigen-presenting cells

Antigens are not presented as intact peptides to the T-cell, but have to undergo processing by antigen-presenting cells (APC's). In these cells peptides are broken down into peptide fragments which bind to MHC class II molecules and are transported to the cell surface. APC's belong to the monocyte/macrophage system. In addition, also B lymphocytes and epithelial cells can exert antigen-presenting properties for T-lymphocytes. Endothelial cells can also act as accessory cells for antigen presentation (52).

Godthelp et al (53) demonstrated that the number of Langerhans' cells in the epithelium and lamina propria in the nose increased significantly during an allergen provocation period of 2 weeks. IgE positive Langerhans' cells were present in the nasal mucosa and the number increased after allergen provocation.

Antigen-presenting cells can play an important role in directing the immune response (54, 55). The type of antigen-presenting cell can influence the differentiation into Th1 or Th2 type T-lymphocytes (56, 57). For instance the production of prostaglandin E_2 from antigen-presenting cells has been reported to favour the differentiation of Th2 responses (58), while IL-12 production by monocytes and macrophages favour Th1 (IFNγ) responses (54, 59). Expression of IL-10 by antigen-presenting cells may have a role in reducing allergic inflammation through its ability to inhibit the synthesis of non-specific proinflammatory cytokines such as IFNγ, IL-1, IL-6, and TNFα, as well as cytokines associated with allergic inflammation including IL-4 and IL-5 (60). In addition IL-10 has the ability to induce tolerance to allergens (61) and to inhibit eosinophil survival (62) and IgE synthesis (63). Recently Borish et al (64) demonstrated a diminished IL-10 secretion in the BAL fluid of patients with asthma compared to normal subjects. Also peripheral blood mononuclear cells from patients with asthma demonstrated decreased spontaneous and stimulated IL-10 production compared to normal subjects.

Not much is known about the occurrence of allergen-specific CD8+ T suppressor cells in man and their relationship to antigen-presenting cells. For viruses and viral antigens, dendritic cells are potent inducers of CD8+ cytotoxic cell T cells (65). McMenamin et al (66) found that inhaled soluble protein antigens may be processed by a subpopulation of spleen APC's, resulting in priming antigen-specific CD8+ T cells.

In conclusion, antigen presenting cells are necessary for the initiation of allergen specific T-cell responses. In addition APC-derived products can direct the immune response.

Fibroblasts, epithelial and endothelial cells

Tissue structural cells such as fibroblasts, epithelial and endothelial cells respond to cytokines and inflammatory mediators from infiltrating inflammatory cells as target cells. However recently it has been shown that the tissue structural cells are capable of releasing many of these same cytokines and mediators and therefore may contribute directly to the chronic inflammatory response.

Fibroblasts can produce a panel of cytokines, including IFN-β_1, IL-6, IL-8, colony-stimulating factors, IL-1, and TNF through which fibroblasts can influence a number of other cells in the tissue. The chemotaxis of neutrophils and monocytes is influenced by fibroblast derived IL-8 and Monocyte Chemotactic and Activating Factor (MCAF), while GM-CSF mediate neutrophil, monocyte and eosinophil accumulation and activation in tissues (67).

Epithelial denudation is a feature of asthma. Increased numbers of *epithelial cells* have been found in the Bronchoalveolar lavage fluid and sputum in patients with asthma (47, 68), and the increased recovery of epithelial cells in the bronchoalveolar lavage fluid correlates inversely with the level of bronchial reactivity (47). Epithelial denudation is immediately followed by a restitution process (69), and repeated denudation-restitution processes in previously normal airways result in a thickening of the reticular basement membrane. Eosinophils, which are largely involved in allergic reactions, probably play an important role in epithelial denudation. The traditional view has been that epithelial shedding is secondary to cytotoxicity from eosinophil products such as ECP and MBP (70). However it is also possible that proteolytic cleavage occurs by eosinophil derived proteolytic enzymes (71, 72). Airway epithelial cells in vitro generate and release cytokines, particularly IL-6, IL-8 and GM-CSF (73, 74). IL-8 and GM-CSF will contribute to eosinophil airway recruitment.

The selective recruitment of cell types is determined at the level of leucocyte adhesion and chemotaxis, and is modulated by cytokines.

Endothelial cell activation, as evidenced by the induction of leucocyte adhesion molecules, is a well characterised phenomenon. Treatment of endothelial cell monolayers (ECM) with IL-1, TNF or lipopolysaccharide (LPS) results in enhanced binding of basophils, eosinophils and neutrophils to endothelium (75, 76). Antibody

inhibition studies demonstrate that intercellular adhesion molecule 1 and endothelial leucocyte adhesion molecule 1 are involved in the binding of all three granulocyte types. VCAM-1 participated in binding of basophils, eosinophils, monocytes and lymphocytes to ECM, but has no role in neutrophil binding (77, 78) . VLA-4 (very late antigen 4) is the cellular receptor for VCAM-1 and is expressed on eosinophils, basophils, monocytes and lymphocytes (78) . The VCAM-1/VLA-4 pair may have special relevance in allergic asthma by affecting the specific adhesion of eosinophils to the pulmonary capillary endothelium.

2.2.3 Infiltrating cells

Eosinophils

Eosinophils are evident in nasal mucosal biopsies within the submucosa and epithelium in active rhinitis (50, 79). Also in asthma eosinophils are found in sputum (80), lavage (81, 82) and biopsy samples (47, 83). The eosinophils are activated, as evidenced by morphological changes on transmission electronmicroscopy (47, 83). After experimental bronchial allergen challenge a raised number of blood eosinophils after 24 hours (21) and a raised eosinophil number in the bronchoalveolar lavage fluid after 3,6 and 24 hours (25, 84) was found in those patients who developed a late phase bronchial reaction. Also in the nose late phase allergic reactions after experimental allergen challenge are associated with an increase in the number of eosinophils in nasal washings and biopsies (85, 86). Natural exposure to allergens induces blood, bronchial and nasal eosinophilia (87-89).

Apart from the release of several vasoactive and bronchoactive mediators, the eosinophils have the capacity to produce cytotoxic proteins, such as major basic protein (MBP), eosinophil peroxidase, eosinophil-derived neurotoxin and eosinophil cationic protein (ECP) (83). These cationic proteins are toxic to the epithelium in vitro and have been proposed to cause desquamation of the airways in vivo. Eosinophils in the airways in asthmatic patients and in rhinitis patients have also been demonstrated to contain IL-5 (50, 51).

IL-3, IL-5 and GM-CSF play an important role in the enhancement of survival, maturation and activation of eosinophils (90-93). Eosinophil survival and cytokine production is inhibited by IL-10 (see antigen presenting cells) (94)

In conclusion, eosinophils are important effector cells in the allergic inflammation. Their activation and survival in controlled by various cytokines derived from CD4+ T-cells and antigen-presenting cells.

Basophils

The role of the basophil in the mediation of allergic inflammation has not been clearly elucidated. They express high affinity receptors for IgE on their surface. When activated, basophils release histamine and leukotriene C_4, but unlike mast cells they generate only very small quantities of tryptase and no detectable prostaglandin D_2 (95). Late phase reactions in the bronchi (96), skin (97) and nose (98) are associated with an influx of inflammatory cells, including basophils. The finding that in nasal washings in allergen-induced rhinitis histamine and leukotriene C_4 , but not prostaglandin D_2, is found suggests a role for the basophil, rather than mast cells in late phase nasal reactions (99). Later on the presence of basophils were demonstrated in late phase nasal reactions (100). Recently Naclerio et al (101) demonstrated that during late phase nasal response the increase in symptom scores coincide with an increase in histamine, but not in tryptase and prostaglandin D_2, in the nasal lavage. This finding excludes a mast cell source for the late phase histamine rise. Basophils from peripheral blood are capable of synthesising IL-4 and IL-8 (102, 103), however the relevance of this cell population to cytokine synthesis within the tissue is uncertain.

Basophil priming is induced by IL-3, IL-5 and GM-CSF. The releasibility of basophils is increased in the presence of IL-3 (104).

In conclusion, although the role of the basophil in allergic inflammation in the lower airways is largely unknown, in nose and skin they are often associated with late phase allergic reactions.

Neutrophils

The role of the neutrophil in the allergic reaction seems to be controversial.

A neutrophilic influx into the airways was observed in association with a late phase allergic reaction and a subsequent increase in airway reactivity in patients with asthma (105). Also Diaz et al (106) found an increased number of neutrophils in the BAL 6 hours after challenge in dual responders. Segmental bronchial challenge with increasing amounts of allergen in atopic subjects resulted in an increased number of inflammatory cells in the BAL fluid, including neutrophils, although there was no direct relationship between the dose of allergen and the number of neutrophils recruited (107). Boulet et al (108) demonstrated that seasonal exposure in pollen allergic asthmatics resulted in a significant change in epithelial disruption and an increased neutrophil count in biopsies.

In other studies the role of the neutrophil in allergic asthma seems limited. In patients with newly diagnosed asthma, Laitinen et al (109) found no significant difference in the number of neutrophils compared to healthy controls. In bronchial biopsy specimens no significant difference in the number of neutrophils were found between patients with atopic asthma, atopic patients without asthma and normal controls (110). Beasley et al (47) did not find significant changes in neutrophil numbers in bronchial biopsies and lavages before and after bronchial provocation with allergen in mild asthmatics, although this might be due to the rather late time point of the bronchoscopy (18 hours after challenge). In control specimens neutrophils were more numerous and were accompanied by intravascular neutrophils, suggesting that this was an acute response to bronchoscopy. In addition Von Essen et al (111) found a significant increase in bronchial and alveolar neutrophil numbers after sequential bronchoscopy with BAL in 30 subjects which was accompanied by peripheral blood neutrophilia. In a recent study, Teran et al (112) also demonstrated that neutrophil infiltration after segmental allergen challenge is a nonspecific response to the procedure of bronchoscopy and lavage.

In the nose Fokkens et al (113) found more neutrophils in the lamina propria of allergic patients and non-allergic patients with polyps than in healthy controls, suggesting that neutrophils play a role in allergic and non-allergic inflammation. Takasaka et al (114) found more neutrophils in polyps of patients with chronic sinusitis than in allergic patients.

In conclusion, neutrophils are often found in association with late phase allergic reactions and chronic allergen exposure. The fact that not all studies are conclusive might be due to the time related dynamics of neutrophil influx.

T-lymphocytes

T-lymphocytes control the production of IgE (115) and can regulate inflammatory reactions. T-lymphocytes can be phenotypically and functionally subdivided in CD4+ and CD8+ T- cells. CD4+ T-lymphocytes recognise protein-derived antigens bound to Major Histocompatibility Complex (MHC) molecules on the surface of antigen-presenting cells. Antigen recognition of CD4+ cells is generally MHC-class II restricted. CD8+ cells generally recognise antigens in the context of MHC I class proteins.

In this chapter only the role of the T-lymphocyte in regulating the inflammatory response is described.

CD4+ T-lymphocytes

It is now well established that CD4+ T-lymphocytes play an important role in the development and maintenance of allergic diseases (45). Through the production of various cytokines, these T-cells have the capacity to enhance the activity of effector cells, such as mast cells and eosinophils, by acting upon their recruitment, maturation, survival and activity. An increase in activated CD4+ T-lymphocytes has been demonstrated in both active asthma (116-118) and rhinitis (119).

Table 1: Cytokine production profiles of human "Th1" and "Th2" type lymphocytes.

	Th1	**Th2**
IL2	++	+
Interferon γ	++	-
(TNF-β)	++	+
IL3	++	++
GM-CSF	++	++
TNF-α	++	++
IL6	+	+
IL4	-	++
IL5	-	++
IL10	+	++
IL13	+	++
IL9	-	+

CD4+ T-lymphocytes can be divided into 2 sub-populations according to their profile of cytokine production (120, 121)(Table 1). Naive CD4+ cells produce modest amounts of an unrestricted range of cytokines when stimulated (Th0 pattern). The cytokine production profiles of naive cells are determined during their differentiation.. After activation they may differentiate either into cells that produce large amouts of IL-2 and IFNγ and support cell-mediated immunity ("Th1" cells) or differentiate into cells which produce IL-4 and IL-5 , but little IFNγ, and induce IgE and eosinophilia ("Th2" cells). Functional characterisation of enhanced cytokine production may not represent a firm state of differentiation, but rather the functional consequences of the milieu in which the cell is being stimulated. Several factors, including cytokines, may specifically promote Th1 or Th2 outgrowth. IFNγ and IL-12 strongly promote the generation of Th1 cells, while IL-4 and PGE_2 promote the generation of Th2 cells (54, 122-125). Because IL-12 and PGE_2 are factors derived from antigen-presenting cells, these cells may play an important role in the induction of Th1 or Th2 type cells.

Using in-situ hybridisation techniques, enhanced expression of mRNA for different cytokines can be linked to disease activity. An increase in IL-5 mRNA positive cells in bronchial biopsies and an increased expression of IL-3, IL-4, IL-5 and GM-CSF mRNA in BAL cells were found in atopic asthmatics (126, 127). Using a segmental allergen challenge model, Huang et al (128) reported a significant enhancement of both IL-13 transcripts and secreted proteins in allergen challenged bronchoalveolar lavage compared with saline-challenge control sites in asthmatic and rhinitis patients. In acute asthma a significant increase in mRNA expression for IL-5 in peripheral blood CD4+ cells is found, compared to stable asthma and controls (129). Following nasal challenge, an increase in IL-4, IL-5 and GM-CSF mRNA positive cells has been described in association with a mucosal eosinophilia (130). A significant increase in IL-4 mRNA was found after allergen challenge in patients with seasonal rhinitis compared to controls and during natural seasonal provocation(131). Pawankar et al (132) demonstrated an increased IL13 gene expression in the epithelial compartment of the nasal mucosa in patients with perennial rhinitis compared to controls.

CD8+ T cells

CD8+ cells are either cytotoxic effector cells that lyse target cells, or suppressor cells that can inhibit CD4+ T cell responses as well as antibody production by B-lymphocytes. In contrast to the role of the CD8+ T cells in controlling IgE synthesis (115), the role of these cells in the regulation of allergic inflammation is largely unknown.

In the eighties CD8+ cells were linked to suppression of allergy, because of their possible role in successful allergen immunotherapy. Using a suppressor-cell assay, Rocklin et al (133) found that blood mononuclear cells from ragweed allergic patients specifically suppressed a ragweed proliferative response after treatment with immunotherapy. Also in other studies allergen immunotherapy has been associated with an increase in allergen-specific CD8+ cells (134, 135).

A revival of interest in CD8+ suppressor cells can be seen in the last few years. CD8+ T-cells seems be associated with a diminishing of a subsequent late-phase response.(136, 137). It now appears that there are distinct subsets of CD8+ T cells that produce different combinations of cytokines and may play an important role in the growth and differentiation of CD4+ cells and IgE production. Recent studies indicate that CD8+ T cells can inhibit IgE production (138-140), possibly acting indirectly by modulating the differentiation and function of Th2-type CD4+ T cells (115). Elimination of IgE regulatory CD8+ T cells in rats resulted in an increased expression of IL-4, IL-5 and IL-10 (115, 141). Furthermore a subset of CD8+ T cells,

bearing $\gamma\delta$-receptors, can produce IFNγ and IL-2 (142, 143). Depletion of CD8+ T cells in rats is associated with an enhancement of airway inflammation (144).
In conclusion, apart from controlling IgE production, different subsets of T-lymphocytes play an important role in regulating allergic inflammation. Cytokines from "Th2" type CD4+ T-cells favour inflammation, while cytokines derived from "Th1" type cytokines support cell-mediated immunity. Although there is indirect evidence that CD8+ T-cells are involved in suppression of allergic reactions, the precise role of these cells is not yet elucidated.

2.3 Neuroregulation of the nose and bronchi
The sympathetic and parasympathetic components of the nervous system control airway vasculature, secretory glands and airway smooth muscle, the primarily effector tissues in nose and bronchi. Also reflex control via sensory (mainly C-fibre) receptors play an important role in the neuroregulation in the airways (145).
Sympathetic and parasympathetic nerve endings both release many neurotransmitter substances, resulting in complex interactions and diverse effects in the various target organs. In addition to classic cholinergic and adrenergic innervation of the airways, there are neural mechanisms that are not blocked by cholinergic or adrenergic antagonists. These nonadrenergic noncholinergic (NANC) mechanisms result in both bronchodilatation and bronchoconstriction, vasodilatation and vasoconstriction, and mucus secretion, indicating that several types of neurotransmitters are involved (146).
Parasympathetic nerve activity in the nasal and bronchial mucosa results in a vasodilatory effect. Parasympathetic neuromediators, such as acetylcholine, vasoactive intestinal polypeptide (VIP) and nitric oxide, cause reduced vascular resistance and thus increased blood flow. Sympathetic neuromediators such as noradrenaline and neuropeptide Y, cause an increased vascular resistance and thus a reduced blood flow. Mucosal thickness and airflow resistance can be affected by neurally induced changes in the airway vascular bed. This is best demonstrated in the nose, where nasal patency is increased during vasoconstriction due to thinning of the mucosa (147). Although functionally less important, a similar process can be demonstrated for the trachea (148). The airway vascular beds are also under reflex control (149). Stimulation results in reflex mucosal vasodilatation of the lower airways and nose.
Mucus secretion at all airway sites is caused by parasympathetic nerves. Although there is little evidence that sympathetic nerves have an important role in controlling

airway secretion, it is possible that they may control the release and composition of gland secretion. Also reflex activation of the airway submucosal glands is possible. Irritation of the nose and lower airways stimulates C-fibre and rapidly adapting receptors and causes subsequent secretion (145).

The dominant motor control of the tracheobronchial smooth muscle is parasympathetic and cholinergic (145, 150). There is less evidence that the sympathetic nerves regulate airway smooth muscle tone in humans (151). Reflex actions play an important role in the control of the airway smooth muscle tone (150). Receptors may be activated in airway inflammation, and thus resulting in bronchoconstriction.

Neurogenic inflammation is a further component of neural control, which occurs when sensory nerves are activated (145). Sensory peptides, such as substance P, neurokinin A and calcitocine gene-related peptide (NANC system) are released from nerve endings. The responses include submucosal gland secretion, mucosal vasodilatation with transudation and actions on the epithelium leading to its permeability. There may also be contraction of airway smooth muscle (145).

2.4 Differences between allergic manifestations in nose and bronchi

Rhinitis is a usually clinically benign condition, although often resulting in much discomfort to the patients. This is evidenced by an impaired quality of life in rhinitis patients (152). In contrast the natural course of asthma may be quite severe and potentially fatal.

Due to its important filter function, allergen exposure in the nose is often more pronounced than in the lower airways: this might partly explain a higher prevalence of allergic rhinitis than of allergic asthma.

The *symptomatic response* to allergen challenge in the nose is characterised by sneezing, itching, rhinorrhoea and blockage. In the lower airways this response is characterised by wheezing and sometimes coughing. The allergic responses in both nose and bronchi are caused by mediators derived from inflammatory cells and neurogenic mechanisms. Whether there are differences in the relative contribution of these 2 components between nose and bronchi is not clear; the density of epithelial nerve fibres has not been compared in the upper and lower airways.

Although most *inflammatory cells* that have been described in the lower airways are also apparent in the nose, there are some differences in relative contribution and activity. Although there is no evidence to suggest that there are fundamental differences between nose and bronchi with regard to IgE antibodies, biochemical

mediators, mast cell subsets and their releasability, the high efficacy of H_1-antagonists in rhinitis and their marginal effect in asthma suggests a more important role of histamine in the early phase nasal reaction. Lymphocyte aggregates, similar to the bronchus-associated lymphoid tissue (BALT) have not been found in the nose (153). Probably the adenoid has a function that corresponds to that of BALT (154). There are few macrophages in the lamina propria of the nose and these cells have not been identified in nasal smears and lavage fluid. In contrast the BAL fluid contain many macrophages (154). Macrophages in the lower airways bear low affinity IgE receptors (155, 156), while macrophages in the nasal mucosa do not express IgE recepors (53).

Possible differences in inflammatory cell composition have been described by Djukanovich et al (157). Although mucosal eosinophilia is a characteristic of both allergic rhinitis and asthma, there are some differences in effects. The epithelial disruption which is evident in bronchial biopsies from asthmatics, due to toxic proteins from eosinophils, is not observed in nasal biopsies in seasonal allergic rhinitis despite prominent eosinophil infiltration (157). Furthermore in asthma the degree of infiltration with activated eosinophils in the bronchial mucosa has been correlated with the degree of airway hyperresponsiveness (110) but no evidence for a similar association has been shown in allergic rhinitis. Also T cell activation in rhinitis is less marked than in asthma. Although increased T-cell activation can be demonstrated following allergen challenge in the nose, in perennial rhinitis T-cell activation is not a prominent finding.

The most important difference between allergic response in nose and bronchi is the difference in *primary effector tissue*. Although the primary effector tissues in both nose and bronchi are vasculature, secretory glands and airway smooth muscle, the contribution of these different components in nose and bronchi is essentially different. In the lower airways the primary effector tissue is smooth muscles in the bronchial wall, while in the nose vascular congestion is the major cause of obstruction. The nasal vasculature is complex and consists of arterial vessels which give rise to subepithelial and glandular capillary beds. These capillary beds consist of fenestrated capillaries, draining into venous sinusoids which act as capacitance vessels (157). Prominent arterial-venous anastomoses shunt blood from the arterial to the venous system, thus bypassing the capillary bed (158, 159). Engorgement of the capacitance vessels is the main contributor to nasal obstruction, whereas smooth muscle constriction is the major determinant of acute lower airway narrowing (157).

The insignificant effect of alpha adrenoreceptor agonists in asthma indicates that vasodilatation is unimportant for bronchial symptoms, while β_2-adrenergic drugs are effective in reducing bronchoconstriction by relaxing smooth muscle. On the other hand nasal responses to allergen challenge can be blocked by using vasoconstrictive agents, such as oxymetazoline hydrochloride.
Increased production of secretions after allergen exposure is apparent in both nose and bronchi, however in the nose secretions can be blown out, while the lower airways become plugged with mucus. Although edema formation in nose and lung is not fundamentally different, the aerodynamic consequence is more prominent in small than in large airways.
In conclusion, the manifestation of allergic reactions in nose and bronchi is rather different, which probably can be explained by a different "translation" from inflammatory processes into physiological changes (Table 2).

2.5 The early and late allergic reaction in the skin
Macroscopy
Skin testing with allergen is often used to confirm the diagnosis of allergy. For this purpose the immediate reaction, measured 15 minutes after dermal or intradermal challenge is used. This early reaction is visible as a wheal and flare, and is primary caused by IgE dependent mast cell degranulation . For the documentation of the response, the wheal is measured in two perpendicular diameters, 15 minutes after challenge; the mean value of the two measurements is recorded. Although a positive skin test often predicts the existence of allergic manifestations in the airways, the magnitude of allergic reactions in skin and airways are only weekly correlated (160-162).
When sufficient amount of allergen is injected, a late phase skin reaction can be evoked. After a peak of 6-12 hours the reaction begins to subside, but is often still detectable up to 48 hours after challenge. Late phase reactions are characterised by ill-defined erythema and induration, often accompanied by burning discomfort. Umemoto et al (163) found that when the early response has a wheal diameter of 8 mm or more, a late response generally occurred. The size of the late response is directly related to the size of the early response.
In contrast to the early response, the documentation of the late response is more difficult to perform. With the customary technique of palpation the margins of the indurated area are difficult to define. When measuring indurations of tuberculin reactions, individual readers reproduced their own measurements quite well, however

a large variability was found between different readers (164). Sokal et al (165) developed a technique for measuring indurations in delayed skin test responses, evoked by tuberculin. A line is drawn with a "medium" ball-point from a point 1 to 2 cm away from the margin of the skin test reaction, towards its centre. When the ball-point reaches the margin of the indurated area, a definite resistance is felt; the pen is then lifted. This procedure is repeated from other sides of the reaction. When the different end points are connected, the margins of the induration are visible.
The induration is measured in two perpendicular diameters and the mean value of the two measurements is recorded. Although this technique is developed for the measurement of delayed type skin reactions, it can also be used for the documentation of late phase skin responses after allergen challenge.
Late skin reactions after allergen challenge are not used as a diagnostic tool. Because of the simple accessibility of the skin, the late phase skin response is often used as a model to study inflammatory changes during late phase reactions in the airways.

Immunohistology of the allergic skin reaction
The early skin reaction, which is characterised by a wheal and flare, is primarily caused by mast cell derived mediators, such as histamine, prostaglandin D_2, leucotrienes and Platelet Activating Factor (PAF). The release of these mediators takes place as a result of IgE-mediated and non-IgE-mediated reactions. Using skin chambers Ting et al (166) demonstrated a rapid rise in the skin chamber of histamine, which peaks by 30 minutes and which is followed by a decline during the subsequent 4 hours . The total amount of histamine released during this initial phase correlated significantly with the intensity of the subsequent late phase reaction as measured by skin testing (167). Although histamine is most prominent during the early phase reaction, a slow but persistent rise of histamine was also demonstrated during late phase reactions (168). This rise in histamine usually peaks at 11 or 12 hours and is followed by slow decline to baseline. It is suggested that the basophil, rather than the mast cell may be the source of this late phase histamine release. The early pattern of PGD_2 production without late production and the absence of mast cell derived tryptase during late phase reaction tends to support this hypothesis. Bochner et al (169) demonstrated that the increased level of histamine in the skin chamber fluid during the late phase reaction coincided with an increase in basophils in the skin chamber. Other mediators that play a role in the late phase skin reaction are leucotrienes, especially LTC_4. Leucotrienes during late phase reaction are probably eosinophil derived. It is likely that the production of LTC_4 by activated

eosinophils, in concert with other mediators, significantly contributes to the clinical late phase reaction (170). Also PAF, one of the most potent chemotactic factors known for eosinophils (171), may play an important role in the late phase skin reaction. Using skin chambers, Shalit et al (172) demonstrated the presence of PAF in the skin chamber fluid of allergic subjects who were challenged with continuous low dose of antigen. The production was first detected during hour 3 and was continued for 9 hours. PAF results in increased vascular permeability, increased tissue edema and enhanced eosinophil diapedesis(173) and is capable to cause release of LTC_4 from eosinophils (171).

The histopathology of the late phase skin reaction is characterised by infiltration of mononuclear cells, basophils, neutrophils and notably eosinophils (97, 174, 175). The same cellular pattern, however, can be found after an immediate wheal and flare reaction that did not lead to a macroscopic late phase reaction (176). Neutrophils are numerically the most prominent cells (170, 177-180) , but they are also present in control biopsies in response to non-specific trauma (168). In a non-traumatic test situation, such as the atopy patch test, very low number of neutrophils were found (180).

Basophils are demonstrated in cutaneous late phase reactions in biopsies and skin chamber cytology (168). Basophils were clearly increased in the antigen challenges chambers, but were fewer than eosinophils. The eosinophil and basophil were selective for the antigen challenged side in the chamber study, suggesting that these two cell types are preferentially recruited to the late phase reaction (168). Earlier it is suggested that the basophil may be the source of the late phase histamine release. Basophils can also release LTC_4 and cytokines.

Frew et al(181) demonstrated a significant increase in CD4+ T-cells and activated eosinophils, but not in CD8+ T-cells, in biopsies of late phase skin reactions at 6, 24 and 48 hours compared to control sites. The late phase reaction diameter at 6 hours correlated with the number of activated eosinophils at 48 hour, but not with infiltration of T-cells. There was a strong correlation between the numbers of CD4+ cells and activated eosinophils at 24 hours. The role of the eosinophil in the late phase skin reaction was studied by Leiferman et al (178), who investigated the time course for the deposition of extracellular major basic protein (MBP), one of the toxic proteins derived from the activated eosinophil. Allergen challenge in the skin resulted in an extensive deposition of MBP in the dermis by the third hour, while deposition of cationic proteins persisted for up to 56 hours. So eosinophils seems to

come in early and have a long persisting effect. Apart from toxic proteins, eosinophils are also capable of releasing other products such as leucotrienes, PAF and cytokines. Various cytokines have been studied in relation to the allergic skin reaction. IL-6 release at various time points after allergen challenge was demonstrated in skin chambers in atopic individuals and not in controls (182). Early IL-6 release correlated with late histamine production and late IL-6 influx correlated with eosinophil influx. IL-2 release was not different between allergen challenge and control sites. IL-1 bioactivity was detected in skin chamber fluids 10 to 12 hours after challenge in allergen challenge sites, but not in control sites. Analysis of IL-1β levels by RIA revealed an initial peak at 1 hour and a second elevation after 7-11 hours after challenge (169).

Using hybridisation techniques, Kay et al (183) demonstrated increased expression of IL-3, IL-4, IL-5 and GM-CSF, but not IL-2 or IFNγ in the cutaneous late-phase reactions to grasspollen. There was also an increase in the number of CD4+, CD3+ and EG2+ cells, with a significant correlation between transcripts for IL-4/5 and EG2+ cells. An enhanced expression of TNFα mRNA was demonstrated in late phase reactions in the skin, and also in nose and BAL fluid, compared to controls (184). TNFα is produced by a wide range of cells such as lymphocytes, macrophages, mast cells, neutrophils and epithelial cells.

In conclusion, the late allergic reaction after intradermal challenge is caused by products derived from infiltrating effector cells such as eosinophils, neutrophils and basophils. In addition, T-lymphocytes play an important role in regulating the inflammatory response.

2.6 The allergic skin reaction as a model for allergic reactions in the airways

Because of the easy accessibility of the skin, the allergic skin reaction is often used as a model for the allergic reactions in the airways. For this purpose the reaction after intradermal challenge of the non-disrupted skin is used.

The symptomatic response to allergen challenge in the skin is characterised by burning discomfort and itching, in the nose by sneezing, itching, rhinorrhoea and blockage and in the lower airways by wheezing and sometimes coughing. The registration of the allergic responses in skin and airways show large differences: The physiological response to allergen challenge in the airways is measured as a fall in lungfunction or an increase in airway resistance and is determined by a decrease in airway diameter. So the expansion of the reaction is measured as a decrease in airway diameter. This in contrast to the skin were the reaction can be visualised and directly

Table 2: Comparison of the allergic reaction in the skin (after intracutaneous challenge),nose and lower airways

		skin	nose	lower airways
immediate response	**cells**	Mast cells (MC_{TC})	Mast cells (MC_{TC} MC_T)	Mast cells (MC_T)
	mediators	Histamine (prostaglandins, leucotrienes etc)	Histamine (prostaglandins, leucotrienes etc)	Histamine (prostaglandins, leucotrienes etc)
late response	**cells**	Eosinophils T-Lymphocytes (neutrophils, basophils)	Eosinophils T-Lymphocytes (basophils, neutrophils)	Eosinophils T-Lymphocytes (neutrophils, basophils)
	mediators	Toxic proteins (ECP, MBP etc) LTC4, (histamine)	Toxic proteins (ECP, MBP etc) LTC4, (histamine)	Toxic proteins (ECP, MBP etc) LTC4, (histamine)
Translation	**effector tissue**	subcutaneous vessels	sinusoids, secretory glands	muscles, submucosal vessels, secretory glands
	effect	vasodilatation /edema	vasodilatation/edema secretion	spasm, vasodilatation/edema secretion
	registration	directly: diameter	indirectly: nasal airways resistance symptom scores	indirectly: lungfunction

measured as a reaction diameter. This difference in registration of the response may partly explain why the magnitude of allergic reactions in skin and airways are only weekly correlated (160-162, 176).

In contrast to the macroscopy, there are many similarities in the microscopic appearance between the allergic reactions in skin and airways. There are no studies in which inflammatory changes in biopsies after allergen challenge in the different

organs are compared in the same subjects at the same time point. Recently such a study was performed in order to investigate whether the late skin reaction is a useful model to study the kinetics of the inflammatory response in atopic dermatitis (180). The results of this study show important differences in inflammatory cell infiltrate between the late phase response on one hand and the atopy patch test and lesional skin on the other hand and therefore the late skin reaction is not a suitable model to study atopic dermatitis. The comparison between the inflammatory cell infiltrate in late phase reactions after intradermal challenge and allergic reactions in the airways can be accomplished by comparing studies on inflammatory changes after allergen challenge in the different target organs. Looking at these data it seems that there are only small differences in the quality of infiltrating inflammatory cells between the different organs. Mast cell dependent mediators are primarily responsible for the early reaction in both skin and airways, although the skin mast cell is different from mast cells in the airways. As was discussed earlier the predominant mast cell in the skin contains tryptase and chymase (MC_{TC},) while the mast cell present in the lung mucosa contains merely tryptase (MC_T). Skin mast cells differ from the lung in producing prostaglandin D_2, but little or no leukotriene C_4 and are less influenced by IL-3 . Eosinophils, neutrophils, basophils and T-lymphocytes are present during late phase responses in all organs, although there may be small differences in relative distribution.

An important difference between the allergic reaction in skin and airways after experimental allergen challenge is the absence of epithelial damage as a result of allergen exposure in the skin. Because the allergen is applied intradermally, the inflammatory process takes place beneath the epithelium, without affecting it. This in contrast to the airways, where the epithelium is actively involved in the inflammatory process. Epithelial damage after allergen exposure takes place with subsequent changes in permeability.

Differences in manifestation of the allergic reaction in skin and airways can be partly explained by differences in primary effector tissue. As was discussed earlier, in the lower airways the primarily effector tissue is smooth muscles in the bronchial wall, while in the nose vascular congestion in the major cause of obstruction. In the skin in the absence of smooth muscle and mucus producing and secreting cells such as Goblet cells, the primarily effector tissue is the vasculature. This is most prominent during the early phase reaction, but also during late phase reaction oedema and erythema are present. Late phase skin reactions are also characterised by depositions of proteins in the dermis.

In conclusion, the microscopic changes during the late allergic skin reaction after intradermal challenge seem to resemble the microscopic changes during allergen-induced inflammatory responses in the airways. However the macroscopic expression of allergic reactions in airways and skin is quite different (Table 2).

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Chapter 3

Dynamics of the allergic reaction

3.1 Introduction

Allergic reactions in skin and airways can be influenced by various factors, resulting in an increased or a decreased expression of early and/or late responses. In this thesis two intervention methods are used to study the dynamics of the allergic reaction: Repeated experimental allergen challenge and systemic allergen immunotherapy. We speculated that repeated allergen challenge in the different target organs would lead to increased responses, while systemic allergen immunotherapy would lead to a diminished response.

3.2 Priming

In this chapter priming as a physiological phenomenon is described; priming as a immunological phenomenon is not is not discussed here.

3.2.1. Nose

The priming phenomenon was first described in the nose by Connell and was defined as an increased nasal reactivity following repeated allergen exposure (1, 2). Connell found that when daily challenges with ragweed were performed, lower doses of pollen were required on successive days to increase nasal airway resistance. Thus it was postulated that the reaction threshold for allergen is lowered as a result of repeated allergen exposure. The priming effect disappeared by stopping challenges for several days. Connell also demonstrated that environmental exposure to ragweed pollen during the season, also resulted in priming, which was reversible several weeks after the pollen season had ceased (1). Although Connell's experiments were based on a limited number of patients, the priming concept has become a well accepted phenomenon. Several years later a number of studies have used the experimental model of repeated allergen challenge. These studies show large differences in the set-up of the study, such as differences in allergen doses, number of challenges, intervals between the challenges and differences in registration of the response.

Wachs et al (3) studied the effect of repeated allergen challenge on symptomatic responses, mediators and inflammatory cells in nasal lavages. In this study 3 successive daily challenges were performed with the same dose of allergen. Six of the 10 patients studied showed an enhanced symptomatic response, while the other 4 subjects had little change in response. The levels of histamine, TAME esterase, kinins and prostaglandin D_2 were significantly increased after challenge 3 compared to challenge 1, whereas there was no significant change in the level of leucotriene C_4. An increased number of eosinophils and neutrophils were found in the nasal lavages

24 hours after challenge 1 and 2 compared to before; there was however no further increase in cell numbers after challenge 2 compared to challenge 1. The authors suggested that although there is evidence of priming in the total group of patients, not every subject may have a priming response, and those subjects who do may become ill during seasonal exposure. Pipkorn et al (4) found an increased number of eosinophils in nasal lavages 24 hours after daily challenges compared to before. The increased number of eosinophils was remained throughout the whole challenge period, but did not show a further increase after the successive challenges. Nasal peak flow measurements before and after allergen challenge showed only minor differences between the challenge days and a significant increase in composite symptom score was reached after challenge 5, 6, and 7 compared to before. Iliopoulos et al (5) rechallenged 10 hours after the first challenge, using a lower dose of allergen. An enhanced reaction based on mediators in nasal lavage fluid was found in 21 of 55 subjects and another 10 subjects showed an enhanced symptomatic response without changes in the levels of mediators.

Absence of priming after repeated experimental allergen challenge with an interval of 48 hours using the same dose of allergen was reported by Malmberg et al (6).

In 10 patients with isolated birch-pollen allergy natural seasonal exposure to allergen resulted in an increase in percentage and total number of eosinophils in nasal imprints compared to preseason. This increase in eosinophils closely followed the nasal symptoms and pollen counts (7). There was also an increase in the number of mast cells in nasal imprints and an increase in TAME esterase in the lavage fluid. Also in earlier studies with secretion smears, a seasonal increase in eosinophils was observed(8). In contrast in studies on the effect of local nasal immunotherapy Passalacqua (9) and Nickelson (10) did not find increases in nasal sensitivity after the season compared to before in the placebo groups, which might indicate the absence of priming after natural allergen exposure.

3.2.2 Lower airways

Only a limited number of studies have been performed on the effect of repeated allergen challenge in the lower airways. Four consecutive bronchial allergen challenges in ragweed allergic patients did not result in significant changes in early allergic reaction (11). Using an interval of 14 days Rasmussen (12) did not find significant changes in early response, however a significant increase in late allergic response was reported. This in contrast with a study of Bel et al , in which reproducible early and late phase reactions were found after allergen challenges with

an interval of 2 weeks (13, 14). Ihre et al (15) found a significant increase in non-specific airway responsiveness after repeated low doses of allergen, however there are no data on effects on allergen sensitivity.

Natural allergen exposure can also influence the allergen sensitivity of the lower airways. Crimi et al (16) found an increase in early and late bronchial response to allergen challenge in 5 birch pollen allergic patients, who did not use inhaled steroids, after the pollen season compared to before. In another study allergen specific priming effect on the late phase bronchial reaction was reported (17). In patients with mild allergic and pollen-induced asthma seasonal allergen exposure was associated with a mild increase in baseline airway inflammation (18). Djukanovic et al (19) demonstrated that natural allergen exposure in grasspollen sensitive asthmatic patients resulted in an increase in symptoms , which was accompanied by an increase in T-cell activation in the BAL fluid, an increase in CD4+ T cells in the submucosa and an increased number of mucosal mast cells staining for IL-4 .

3.2.3 Skin

Using several allergens and anti-IgE, Shaikh et al (20) found no change in early reaction after repeated intracutaneous challenges with intervals of 1, 2 and 3 weeks in a limited number of patients. The late phase reactions appeared to be smaller. When performing 2 intracutaneous challenges with pollen with an interval of 12 hours, Andersson et al found a decrease in early reactions at rechallenge with unaltered histamine reactions (21, 22).

3.3 Systemic allergen immunotherapy

3.3.1 Introduction

Immunotherapy with allergen extracts traditionally involves a series of injections of allergen extract, with the aim of reducing the patient's sensitivity to that allergen. This treatment was first introduced by Noon in 1911 (23) and has been widely used for many years. Immunotherapy can be used for the treatment of allergies to inhalant allergens, hymenoptera venoms or type I drug hypersensitivity. Because the studies in this thesis are focused on immunotherapy with grasspollen and housedustmite, immunotherapy with other inhalant allergens, hymenoptera venoms or drugs will not be discussed here. Also other forms of therapeutic allergen administration, such as local and oral administration, will not be discussed here.

Treatment with immunotherapy can be considered in patients with elevated specific IgE and/or positive skin tests to one or more inhalant allergens and in which signs

and symptoms are related to allergen exposure. In the past the indications for the treatment with immunotherapy were often confined to patients not responding adequately to drug treatment, or presenting with side effects (24). The disadvantage of using immunotherapy in such a late course of the disease is that the capacity of immunotherapy to change the progression of allergic diseases may be limited because of the tissue damage that may have occurred (25). In such cases immunotherapy does not seem to have a "fair chance". Therefore in the last EAACI Position Paper on Immunotherapy (25) it is suggested that immunotherapy should be seriously considered in the following patients:

1- Patients with rhinitis or asthma caused by allergens for which clinical efficacy and safety of immunotherapy have been documented by placebo-controlled, double blind studies
2- Patients sensitive to allergens which cannot be sufficiently eliminated
3- Patients with a need for daily pharmacotherapy for longer periods (prophylactic drug treatment during a pollen season or perennially).

Immunotherapy should not be commenced without trying to eliminate the allergen whenever technical feasible (26).

Not everyone agrees with the above suggested indications for immunotherapy. Recently Barnes stated that there are only a few indications for the use of immunotherapy in the routine management of asthma in adults and children (27).

3.3.2 Clinical efficacy

Rhinitis

Clinical efficacy of immunotherapy has been shown by double-blind placebo-controlled studies with standardised, and sometimes polymerised allergen extracts in rhinitis caused by grass, ragweed and tree pollen (28-36). In the study of Varney et al (35) immunotherapy with a depot grasspollen extract was effective in patients with severe hay fever, uncontrolled with anti allergic drugs. A 4-year follow-up study of the same group showed that clinical efficacy was maintained throughout the whole study period (37).

In perennial rhinitis due to housedustmite immunotherapy can be effective, although evaluation is complicated by the heterogeneous and multifactorial nature of perennial rhinitis symptoms, in which non-specific factors are frequently involved (38). Recent double-blind placebo-controlled studies have all shown efficacy (39-41).

Asthma

The use of immunotherapy in the treatment of asthma is still controversial. In the past many studies have been performed on the efficacy of immunotherapy in asthmatic patients, but many studies have been open and poorly controlled. In review articles Ohman (42) and Bousquet (43) give an overview of several studies concerning the efficacy of immunotherapy in asthma. Differences in set-up of the study and the large variety in allergen extracts that are used makes comparisons between the different studies difficult . Although a number of double-blind placebo-controlled trials on the efficacy of immunotherapy in grasspollen asthma show beneficial results (44, 45) the benefit in housedustmite asthma is less certain. In order to try to establish the parameters predicting the efficacy of immunotherapy before the start Bousquet et al (46) investigated 215 housedustmite allergic patients with asthma in age ranging from 3 to 72 years. Rush immunotherapy was given to 171 patients and 44 patients served as a control group. Patients who presented another perennial allergy than housedustmite allergy, and/or a sinusitis, and/or aspirin intolerance were not improved after immunotherapy. The group of patients with only housedustmite allergy and no sinusitis or aspirin intolerance showed a significant decrease in mean symptom and medication scores and a significant increase in FEV_1 value after immunotherapy compared to before and compared to the control group. The best improvement was found in younger patients and in patients with less severe asthma and high FEV_1 values. In this group of patients there was a significant relation between the improvement after immunotherapy and age, severity of asthma before treatment and FEV_1 values. In a study of 40 patients with asthma and housedustmite allergy, who were treated with immunotherapy for 12-96 months, Des Roches et al (47) demonstrated that 3 years after cessation of the treatment 45% of the patients did not relapse. The duration of efficacy of immunotherapy after its cessation was dependent upon the duration of the treatment. Creticos et al studied the efficacy of immunotherapy in adult patients with asthma, exacerbated by seasonal ragweed exposure (48). Objective measures of asthma and allergy were improved, however the clinical effects were limited and many were not sustained for two years.

3.3.3 Serological changes

Immunotherapy is often associated with an increase in serum-specific IgG_1 and IgG_4 (32, 36, 49-56). IgG antibodies might exert a blocking effect on IgE-mediated allergic reactions (56). Peng et al (57) demonstrated in ragweed sensitive patients, who were treated with immunotherapy, that IgG_1 is dominant in the early immune response of

immunotherapy and disappears relatively slowly when immunotherapy is stopped. In contrast IgG_4 appears in significant quantities only after prolonged immunotherapy and disappears rapidly when immunotherapy is discontinued.
The clinical implication of changes in IgG_1 and IgG_4 are not clear. Although positive correlations between increased IgG_1(58) or IgG_4 (59, 60) and clinical improvement after immunotherapy have been reported, other studies failed to find significant correlations between the clinical improvement and the rise in IgG antibodies (32, 51-53).
IgE antibodies usually rise initially, and often show a slow decline to pretreatment levels or even lower levels after several years of treatment (52, 55, 57).The rise in IgE antibodies, which usually occurs during seasonal exposure to allergens in sensitive patients, is often blocked by immunotherapy (36, 57, 61, 62). Suppression of IgE antibodies alone can not explain the clinical efficacy of allergen immunotherapy. Several studies have shown that changes in IgE serum level are not correlated with clinical efficacy (32, 53).

3.3.4 Effects on allergic reactions after experimental allergen challenge
Immunotherapy is often associated with a reduction in late phase allergic reactions, suggesting an anti-inflammatory effect. In the *skin* a reduction in late phase reaction has been described by several authors, while effects on early response are more controversial (63-69). The reduction in late phase response is sometimes associated with a clinical improvement (66, 68) or with an increase in allergen specific IgG (64-66).
In the *lower airways* Warner et al (70) first described a reduction in late asthmatic response in children. Several years later this finding was also found by van Bever et al (71-73). In adult patients with asthma a reduction in late phase asthmatic reaction has also been described, although not all studies are conclusive (43, 74).
The effect of immunotherapy on early, late and rechallenge *nasal* reactions after allergen challenge was described by Iliopoulos et al (67). The symptom scores and mediator release during early phase reaction were significantly reduced after immunotherapy compared to placebo. The late phase reaction, measured as an increase in mediators (histamine, TAME esterase and kinins) was significantly reduced, while there was no significant decrease in late phase symptoms. A significant reduction in early and late symptomatic response after allergen challenge in the nose in the immunotherapy treated patients compared to placebo was described by Durham et al (75).

3.3.5 Immunological changes

Tissue changes

A decrease in skin chamber cellular influx after immunotherapy was described by Nish et al (69). Biopsy specimens taken 8 hours after intradermal challenge did not show consistent changes in dermal cellular infiltrate. In a study in 40 grasspollen allergic rhinitis patients, Varney et al (68) found that immunotherapy resulted in a significant decrease in the number of CD3+ and CD4+ cells in skin biopsies taken 24 hours after intradermal challenge compared to the placebo group. In addition a significant increase in the number of CD25+ cells and HLA-DR+ cells in the biopsy specimens were found after immunotherapy compared to placebo.

In the nose immunotherapy is associated with a decrease in eosinophils (76) and metachromatic cells (77) in nasal washings. However in the study of Iliopoulos et al (67) immunotherapy did not change the cellular infiltrate in nasal lavages at several time points after allergen challenge compared to placebo. When performing nasal biopsies 24 hours after allergen challenge, Durham et al (75) reported a significant decrease in infiltrating CD4+ cells and activated eosinophils in the nasal mucosa after immunotherapy compared to placebo.

In both nose and skin immunotherapy is associated with a significant increase in cells expressing mRNA for IFNγ 24 hours after allergen challenge (68, 75) . In the nose the increase in the number of cells expressing mRNA for IFNγ correlated inversely with seasonal symptoms. In the skin also a significant increase in cells expressing mRNA for IL-2 was reported. These data suggest that successful immunotherapy possibly acts through a mechanism involving protective local increases in Th1-type cells (75).

Peripheral blood changes

After immunotherapy a decrease in the proliferative responses of lymphocytes to antigen stimulation have been demonstrated (78). Hiratani et al (79) found a significant lower lymphocyte responsiveness to Dermatophagoides farinae extract in mite-sensitive asthmatic patients receiving long term hyposensitisation than in untreated patients. When comparing children with newly diagnosed asthma with normal individuals and children who were successfully hyposensitized , Hsieh (80) found that lymphocytes from newly diagnosed patients produced a much greater amount of IL-2 when stimulated with allergen than did those of both hyposensitized patients and normal subjects. IL2-receptor secretion of activated lymphocytes in serum was lower in patients treated with immunotherapy compared to untreated patients (81, 82). A decrease in histamine-releasing factor production after

immunotherapy have been described by Kuna et al (83), and in a study of Wang et al immunotherapy resulted in a suppression of the secretion of tumour necrosis factor (TNF) by monocytes stimulated with allergen in-vitro (84).
Rocklin et al (85) demonstrated that mononuclear cells from 10 patients with ragweed hayfever, who were treated with immunotherapy, specifically suppressed a ragweed proliferative response. These suppressor mononuclear cells were not detected in untreated patients and normal control subjects. Several years later Nagaya (86) also described induction of cells capable of suppressing antigen-stimulated lymphocyte proliferation in patients who were treated with immunotherapy. These cells were characterised as CD8+ cells. An enhanced mitogenic response of CD8+ suppressor cells to antigen stimulation after immunotherapy was described by Hsieh (87), while the mitogenic response of CD4+ helper T-lymphocytes was diminished . In a study of 28 housedustmite sensitive children with asthma Bonno et al (88) investigated Dermatophagoides farinae (Df)-specific IL- 2 receptor (CD25+) induction on T-lymphocytes. Only in patients who received antigen-specific immunotherapy, CD25 was induced on CD8+ T lymphocytes. The level of CD8+CD25+ cells correlated significantly with Df-specific IgG_4 and the cumulative doses of the allergen extract. The levels of CD25 induction on CD4+ T lymphocytes in immunotherapy treated patients were similar to the levels in patients who never received immunotherapy.
In a retrospective study Secrist et al (89) demonstrated that treatment of patients suffering from allergic rhinitis with allergen immunotherapy was associated with a significant reduction in allergen-induced IL-4 synthesis of peripheral blood mononuclear cells in vitro compared to matched untreated controls. The reduced IL-4 synthesis was allergen-specific and associated with a clinical improvement and the IL-4 production by T-cells varies inversely with the length of time of immunotherapy. In a follow-up study Secrist et al (90) demonstrated that CD4+ T cells from allergic donors produced high levels of IL-4 when stimulated with low concentrations of allergen, while the same responding CD4+ T cell population produced little IL-4 when stimulated with high concentrations of allergen.
Although the exact mechanism underlying immunotherapy remains unclear, there is evidence to suggest that immunotherapy acts through a switch towards Th1-type lymphocytes. It is also possible that CD8+ suppressor cells are involved in the suppression of the inflammatory changes after immunotherapy.

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Chapter 4

Aim of the study

4.1 Introduction

As was reviewed in the preceding chapters, allergen challenges in lower airways, nose and skin (intradermal challenge) result in an inflammatory cell infiltrate. Cells that act as effector cells, such as eosinophils, basophils and neutrophils, and regulatory cells, such as T-lymphocytes are present in this infiltrate. However it remains uncertain what the significance of this infiltrate is for renewed allergen contact.

From recent literature it seems that immunotherapy induces changes, especially T-cell changes, in allergen-induced cellular infiltrate. However again the significance of these changes for renewed allergen contact remains uncertain?

4.2 The aim of the present study was to investigate the dynamics of the allergic reaction in the airways and skin by using 2 intervention methods: Repeated allergen challenge and systemic allergen immunotherapy. In addition we studied the interrelationship between allergic reactions in airways and skin.

The following research questions are central in this thesis:

1- Dynamics of the allergic reaction:

a) What is the effect of repeated allergen challenge on allergic reactions in the nose and skin in individual patients?
b) What is the effect of immunotherapy on the response pattern to repeated allergen challenge?
c) Does repeated allergen challenge provide information on the regulation of the allergic reaction?

2- Can the allergic skin reaction be used as a model for allergic reactions in the airways in the evaluation of immunotherapy.

Chapter 5

Bronchial challenge tests with inhalant allergens
Technical and methodological aspects.

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Part of this chapter will be published in **Allergy.**

5.1 Introduction.

Inhalation of allergen in allergen-sensitive patients often results in an early asthmatic reaction (EAR). The early reaction starts within minutes after allergen challenge, reaching its maximum after 15-20 minutes and usually resolves after 1-2 hours. The severity of the early reaction is dependent on the level of specific IgE, the degree of bronchial hyperreactivity and the amount of allergen delivered (1, 2). In 50 to 80% of the individuals tested the early response is followed by a late asthmatic response (LAR), usually starting 3-4 hours after allergen challenge and reaching a maximum response after 7-12 hours. In many cases FEV_1 is still depressed 24 hours after challenge.

When comparing groups of patients with single or dual response in the latter group usually a more severe type of disease is found. While the pathology of the early reaction can mainly be ascribed to the effects of mediators, such as histamine, prostaglandins and leucotrienes, the late phase reaction is characterised by a cellular infiltrate of eosinophils, lymphocytes, basophils and sometimes neutrophils (3-7).

In laboratory setting bronchial challenge tests with allergen are often used to induce asthmatic reactions. Although this setting is different from the situation of natural exposure in many aspects (single challenge vs chronic repeated exposure, aerosol inhalation vs inhalation of dry material) , the bronchial challenge test has proved to be a useful tool in research and to certain extent also in the management of allergic asthma.

5.2 Indications

In recent years the indication for bronchial challenge tests in clinical practise has changed. In the past the test was used for the diagnosis of atopic asthma. Currently skin tests and/or specific IgE tests combined with tests for bronchial hyperresponsiveness have largely replaced the bronchial challenge test with allergen. However some indications remain for this test.

In case of discrepancy between the history of the patient and the outcome of skin tests/specific IgE bronchial challenge with allergen can be performed. In house dust mite allergic patients Mosbech et al (8) found large differences in response to house dust mite of skin, nose, conjunctiva and bronchi, so it seems that apart from systemic conditions , local conditions determine the response to allergen. Bronchial challenge tests can also be used to show presence of late phase reactions or to motivate patients to eliminate allergen sources from their environment. In occupational allergy natural exposure to allergens can be used as challenge tests, for example

lungfunction measurements at the workplace and measurement of bronchial hyperresponsiveness before and after working.
For scientific purposes the bronchial challenge test with allergen is indispensable. Both for basic pathophysiological research as for investigations into the specific effects of drugs, bronchial challenge tests have shown to be extremely useful.

5.3 Patients

The technique of performing bronchial provocation test is critical in order to obtain reproducible results and cause minimal discomfort to the patient. Patients should be coöperative and be able to understand some basic facts about their disease. Although bronchial challenge tests with histamine and methacholine have been performed in young children, bronchial provocation tests with allergen have been performed in children from 7 years of age (9, 10). Patients must be stable without asthma medication and have FEV_1 values $\geq$ 70% of predicted value at the start of the test. Anti-inflammatory medication should ideally be stopped 4-6 weeks before the test, this however can not be achieved in the majority of patients attending an asthma clinic. In many studies inhaled steroids are stopped 1-2 weeks before the test; a slight carry over effect of these drugs is to be expected in such cases. Theophylline drugs, oral β_2-adrenergic drugs and antihistamine drugs must be stopped 48 hours before the test (astemizole 6 weeks); inhaled β_2-adrenergic drugs 12 hours (short-acting) or 24 hours (long-acting) before the test. Bronchial challenge tests with allergen should not be done in patients with severe asthmatic attacks in the previous 6 months or respiratory tract infections in the previous 6 weeks.
Severe airway responsiveness to histamine is to our opinion not a contra-indication for bronchial challenge with allergen. In a recent study (11) we have performed allergen challenges in 20 atopic asthmatic patients with a variable degree of histamine-induced airway responsiveness (PC_{20} histamine varying from 0.47 to 32 mg/ml (30 second method). No rescue medication was necessary. In this group of patients we could not find significant (inverse) correlations between the PC_{20} histamine before allergen challenge and the severity of EAR and LAR. We did find a significant inverse correlation between the PC_{20} histamine before allergen challenge (baseline PC_{20} histamine) and the allergen-induced PC_{20} histamine, suggesting that allergen-induced increases in airway responsiveness mainly occur in patients with relatively high baseline PC_{20} histamine. Thus it seems to be safe to perform bronchial challenge with allergen in patients with severe histamine-induced

hyperresponsiveness, when the test is carefully performed and all necessary precautions are adhered to.

5.4 The technique of the bronchial provocation

Introduction:

Bronchial challenges with allergen are preferable performed in the clinical setting, since it is important that the patient is not exposed to allergens during the test and several hours after the test. Also for safety reasons tit is preferable that the patient should be kept in the hospital. Severe late responses can occur 10-12 hours after challenge. Figure 1 shows the effect of bronchial challenge with allergen in 20 atopic patients with asthma, who were monitored until 11 or 12 hours after challenge. Even 24 hours after challenge 6 of the 20 patients had a fall in FEV_1 value ≥ 15% of baseline. When patients go home after the test they should be treated with inhaled corticosteroids and bronchodilators prior to leaving the hospital to avoid severe reactions at home. In this way it is not possible to monitor the entire late response.

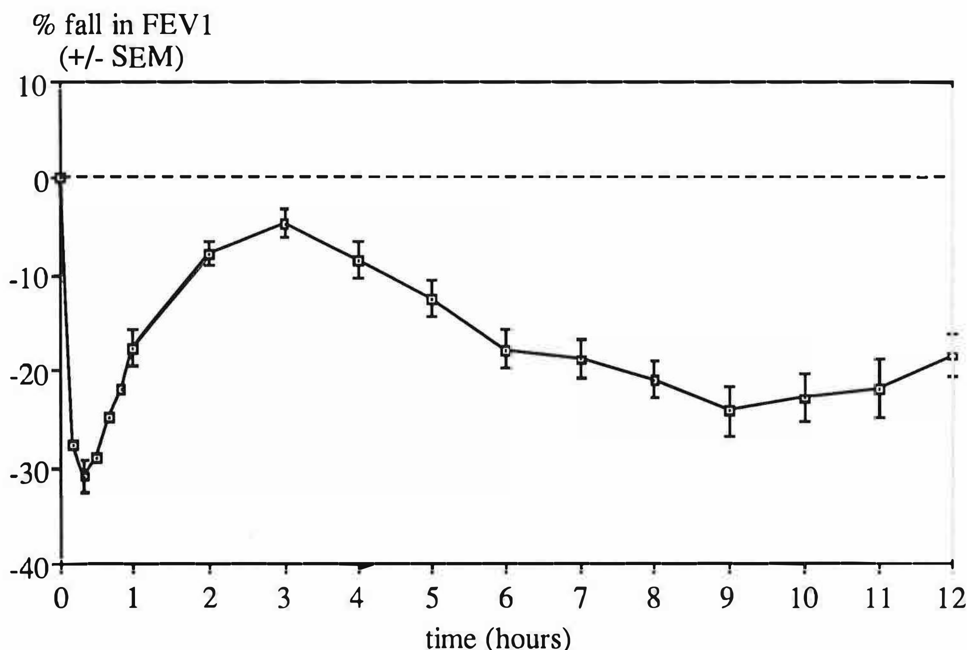

Figure 1: The time course of the early and late asthmatic reaction following allergen challenge in 20 patients, allergic to housedustmite or grasspollen. All patients were mild asthmatics and had baseline FEV_1 values ≥ 70% predicted.

Prior to the allergen challenge a control day is performed on which a phosphate buffer aerosol is inhaled 3-4 times with intervals of 15 minutes. Lungfunction tests are performed at the same moments as during the day on which allergen challenge takes place. The control day is necessary for several reasons:

- At first it is important to estimate individual diurnal variation in lungfunction. Lungfunction parameters, measured on the allergen challenge day, must be corrected for the parameters measured on the same time on the control day. A modest increase in lungfunction during the day is seen in most asthmatic patients, often followed by a gradual decrease in the evening and night. However other individual diurnal patterns in lungfunction are possible. For instance figure 2 shows the effect of diurnal variation in lungfunction in an individual patient. The same pattern was measured at two different occasions. When FEV_1 values on the allergen challenge day were corrected for the FEV_1 values on the control day, the patient did not show a late asthmatic response.
  Arbitrarily allergen challenge should not be performed when diurnal variation is $\geq$ 15% of initial value.
- Another reason why a control day should be performed is to see whether the inhalation of phosphate buffer aerosol results in a fall in FEV_1 value. When there is a fall in FEV_1 value $\geq$ 15% from baseline after inhalation of buffer aerosol no allergen challenge should be performed
- On the control day it is important to check for the presence of spirometer-induced asthma. Before the start of the test minimal 3 measurements of FEV_1 value should be performed with a variation $\leq$ 5%.

Allergen challenge:

1- Test solution.

Preferably a well standardised allergen preparation is used to induce reproducible test results and a minimum of side effects. Allergen should be stored freeze dried at -30°C or in solution at ± 4°C, when stability is guaranteed. The testing material should not contain glycerol. Immediately before the provocation the relevant dilutions are prepared from the stock solution; diluted material should not be stored.

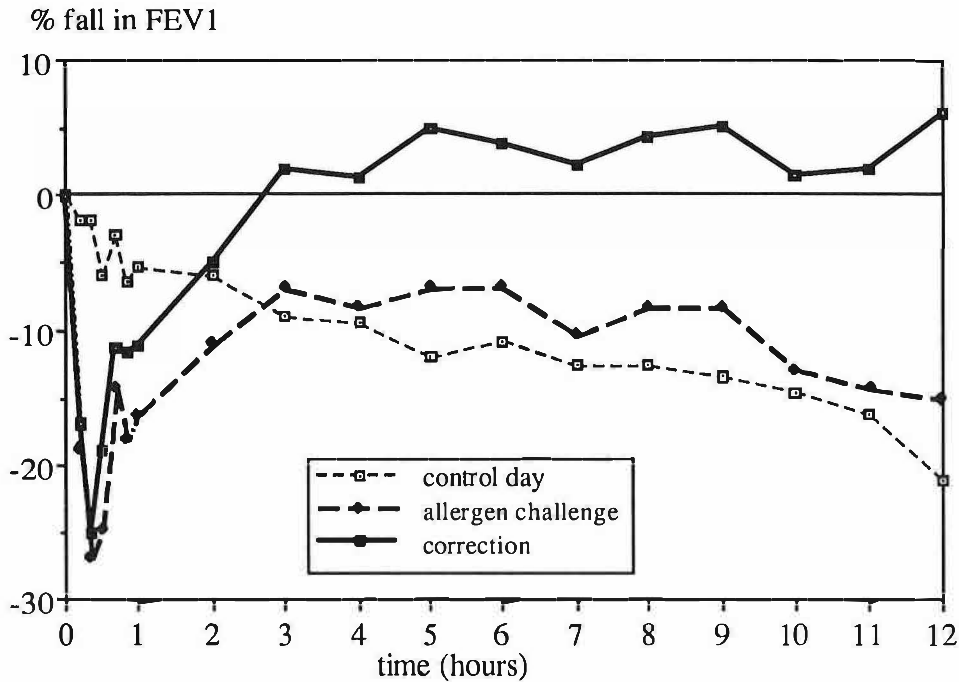

Figure 2: The effect of correction for diurnal variation on the allergen challenge test in a patient who showed a progressive decrease in lungfunction during the control day.

2- Updosing.

a) Starting from a fixed concentration and applying four 5 fold increasing doses. With this method the starting concentration is usually 80 biological units per millilitre (BU/ml). The solution is inhaled during 60 seconds with continuous nebulization during tidal breathing. The dose of allergen is based on a jet nebulizer with an output of 0.13 ml/min. The nebulizer can be mounted to a valvebox with aerosol filter. Lungfuntion is measured immediately after the inhalation and 15 minutes after inhalation. In the 21 atopic asthmatic patients who underwent bronchial challenge with allergen in the previously mentioned study (11), only 6 patients reached their maximal fall in FEV_1 value within 15 minutes; all other patients had further decrease after 20 and 30 minutes. To avoid severe bronchoconstriction it is important to use an interval of at least 20 minutes between the different allergen concentrations when the FEV_1 value tends to decrease after 15-20 minutes. When the fall of FEV_1 has reached 15% from

baseline value no further dose is given. If no response is observed, concentrations of 400, 2000 and 10.000 BU/ml are inhaled for 60 seconds.

b) Based on variable starting concentrations and increasing with doubling dose steps.

The starting concentration is the dosis three steps below a calculated response concentration. This calculation is based on the end point concentration of the pricktest and the PC_{20} histamine value (2-minute method)(12). The formula is:

Log PC_{20} allergen = 0.69 x Log PC_{20} histamine x end point concentration.

All concentrations are inhaled during 2 minutes. The interval between the doubling concentrations is 10 minutes. (To our opinion however an interval of 20 minutes is preferable for the above mentioned reason). After a 15% fall in FEV_1 value from baseline no further dose is given. The highest concentration may consist of 5000 biological units.

Also with this method a jet nebulizer with an output of 0.13 mg/ml is advised.

c) An alternative method is based on a dosimeter delivery.

Since the amount of allergen delivered and deposition in the lungs is different the above mentioned concentrations have to be adapted for dosimeter. When allergen concentrations in the range of 80-10.000 BU/ml are used, 10 inhalations of the lowest concentration usually is a safe starting point. In children lower concentrations may be necessary.

5.5 Quantification and follow up of early and late reaction

Clinical follow-up

a) Follow-up parameters

A useful parameter for the follow-up of the reaction is de FEV_1 value. To get more information about the level bronchoconstriction additional parameters, such as vital capacity (VC) and FEV_1/VC can be taken in account, however these manoeuvres take much effort from the patient. At critical points in the test period, for example when the allergen threshold is reached, it is important to evaluate both the FEV_1 and the VC, in order to check the effort of the patient. In patients with spirometer-induced asthma, or in patients who have technical problems in performing FEV_1 /VC manoeuvres, it is also possible to use the airway resistance as follow up parameter (13, 14). The reproducibility of a simplified oscillometer (Siemens Siregnost FD 5) in comparison to IVC and FEV_1 was tested in healthy subjects and patients with COPD by Gimeno et al. The results of this study indicate that the effort-depending tests,

such as FEV_1, are more reproducible than the effort-independent tests, such as airway resistance (15).

b) Quantification of the response
Several parameters are often used to quantificate the EAR and LAR. A simple method is to calculate the maximum fall in FEV_1 value during the early and late response as compared to the initial value. As mentioned above a correction has to be made for the control day. The disadvantage of this method is that the quantification of the response is based on one single value, which is not always representative for the severity of the reaction. Therefore additional information is offered by calculating the mean fall in FEV_1 value during the response or the area above the FEV_1 curve.
When comparing allergen challenge tests in which different doses of allergen have been used, the PD_{15} or PD_{20} value for allergen can be calculated both for early and late phase reactions. Since the dose response curve for allergen is not completely linear the following formula can be used:

$$PD_{20} = \frac{(0.223 \times \text{dose})}{LN\left(1 - \frac{\Delta FEV1\ max}{100}\right)}$$

ΔFEV_1 max is the maximal difference in FEV_1 between control day and after allergen challenge (corrected for initial challenge) (16).
c) Follow-up period.
We performed a study on the time course of the early and late asthmatic reaction. The aim of this study was to determine the shortest period after allergen challenge during which the FEV_1 value should be measured to get accurate information on both the occurrence and the severity of the late phase reaction. Bronchial challenges with allergen were performed in 20 atopic patients, allergic to housedustmite or grasspollen. All patients had shown a late phase bronchial reaction after allergen challenge. FEV_1 values were followed until 12 hours after the last inhalation on the control day and on the allergen challenge day. All FEV_1 values on the allergen challenge day were corrected for diurnal variation.
When the registration period of the FEV_1 value was shortened from 12 to 10, 8 or 6 hours after challenge, the number of patients with a documented late phase reaction was 20, 18 and 12. In order to study the magnitude of the late phase reaction, the mean fall in FEV_1 value (% fall of post PBS baseline) was measured during 3-6, 3-8, 3-10 and 3-12 hours after challenge (period I, II, III, III and IV respectively). The mean fall in FEV_1 value measured in the period II was significant higher compared with

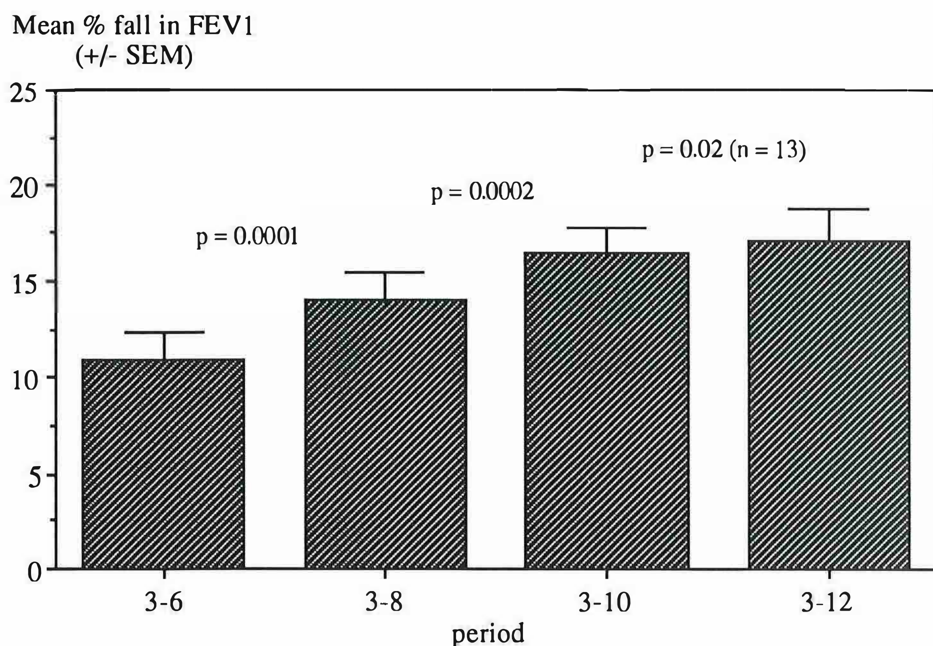

Figure 3: The mean fall in FEV_1 value measured in different periods after allergen challenge in the same groups of patients as in Figure 1.

period I (p = 0.0001). This was also true comparing period III with period II (p = 0.0002) and period III with period IV (p = 0.02, n = 13) (Figure 3). Therefore we recommend the follow-up of the late allergic reaction of minimal 10, and preferable 12 hours.

In vitro follow-up

In recent years an increased emphasis has been put on in vitro correlates of bronchial challenge tests. These in vitro correlates can consist of serum parameters such histamine and tryptase for the early response and eosinophil numbers, Eosinophil Cationic Protein (ECP), Eosinophil Chemotactic Activity (ECA), and Neutrophil Chemotactic Activity (NCA) for the late response (7, 17 , 18-21). Detection of cytokines in peripheral blood after allergen challenge is still cumbersome because of very low concentrations. Urine samples may show an increased level of urinary methyl histamine following the early reaction and leucotriene D_4 (LTD_4) during the late phase reaction (22). Both urinary parameters however lack in sensitivity. Broncho-alveolar lavage and mucosal biopsies have shown to be an important

additional tool to investigate the changes in the bronchi after bronchial challenge (3, 23). Detection of cytokines in bronchoalveolar lavage fluid and the use of in-situ hybridisation in bronchial biopsies have made it possible to study immunological mechanism that are relevant in the expression of the inflammatory response (24-26). Although bronchoscopy and bronchoalveolar lavage can cause side effects, such as alveolar infiltration, wheezing and bronchospasm (27), many studies have been carried out in stable asthmatics before and after bronchoprovocation with allergens or occupational agents without major complications. However special care and careful monitoring is advised in asthmatic patients with marked bronchial hyperresponsiveness (28).

5.6 Safety aspects

Bronchial provocation tests with allergen should preferably be performed in a clinical setting, since severe late reactions can occur in the evening and during the night. When challenge tests are performed in asthmatic patients, their asthma must be stable without maintenance asthma medication and patients must have FEV_1 values $\geq 70\%$ predicted value. In our experience patients with outspoken histamine-induced hyperresponsiveness are not at risk to develop severe reactions provided the test protocol in rigidly adhered to. To avoid serious side effects a well standardised extract should be used. Challenge tests should be performed by well trained staff and always under supervision from a medical doctor, who has experience in the treatment of anaphylactic reactions and severe obstructive reactions; it is important to have rescue medication for the treatment these reactions at hand.

In conclusion, although bronchial challenge test with allergen does not closely resembles the situation of natural allergen exposure, it is a useful test for scientific purposes and for some clinical indications. When all precautions are adhered to the test is safe to perform. Additional information can be obtained by following spirometry in combination with immunological and biochemical parameters measured in blood, urine, bronchoalveolar lavage fluid or bronchial biopsies.

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Chapter 6

Early and late allergic reaction in the nose assessed by whole body plethysmography

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6.1 Abstract
Physiological changes during late phase nasal responses after allergen challenge are difficult to establish and different criteria are used for the definition of a positive late phase nasal reaction. The objective of our study was to assess the value of whole body plethysmography in detecting changes in nasal airway resistance after allergen challenge and to suggest criteria for the definition of early and late phase nasal reactions.
Nasal challenge with allergen was performed in 15 allergic patients. Nasal resistance was followed until 10 hours after allergen challenge and on a control day using whole body plethysmography.
The mean percentage changes in the inspiratory nasal resistance during the early phase period (0.25-2 hours) and the late phase period (4-10 hours) were significantly higher on the allergen challenge day than on the control day (p=0.001; p=0.01 respectively). The mean percentage change in the inspiratory nasal resistance during the early and late phase period on the control day plus 2 times the standard deviation served as cut-off point for a positive reaction. Using this definition all patients had early reactions and 7 of 15 patients (47%) also had late reactions.
We conclude that whole body plethysmography is a useful, non-invasive method for the measurement of the physiological changes in the nose following allergen challenge.

6.2 Introduction
Allergen challenge in the nose in allergic patients often results in an early reaction . This reaction can be detected by various clinical methods, such as evaluation of symptom scores, rhinomanometry and peak inspiratory nasal flow (1-4). The early reaction can also be detected by the influx of inflammatory cells in nasal secretions (5, 6) or by an increased level of mediators, such as histamine, prostaglandin D_2, p-toluenesulfonyl-L-arginine methylester (TAME) esterase activity and kinins in nasal lavage fluid (7, 8).
Nasal late phase reactions have been reported in 3% to 75% of the allergen-challenged allergic patients (8, 9, 10-13). In contrast to the late phase bronchial reaction, no clear criteria have been developed for the definition of the late nasal response. Although many authors have described histological and biochemical changes during nasal late responses (8, 14, 15), the follow-up of the nasal late response using physical methods appeared to be very difficult. In contrast to the changes during the nasal early response, the changes in nasal obstruction during

nasal late response are rather subtle. The influence of various factors, such as nasal cycle, posture and unphysiological stimulation by nasal instruments, can disturb the registration of the nasal late response (16, 17).

This is the first study in which the early and late reactions after nasal allergen challenge were monitored with whole body plethysmography. The aim of the study was to assess the value of whole body plethysmography in detecting changes in nasal resistance after allergen challenge. Furthermore criteria for the definition of early and late phase nasal reactions are suggested and discussed.

6.3 Patients and methods

Patients

Fifteen atopic, non-smoking patients with rhinitis (5 male, 10 female, age 21-55 years) participated in the study. Twelve of the 15 patients also had asthma; 10 patients regularly used inhaled steroids. The clinical data are presented in Table 1. Three patients had positive skin tests (≥ ++) and/or elevated specific IgE (Pharmacia Cap > 0.7 PRU/ml) to house dust mite (HDM), 12 to grasspollen (GP). The pollen allergic patients were tested outside the pollen season. None of the patients gave a history of respiratory tract infections in the 6 weeks prior to the study. All patients gave informed consent. The study was approved by the medical ethics committee of Asthmacenter Heideheuvel, The Netherlands.

Nasal challenge

Allergen solutions were prepared from stock solutions of *Dermatophagoides pteronyssinus* and mixed grasspollen, diluted in PBS with 0.03% human serum albumine and containing 0.0125% benzalkonium chloride (SQ 503 resp. SQ 293, ALK Benelux), to produce a range of tenfold increasing concentrations from 100 to 10.000 BU/ml. The allergen solutions were administered with a pump spray delivering a volume of 0.102 ml (ALK Benelux). Both nostrils were challenged. Before allergen challenge, patients waited 15 minutes to allow the nasal mucosa to become acclimatised. Increasing doses of allergen were given at 15 minutes intervals; no further dose was given when the nasal resistance, measured with whole body plethysmography (see below), had doubled. After the final dose nasal resistance was measured 15 minutes and thereafter every hour until 10 hours after challenge. On a control day nasal resistance was recorded until 10 hours after the inhalation of a control solution, a phosphate buffer solution with 0.03% human serum albumine and containing 0.0125% benzalkonium chloride.

Table 1: Clinical data of the patients

patient	age	sex	allergy	asthma	smokers	medication rhinitis*	medication asthma**
1	30	M	HDM	+	-	S	800
2	27	F	HDM	+	-	-	400
3	25	F	HDM	+	-	S	-
4	36	F	GP	-	-	-	-
5	23	M	GP	-	-	-	-
6	21	F	GP	+	-	S, A	800
7	34	F	GP	+	-	-	100
8	23	M	GP	+	-	-	1600
9	23	F	GP	+	-	-	800
10	21	F	GP	+	-	S,A	1000
11	42	F	GP	+	-	S,A	200
12	55	M	GP	-	-	A	-
13	34	F	GP	+	-	-	1000
14	23	M	GP	+	-	S	400
15	34	F	GP	+	-	-	-

* Medication used for rhinitis; **dose of inhaled steroids
S: nasal steroids. A: antihistamines. HDM: housedustmite, GP: grasspollen

On the allergen challenge day and on the control day the mean percentage changes in inspiratory nasal airway resistance compared to before, in the period 0.25-2 hours and in the period 4-10 hours were calculated in order to characterise early and late allergic reaction.

Whole body plethysmography

Whole body plethysmography is often used to determine the airway resistance in routine practice (18). We used a volume constant body plethysmograph (Jaeger, Wuerzburg Germany). The technique in short: In this procedure airway resistance (Raw) is measured with open airways, and is defined as the quotient of mean alveolar pressure (Pa) and the corresponding gas flow (V') so that Raw=Pa/V'. The gas flow (V') is measured at the mouth with a pneumotachometer. The conversion factor for the determination of alveolar pressure from box pressure fluctuations follows from measurements of mouth pressure and box pressure changes during panting through

the mouth against a closed shutter. Mouth pressure in conditions of no flow is than considered equal to alveolar pressure.
For the measurement of nasal resistance with whole body plethysmography the mouthpiece, connected with the pneumotachometer, was replaced by a solid nasal mask (nasal CPAP mask). The mask was supported by both hands of the patients, keeping the mask fixed on the face of the patients without leakage of air, and preventing the patients to blow up the cheeks. The patients were instructed to breath quietly through the nose, keeping the mouth closed. Nasal flow through both nostrils was recorded. When 5 reproducible measurements of nasal flow were recorded, Pa was determined by the manoeuvre described above. Nasal resistance (Rn) in both nostrils was then calculated from the nasal flow and the Pa according to the formula Rn=Pa/V'n.
In contrast to the pattern of the Pa-V' curve during measurement of airway resistance, the S-shape of the Paw-V' diagram of the nose is most pronounced in the inspiratory phase: During inspiration the patency of the nose is less than during expiration and more sensitive for detection of nasal obstruction due to allergen challenge. Therefore we used the inspiratory nasal resistance ($Rn_{(insp)}$)for the monitoring of the allergic reaction. The initial value of $Rn_{(insp)}$ on the control day and on the allergen challenge day was considered as 0%. The other values of
$Rn_{(insp)}$ were expressed as percentage changes compared to the initial value.

Rhinomanometry
Active anterior rhinomanometry was performed with a rhinomanometer, developed by Bachmann (Nasaltest WB, NT-Vertrieb, Mannheim Germany). Nasal flow (cm^3/s) measured at a pressure of 150 Pa is recorded. Mean flow of both nostrils was used for the follow-up of the allergic reaction.

Peak nasal inspiratory flow
Peak nasal inspiratory flow was measured with the Peak Nasal Inspiratory Flow Meter (Youlten, Clement Clarke; London UK). From 3 measurements the highest value was recorded.

Study design
Subjects visited the hospital for 2 separate days, with at least 3 weeks interval. Medication for the treatment of allergic rhinitis was stopped before the tests: Nasal steroids and cromoglicate were stopped 1 week before the tests; antihistamines were

stopped 48 hours before the tests. None of the patients received oral corticosteroids or immunotherapy. Inhalation medication for the treatment of asthma was continued.
During the first study day nasal resistance was registered 15 minutes after the inhalation of control solution and after that every hour until 10 hours after the inhalation.
On the second day allergen challenge was performed. In all 15 patients nasal resistance was measured 15 minutes after challenge and then every hour until 10 hours after challenge. Measurements were performed on the same time of the day as on the control day. In 11 patients resistance in the lower airways was also recorded. In 10 patients nasal flow and peak nasal inspiratory flow were also measured.

Data analysis
The mean percentage change in inspiratory nasal resistance during early and late reaction was compared with the mean percentage change in inspiratory nasal resistance during the same period on the control day, using the Wilcoxon test. Spearman's rank correlation was used for correlation studies. P values less than 0.05 were considered significant.

6.4 Results

Whole body plethysmography, definition of early and late responses
Figure 1 shows the follow-up of inspiratory nasal resistance ($Rn_{(insp)}$) of 15 patients, measured with whole body plethysmography, after allergen challenge and during the control day. The initial value of $Rn_{(insp)}$ on control day (0.63 kPa*s/l) and on allergen challenge day (0.61 kPa*l/s) were defined as 0%. The mean percentage change in $Rn_{(insp)}$ +/- SD in the period 0.25-2 hours after allergen challenge (early reaction) was 165+/- 85 %; in the period 4-10 hours after challenge (late reaction) 46 +/- 50% (Figure 2).
On the control day the $Rn_{(insp)}$ showed minor variation; no effect of challenge with control solution on $Rn_{(insp)}$ was found. The mean percentage change in $Rn_{(insp)}$ +/- SD in the period 0.25-2 hours was -6+/- 14%; in the period 4-10 hours 1 +/- 14%. When the mean percentage change in $Rn_{(insp)}$ in the period 0.25-2 hours on the challenge day was compared with the mean percentage change in $Rn_{(insp)}$ in the same period on the control day a significant difference was found (p=0.0007). This was also true, although to a lesser extent, during the late phase response (4-10 hours) (p=0.007).

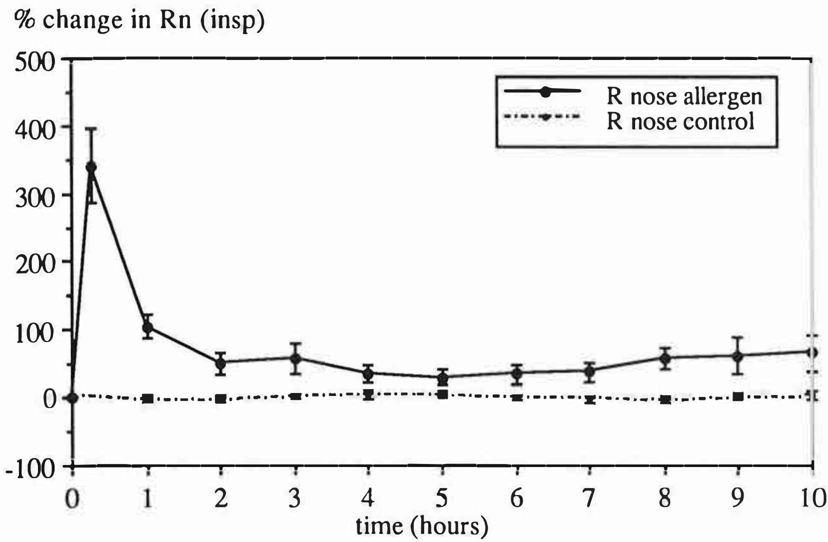

Figure 1: Follow-up of inspiratory nasal resistance after allergen challenge and on control day in 15 patients (+/- SEM).

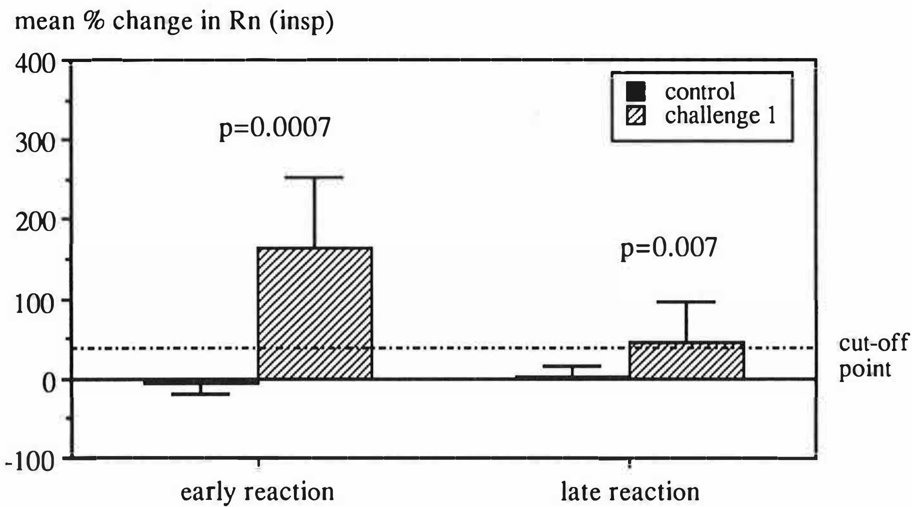

Figure 2: Mean percentage change in inspiratory nasal resistance during early and late phase period on allergen challenge day and on control day in 15 patients (+/- SD)

In order to define the cut off point for early and late nasal responses the mean percentage changes in $Rn_{(insp)}$ on the control day in the above mentioned periods plus 2 times the standard deviation were calculated (early phase 23%; late phase 29%). For practical purposes an early reaction was defined as a mean percentage increase in $Rn_{(insp)}$ >30 % in the period 0.25-2 hours after challenge, when the $Rn_{(insp)}$ before challenge was defined as 0% ; a late reaction was defined as a mean percentage increase in $Rn_{(insp)}$ > 30 % in the period 4-10 hours after challenge. When the above mentioned criteria were used, all 15 patients had early reactions, while 7 patients also showed late responses (Table 2). We also calculated the number of late responders based on the maximal percentage change in $Rn_{(insp)}$ in the period 4-10 hours after challenge. On the control day the mean percentage change in nasal resistance for each individual time point during the late phase period (4-10 hours) plus 2 times the standard deviation in 15 patients was calculated. We then found a cut-off point of 43%. Ten of the 15 patients had a maximal increase in nasal resistance > 43% during the late phase period (Table 2).
Figure 3 shows the follow-up of airway resistance in the nose and in the lower airways after nasal allergen challenge. No effect of nasal challenge was seen on the airway resistance in the lower airways (inhalation medication was continued).
In order to study whether the use of inhaled steroids in the asthmatic patients could have influenced the intensity of the allergic reaction in the nose, we studied the relation between the dose of inhaled steroids and the intensity of early and late allergic reaction, calculated as mean percentage increase in $Rn_{(insp)}$, in the nose. However we could not find a significant (inverse) correlation (early reaction: r=0.05, p=0.92. Late reaction: r= -0.20, p=0.39).

Comparison with rhinomanometry and peak nasal inspiratory flow
In 10 patients the nasal reaction to allergen challenge was registered using 3 methods: whole body plethysmography ($Rn_{(insp)}$), active anterior rhinomanometry (mean flow) and peak nasal inspiratory flow (peak flow). Figure 4 shows the results. The early reaction was registered with all 3 methods. When the nasal early response was defined as the percentage change in respectively nasal resistance, flow and peak flow in the period 0.25-2 hours after challenge, a significant correlation was found between the intensity of nasal early response measured with whole body plethysmography and active anterior rhinomanometry (r= -0.68; p=0.04). This was not true when whole body plethysmography was compared with peak nasal inspiratory flow (r= -0.08; p=0.81).

Table 2: Changes in $Rn_{(insp)}$ during early and late phase period on control day and on allergen challenge day.

	Control day		Allergen challenge day			
	Mean % change		mean % change		max % change	
Pt No	Rn (insp) ER	Rn (insp) LR	Rn (insp) ER	Rn (insp) LR	Rn (insp) ER	Rn (insp) LR
1	-26	-9	283	57*	531	157#
2	-8	5	72	10	106	19
3	3	23	110	180*	195	424#
4	-24	-25	209	17	454	39
5	-5	0	86	1	227	20
6	-11	12	44	23	120	61#
7	0	7	116	47*	160	88#
8	15	18	119	17	323	68#
9	0	-1	181	103*	233	176#
10	-33	-4	215	70*	410	110#
11	2	-7	206	71*	440	169#
12	0	5	378	78*	900	321#
13	18	12	183	-2	504	38
14	-1	-2	100	-16	251	2
15	-14	-26	175	28	256	54#
mean	-6	1	165	46	341	116
SD	14	14	88	50	207	119

*: late responder, defined as mean increase in $Rn_{(insp)}$(4-10h) > 30%.
#: late responder, defined as maximal increase in $Rn_{(insp)}$(4-10h) at an individual time-point >43%. Pt: patient; $Rn_{(insp)}$: Inspiratory nasal resistance

In the period 4-10 hours after challenge a slight increase in nasal resistance was seen from 7 hours after challenge. This was not paralleled by a decrease in flow of peak flow (Figure 4). When the nasal late response was defined as the percentage change in respectively resistance, flow and peak flow in the period 4-10 hours after challenge no significant correlation was found between the intensity of the nasal late response measured with whole body plethysmography and active anterior rhinomanometry (r= -0.58; p=0.08) or between whole body plethysmography and peak nasal inspiratory flow (r= -0.26; p=0.44).

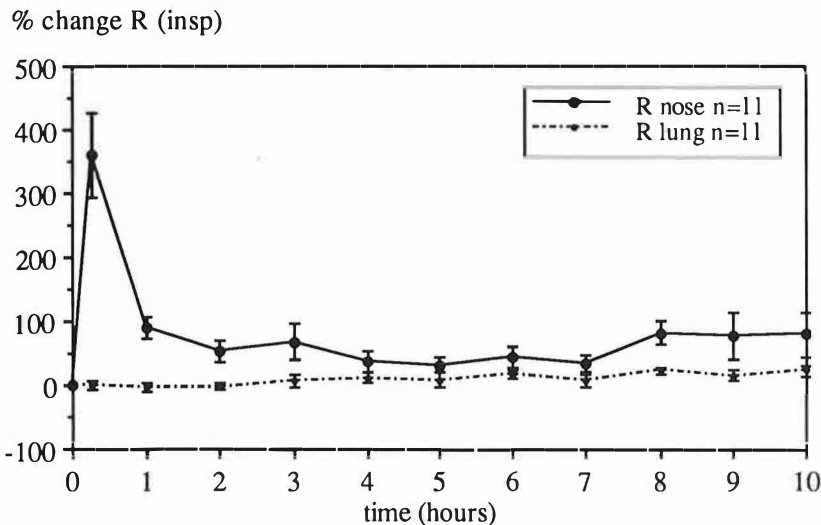

Figure 3: Follow-up of inspiratory airway resistance measured in the nose and in the lower airways after allergen challenge in the nose in 11 patients (+/- SEM).

6.5 Discussion

In contrast to the lower airways, physiological changes during late nasal responses after allergen challenge are difficult to establish. Furthermore different criteria are used to define late responses in the nose. In order to detect minor physiological changes in the nose during the late phase period we used whole body plethysmography, a very sensitive method for the measurement of airway resistance. This method has been used for the follow-up of nasal surgery (19, 20), but no other data are available about its use for the follow-up of the allergic reaction in the nose. This technique has the advantage that no mechanical irritation or deformation of the upper airways occurs. Both nostrils are considered as a physiological unit.

On the control day the variation in nasal resistance measured with whole body plethysmography was small, indicating no interference of disturbing factors. The stable control day and the minor variations in values after repeated measurements at the same time point indicate a good reproducibility of the method.

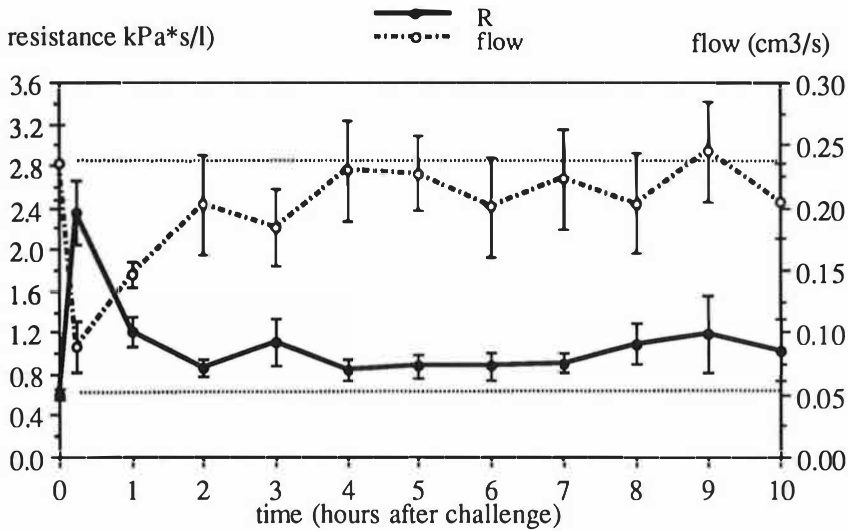

Figure 4a: Follow-up of early and late allergic reaction in the nose using whole body plethysmography and rhinomanometry in 10 patients (+/- SEM).

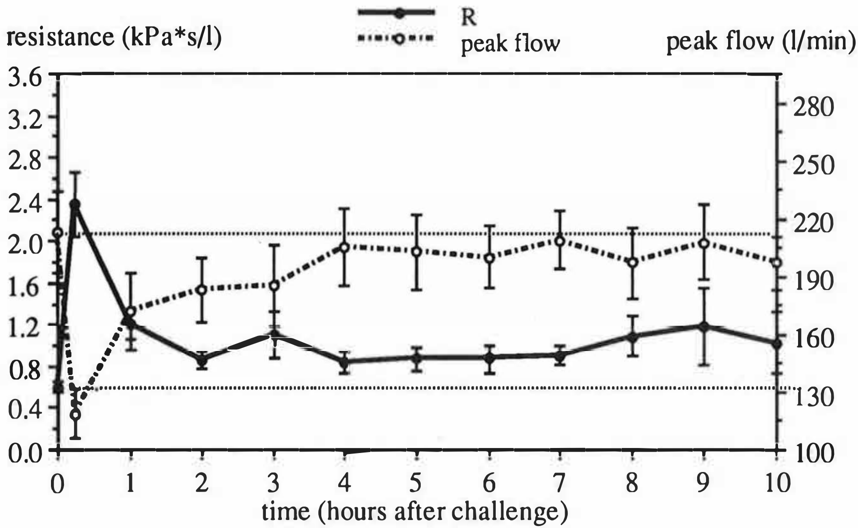

Figure 4b: Follow-up of early and late allergic reaction in the nose using whole body plethysmography and inspiratory nasal peak flow in 10 patients (+/-SEM).

On the allergen challenge day the large changes in nasal patency during the early phase period were detected by whole body plethysmography, active anterior rhinomanometry and peak nasal inspiratory flow. The severity of the nasal early response measured with whole body plethysmography showed only a positive correlation with the severity measured with active anterior rhinomanometry. During the quiet period between 4 and 7 hours after challenge the standard error of the mean of the $Rn_{(insp)}$ was relatively small compared to the flow and inspiratory peak flow. During the late phase period the changes in nasal resistance were relatively small compared to the changes measured during the early phase period, but a significant difference was found between the allergen challenge day and the control day.
The order of challenge (control challenge and allergen challenge) was not randomised in this study, because when an allergen challenge is followed by a control challenge the interval between the 2 tests should be more than 3 weeks to avoid interference of the allergen challenge on the control challenge. This large interval has the disadvantage that differences in test conditions, especially patients conditions, could occur between the two test days. To exclude a learning effect all patients must be able to produce reproducible values using whole body plethysmography before the tests were started.
In order to define individual early and late responders, the mean percentage changes in $Rn_{(insp)}$ in the period 0.25-2 hours respectively 4-10 hours on the control day were calculated and these value were enlarged by 2 times the standard deviation. When the mean percentage increase in $Rn_{(insp)}$ on the allergen challenge day during the early or late phase period was above this value (30%), patients were defined as early, respectively late responders. Using this definition all patients were classified as early responders and 7 of the 15 patients (47%) as late responders.
The number of late responders largely depend on the definition of the late response. In the literature the maximal percentage change during early and late phase period in often used to define a response. In order to define the number of late responders in the present study based on the maximal percentage increase in nasal resistance during late response (4-10 hours), we calculated for each individual time point the mean percentage change in nasal resistance of the 15 patients on the control day plus 2 times the standard deviation, and found a change of 43% as cut off point. After allergen challenge 10 of the 15 patients had a maximal increase in nasal resistance > 43% during the late reaction and should be classified as late responders (Table 2). Although this definition appears to be mathematically right, we prefer a definition of

the late response based on mean percentage change, because this method is less influenced by outliers and better reflects the intensity of the entire response.

Late phase nasal responses after allergen challenge have been reported in 3% to 75 % of the allergic patients. This variation in reported late reactions largely depends on differences in investigation techniques and different positivity cut-offs (21). Based on symptom scores and mediator release Naclerio et al (13) reported 75% late responders. Pastorelli et al (21) reported a comparable number of late responders as in our study (47%) , using rhinomanometry for the follow-up of the allergic reaction. However when the definition of a late reaction in the same study was based on symptom scores or on a significant influx of inflammatory cells, the percentage of late responders was respectively 13% and 100%. In the study of Iliopoulos et al (8) the number of patients with an increased number of inflammatory cells (especially eosinophils) in nasal secretions during the late phase period (76%) was larger than the number of patients who were classified as late responders based on mediator release (47%) or symptom scores (45%). In general the reported number of nasal late reactions, based on inflammatory changes in the nasal secretions, is higher than the number of late reactions based on physiological methods. It seems that an influx of inflammatory cells in the nose during the late phase period is common in all patients, but these changes are not always detectable with physiological methods.

In the present study we report on a sensitive, non-invasive method for the follow-up of nasal resistance after allergen challenge. Furthermore we defined criteria for a positive nasal early reaction and nasal late reaction. Although most of the knowledge concerning nasal early reactions and nasal late reaction is based on cytological and biochemical changes, the detection of physiological changes after allergen challenge may still be relevant. Physiological parameters are usually more related to clinical symptoms and because of the non-invasive character of the detection technique, repeated measurements are possible for longitudinal follow-up of the allergic reaction. We conclude that the presented method is a useful, non-invasive method for the measurement of physiological changes in the nose following allergen challenge.

6.6 Acknowledgements

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Chapter 7

Lack of effect of cetirizine on early and late asthmatic response after allergen challenge.

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7.1 Abstract

Background: Cetirizine hydrochloride has proved to be effective in reducing allergic symptoms and can inhibit the infiltration of eosinophils in allergic late-phase responses in the skin. Because eosinophils are likely to play an important role in allergic late phase reactions, we studied the effect of Cetirizine on early and late asthmatic reactions and on eosinophil cationic protein (ECP) bloodlevel after allergen challenge.

Methods: The effect of 15 mg Cetirizine given twice daily, was studied in 16 patients allergic to housedustmite in a double-blind, placebo controlled study. Patients were treated for three weeks. Before and after treatment bronchial challenges with housedustmite were performed. Blood ECP levels were measured six hours after challenge. Methacholine provocation was performed 72 hours before and 24 hours after each challenge.

Results: Early and late asthmatic response, measured as mean maximal fall in forced expiratory volume in one second (FEV_1) and the provocative dose of allergen which causes a 15% fall in FEV_1 (PD_{15} allergen), were not significantly reduced after treatment with Cetirizine compared with placebo. There was also no significant effect on the concentration of methacholine, which causes 20% fall in FEV_1(PC_{20} methacholine). The increase in ECP bloodlevel after allergen challenge was reduced after Cetirizine treatment compared with placebo, but this difference failed to reach statistical significance.

Conclusion: 18 days of treatment with Cetirizine did not significantly reduce the intensity of the early and late asthmatic response.

7.2 Introduction

Cetirizine, the principal metabolite of Hydroxyzine, is a member of a new therapeutic class of H_1 antagonists, which have little or no adverse CNS effects. This can be attributed to their selectivity for the H_1 receptor and the limited capacity of these agents to cross the blood-brain barrier (1-4).

Clinical trials indicate that Cetirizine effectively reduces symptoms in patients suffering from allergic rhinitis (5-7) and chronic urticaria (8, 9). In asthmatic patients Cetirizine effectively protects against bronchial histamine challenge (10, 11) and may be effective in severe birch pollen asthma (12). Dijkman et al (13) found a significant reduction of asthma symptoms and escape medication and increase in morning peak

flows in patients with graspollen-induced asthma using Cetirizine during the graspollen season.
An interesting property of Cetirizine is its ability to inhibit the infiltration of eosinophils in allergic skin response. Several studies have demonstrated that Cetirizine can inhibit eosinophil migration by 60% - 84% in allergic late phase responses (14-17). Eosinophils are likely to play an important role in the late phase allergic reaction and induction of bronchial hyperreactivity (18-21). They contain substances such as major basic protein and eosinophil cationic protein (ECP), which may cause respiratory epithelial cell damage, characteristic of asthma (22). Bronchoalveolar lavage after allergen challenge demonstrates accumulation of eosinophils and elevated ECP levels in patients showing late phase asthmatic reactions (20).
The aim of our study was to investigate if an 18 days treatment with Cetirizine could protect against allergen-induced early and late phase bronchial reactions in atopic patients with asthma. We also examined the effect of Cetirizine on eosinophil activation after allergen challenge.

7.3 Patients and methods

Patients

Sixteen atopic, non smoking patients with mild to moderate asthma (ten female and six male, age 17 -31 years) participated in the study. The clinical data are presented in Table 1. The patients were selected on the basis of positive skin tests and/or elevated IgE for housedustmite (HDM) allergen and increased bronchial responsiveness to methacholine inhalation ($PC_{20} < 16$ mg/ml, 30' method). All patients had FEV_1 values ≥ 70 % of the predicted values (Table 1) and had shown a late phase bronchial reaction after HDM challenge. A late phase reaction was defined as a fall in the FEV_1 value of ≥ 15 % from the baseline value. Pollen allergic patients were tested outside the pollen season. None of the patients gave a history of respiratory tract infections in the previous six weeks or severe asthmatic attacks in the previous six months. None had received oral corticosteroids. All the patients gave informed consent. The study was approved by the medical ethics committee of Asthmacenter Heideheuvel, the Netherlands.

Table 1: Clinical data of the patients

Patient	Age	Sex	FEV_1 (l)	FEV_1 % pred.	PC_{20} meth mg/ml	Medication
Placebo						
1	24	F	2.96	87.40	0.52	A,B,C,S
2	17	F	3.56	86.10	0.63	B,S
3	26	F	2.32	70.40	0.43	B,C,S,T
4	20	F	3.19	84.20	0.85	C,S
5	19	M	4.30	76.10	2.42	B,S
6	22	F	3.09	98.80	0.93	A,B,C,S
7	24	M	4.05	99.30	0.83	B,C,S
8	20	M	3.99	100.00	1.66	B
Mean	21.5		3.43	87.80	1.03	
Cetirizine						
1	29	F	3.23	98.00	0.47	A,B,C,S
2	21	F	2.57	82.20	7.59	A,C
3	23	F	2.56	65.50	0.29	A,B,C,S
4	19	F	3.27	80.50	0.83	A,S
5	31	M	3.17	78.30	0.41	B,S
6	21	F	3.14	109.30	1.37	B
7	21	M	4.40	108.70	12.08	B,S
8	23	M	3.95	98.50	14.36	C,S
Mean	23.5		3.29	90.10	4.68	

A, Antihistamine; B, Beclomethasone or Budesonide; C, Cromoglycate; S, Salbutamol; T, Theophylline

Methacholine provocation

Methacholine chloride was prepared in 0,9 % sodium chloride solution to produce a range of doubling concentrations from 0,25 to 32 mg/ml. The solutions were administered through a De Vilbiss 646 nebulizer with a gauged output of 0,13 mg/ml. The nebulizer was mounted to a valvebox with aerosol filter. The nebulation time was 30 seconds, during which the patient was instructed to breath quietly. The test was started with inhalation of a phosphate buffer aerosol. Prior to the inhalation three measurements of VC and FEV_1 were performed using the portable electronic Printer Spirometer II (Micro Medical Ltd, Rochester, Kent England): Calibration of the spirometer was performed every two weeks. No adjustments were necessary. After

each concentration the FEV_1 value was measured. The PC_{20} methacholine was derived by linear interpolation.
Two PC_{20} methacholine ratio's were used to study the treatment effect: The PC_{20} before bronchial challenge after treatment/PC_{20} before challenge before treatment (ratio 1) and the PC_{20} after bronchial challenge after treatment/PC_{20} after challenge before treatment (ratio 2).

HDM inhalation

Allergen solutions were prepared from stock solutions of Dermatophagoides pteronyssinus, diluted in PBS with 0.03 % human serum albumin and containing 0.5 % phenol (Diephuis Laboratories, Groningen, The Netherlands) to produce a range of fivefold increasing concentrations from 80 to 10.000 BU/ml. The allergen solutions were administered through a De Vilbiss nebulizer mounted to a valvebox with aerosolfilter (output 0.13 mg/ml, see methacholine provocation). The patients were instructed to breath quietly during one minute for each dose. Increasing doses were given at 15 minute intervals. The challenge procedure was terminated when the FEV_1 value fell ≥ 15% below baseline value. After the last inhalation FEV_1 was recorded at 10-minute intervals for the first hour and hourly thereafter until 12 hours after the last inhalation.
The early asthmatic response (EAR) was characterised by the maximum percentage decrease in FEV_1 from initial value during the first hour after allergen challenge; for the late asthmatic response (LAR) the maximum percentage decrease from initial value in the period 3-12 hours after challenge was calculated. Because it is possible that the dose of allergen necessary to induce a fall in FEV_1 value of ≥15% is different in the first and second allergen challenge test, the provocation cumulated dose of allergen necessary to induce a fall in FEV_1 value of 15 % from baseline was also calculated to characterize EAR and LAR (24).

ECP

Serum samples were stored at -20 °C. ECP was assessed by Dr P. Venge using a radioimmunoassay method, which has been described previously (23).

Study design

The study was performed in a randomized placebo-controlled, double blind, parallel group design with two groups of eight patients, comparing Cetirizine, 15 mg twice daily during 18 days, with matching placebo. Patients were stratified according to

sensitivity to allergen in the initial bronchoprovocation into a group of low-dose responders, reacting to 80 or 400 Bu/ml, and a group of high-dose responders, reacting to 2000 or 10.000 Bu/ml.

The study consisted of three visits: A selection visit (V1), a visit for the HDM provocation before treatment (V2) and a visit for the HDM provocation after treatment (V3). Medication was withheld before the study period: Inhaled steroids and sodium cromoglycate were stopped one week before the study (= 4 weeks before the allergen challenge after treatment); theophylline drugs, oral β_2- adrenergic drugs and antihistamine drugs were stopped 48 hours before the study; inhaled β_2-adrenergic drugs were stopped six hours before the tests.

At the selection visit patients were assessed clinically. FEV_1 and VC values were measured and patients underwent methacholine provocation to determine PC_{20}.

Seventy-two hours after the selection visit patients were admitted to the hospital for three consecutive days. On the first day, starting at 9.00 AM, control solution was inhaled four times at 15-minute intervals. FEV_1 values were measured immediately and 15 minutes after each inhalation. After the fourth inhalation FEV_1 was recorded at 10-minute intervals for the first hour and was followed every hour until 12 hours after the last inhalation. Six hours after the last inhalation blood sampling was performed for ECP and Cetirizine measurements. The second day at 9.00 AM subjects underwent allergen challenge with HDM; spirometry was performed as on the first day. Blood sampling was performed 6 hours after the last inhalation for ECP and Cetirizine measurements. On the third day patients underwent a second methacholine provocation .

Patients were then treated with Cetirizine or placebo for 18 days. At the end of the treatment period PC_{20} Methacholine was determined. Seventy-two hours later patients were again admitted to the hospital for three consecutive days. The same tests as during visit 2 were performed.

Data analysis.

Statistical analyses were performed with an SAS statistical package (SAS Institute, Cary, N.C.). In all statistical tests p-values below 0.05 were considered significant.

The two treatment groups were compared with regard to patients characteristics at selection using Fisher's exact test for the dichotomous variables, t-test for the continuous variables or Mann-Whitney rank test for the ordinal variables.

The parameters of EAR and LAR were compared using the analysis of covariance with the value measured before treatment as covariate. The means after treatment

adjusted for initial difference between the treatment groups were calculated. A logarithmic transformation was applied prior to the statistical analysis of PD_{15} allergen measurements.
The PC_{20} methacholine ratio's of the two groups were compared by one-way analysis of variance.
The provocation induced change in ECP blood level in the two treatment groups was compared using an analysis of covariance.
For evaluation of safety the number of patients experiencing adverse reactions was compared between the two treatment groups using the Fisher's exact test.

7.4 Results

Demographic characteristics were very similar between the two treatment groups. The (log-transformed) mean values of PC_{20} methacholine showed no significant difference between the two groups (Table 1).

Early and late asthmatic responses

Figure 1 shows the effect of allergen challenge on FEV_1 value in the placebo and Cetirizine group before and after treatment. Allergen challenge resulted in a mean maximum fall in FEV_1 value between 0 and 1 hour of 37.9 ± 16.7 % in the placebo group and 40.2 ± 12.2 % in the Cetirizine group before treatment. After treatment these values were 33.2 ± 11.0 % in the placebo group and 30.8 ± 11.5 % in the Cetirizine group; after adjustment for baseline difference, the mean values were 33.8 % in the placebo group and 30.1 % in the Cetirizine group. This difference was not statistically significant (p = 0.46) (figure 2). During the late response the mean maximal fall in FEV_1 value, 3 - 12 hours after challenge, was 41.0 ± 12.1 % in the placebo group and 32.0 ± 13.8 % in the Cetirizine group before treatment. After treatment these values were 34.8 ±9.0 % in the placebo group and 26.8 ± 11.3 % in the Cetirizine group; after adjustment for baseline difference 31.6 % in the placebo group and 29.9 % in the Cetirizine group. This difference was not statistically significant (p = 0.64) (figure 2).

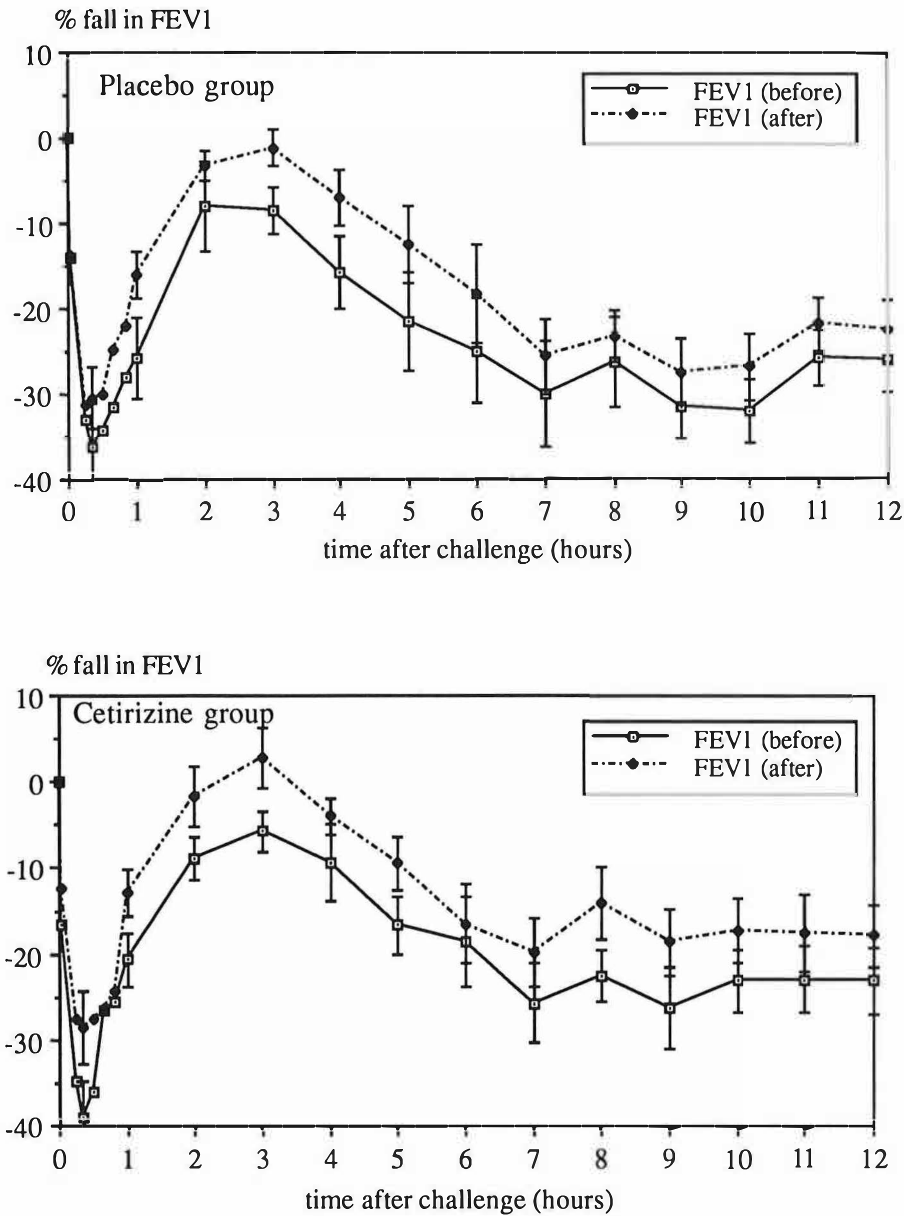
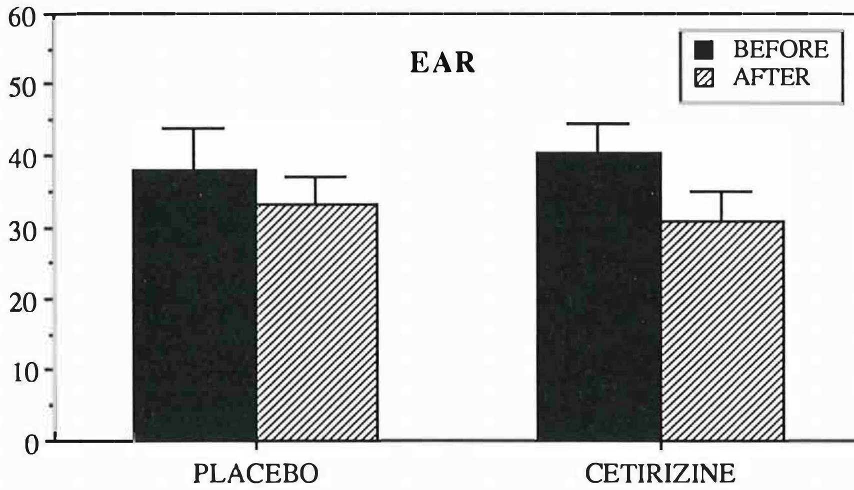


Figure 1: The effect of allergen challenge on FEV_1 before and after treatment (mean percentage fall in FEV_1 +/- SEM). Top, Placebo; bottom, Cetirizine group



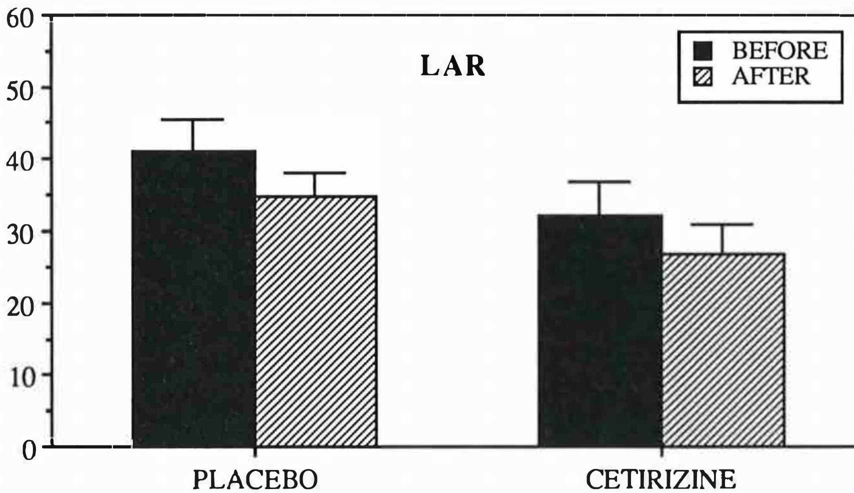


Figure 2: The mean maximal fall in FEV_1 value 0 to 60 minutes (EAR) and 3-12 hours (LAR) after allergen challenge in both groups (+/- SEM). There was no significant difference between the groups (EAR; p=0.46) (LAR; p=0.64).

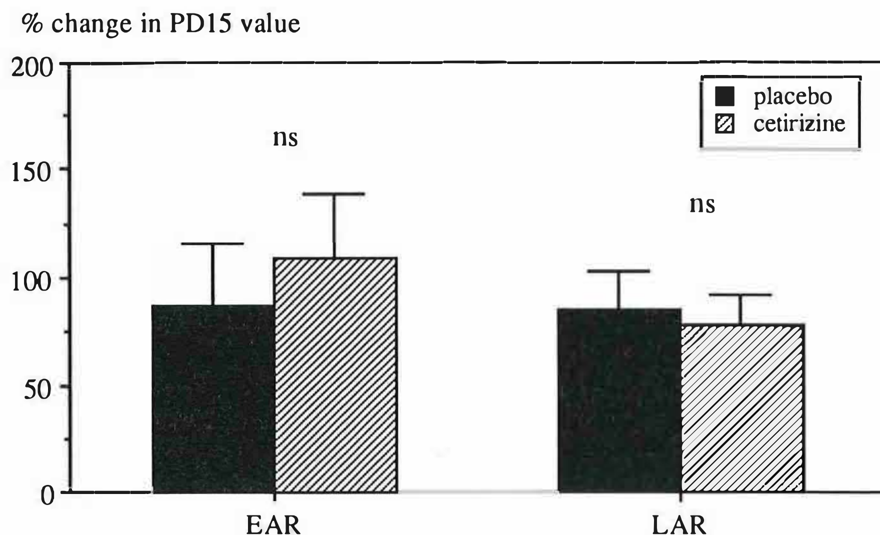


Figure 3: Percentage change in PD_{15} value (+/- SEM) of EAR and LAR after treatment in both groups. There was no significant difference between the groups (EAR; p=0.53)(LAR; p=0.85).

Since in 6 patients (3 in each group) the allergen concentration required to induce a fall of $FEV_1 \geq 15\%$ was lower after the treatment than before, the PD_{15} allergen for early and late response were also calculated. The geometric mean of PD_{15} allergen 0-1 hour before treatment was 611 BU/ml in the placebo group and 800 BU/ml in the Cetirizine group. After treatment these values were 386 and 627 BU/ml, respectively. The adjusted geometric means were 427 and 568 BU/ml after treatment with placebo and Cetirizine, respectively. This difference was not statistically significant (p = 0.53). The geometric mean of PD_{15} allergen 3-12 hours before treatment was 511 BU/ml in the placebo group and 1126 BU/ml in the Cetirizine group. After treatment these values were 353 and 772 BU/ml, respectively. The adjusted geometric means were 503 BU/ml in the placebo group and 542 BU/ml in the Cetirizine group. This difference was not statistically significant (p = 0.85) (figure 3).

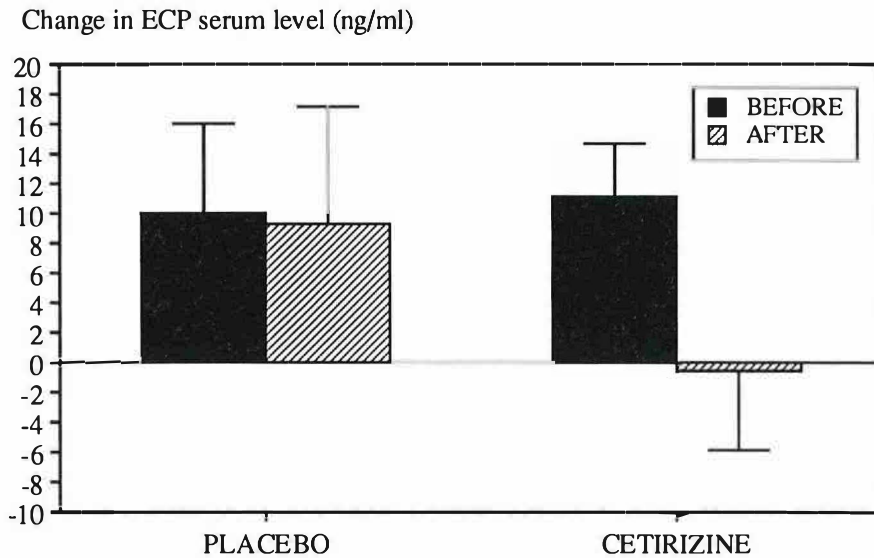


Figure 4: Allergen-induced changes in ECP serum level (+/- SEM) before and after treatment in both groups. There was no significant difference between the groups (p=0.34)

PC_{20} Methacholine

To study the effect of treatment on the hyperresponsiveness two ratio's of PC_{20} methacholine were calculated in both treatment groups. The mean ratio of PC_{20} methacholine before allergen challenge (ratio 1) was 1.22 in the Cetirizine group and 1.29 in the placebo group. Again this difference was not statistically significant ($p = 0.89$). The mean ratio of PC_{20} methacholine after allergen challenge (ratio 2) was 1.13 with Cetirizine and 1,71 with placebo, no significant difference ($p = 0.58$).
For the group as a whole (N=16) before treatment allergen challenge did not significantly change the PC_{20} methacholine (mean ratio of PC_{20} after/mean ratio PC_{20} before = 1.03).

ECP

Allergen challenge resulted in a significant increase in ECP value before treatment (p=0,01). After treatment the adjusted differences were -0.4 ng/ml in the Cetirizine group and +9.2 ng/ml in the placebo group (figure 4). This difference was not statistically significant (p=0.34).

Evaluation of safety

There was no significant difference in occurrence of adverse events, symptoms (dyspnea, wheezing, cough) and abnormalities in the blood, between the two treatment groups.

7.5 Discussion

Cetirizine has proved to be effective in inhibiting early responses in the skin, as measured by wheal and flare reactions (14, 15, 25), and in the nose, as measured by symptom scores (5, 6). Several authors have studied the effect of Cetirizine on early mediator release in skin blister models. Charlesworth et al (16) found a significant reduction in prostaglandin D_2 production until 5 hours after challenge, but no effect on histamine release after the use of 20 mg Cetirizine during 3 days. However Michel et al (15) found a significant reduction in immediate histamine release after 10 mg Cetirizine during 4 days. In a nasal challenge model Cetirizine also significantly decreased TAME-esterase activity and leukotriene C_4 production, but had no effect on histamine and prostaglandin D_2 release (26). Thus Cetirizine seems to be effective in inhibiting early responses in the nose and skin.

We have demonstrated that 18 days of treatment with Cetirizine did not reduce the intensity of the early asthmatic response, measured by maximal fall in FEV_1 value and PD_{15} allergen during the first 30 minutes after challenge. These findings are in accordance with the study of Rédier et al (27) and Wasserfallen et al (28). Although there is little doubt about the antihistaminic effects of Cetirizine, its anti-allergic properties in the airways are not proven. In our study we used a high dose (30 mg Cetirizine a day) in order to study such possible anti-allergic effects. In contrast to the nose and skin Cetirizine is not effective in inhibiting early responses in the lower airways. It is possible that the tissue concentration of Cetirizine in the lungs is to low to be effective. In patients with exercise induced asthma, Ghosh et al (29) found that Cetirizine was effective in inhibiting the early fall in FEV_1 after exercise after a single inhaled dose, whereas there was no effect after a one week course of oral Cetirizine. This difference may be due to differences in local tissue concentrations in the airways.

Our data also failed to show a significant effect of Cetirizine on the late asthmatic response after allergen challenge, as measured by the maximal fall in FEV_1 and the PD_{15} allergen during late response. These results are in agreement with the results of a study by Rédier et al (27), who also found no significant effect on late response after an eight day course of Cetirizine. However Wasserfallen et al (28) did find a

significant reduction in LAR after Cetirizine treatment during 7 days. A possible explanation for these different outcomes may be a difference in severity of asthma in the patients. In our study most patients used inhaled steroids, often in combination with other drugs, for the control of their asthma prior to the study and had rather low PC_{20} methacholine (Table I). In the study of Wasserfallen however, two patients in each group used no medication for their asthma at the time of the study; the use of medication of the other patients and the degree of hyperresponsiveness was not presented. There is also a difference in patient selection with respect to the type of allergy. Wasserfallen studied mostly cat allergic patients (12 out of 16 patients); the degree of exposition to cat allergen at home during the study in the two groups was not presented. In our study and in the study of Rédier most of the patients were allergic to HDM (16 out of 16, 8 out of 12 respectively).

Late phase allergic reactions, which are seen in the nose, conjunctiva, skin and lower airways are associated with the infiltration of various inflammatory cells such as eosinophils, mononuclear cells and possibly neutrophils (21, 30). Eosinophils are supposed to play an important role in the pathogenesis of the late phase reaction (31-33). Release of basic proteins such as major basic protein and cationic protein may cause damage to the bronchial epithelium and is associated with an increase in bronchial hyperresponsiveness (34, 35). In order to study the activation of eosinophils we measured ECP blood levels during allergen-induced late phase response before and after treatment. We found a marked, but not significant effect of Cetirizine on ECP blood levels 6 hours after allergen challenge. Rédier et al (27) found that Cetirizine inhibited the recruitment of eosinophils into the bronchoalveolar lavage fluid 24 hours after allergen challenge. In a study of Kyan-Aung et al (36) it was demonstrated that Cetirizine significantly inhibited the adhesion of eosinophils to stimulated endothelial cells. Several authors have demonstrated an inhibiting effect on infiltration of eosinophils during the late phase response in the skin (15-16). Despite of these findings, there was no reduction in the clinical manifestation of the LAR in our study and in the study of Rédier et al. This discrepancy in effect of Cetirizine on recruitment of eosinophils and on the late phase reaction measured by lungfunction is difficult to explain. Perhaps the role of the eosinophil in the late phase response is more limited than previously thought. Other cells, such as lymphocytes, macrophages, mast cells or basophils may be more important in the generation of the late-phase response (21). It is also possible that the tissue concentration of Cetirizine in the lower airways is too low to be effective (29).

In our study we did not find a significant increase in bronchial hyperresponsiveness following the late phase bronchial response. This is in contrast with several other studies in which allergen-induced asthmatic responses are associated with an increase in bronchial responsiveness to histamine or methacholine (37, 38). The methacholine tests before allergen challenge were performed in an outpatient setting during the selection visit, whereas the PC_{20} measurements after challenge were performed after 2 days of hospitalisation. In our hospital the concentration of housedustmite is very low compared to the average. This low exposure may cause a rapid improvement in pulmonary symptoms after only 1 day in hospital . It is possible that the effect of allergen challenge on the PC_{20} methacholine is obscured by the effect of hospitalisation.
We also failed to find a significant effect of Cetirizine on the baseline hyper-responsiveness (ratio 1) and on the allergen-challenge induced hyper-responsiveness (ratio 2). This is in accordance with the fact that there was no significant effect on the late phase response. Because both late phase response and non-specific hyper-responsiveness are manifestations of airway inflammation, it seems that Cetirizine has no inhibiting effect on the inflammatory process in the lower airways.
In conclusion, although Cetirizine can be effective in the treatment of allergic disorders in nose and skin, we failed to find any effect on bronchial early and late response after allergen challenge. This lack of effect can be due to low tissue concentration in the lower airways, although we used a daily dose three times higher than usually recommended. Our data, combined with those of Rédier et al, suggest a discrepancy between the clinical response during the LAR and eosinophil dynamics. This could imply that the role of the eosinophil in the development of the LAR has been overestimated. On the other hand a stronger reduction in eosinophil chemotaxis than is achieved with this drug after oral gift may be neccesary to suppress the LAR.

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Chapter 8

Allergen-induced changes in airway responsiveness are related to baseline airway responsiveness.

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8.1 Abstract

In the literature bronchial allergen challenge usually is reported to result in an increase in histamine-induced airway responsiveness (AR). In the present study the relation between baseline AR and allergen-induced changes in AR was investigated.

The effect of allergen challenge on AR was investigated in 21 atopic asthmatic patients. Allergen challenge resulted in a significant decrease in PC_{20} histamine after 24 hours. When the group was divided into 3 subgroups, depending on baseline PC_{20} histamine, a significant decrease in PC_{20} histamine was only found in patients with relatively high baseline PC_{20} histamine (group 1 and 2). A significant inverse correlation was found between baseline PC_{20} and allergen-induced change in PC_{20} histamine.

The effect of repeated allergen challenge on AR was studied in 8 patients. The first allergen challenge resulted in a significant decrease in PC_{20} histamine; no further decrease in mean PC_{20} histamine was seen after the second allergen challenge.

These results suggest, that allergen-induced changes in AR occur mainly in patients with relatively high baseline PC_{20} values. Once an increase in AR is induced, further allergen challenge not always results in further increase in AR .

8.2 Introduction

The bronchial response to inhaled allergen and the degree of histamine-induced airway responsiveness (AR) are considered to be interrelated. An increase in AR is found after natural exposure to allergen (1) and at various intervals after experimental allergen challenge (2-4). Cartier et al (4) found a significant correlation between the allergen-induced increase in AR and the severity of the late asthmatic response (LAR), suggesting the same underlying mechanisms. These mechanisms may include inflammatory changes in the airways, as described by several authors during late responses and allergen-induced increases of AR (5-8). More recent studies show that the increase in AR precedes the development of the LAR (9, 10).

In an earlier study on the effect of Cetirizine on early and late asthmatic reactions we did not find a significant increase in AR after allergen challenge, despite the fact that all patients had late phase responses (11). It was discussed that this could be related to the design of the study. Since in this study many included patients had a relatively low baseline PC_{20} histamine, we also considered the possibility that this patient characteristics might have influenced the results. The aim of the present study was to investigate whether allergen-induced changes in AR are related to the pre-existing level of AR .

8.3 Patients and methods

Patients

Twenty-one atopic, non smoking patients with mild to moderate asthma (15 female and 6 male, age 17-44 years) participated in the study . The clinical data are presented in Table 1. Nineteen patients had positive skin tests and/or elevated specific IgE (Pharmacia Cap > 0.7 PRU/ml) to housedustmite (HDM) , 2 to grasspollen (GP). The pollen allergic patients were tested at the end of the pollen season. All patients had asthma, well controlled by inhaled anti-inflammatory therapy, and FEV_1 values ≥ 70 % of the predicted value. None of the patients gave a history of respiratory tract infections in the 6 weeks prior to the study or had received oral corticosteroïds. All patients gave informed consent. The study was approved by the medical ethics committee of Asthmacenter Heideheuvel, The Netherlands.

Histamine challenge

Histamine phosphate solutions (doubling concentrations from 0.25 to 32 mg/ml) were administered through a De Vilbiss 646 nebulizer with a gauged output of 0.13 mg/ml. The nebulizer was mounted to a valvebox with aerosol filter. The nebulation time was 30 seconds, during which the patient was instructed to breath quietly. The test was started with inhalation of a phosphate buffer aerosol. Prior to the inhalation three measurements of VC and FEV_1 were performed using the portable electronic Printer Spirometer II (Micro Medical Ltd, Rochester, Kent England): Calibration of the spirometer was performed every two weeks. No adjustments were necessary. After each concentration the FEV_1 value was measured. The PC_{20} histamine was derived by linear interpolation.

Bronchial allergen challenge

Allergen solutions were prepared from stock solutions of *Dermatophagoides pteronyssinus* and mixed grasspollen, diluted in PBS with 0.03 % human serum albumin and containing 0.5 % phenol (SQ 503 resp SQ 293, ALK Benelux) to produce a range of fivefold increasing concentrations from 80 to 10.000 BU/ml. The allergen solutions were administered through a De Vilbiss nebulizer mounted to a valvebox with aerosol filter (output 0.13 mg/ml, see histamine challenge). The patients were instructed to breath quietly during one minute for each dose. Increasing doses were given at 15 minute intervals.

Table 1: Clinical data of the patients

Patient	Age	Sex	FEV_1	FEV_1 % pred.	PC_{20} hist mg/ml	Allergy	Regulary steroids	Smokers
Group 1								
1*	44	F	2.80	89	8.00	GP	+	-
2*	20	F	2.92	103	7.74	HDM	+	-
3	21	M	4.40	109	12.08	HDM	+	-
4*	34	F	3.04	90	32.00	HDM	+	-
5	21	F	2.57	82	7.59	HDM		-
6	23	M	3.95	99	14.36	HDM		-
7*	26	F	3.20	96	5.50	HDM	+	-
Mean	27		3.27	95	12.47			
SD	9		0.66	9	9.13			
Group 2								
8*	27	F	2.80	88	1.45	GP	+	-
9*	24	F	3.72	99	3.00	HDM	+	-
10*	23	M	4.80	96	3.25	HDM	+	-
11	23	M	4.00	89	3.76	HDM	+	-
12	19	M	4.30	76	2.42	HDM	+	-
13	33	F	2.68	79	1.74	HDM	+	-
14	39	F	2.36	81	3.99	HDM		-
15	21	F	4.68	119	3.12	HDM	+	-
Mean	26		3.67	91	2.84			
SD	7		0.95	14	0.91			
Group 3								
16	19	F	3.27	81	0.83	HDM		-
17	22	F	3.09	99	0.93	HDM	+	-
18	32	F	3.36	92	0.77	HDM	+	-
19	17	F	3.56	86	0.63	HDM	+	-
20*	20	M	3.56	84	0.82	HDM	+	-
21	29	F	3.23	98	0.47	HDM	+	-
Mean	23		3.35	90	0.74			
SD	6		0.19	8	0.17			

* Patients who were rechallenged with allergen after 48 hours

The challenge procedure was terminated when the FEV_1 value fell ≥ 15 % below baseline value. After the last inhalation FEV_1 was recorded at 10-minute intervals for the first hour and every hour thereafter until 10 hours after the last inhalation. FEV_1 values were corrected for diurnal variation; a fall in FEV_1 value ≥ 15 % from baseline value between 3-10 hours after challenge was considered as a late phase reaction.
The EAR and LAR were expressed by the mean fall in FEV_1 value during the early response (1-60 minutes after challenge) and during the late response (3-12 hours after challenge).

Study design
The group of 21 patients was divided into 3 subgroups depending on the initial PC_{20} histamine: Group 1 with PC_{20} histamine > 4 mg/ml (n=7) ; group 2 with PC_{20} histamine > 1 mg/ml and ≤ 4 mg/ml (n=8); group 3 with PC_{20} histamine ≤ 1 mg/ml (n=6)(Table 1).
All patients underwent a bronchial challenge with allergen. Subjects were admitted to the hospital for 3 successive days. Seventy-two hours before admission histamine challenge was performed to determine the PC_{20} histamine before allergen challenge. Medication was withheld before the study period: Inhaled steroids and sodium cromoglycate were stopped one week before the study; theophyllines, oral β_2-adrenergic drugs and antihistamines were stopped 48 hours before the study; inhaled β_2-adrenergic drugs were stopped 12 hours before the tests. On the first admission day control solution was inhaled four times at 15-minute intervals. FEV_1 was measured immediately and 15 minutes after each inhalation. After the fourth inhalation, FEV_1 was recorded at 10-minute intervals for the first hour and was followed every hour until 11 hours after the last inhalation. The second day subjects underwent the first allergen challenge with HDM or GP. Spirometry was performed as on the first day. On the third admission day patients underwent a second histamine challenge to determine the PC_{20} histamine after allergen challenge.
In order to study the dynamics of allergen-induced changes in AR a subgroup of 8 patients (Table 1) was rechallenged with allergen 48 hours after the first allergen challenge with the same dose of allergen. Twenty-four hours after the second allergen challenge a third histamine challenge was performed to determine the PC_{20} histamine after the second allergen challenge. This group of patients stayed in the hospital for 5 successive days.

We chose an interval of 48 hours between the allergen challenges in order to secure baseline FEV_1 values comparable to those obtained before the first challenge, with probably inflammatory changes still present (12, 13).

Data analysis

Comparisons were made with Wilcoxon signed rank test (WSR). Spearman's rank correlation test was used for correlation studies. P values less than 0.05 were considered significant.

8.4 Results

Allergen challenge resulted in an EAR in all 21 patients (mean fall in FEV_1 23.9 +/- 7.4%) and a LAR in 19 patients (mean fall in FEV_1 15.8 +/- 8.8%). Before allergen challenge the PC_{20} histamine of the patients varied from 0.47 to 32 mg/ml; the mean value was 5.45 +/-7.20 mg/ml . Allergen challenge resulted in a significant decrease in PC_{20} histamine, measured after 24 hours (WSR log-transformed data, p=0.006).

The effect of allergen challenge on PC_{20} histamine in the three subgroups is shown in Figure 1. In group 1 and 2 allergen challenge resulted in a significant decrease in PC_{20} histamine (WSR log-transformed data, p=0.03; p=0.01 respectively). However in group 3 no significant change in PC_{20} histamine was found after allergen challenge.

When the entire group was evaluated no significant correlation was found between the allergen-induced change in PC_{20} histamine and the mean fall in FEV_1 (EAR) (Spearman, r=.214; p=0.34: Figure 2A). When the change in PC_{20} histamine was compared with the mean fall in FEV_1 (LAR) a significant correlation was found (Spearman, r=.474; p=0.03: Figure 2B). The baseline PC_{20} histamine (before allergen challenge) showed a significant *inverse* correlation with the allergen-induced change in PC_{20} histamine (Spearman, r=-.552; p=0.01: Figure 2C).

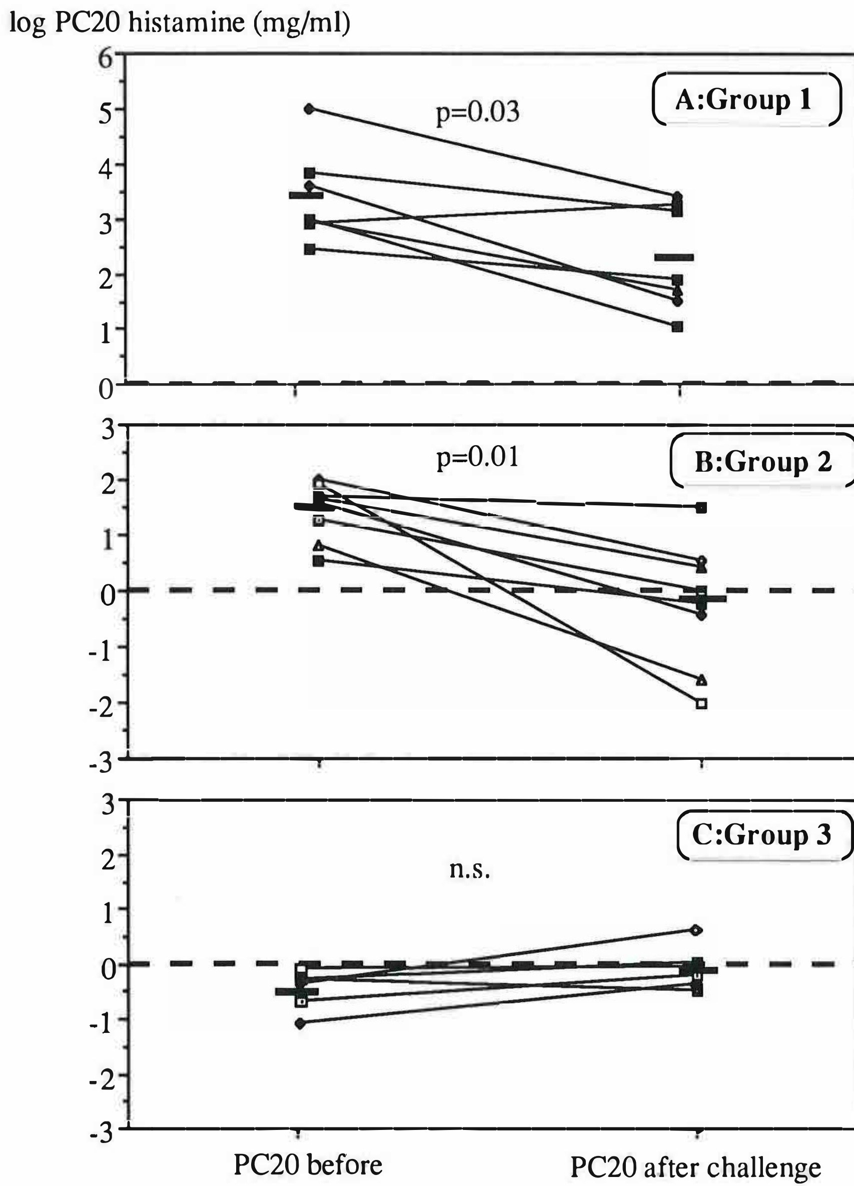

Figure 1: PC_{20} histamine before and after allergen challenge (log transformed data)
A: Group 1; B: Group 2; C: Group 3

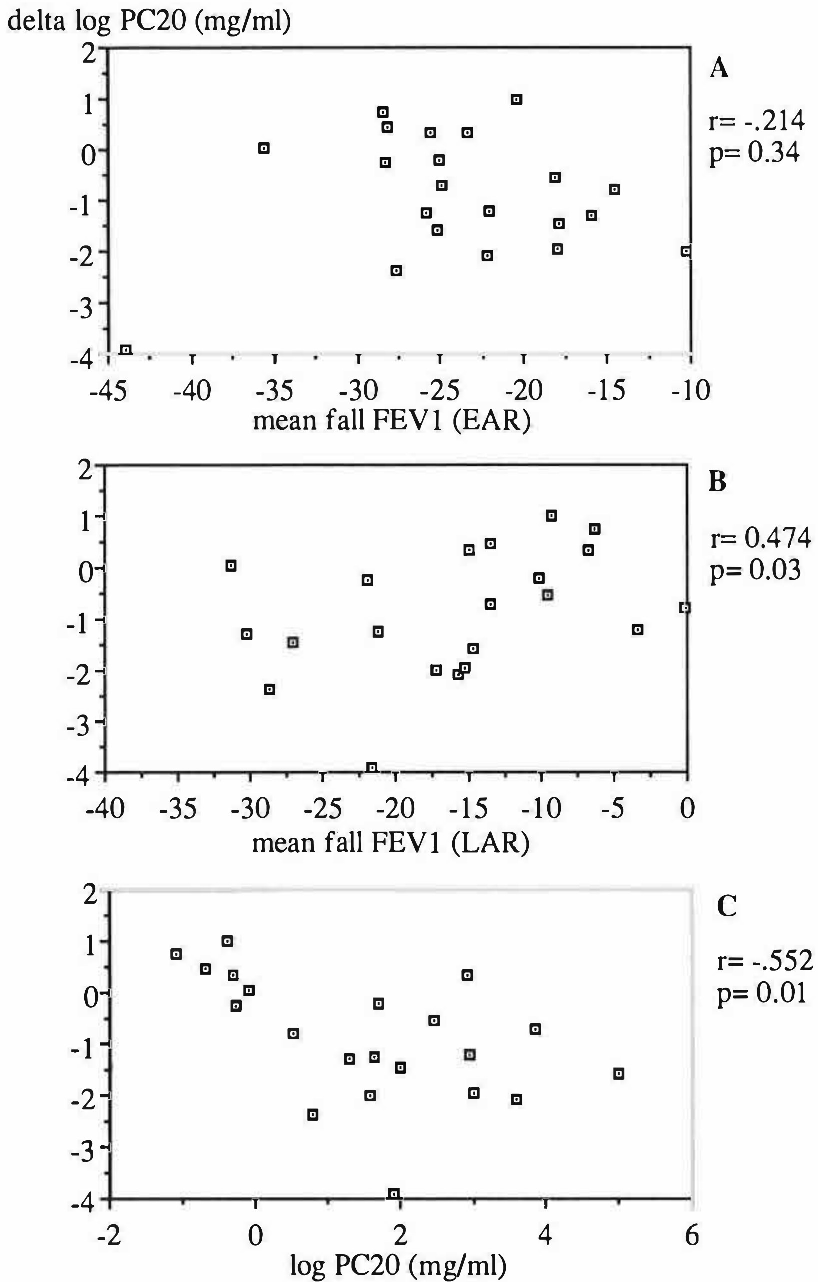

Figure 2: Correlation between the allergen-induced change in PC_{20} histamine (delta PC_{20}) and the mean fall in FEV_1 (EAR) (A), mean fall in FEV_1 (LAR) (B) and baseline PC_{20} histamine (log-transformed data) (C)

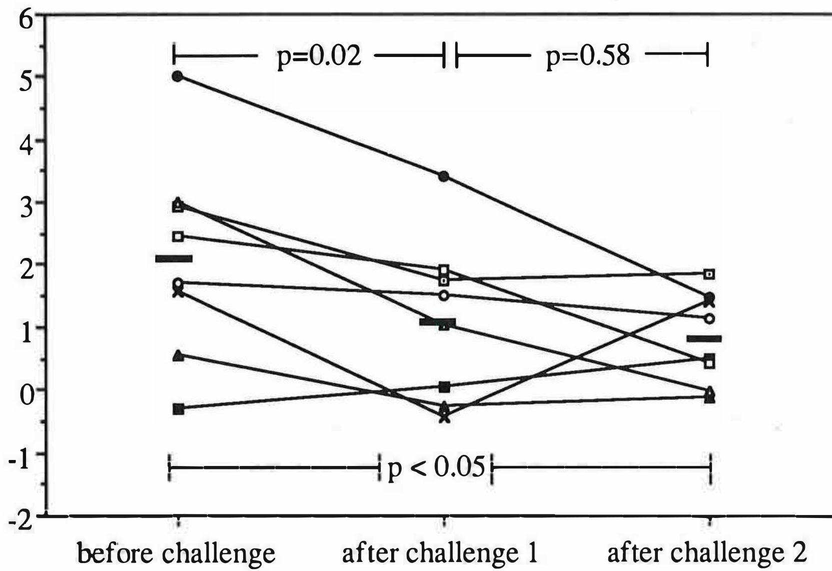

Figure 3: PC_{20} histamine before and after allergen challenge 1 and 2 (log-transformed data)

Table 2: PC_{20} histamine (mg/ml) before and after repeated allergen challenge

Patient	PC_{20} 1	PC_{20} 2	PC_{20}3
1	8.00	2.05	0.99
2	7.74	3.32	3.64
4	32.00	10.68	2.79
7	5.50	3.73	1.34
8	1.45	0.84	0.91
9	3.00	0.74	2.62
10	3.25	2.80	2.19
20	0.82	1.03	1.40
mean	7.72	3.15	1.99
SD	10.17	3.25	0.98

In a subgroup of 8 patients repeated allergen challenge was performed with an interval of 48 hours using the same dose of allergen. The first allergen challenge in this subgroup of patients resulted in an EAR in all patients (mean fall FEV_1 20.4 +/- 4.9%). This was followed by a definite late response in 6 patients. The mean fall in FEV_1 of all 8 patients during late phase period was 9.9 +/- 8.0%. The second allergen challenge resulted in an EAR in all patients (mean fall FEV_1 22.3 +/- 8.0%) and a LAR in 7 patients. The mean fall in FEV_1 of all 8 patients was 12.8 +/- 8.6 %. Repeated allergen challenge did not result in significant changes in the severity of EAR and LAR, calculated as mean fall in FEV_1 value during early and late response (WSR, p=0.33 resp 0.33).
The PC_{20} histamine before the first allergen challenge varied from 0.82 to 32 mg/ml; the mean value was 7.72 +/- 10.17 mg/ml. The first allergen challenge resulted in a significant decrease in PC_{20} histamine (WSR log-transformed data, p=0.03). The second allergen challenge did not result in a significant change in PC_{20} histamine (WSR log-transformed data, p=0.58: Figure 3).

8.5 Discussion

The present study was performed in order to investigate whether allergen-induced changes in AR are dependent on baseline AR. In an earlier study (11) we did not find a significant increase in AR after allergen challenge in a group of atopic asthmatic patients, and we speculated that this could be related to the rather low baseline PC_{20} histamine (mean value 2.85 +/- 4.43 mg/ml) of the patients in this group. In the present study 21 atopic asthmatic patients with a higher baseline PC_{20} histamine (mean value 5.45 +/- 7.20 mg/ml) compared to the former study were included, using the same study design including 3 days of hospitalization. In contrast to the former study, allergen challenge in these 21 patients resulted in a significant decrease in PC_{20} histamine 24 hours after allergen challenge. The change in PC_{20} histamine after allergen challenge showed a significant inverse correlation with baseline PC_{20} histamine, suggesting a more pronounced increase in allergen-induced AR in patients with an initially relatively high PC_{20} histamine.
To further explore the relationship between baseline PC_{20} histamine and allergen-induced changes in AR we divided the group of 21 patients into 3 subgroups, depending on initial PC_{20} histamine. In group 1 and 2 (PC_{20} histamine > 1 mg/ml) allergen challenge resulted in a significant decrease in PC_{20} histamine, although the differences in group 1 (PC_{20} histamine >

4 mg/ml) were rather small. The patients with the lowest baseline PC_{20} histamine (group 3, PC_{20}histamine ≤ 1 mg/ml) did not show a significant change after allergen challenge. This could not be attributed to a limited measuring range of the group with the lowest baseline PC_{20} histamine. As shown in Table 1, in all patients PC_{20} values were above the lowest histamine phosphate concentration (0.25 mg/ml) that was used. Also differences in allergen dose administered in the 3 groups could not explain for the lack of significant change in PC_{20}histamine after allergen challenge in group 1. Although there was a small difference between the mean allergen dose administered in the 3 groups, the patients with the lowest baseline PC_{20} histamine did not receive the lowest dose of allergen. The mean dose of allergen in group 1, 2 and 3 were 5.1 BU/ml, 4.3 BU/ml and 5.0 BU/ml respectively (log transformed data). In order to study a possible relation between the dose of anti-inflammatory treatment prior to the tests and the allergen-induced change in PC_{20} histamine we calculated the correlation between the dose of inhaled steroids before the tests and the allergen-induced change in PC_{20} histamine. We could not find a significant correlation (r=0.27). The mean dose of inhaled steroids in group 1, 2 and 3 were 514, 487 and 800 microgram respectively, however there were large differences within the groups.
In the past many studies have been performed on the effect of allergen challenge on the induction of AR. A significant decrease in PC_{20} histamine 24 hours after allergen challenge, as we found in our group of 21 patients, was reported by several other authors (2-4, 9, 10). A significant correlation between the allergen-induced increase in AR and the severity of the LAR has earlier been reported in literature (4, 10). No data have been reported concerning the relation between baseline AR and allergen-induced changes in AR.
In the second part of the present study the relation between baseline AR and allergen-induced change in AR was studied in a longitudinal design. A subgroup of 8 patients, who were willing and able to stay in hospital for 5 successive days, underwent 2 allergen challenges with an interval of 48 hours using the same dose of allergen. The first allergen challenge resulted in a significant decrease in PC_{20} histamine. This significant lower PC_{20} histamine after the first allergen challenge served as baseline value for the second allergen challenge. No significant further decrease in mean PC_{20} histamine was found after the second allergen challenge, despite the fact that the intensity of the allergic reaction, expressed as mean fall in FEV_1 during EAR and LAR, did not differ significantly from the first. As shown in Table 2, in all patients PC_{20} values after the first challenge were above the lowest histamine phosphate concentration (0.25 mg/ml) that was used. Thus, the lack of a

further decrease in PC_{20} values after the second allergen challenge could not be attributed to a limited measuring range.
This finding also suggests that in patients with relatively low baseline PC_{20} histamine no further fall in PC_{20}value may occur.
The effect of repeated allergen challenge on AR in allergic patients was also studied by Ihre et al (14), who found a significant increase in AR after repeated inhalation of low doses of allergen. AR was not measured during the course of these repeated inhalations, so it is possible that the increase in AR was already maximal after the first allergen challenge as in our study.
Another finding in the second part of our study was that repeated allergen challenge did not lead to significant changes in the intensity of early and late asthmatic responses. Our findings concerning the EAR are in accordance with the findings of Rosenthal et al (15), who performed repeated allergen challenges for 4 consecutive days in 13 ragweed allergic patients out of the season and also could not find significant changes in the intensity of the EAR. The effect of repeated allergen challenge on EAR and LAR was studied by Rasmussen (16), who used an interval of 14 days between the challenges. No significant changes in EAR were found, but a significant increase in the intensity of the LAR was found, which was thought to be related to an enhanced specific reactivity. These findings are in contrast with a study performed by Bel et al (17), who found reproducible EAR and LAR after allergen challenge with an interval of 2 weeks in 10 atopic asthmatic patients (18).
Our finding that allergen-induced change in AR shows an inverse correlation with baseline AR also has practical implications. For instance when selecting patients for controlled medication studies in which the effect on allergen-induced changes in AR are studied, baseline AR should be comparable in each group.
In the second part of the study we found that repeated allergen challenge did not induce further significant increase in AR, despite the fact that the LAR after both challenges was not statistically different. It seems that some patients have reached their individual maximal level of AR due to the first allergen challenge. In the literature the occurrence of a LAR is often associated with a subsequent increase in AR, which in turn could lead to a more severe LAR. The fact that in our study an increase in AR did not lead to an increase of the subsequent LAR suggests that not all patients will deteriorate after repeated allergen exposure. Further studies in which AR is measured after several successive allergen challenges are needed to elucidate this problem.

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Chapter 9

Effect of local allergen priming on early, late, delayed phase and epicutaneous skin reactions.

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9.1 Abstract
Background: Allergic disease is reflected in a chronic inflammatory response to an allergen. It is thought that local allergen priming underlies this chronicity.
Objective: To assess the effect of allergen priming on the magnitude and histology of the allergic reaction.
Methods: Four sequential intracutaneous skin tests were done with 48 hour intervals in thirteen patients allergic to housedustmite (Dpt). Reactions were measured at 15 minutes, and at 6, 24 and 48 hours. Subsequently epicutaneous tests were done on Dpt-primed spots (n=5).
Results: At 6, 24 and 48 hours reactions increased after priming ($p<0.006$) , with unaltered early reactions. Epicutaneous reactions to Dpt on primed spots were larger, compared with epicutaneous controls on similarly primed skin.
Conclusion: Local intradermal priming results in greater inflammatory responses at both intra- and epicutaneous challenge. This mechanism may offer an explanation for the chronicity of allergic reactions at epithelial surfaces

9.2 Introduction
The chronic nature of both allergic asthma, rhinitis and atopic dermatitis is well established. This chronicity suggests that a continuing allergic reaction may have some permissive effect for further allergen-driven inflammation. This is illustrated both in pollen allergic rhinitis and allergic asthma where the threshold of specific reactions of the nose and lower airways is lowered in the pollen season (1). Frequent intranasal challenges also lead to an increased sensitivity to allergen (2, 3).
Intradermal skin reactions are widely used as a model for allergen induced inflammation of the upper and lower airways. Usually the skin is challenged a single time before evaluating the inflammatory response. Such an approach evidently does not take into account the chronic nature of the reactions in the airway mucosa. The chronicity in allergen exposure may influence both the clinical magnitude and the histological quality of the reaction. Repetitive challenge of the skin has earlier been found to induce diminishing immediate reactions, with unaltered histamine responses (4, 5). Little is known, however, about the late-phase and delayed-phase reactions at rechallenge, the duration of the reaction and the effect of allergen priming on epicutaneously induced allergic reactions.
We deal with these points in this study. Thirteen subjects allergic to housedustmite were sequentially challenged four times with intradermal application of housedustmite allergen. Reactions were measured at time intervals up to 96 hours

after challenge. After sequential intradermal challenge, epicutaneous reactions with housedustmite were assessed.

9.3 Materials and methods

Subjects

The study was approved by the ethical committee. The subjects (n=13) were student volunteers, who suffered from bronchial asthma. All showed a positive skin test reaction (diameter ≥ 5 mm) to housedustmite allergen (ALK, Copenhagen). None of the patients suffered from atopic dermatitis.

Skin tests

Intracutaneous tests: We induced skin reactions with housedustmite allergen (ALK, Copenhagen) by injecting 30 BU/ml (0.03 ml) intracutaneously in the back of all 13 patients at four different locations (1-4; table 1). We repeated the testing three more times with Dpt at the Dpt spots. Intervals between the tests were 48 hours.

We read the reactions at 15 minutes, 6 hours, 24 hours and 48 hours after challenge and scored the wheal diameters as a measure of the early responses. Indurations were determined for the later phases of the reactions according to a procedure suggested by Sokal (6), and the maximal diameter of each reaction was expressed in millimetres (mm). In case of similar challenge patterns the average diameters were used for subsequent analysis.

Epicutaneous tests: In 5 of the patients Dpt was given epicutaneously 48 hours after the four intracutaneous tests at location 1 (Dpt-Dpt-Dpt-Dpt) and at a non-prechallenged control spot (location 5). Plasters with control solution were put on Dpt prechallenged (Dpt-Dpt-Dpt-Dpt; spot 2) and at a control spot (6). All 4 epicutaneous reactions were measured 48 hours later.

Immunohistological analysis

Monoclonal antibodies: For immunophenotyping of distinct cell types a series of mouse monoclonal antibodies was used including OKT1, OKT11, OKT8 (aCD8), OKIa (aHLA-DR), (Ortho Pharmaceutical Corp.) and Leu3a (aCD4) (Becton Dickinson). An indirect, two-step immunoperoxydase method was used on frozen tissue sections (4-6 μm).

Table 1: Pattern of repeated intra- and epicutaneous challenges with housedustmite (Dpt).

POSITION	TEST 1	TEST 2	TEST 3	TEST 4	TEST 5
1	Dpt IC	Dpt IC	Dpt IC	Dpt IC	Dpt epi
2	Dpt IC	Dpt IC	Dpt IC	Dpt IC	Contr epi
3	Dpt IC	Dpt IC	Dpt IC	Dpt IC	
4	Dpt IC	Dpt IC	Dpt IC	Dpt IC	
5					Dpt epi
6					Contr epi
7				Dpt IC	
8				Dpt IC	

Position: Skin position at the patients' back, at which the repeated challenges were done
Dpt IC: Intracutaneous skin test with Dpt (30 BU/ml)
Dpt epi: Epicutaneous application of Dpt (for a period of 48 hours beginning at 48 hours after the fourth IC test)
Contr epi: Epicutaneous application of a control solution

Statistics

Non-parametric tests were used for statistical analysis: Mann-Whitney's for comparisons in reaction diameters and Spearman's rank correlation test was used for correlation studies. Only p values less than 0.05 were considered significant.

9.4 Results

Macroscopic appearance of the skin reactions at sequential testing

Intracutaneous tests: Figure 1 shows the diameters of the reactions at 15 minutes, 6 hours, 24 hours and 48 hours after testing. The reactions at 15 minutes (early) did not change after rechallenge. The reactions at 6 hours (late) were significantly larger at the third (from 13 (test 1) to 25 mm; p=0.003) and fourth (23 mm; p=0.002) challenge. The reactions read at 24 hours were larger at each rechallenge, approximately doubling in size (test 1: 9 mm, test 2: 13 mm; p=0.006, test 3: 20 mm; p=0.002, test 4: 18 mm; p=0.003). The same pattern was seen for the reaction at 48 hours (test 1: 7 mm; test 2: 11 mm; ; p=0.003, test 3: 12 mm; p=0.002, test 4: 12 mm; p=0.002).

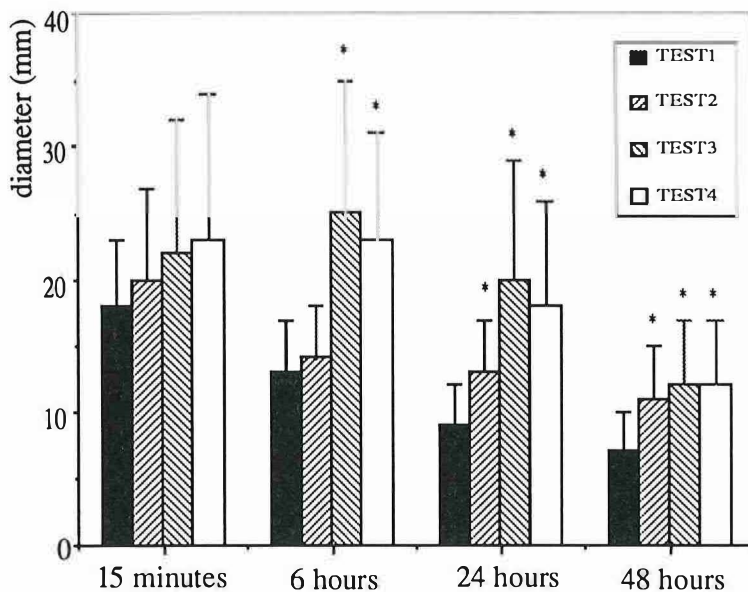

Figure 1: Diameters of skin reactions after repeated challenge with housedustmite (dpt)
* increase in relation to the diameters at test 1 ($p<0.006$)

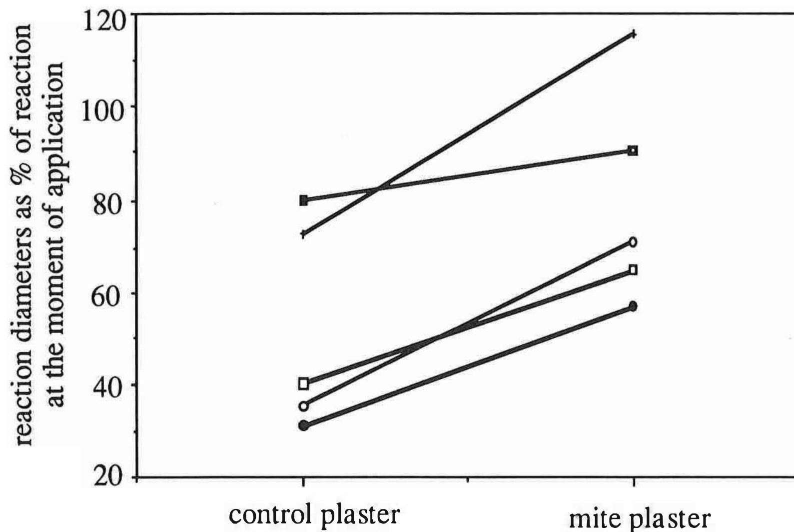

Figure 2: Epicutaneous tests with Dpt and control solution were done at 48 hours after intracutaneous priming with **Dpt** (at position 1:Dpt, position 2:control). Plasters were removed after 48 hours, and the reaction was expressed as % of the local priming reaction (just before application of the plasters).

Epicutaneous tests: Application of housedustmite on skin plasters on unprimed skin failed to elicit any visible or palpable reaction. When the skin was primed intracutaneously with housedustmite (four times), application of these plasters resulted in larger reactions after a 48 hours' exposure period (on average 80% of the reaction before application of the plaster) in all five patients tested, compared to control plasters (52% of the reaction before application of the plaster) on similarly primed skin (figure 2).

Histology

In unchallenged skin small numbers of mononuclear cells are usually present, mainly grouped around organelles as vessels, hair follicles and sweat glands. After challenge the numbers of cells increase considerably. CD4 positive lymphocytes represent the majority of these cells, followed by eosinophils. Cell numbers stayed high for up to 96 hours after the tests.

9.5 Discussion

Repeated intracutaneous challenge with allergen resulted in an increase in late, delayed phase and epicutaneous reactions .

Sequential challenge of the skin was done earlier using different challenge intervals. Shaikh (7) did intracutaneous challenges with intervals of one, two and three weeks, using both anti-IgE and several allergens. The early reaction was unaltered at rechallenge, the late phase reactions however appeared to be smaller. The number of allergen-challenged patients was relatively small. Andersson (4) repeated intracutaneous skin tests with pollen once after an interval of 12 hours. Early reactions at rechallenge were smaller with unaltered histamine reactions, but the effect on late phase reactions was not mentioned. These data underline the importance of the challenge interval in determining the priming effect. We choose an 48 hours' interval because of the observation that at that point of time numerous activated cells are still accumulated at the reaction site but the macroscopic reaction is declining. As the presence of activated cells as lymphocytes, eosinophils, mast cells and possibly Langerhans cells may account for the observed priming effect on the reaction, an interval of weeks is likely to be rather long. Anderssons observation on the decline of early reactions at 12 hours intervals may be explained by depletion of mast cell histamine content. Furthermore we only observed significant increases at the third and fourth challenges. It was noted that the most striking degree of increase occurred after the third challenge. This effect appeared to level off at the fourth

challenge, suggesting that at repetitive exposure reactions grow up to an individual maximum. This is of course compatible with the clinical observation that with continuous exposure reactivity does not increase indefinitely. The effect of intracutaneous priming on epicutaneous reactions was not described before.
Local allergen specific priming is likely to depend on the attraction and activation of cells at the reaction site. We found that CD4+ lymphocytes and eosinophils were increased up to 96 hours after challenge. The role of allergen-specific CD4+ cells in the regulation of allergen-induced inflammation is increasingly understood. Allergen specific T cells resemble the Th2 phenotype in mice, producing IL-4 and IL-5 as cytokines (8). Using in situ hybridisation, Kay showed that there was a predominance of Th2 type lymphocytes 24 hours after intracutaneous challenge with housedustmite (9). IL-5, GM-CSF and IL-3 produced by these cells show potent activities in stimulating the growth of eosinophils, as well as attracting and activating them. Eosinophil degranulation products contribute to the tissue damage caused by allergic inflammation. Mast cells, bearing allergen specific IgE, may also be involved in allergen specific priming. At continuous epicutaneous challenge of the skin during up to 8 days with housedustmite allergen, the number of mast cells showed a time dependent increase (10). This may result from local production of growth potentiating IL-3 by the attracted T-lymphocytes (11). A similar influx of mast cells and basophils and an increased concentration of their mediators (histamine and PGD_2) was observed at repeated nasal challenges (3).
On the basis of these mechanistic considerations some of our observations in the skin might be extrapolated to the mucosal allergic reactions of the airways. These too are dominated by T-lymphocytes and eosinophils. A study of Crimi (12), who reported an allergen specific priming effect on late phase bronchial reactions due to seasonal influences, supports this association. Potentiation of the late, but not the early, response was further observed by Rasmussen (13) in a study in which patients with allergic asthma underwent two bronchial allergen challenges with an interval of two weeks. Inhalation of small amounts of allergen (1-10 BU) was shown to increase non-specific bronchial hyperreactivity in 11 out of 13 patients tested (14). Comparisons with studies concerning nose is difficult as late phase reactions of the nasal mucosa are seldom measured due to technical difficulties. The potentiating effect of the pollen season on the early reactions in patients with allergic rhinitis is well established. Also chronic or recurrent nasal challenge have caused an increased sensitivity of the nasal mucosa to allergen or histamine (15). These data show that

priming of late phase reactions at sequential challenges is both observed in the skin and the lower airways.
The relevance of these observations is twofold. In the first place from a mechanistic point; as allergen is reaching the airway mucosa continuously, any priming effect is likely to determine the severity of the allergic reaction and symptoms. Secondly, repetitious skin testing can be used as an extension of the conventional screening of allergy to provide additional information on the regulation of the allergic reaction. This may very well be useful to assess effects of immunomodulating therapy.

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Chapter 10

Repeated nasal allergen challenge does not always lead to priming

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Submitted for publication

10.1 Abstract.

Background: Repeated allergen challenge in the nose is often associated with "priming" of the early phase reaction, which is defined as an increased nasal reactivity following repeated allergen exposure. However little is known about the effect of repeated allergen challenge on changes in late phase reaction.

Objective: In the present study the effect of repeated allergen challenge on early and late allergic reactions in the nose was investigated in 11 grasspollen allergic patients out of the season. In addition we studied the interrelationship between the response to repeated challenge in the nose and the skin.

Methods: Repeated nasal challenges with grasspollen were performed in 11 patients three times with an interval of 48 hours: early and late responses were recorded after challenge 1 and 3 using whole body plethysmography. Repeated intradermal challenges with grasspollen were performed in the same patients on the same days as the nasal challenges; early and late responses were recorded.

Results: Repeated allergen challenge did not result in significant overall changes in early and late allergic responses in the nose and in the skin. Large individual differences in response patterns were found. In the nose a significant inverse correlation was found between the priming response and response to the first challenge for early and late reaction. A significant correlation was found between the effect of repeated allergen challenge on the late response in the nose and the skin.

Conclusion: Repeated allergen challenge in the nose can lead to priming as well as to down-regulation of early and late responses. The type of response partly depends on the intensity of the reaction to the first challenge but also seems to be a characteristic of the individual patient.

10.2 Introduction

Allergen challenge in the nose in allergic patients often results in an early allergic reaction. Late reactions in the nose have been reported in 3% to 75% of the allergen challenged patients (1-7). Although nasal challenge with allergen is a useful model in studies on the pathogenesis and treatment of allergic rhinitis, this model does not take into account the chronicity of natural allergen exposure to inhaled allergens. Therefore repeated experimental allergen challenges may provide additional information on the pathogenesis of allergic rhinitis.

In the past several studies have been performed on the effect of repeated allergen exposure in the nose on the early response and most studies report on an increased allergen specific reactivity, the so called "priming effect" (6, 8-11) .This priming effect

seems to be associated with an influx of inflammatory cells in the nose and an increase in locally produced mediators (10, 11). However there are no data available about the effect of repeated allergen challenges on physiological changes during the late phase reaction in the nose. Since the late phase reactions may better reflect the tissue changes in day to day allergic rhinitis than the early reactions (12-14), we investigated in the present study the effect of repeated allergen challenge on the nasal patency during early and late allergic reaction using a previously described whole body plethysmographic method (7). In order to study whether the response on repeated allergen challenges is a local phenomenon or a generalised immunologic characteristic of the individual patient, we compared nasal reactions with skin reactions after repeated allergen challenge.

10.3 Patients and methods

Patients.

Eleven atopic, non-smoking patients with seasonal rhinitis (3 male, 8 female, age 21-42 years) participated in the study. Nine of the 11 patients also had asthma. The clinical data are presented in Table 1. All patients had positive skin tests (≥ ++) and/or elevated specific IgE (Pharmacia Cap > 0.7 PRU/ml) to grasspollen. The patients were tested outside the pollen season. None of the patients gave a history of respiratory tract infections in the 6 weeks prior to the study. All patients gave informed consent. The study was approved by the medical ethics committee of Asthmacentre Heideheuvel, The Netherlands.

Table 1: Clinical data of the patients

patient	age	sex	allergy	asthma	smoker	medication* rhinitis	Skin test early reaction	Skin test late reaction
1	36	F	GP	-	-	-	25	23
2	23	M	GP	-	-	-	22	18
3	21	F	GP	+	-	S, A	14	10
4	34	F	GP	+	-	-	23	18
5	23	M	GP	+	-	-	14	23
6	23	F	GP	+	-	-	14	16
7	21	F	GP	+	-	S,A	21	27
8	42	F	GP	+	-	S,A	11	17
9	34	F	GP	+	-	-	17	14
10	23	M	GP	+	-	S	12	0
11	34	F	GP	+	-	-	28	33

* Medication used for rhinitis: S = nasal steroids; A = antihistamines

Study design
Subjects visited the hospital for 4 separate days. Medication for the treatment of allergic rhinitis was stopped before the tests: Nasal steroids and cromoglycate were stopped 2 weeks before the tests; antihistamines were stopped 48 hours before the tests. None of the patients received oral steroids or immunotherapy for grasspollen. Inhalation medication for the treatment of asthma was continued.
During the first study day nasal resistance was recorded 15 minutes after the inhalation of control solution and after that until 10 hours after the inhalation using whole bodyplethysmography (see below).
On the second study day nasal challenge with grasspollen was performed and nasal resistance was recorded until 10 hours after challenge. Intradermal challenge with grasspollen was performed; reactions were measured 15 minutes and 6 hours after challenge. Skin challenges with histamine and phosphate buffer diluent were also performed.
On the third study day (48 hours after the second day) nasal challenge with grasspollen was repeated, using the same dose of allergen as was used for the first challenge. Intradermal challenge with grasspollen was repeated on the same location as the previous challenge. On the fourth study day (48 hours after the third day) nasal challenge with grasspollen was again repeated, using the same dose of allergen; nasal resistance was followed until 10 hours after challenge. Intradermal challenge with grasspollen was repeated on the same location as the previous challenges; reactions were measured 15 minutes and 6 hours after challenge.
The reaction to repeated allergen challenge in nose (RACn) and skin (RACs) were expressed as the difference between the reaction after challenge 3 and challenge 1 (RAC value nose and RAC value skin)

Nasal challenge.
Allergen solutions were prepared from stock solutions of mixed grasspollen, diluted in PBS with 0.03% human serum albumine and containing 0.0125% benzalkonium chloride (SQ 293, ALK Benelux), to produce a range of tenfold increasing concentrations from 100 to 10.000 BU/ml. The allergen solutions were administered with a pump spray delivering a volume of 0.102 ml (ALK Benelux). Both nostrils were challenged. Before allergen challenge, patients waited 15 minutes to allow the nasal mucosa to become acclimatised. Increasing doses of allergen were given at 15 minutes intervals; no further dose was given when the nasal resistance, measured with whole body plethysmography (see below), had doubled. After the final dose

nasal resistance was measured at 15 minutes and thereafter every hour until 10 hours after challenge.
On a control day nasal resistance was recorded until 10 hours after the inhalation of a control solution, a phosphate buffer solution with 0.03% human serum albumine and containing 0.0125% benzalkonium chloride.
The early nasal response was expressed as the maximal % change in $Rn_{(insp)}$, measured 15 minutes after challenge and the mean % change in $Rn_{(insp)}$ measured in a time period (0.25-2 hours after challenge). The late response was expressed as maximal % change in $Rn_{(insp)}$, measured 8 hours after challenge and the mean % change in $Rn_{(insp)}$ measured in a time period (4-10 hours after challenge).

Whole body plethysmography
Whole body plethysmography is often used to determine the airway resistance in routine practice. In a previous article we described this technique for the detection of early and late reactions in the nose after allergen challenge (7). The technique in short: For the measurement of nasal resistance the mouthpiece, normally connected with the pneumotachometer, was replaced by a solid nasal mask (nasal CPAP mask). The mask was supported by both hands of the patients, keeping the mask fixed on the face of the patients without leakage of air, and preventing the patients to blow up the cheeks. The patients were instructed to breath quietly through the nose, keeping the mouth closed. Nasal flow through both nostrils was recorded. Nasal resistance (Rn) in both nostrils was then calculated from the nasal flow (V'n) and the alveolar pressure (Pa) according to the formula Rn=Pa/V'n. Because in the nose the inspiration phase is most sensitive for detection of nasal obstruction to allergen challenge, we used the inspiratory nasal resistance (Rn insp) for the monitoring of the allergic reaction. The initial value of $Rn_{(insp)}$ on the control day and on the allergen challenge days were considered as 0%. The other values of $Rn_{(insp)}$ were expressed as percentage changes compared to the initial value.

Skin tests
Intradermal challenges (30 BU/ml, 0.03 ml) were performed at the back of the patients with a standardised grasspollen extract (ALK Benelux, SQ-293). The early response was scored as the wheal diameter 15 minutes after challenge. Late responses were measured 6 hours after challenge: indurations were determined according to a procedure suggested by Sokal (15). The reaction on histamine and phosphate buffer diluent served as positive and negative controls.

Data analysis
Comparisons were made with the Wilcoxon Signed Rank test (WSR). Spearman's rank correlation test was used for correlation studies. P values less than 0.05 were considered significant.

10.4 Results

Early and late reactions in the nose
Figure 1 shows the follow-up of inspiratory nasal resistance ($Rn_{(insp)}$) of 11 patients on the control day and after challenge 1 and 3. The initial value of $Rn_{(insp)}$ on the control day and on both allergen challenge days were defined as 0%. On the control day the mean percentage changes in $Rn_{(insp)}$ of the 11 patients on the different time points showed minor variation (-7 to 2%). The first allergen challenge resulted in an early response with a maximal percentage change in $Rn_{(insp)}$ of 287 +/- 131 % (mean value in 11 patients), measured 15 minutes after challenge. During the late phase period the maximal percentage change of $Rn_{(insp)}$ was 46 +/- 48 %, 8 hours after challenge. After the third allergen challenge the mean maximal percentage change in $Rn_{(insp)}$ during the early response was 266 +/- 254 %, 15 minutes after challenge; during the late response 26 +/- 54 %, 8 hours after challenge.
To evaluate the effect of repeated allergen challenge on the magnitude of the early and late nasal reactions, the mean percentage changes in $Rn_{(insp)}$ during early phase period (0.25-2 hours) and during late phase period (4-10 hours) were calculated for the whole group after challenge 1 and 3 (Figure 2). For the early phase (NER) the mean percentage change in $Rn_{(insp)}$ after the first challenge was 134 +/- 53%; after the third challenge 127 +/- 151%. The difference between challenge 1 and 3 was not significant. During the late phase (NLR) the mean percentage change in $Rn_{(insp)}$ after the first challenge was 24 +/- 26%; after the third challenge 13 +/- 23%. The difference between challenge 1 and 3 was not significant. The response on repeated allergen challenge showed a considerable variation between the patients.
The mean percentage change in $Rn_{(insp)}$ during "early and late phase period" on the control day was calculated; these values were -5 +/- 15% and -1 +/- 14% respectively. For the early response the mean percentage changes $Rn_{(insp)}$ after challenge 1 and 3 were significantly higher than on the control day (WSR, p=0.003; p=0.008 respectively). For the late response only the mean percentage change in $Rn_{(insp)}$ after challenge 1 was significantly higher than in the mean percentage change in $Rn_{(insp)}$ on the control day in the same period (WSR, p=0.04).

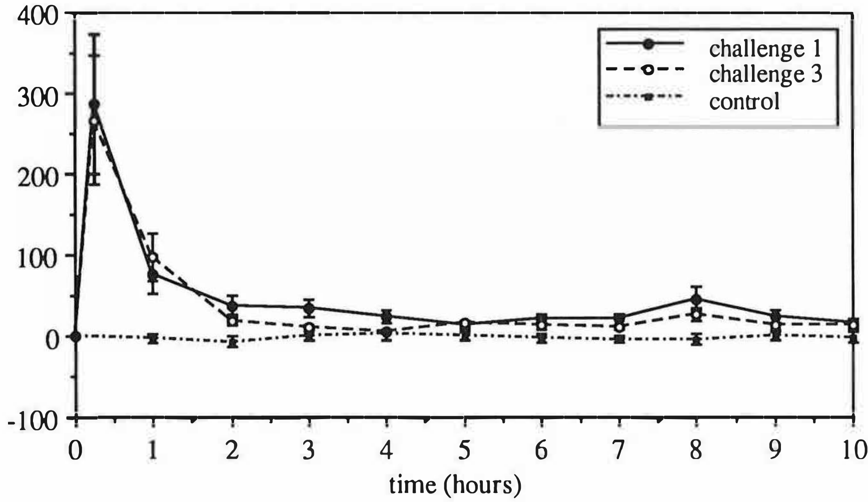

Figure 1: The effect of repeated allergen challenge on early and late allergic reaction in the nose in 11 patients (+/- SEM)

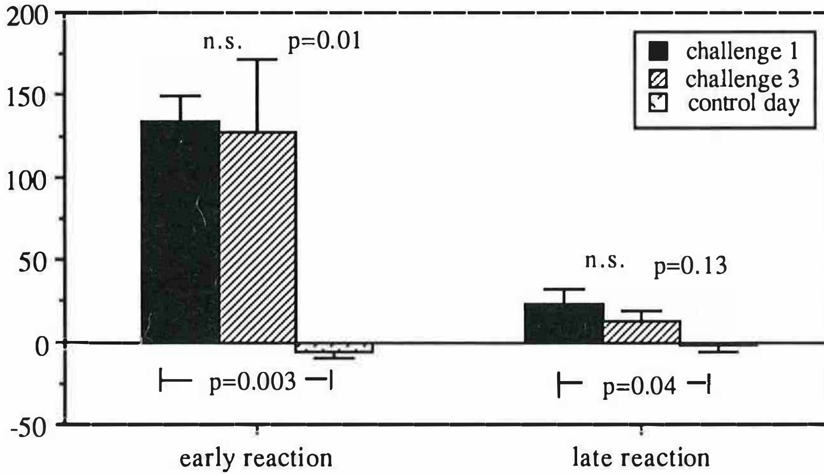

Figure 2: The effect of repeated allergen challenge in the nose on the mean percentage change in nasal resistance during early and late allergic reaction in 11 patients (+/- SEM)

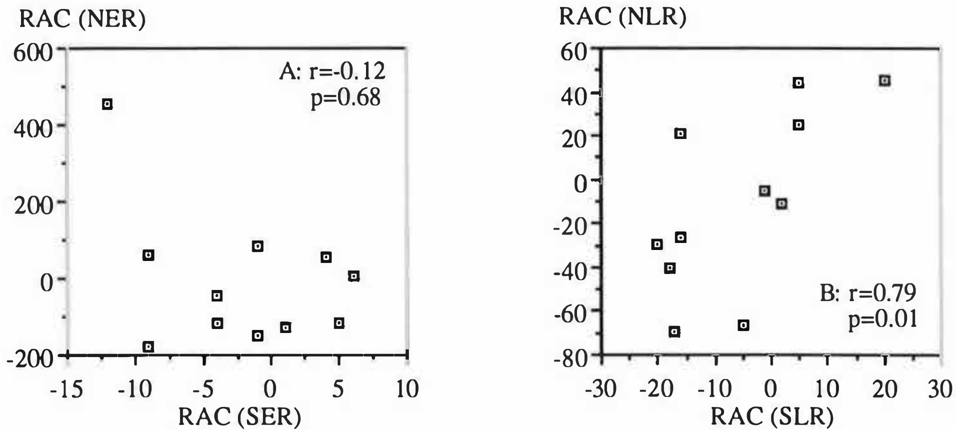

Figure 3: Correlations between the effect of repeated allergen challenge (RAC) and the reaction after challenge 1 for early (A) and late (B) response in 11 patients

A diminished response after repeated allergen challenge in patients with a strong response after the first challenge and a enhanced response in patients with a mild initial response was found. This is evidenced by a significant inverse correlation between the effect of repeated allergen challenge on the early response in the nose expressed as RAC value$_{(NER)}$ and the early reaction after the first challenge (Spearman r= -0.82, p=0.01; Figure 3a). There was also a significant inverse correlation between the effect of repeated allergen challenge on the late response in nose expressed as RAC value$_{(NLR)}$ and the late response after the first challenge (Spearman r=-0.88, p=0.005; Figure 3b).
No significant correlations were found between the provocative dose of allergen and the effect of repeated allergen challenge on the early (Spearman r= -0.18, p=0.57) and late response in the nose (Spearman r=-0.10, p=0.75)

Early and late reactions in the skin
The effect of repeated allergen challenge on early and late skin reaction is shown in Figure 4.
The mean diameter of the early skin reaction after challenge 1 was 18 +/- 6 mm; after challenge 3; 16 +/- 7. The difference between challenge 1 and 3 was not statistically significant. The mean diameter of the late reaction after challenge 1 was 18 +/- 9 mm; after challenge 3; 13 +/- 10 mm. Again the difference between challenge 1 and 3 was not statistically significant. As in the nose the response on repeated allergen challenge showed a large variation between the patients.

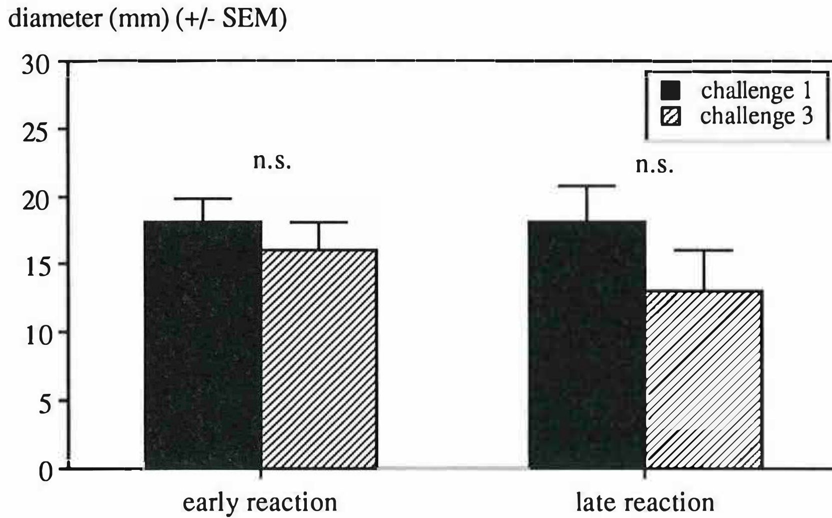

Figure 4: The effect of repeated allergen challenge on early and late allergic reaction in the skin in 11 patients (+/- SEM)

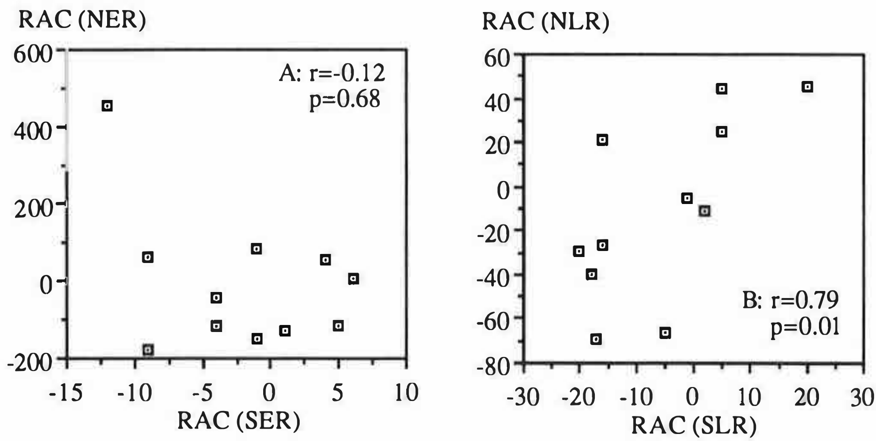

Figure 5: Correlations between the effect of repeated allergen challenge (RAC) in the nose and the skin for the early response (A) and the late response (B) in 11 patients

Correlations between the effect of repeated allergen challenge in the nose and the skin

For the late response a significant correlation was found between the effect of repeated allergen challenge in the nose (RAC $value_{(NLR)}$) and the skin (RAC $value_{(SLR)}$)(Spearman r=0.79, p=0.01; Figure 5b). No such correlation was found for the early response (Spearman r=-0.12, p=0.68; Figure 5a).

10.5 Discussion

In the present study the effect of repeated allergen challenge on early and late reaction in the nose was studied. Surprisingly repeated allergen challenge with an interval of 48 hours did not result in overall significant changes in early and late nasal responses. There was, however a large variation in individual responses to the repeated challenges, varying from strongly enhanced responses to complete diminution of the response. In order to investigate whether the variety in response to repeated allergen challenge is just a random variation or rather a characteristic of the individual patient we also performed repeated skin challenges and studied the interrelationship. The fact that we found a significant correlation between the effect of repeated allergen challenge in the nose and the skin for the late response, suggest the former.

Repeated nasal allergen challenge is often associated with "priming", which is defined as an increased nasal reactivity following repeated allergen exposure. Connell (8, 9) found that when daily challenges with ragweed were performed, lower doses of pollen were required on successive days to increase nasal airway resistance. However, these observations were based on a small number of patients. Several years later a limited number studies have used the experimental model of repeated allergen challenge. The results of these studies are difficult to compare because of large differences in the set-up of the study, such as differences in allergen doses, number of challenges, intervals between the challenges and differences in registration of the responses. Wachs et al (10) performed 3 successive daily challenges with the same allergen dose and found an increase in symptomatic response in 6 of the 10 subjects and an increase in the level of mediators such as histamine, TAME esterase, kinins and prostaglandin D2 after challenge 3 compared to challenge 1. An increased number of eosinophils and neutrophils in nasal lavages was found 24 hours after challenge 1 and 2 compared to before; there was no further increase in cell numbers after challenge 2 compared to challenge 1.

Pipkorn et al (11) found that 7 daily challenges with the same dose of allergen resulted in an increased number of eosinophils in nasal lavages 24 hours after challenge compared to before. The increased number of eosinophils was remained throughout the whole challenge period, but did not show a further increase after the successive challenges. A significant increase in composite symptom scores was reached only after provocation 5,6 and 7, whereas nasal peakflow measurements before and after challenge showed only minor differences between the challenge days.
In a study performed by Iliopoulos et al (6) rechallenge was performed 10 hours after the first challenge, using a lower dose of allergen compared to the first challenge. An enhanced reaction based on mediators after rechallenge was found in 21 of the 55 subjects; an enhanced symptomatic response was found in another 10 patients without enhancement in the levels of recovered mediators.
Although in the above studies there is evidence of priming, not all aspects of these studies support the priming hypothesis. In the studies of Wachs and Iliopoulos only a limited number of patients showed an enhanced symptomatic response to rechallenge and in the study of Pipkorn no significant effect of repeated challenges on nasal peak flow measurements before and after challenge were seen. Absence of priming was reported in the study Malmberg et al (16), in which 3 unilateral challenges with equal allergen doses were performed in 9 patients with seasonal allergy with an interval of 48 hours. The reaction after the second and the third challenge, measured as nasal obstruction, weight of nasal secretion and histamine content of the nasal secretion did not change significantly compared to the first challenge. Also Doyle et al report lack of nasal priming after repeated allergen challenge (17).
Repeated allergen challenges have also been used therapeutically in local nasal immunotherapy (LNIT), in which increasing doses of allergen are used. Beneficial effects on nasal symptom/medication scores during the season after preseasonal LNIT have been documented in several studies (18-22). Nasal allergen challenges before and after LNIT showed a significant decrease in nasal sensitivity after treatment in most studies (19-24). In the study of Passalacqua (21) the decreased nasal sensitivity was accompanied by a significant reduction in inflammatory cells in the nose. These observations seem to be in contradiction with the priming concept. Only a few studies report on an increase in specific nasal sensitivity after LNIT, a situation which is conform the priming hypothesis (18, 25, 26).

A surprising aspect in the studies of Passalacqua (21) and Nickelsen (18) is the lack of increase in nasal allergen sensitivity after the season compared to before in the placebo groups, which might indicate absence of priming due to natural exposure. Looking at individual data in Passalacqua 's study, 3/9 patients in the placebo group showed an increase in nasal sensitivity, while 4 patients showed a marked decrease in allergen sensitivity after the season compared to before.

From our data and from observations in the literature it seems that repeated or chronic allergen exposure can lead to an enhanced response (priming) or to a diminished response (down regulation). This brings up the questions what factors determine whether priming or down-regulation occurs and is the response on repeated allergen challenge a patient characteristic or just a coincidence. In the present study we found a significant inverse correlation between the reaction to the first challenge and the effect on repeated challenge for early and late responses. This indicates a diminished response in those patients who had rather strong responses to the first challenge and a priming response in those patients who had mild initial responses. This finding could not be attributed to a limited measuring range of the whole body plethysmography; higher responses could be measured in patients with strong initial responses and lower reactions could be detected in patients with mild initial responses. Maybe every person has a maximal individual level in response and after a mild initial reaction there is still room left for enhancement of the response at rechallenge, while after a strong initial reaction the maximal individual response level is reached.

In our study we could not find a significant correlation between the provocative dose of allergen and the response to repeated allergen challenge. However the occurrence of priming or down-regulation might be dependent on the dose schedule that is used in the repeated challenges: a decrease in allergen dose on successive challenges, as in Connell's experiments, may lead to priming, while increasing allergen doses, as in LNIT, may lead to down-regulation.

The mechanisms underlying priming and down-regulation are still unclear. Experimental nasal allergen challenge results in an increase in inflammatory cells in nasal washings (27, 28) and biopsies (29, 30) during late phase responses and this cellular influx might serve as a connection between late responses and enhanced rechallenge responses (priming)(6). Down-regulation of the response, which in our study especially occurs after a strong initial response, could be explained by an exhaustion of mediators or activation and attraction of inflammatory cells with suppressive characteristics.

In conclusion, repeated allergen challenge in the nose can lead to priming as well as to down-regulation of early and late responses. The type of response partly depends on the intensity of the reaction to the first challenge and seems to be a characteristic of the individual patient.
Further studies are needed to compare the response to repeated allergen challenge with the response to natural seasonal allergen exposure in the same patients and to explore possible underlying mechanisms using nasal biopsies .

10.6 Acknowledgements

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Chapter 11

Down-regulating effect of immunotherapy on repeated intradermal allergen challenge

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Submitted for publication

11.1 Abstract

In the present study we investigated the effect of allergen immunotherapy on the early and late skin reaction after repeated allergen challenge (RAC), a model that supposedly reflects the chronicity of natural allergen exposition. Three intradermal challenges with housedustmite were performed with an interval of 48 hours before and after 1 year of immunotherapy in 10 housedustmite allergic patients. Skin biopsies were taken 24 hours after the first allergen challenge before and after 1 year of immunotherapy.

Immunotherapy resulted in a significant decrease in late skin reaction, but not in early skin reaction, after the first allergen challenge. Repeated allergen challenge after immunotherapy resulted in a further significant decrease in late reaction diameter, when challenge 3 was compared to challenge 1. This was not observed for the early response. In the skin biopsies a significant increase in the percentage of CD8+ and CD25+ cells was found after immunotherapy compared to before, while there was no significant change in mean overall mononuclear cell density.

The conclusion of the study was that immunotherapy with housedustmite has a down-regulating effect on the late phase skin reaction after single and repeated allergen challenge, which may be explained by a suppressive action of CD8+ suppressor T-cells.

11.2 Introduction

In allergic patients allergen challenge in the skin results in an early reaction. This early reaction is frequently followed by a late reaction, which is characterised by inflammatory changes(1, 2). Although intradermal skin reactions after allergen challenge are often used as a model for allergen-induced reactions in the airways, these reactions are only weekly correlated(3-6). This could be due to differences in tissue characteristics or to differences in allergen exposition. In order to develop a test model that takes into account the chronicity of allergen exposition to inhalant allergen such as housedustmite (HDM), we recently studied the effect of repeated allergen challenge (RAC) on early, late and delayed skin reactions (7). Repeated allergen challenge with an interval of 48 hours resulted in a significant increase in reaction diameters, measured at 6 hours, 24 hours and 48 hours after challenge, especially after the third challenge. The increased late response was accompanied by an increasing number of inflammatory cells in 4 of the 5 patients studied. We suggested that the model of repeated allergen challenge may provide additional

information on the regulation of the allergic reaction and might therefore be useful to assess the effects of immunomodulating therapy.
Therefore in the present study we investigated the effect of systemic allergen immunotherapy on repeated allergen challenge (RAC) in the skin. In addition we studied changes in T-cell characteristics in skin biopsies taken 24 hours after the first challenge .

11.3 Patients and methods

Patients

Ten atopic, non smoking patients with mild to moderate asthma (6 female and 4 male, age 19-39 years) participated in the study. The clinical data are presented in Table 1. The patients were selected on the basis of positive skin tests (ALK Benelux, SQ-503), and/or elevated IgE (Pharmacia CAP ≥ class 2) for HDM allergen. All patients had late skin responses (≥ 10 mm) measured 6 hours after allergen challenge. All had asthma, well controlled by inhaled anti-inflammatory therapy , and FEV_1 values ≥ 70 % of the predicted value. Antihistamine drugs were stopped 48 hours before the tests and none of the patients had received oral corticosteroids within 3 months. The patients gave informed consent. The study was approved by the medical ethics committee of Asthmacenter Heideheuvel, the Netherlands.

Study design

Repeated allergen challenge was performed before and after 1 year of immunotherapy. On the first study day intradermal challenge with HDM was performed on 2 skin sites (site a and b); reactions were measured 15 minutes and 6 hours after challenge (see below). Skin challenge with histamine and phosphate buffer diluent were also performed. On the second day a skin biopsy was obtained from the allergen challenged sites (24 hours after challenge, site a). On the third study day allergen challenge with HDM was repeated on site b (48 hours after challenge 1); early and late reactions were measured. On the fifth day allergen challenge was performed on site b (48 hours after challenge 2); early and late responses were measured.
The same test protocol was repeated after one year treatment with immunotherapy.

Table 1: Clinical data of the patients

Patient	Age	Sex	FEV1 (% pred)	Skin reaction to HDM early (mm)	Skin reaction to HDM late (mm)	Medication
1	21	F	92	15	15	B,S,N
2	39	F	87	23	25	C,S,N
3	24	F	99	15	19	B,S
4	23	M	96	20	23	B,S,N
5	20	F	103	18	18	B,N
6	19	M	84	19	17	B,S,N
7	25	M	102	24	21	B,S,N
8	21	F	105	21	19	B,S,N
9	23	M	89	20	27	B,C,S
10	33	F	89	19	19	B,N
mean	25		95	19	20	
SD	6		7	3	4	

B: budesonide of beclomethasone; C: cromoglycate; S: salbutamol; N: nasal steroids

Skin tests

Intradermal challenges (30 BU/ml, 0.03 ml) were performed at the back of the patients with a standardised HDM extract (ALK Benelux, SQ-503). The early response was scored as the wheal diameter 15 minutes after challenge. Late responses were measured 6 hours after challenge: indurations were determined according to a procedure suggested by Sokal (8). The reaction on histamine and phosphate buffer diluent served as positive and negative controls. The reaction to repeated allergen challenge (RAC) was expressed as the difference between the reaction diameter after challenge 3 and after challenge 1 (RAC value).

Skin biopsies

Skin biopsies, taken 24 hours after allergen challenge, were obtained before and after 1 year of immunotherapy using a 5 mm disposable punch. Local anaesthesia was performed with 2 % lidocain. Biopsy specimens for immunohistology were snap frozen in liquid nitrogen and seven-micrometer sections were cut on a freezing

microtome and mounted on 3-aminopropyl tri-ethoxy silaan (Sigma A3648) coated slides.

Immunostaining

The following set of monoclonal mouse antibodies was used: CD3 (Leu 4, Becton Dickinson 668, 1:50), CD4 (Dako, M716, 1:20), CD8 (Dako M707, 1:50), CD25 (Dako M731, 1:50), HLA-Dr (Becton Dickinson, 1:50).
Sections were fixed with dry acetone (p.a.Sigma) for 7 ', air dried, and pre-incubated for 20 ' in 10% Normal Horse Serum (NHoS), 10% Normal Human Serum (HMuS), in PBS, PH 7.4). Primary antibodies were diluted in PBS with 1% NHoS/NHuS, and incubated with the sections for 60'. Slides were washed (3x5', PBS + 0.05% Tween 20 (Sigma P-1379)), after which a second layer of biotinilated Horse-anti Mouse (Ham-bio) (IgG (H+L) Vector BA-2000, 1:800 in PBS 1% NMoS/NHuS) was applied for 30'. After washing with PBS/Tween (3 x, 5') the sections were incubated with Streptavidine conjugated Alkaline Phosphatase (Str-AP) (Dako, D396, 1:300 in PBS 1% NHuS) for 30', and washed in Tris-HCl (3 x, 5' 0.1M, PH 8.5). Alkaline Phosphatase reactivity was demonstrated using Naphtol AS-BI Phosphate (sodium salt, 50 mg/100 ml, Sigma N-2250) as substrate and New Fuchsine (10 mg/100 ml, Merck 1358) as chromogen dissolved in 0.1M Tris-HCl, PH 8.5, resulting in pink/red staining. Endogenous AP activity was inhibited by addition of Levamisole (35 mg/100 ml, Sigma L-9756) to the reaction mixture. Slides were thereafter lightly counterstained with haematoxiline and embedded in gelatine.
Quantification: Two sections of each biopsy were stained with each monoclonal antibody. The mononuclear cell infiltration was scored semiquantitatively using a score from 1+ to 4+. Sections were scored in triplo by 2 investigators independently in a blind fashion. To determine the percentage of CD4+, CD8+, CD25+ and HLA-DR+ cells, differential counts on 100 mononuclear cells were performed 3 times on each section .

Immunotherapy protocol

Immunotherapy was performed with a standardised aluminium-absorbed depot extract (SQ 503, ALK Benelux). Subcutaneous injections were given weekly with increasing doses. When the highest tolerated dose was reached, the interval between the injections was extended till 4 weeks. Before every injection the clinical status of the patient was evaluated. After the injection patients stayed in the hospital under supervision for at least 30 minutes.

Data analysis.
Data were evaluated with Wilcoxon signed rank test (WSR). P values smaller than 0.05 were considered significant.

11.4 Results

Macroscopic appearance of skin reactions.

- Early skin reaction after the first challenge.
  Before immunotherapy the mean diameter of the early skin reaction 15 minutes after the first challenge was 19 +/- 3 mm in the 10 patients tested. After immunotherapy the mean reaction diameter was 16 +/- 3 mm. Immunotherapy did not result in a significant difference in early skin reaction (WSR; p=0.08, Figure 1a).
- Late skin reaction after the first challenge.
  Immunotherapy resulted in a significant decrease in late skin reaction, measured 6 hours after challenge, compared to before. The mean reaction diameter before immunotherapy was 20 +/- 4 mm; after immunotherapy 12 +/- 2 mm (WSR; p=0.005, Figure 1b).
- Early skin reaction after repeated allergen challenge (RAC).
  Before immunotherapy RAC in 10 patients resulted in an increase in mean reaction diameter (RAC value before) of 1.5 +/- 2.6 mm. There was no significant difference between reaction 1 and 3. After immunotherapy RAC resulted in a decrease in mean reaction diameter (RAC value after) of -1.4 +/- 2.8 mm. The difference between reaction 1 and 3 was not statistically significant. The difference between the mean RAC value before and after immunotherapy was not statistically significant (WSR; p=0.07, Figure 2a).
- Late skin reaction after repeated allergen challenge (RAC).
  Before immunotherapy RAC resulted in an increase in mean reaction diameter (RAC value before) of 3.4 +/- 5.9 mm. There was no significant difference between reaction 1 and 3. After immunotherapy RAC resulted in a decrease in mean reaction diameter (RAC value after) of -4.3 +/- 3.8 mm. There was a significant difference between reaction 1 and 3 (WSR: p=0.005). The difference between the mean RAC value before and after immunotherapy was also statistically significant (WSR; p=0.005, Figure 2b).

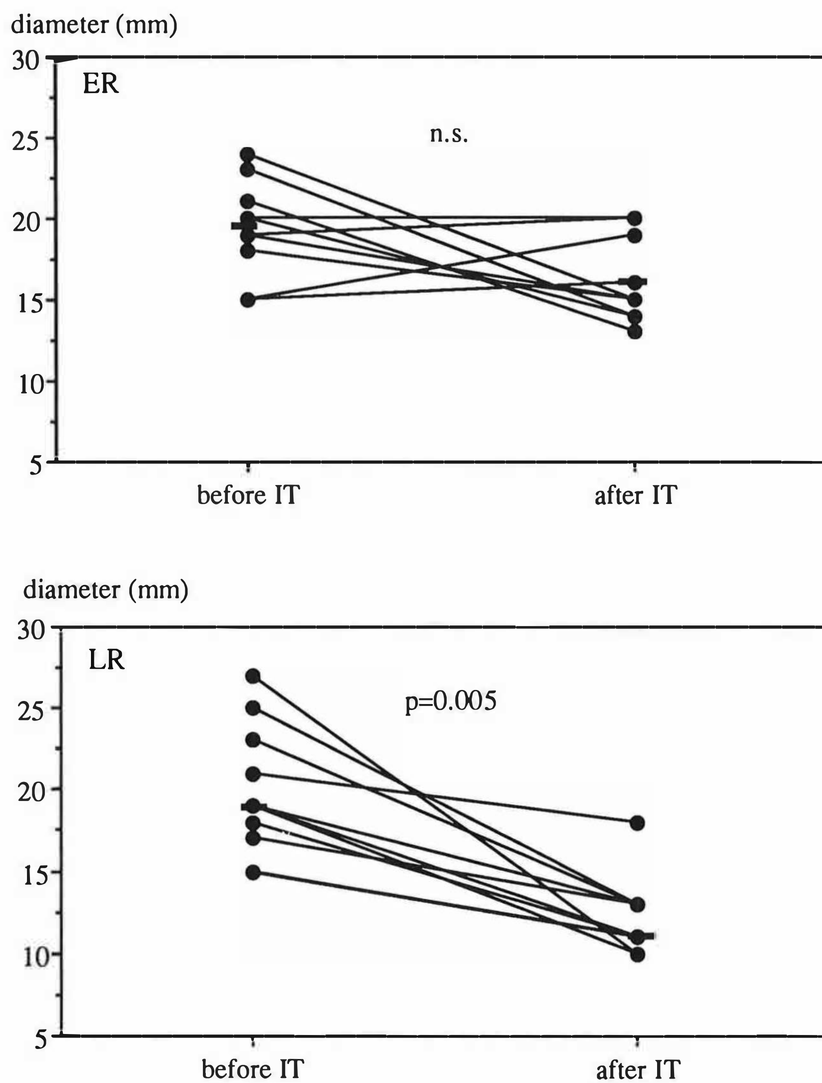

Figure 1: Skin reaction after the first allergen challenge before and after 1 year of immunotherapy. ER: Early reaction. LR: Late reaction

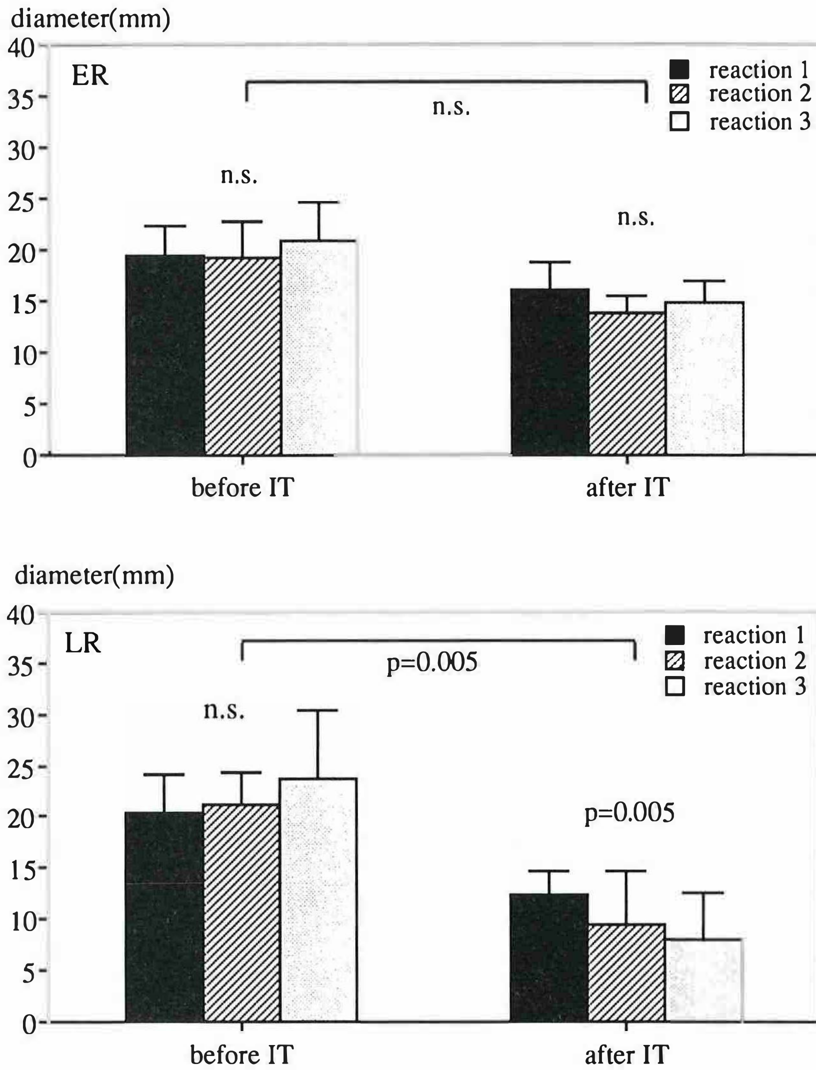

Figure 2: Skin reaction (+/- SD) after repeated allergen challenge (RAC) before and after 1 year of immunotherapy. ER: Early reaction. LR: Late reaction

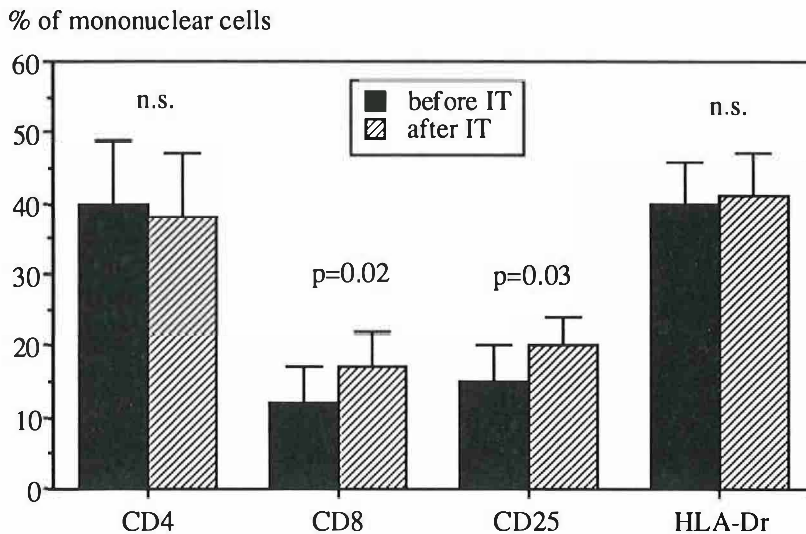

Figure 3: The effect of immunotherapy on the quality of the cellular infiltrate in skin biopsies taken 24 hours after allergen challenge (+/- SD).

Microscopic appearance of the skin reactions

In the skin biopsies, taken 24 hours after the first challenge, no significant change in mean overall density of cellular infiltration was found after immunotherapy compared to before. Immunotherapy did not result in significant changes in the mean percentages CD4+ cells (before IT 40 +/- 9 %; after IT 38 +/- 9 %) and HLA-Dr+ cells (before IT 40 +/- 6 %; after IT 41 +/- 6 %). After immunotherapy a significant increase was found in the mean percentage of CD8+ cells (before IT 12 +/- 5 %; after IT 17 +/- 5 %; WSR p=0.02) and CD25+ cells (before IT 15 +/- 5 %; after IT 20 +/- 4 %; WSR p=0.03) compared to before (Figure 3). A significant decrease in CD4+/CD8+ ratio was found after immunotherapy compared to before (before IT 3.77 +/- 1.87; after IT 2.54 +/- 1.32; WSR p< 0.05).

11.5 Discussion

In the present study the effect of immunotherapy on the early and late skin reaction after single intradermal allergen challenge and after repeated allergen challenge (RAC) was studied. In addition we studied T cell patterns in these reactions.

After single allergen challenge, we found a significant reduction in late skin reaction, but not in early reaction. Although studies concerning the effect of immunotherapy on the early skin reaction are controversial (9-12), a significant decrease in late phase

allergic response in the skin after immunotherapy was found by most authors (9-13). In the nose (11) and lower airways (14, 15) immunotherapy is also associated with a significant decrease in late phase allergic reactions after allergen challenge.
In the present study we also investigated the effect of immunotherapy on repeated allergen challenge in the skin, because we speculated that this test model more takes into account the chronic nature of natural allergen exposure (7). When performing RAC we choose an interval of 48 hours, since the macroscopic reaction has already declined at this time point, whereas inflammatory cells are still present (1, 2). Repeated allergen challenge in the skin before immunotherapy resulted in a slight increase in early reaction diameter and a more pronounced increase in late reaction diameter, however the differences between reaction 1 and reaction 3 were not statistically significant. Our findings concerning the early reaction after repeated allergen challenge are in accordance with the findings reported by Shaikh et al (16) and Andersson et al (17). In an earlier study from our group (7) we performed 4 repetitive allergen challenges with an interval of 48 hours in 13 housedustmite allergic patients. In that study we also did not find significant changes in early reaction, however a significant increase in late phase reaction was found, measured 6, 24 and 48 hours after challenge. The increase in reaction diameter was most pronounced at 6 hours and after the third challenge. Although in the present study the late reaction diameter after repeated challenge measured 6 hours after challenge tended to increase, the differences between challenge 1 and 3 did not reach significance (P=0.08). The reason for the lack of significance in this study could be attributed to the power of the study or the patient selection.
After immunotherapy the late phase skin reaction diameter showed a highly significant decrease after repeated allergen challenge (RAC). This finding may provide interesting additional information about the change in regulation of the inflammatory response after immunotherapy. After treatment all patients had a diminished, but still visible late phase reaction after the first challenge, suggesting the infiltration and activation of inflammatory cells. The fact that rechallenge in all patients led to a decrease in response suggest suppressive activity of these cells. In order to elucidate possible regulatory mechanisms that are responsible for the change in reaction pattern to RAC after immunotherapy, skin biopsies were taken 24 hours after the first challenge. Despite the fact that the macroscopic late reaction was inhibited, we could not find quantitative changes in the mononuclear cell infiltrate. This finding is in accordance with the study of Nish et al (18) who studied skin biopsies taken 8 hours after intradermal challenge before and after 6 months

treatment with immunotherapy. The fact that a decrease in late skin reaction do not coincide with quantitative changes in cell infiltrate in the skin biopsies is not surprising. Skin biopsies in our study and in the study of Nish et al were taken at the centre of the late allergic reaction. Although immunotherapy results in a diminished expansion of the late reaction, this does not necessarily imply quantitative changes in cell infiltrate at the centre of the reaction. Such changes in cell infiltrate could be expected in biopsies, taken in the peripheral area of the reaction. However it is possible that the type and activity, rather than the number of the infiltrating cells in the centre of the reaction determines the expansion of the reaction and the response to repeated allergen challenge.

In our study we found a small, but significant increase in the percentage CD8+ cells in the skin biopsies after immunotherapy compared to before. Although in the past several studies have associated allergen immunotherapy with an increase in CD8+ suppressor T cells in the peripheral blood (19-21), this is the first study in which an increase in CD8+ cells after immunotherapy was found in the allergen challenged skin. Recent studies indicate that a subset of CD8+ T cells, bearing δγ–receptor, can produce IFNγ and IL2 (22-25). The increase in CD25+ cells in our study provides indirect evidence for an enhanced production of IL2 in the skin biopsies after immunotherapy. Evidence for an enhanced production of IL2 and INFγ comes from a study of Varney et al (26), in which a significant reduction of the late skin reaction after immunotherapy was associated with an enhanced expression of mRNA for IL2 and INFγ in skin biopsies, taken 24 hours after challenge. In this study a significant reduction in the recruitment of CD3+ and CD4+ cells and a significant increase in CD25+ cells and HLA-Dr+ cells was found, whereas there was no significant difference in the number of CD8+ cells in the allergen-challenged sites between the treatment and placebo group. Apart from differences in the set-up of the study and type of allergen studied (housedustmite vs grasspollen) , a major difference between our study and the study of Varney et al is the amount of allergen that was used for intradermal skin testing. In our study we injected an allergen solution, containing 0.9 BU (30 BU/ml), while in the study of Varney et all an allergen solution containing 30 BU was used. This difference in amount of allergen injected in the skin (> 30 times) is reflected by far more pronounced late phase reactions after challenge in the study of Varney et al.

In the present study we demonstrated the down-regulatory effect of allergen immunotherapy on the late phase skin reaction in two different ways: Firstly all patients showed a decrease in late phase skin reaction after immunotherapy

compared to before; secondly all patients showed a further down-regulation of the reaction after rechallenge. Although we found a significant increase in the percentages of CD8+ cells and CD25+ cells in the skin biopsies after immunotherapy, it seems unlikely that these small differences alone are responsible for the large changes in macroscopic appearance of the late skin reaction after single and repeated challenge. Probably changes in cell activity rather than changes in cell numbers are responsible for the observed changes in late phase reaction after immunotherapy . Therefore further studies, in which immunohistology and in-situ hybridisation on cytokines are combined are needed to elucidate the mechanism of allergen immunotherapy.

11.6 Acknowledgement

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Chapter 12

Can skin testing predict the effect of allergen immunotherapy on allergic reactions in the airways?

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12.1 Abstract

The *aim* of the present study is to investigate the relationship between the effect of immunotherapy on allergic reactions in the airways compared to the skin.

Methods: Bronchial- and skin challenges with housedustmite were performed in 10 housedustmite allergic patients with asthma before and after 1 year of immunotherapy with a standardised housedustmite extract. Early and late allergic reactions in airways and skin were registered. Bronchial challenges with histamine were also performed before and after treatment to study the effect of immunotherapy on airway responsiveness.

Results: Immunotherapy resulted in a significant decrease in early and late allergic reactions in the airways and in late skin reaction. After immunotherapy there was a small, but statistically significant increase in PC_{20} histamine compared to before treatment. No significant correlation was found between the effect of immunotherapy on early or late allergic reactions in the airways compared to skin reactions. The effect of immunotherapy on allergic reactions in the airways could not be predicted from effects on early and late allergic reactions in the skin, corrected for effects on PC_{20} histamine.

Conclusion: The effect of immunotherapy on allergic reactions in the airways can not be predicted from effects on early or late skin reaction.

12.2 Introduction

Bronchial challenge tests with allergens are often used to evaluate the anti-inflammatory effect of allergen immunotherapy in asthmatic patients. Especially effects on the late asthmatic reaction have been studied, since the pathogenesis of this reaction is more closely related to chronic asthma than is the pathogenesis of the early reaction (1-3). Several authors have shown that immunotherapy is effective in reducing the late asthmatic reaction (4-6). However bronchial allergen challenge tests are time consuming and may be unpleasant or even dangerous to the patients. In addition it is difficult and invasive to obtain tissue from challenged sites for studies on mechanisms underlying allergen immunotherapy. Thus the late allergic skin reaction is often used as a model for the late allergic reaction in the airways. Although it is known that the severity of allergic reactions in skin and airways are not correlated (7), this does not imply that the effect of immunotherapy on airways and skin is not correlated. A reduction in late allergic skin reaction after immunotherapy was found by several authors (8-12), however there are no data on interrelationship between the effect of immunotherapy on late phase reactions in skin and airways. Therefore the

aim of the present study was to investigate the correlation between the effect of immunotherapy on the allergic reactions in the airways compared to the skin. Because airway responsiveness to non-specific stimuli also plays an important role in the response to allergen in the lower airways, we also to tried to predict the effect of immunotherapy on the allergic reactions in the airways from effects on skin reactions corrected for effects on PC_{20} histamine.

12.3 Patients and methods

Patients

Ten atopic, non smoking patients (6 female and 4 male, age 19-39 years) participated in the study. The clinical data are presented in Table 1. All patients had asthma, well controlled by inhaled anti-inflammatory therapy, and had FEV_1 values ≥ 70 % of the predicted value. The PC_{20} histamine values were ≤ 8 mg/ml (30 second method). The patients were selected on the basis of positive skin tests (ALK Benelux, SQ-503), and/or elevated IgE for housedustmite (HDM) allergen (Pharmacia CAP ≥ class 2). None of the patients gave a history of respiratory tract infections in the previous six weeks or severe asthmatic attacks in the previous six months. None had received oral corticosteroids. All the patients gave informed consent. The study was approved by the medical ethics committee of Asthmacenter Heideheuvel, the Netherlands.

Histamine challenge

Histamine phosphate solutions (doubling concentrations from 0.25 to 32 mg/ml) were administered through a De Vilbiss 646 nebulizer with a gauged output of 0.13 mg/ml. The nebulizer was mounted to a valvebox with aerosol filter. The nebulation time was 30 seconds, during which the patient was instructed to breath quietly. The test was started with inhalation of a phosphate buffer aerosol. Prior to the inhalation three measurements of VC and FEV_1 were performed using the portable electronic Printer Spirometer II (Micro Medical Ltd, Rochester, Kent England): Calibration of the spirometer was performed every two weeks. No adjustments were necessary. After each concentration the FEV_1 value was measured. The PC_{20} histamine was derived by linear interpolation.

Table 1: Clinical data of the patients

Patient	Age	Sex	FEV1 (% pred)	PC_{20} histamine mg/ml	Early skin reaction (mm)	Medication
1	21	F	92	1.65	15	B,S,N
2	24	F	99	3.00	15	B,S
3	23	M	96	3.25	20	B,S,N
4	39	F	87	3.99	23	C,S,N
5	20	F	103	7.74	18	B,N
6	19	M	84	0.82	19	B,S,N
7	26	F	98	5.50	19	B,S,N
8	25	M	102	0.84	24	B,S,N
9	23	M	89	3.76	20	B,C,S
10	35	F	89	1.74	19	B,N
mean	26		94	3.23	19	
SD	7		7	2.18	3	

B: budesonide or beclomethasone; C: cromoglycate; S: salbutamol; N: nasal steroids

Bronchial allergen challenge

Allergen solutions were prepared from stock solutions of *Dermatophagoides pteronyssinus* , diluted in PBS with 0.03 % human serum albumin and containing 0.5 % phenol (SQ 503 resp SQ 293, ALK Benelux) to produce a range of fivefold increasing concentrations from 80 to 10.000 BU/ml. The allergen solutions were administered through a De Vilbiss nebulizer mounted to a valvebox with aerosol filter (output 0.13 mg/ml, see histamine challenge). The patients were instructed to breath quietly during one minute for each dose. Increasing doses were given at 15 minute intervals. The challenge procedure was terminated when the FEV_1 value fell ≥ 15 % below baseline value. After the last inhalation FEV_1 was recorded at 10-minute intervals for the first hour and every hour thereafter until 10 hours after the last inhalation. FEV_1 values were corrected for diurnal variation; a fall in FEV_1 value ≥ 15 % from baseline value between 3-10 hours after challenge was considered as a late phase reaction.

The early and late asthmatic reactions were expressed by the area above the FEV_1 curve during early (0.25 -1 hours) and late (3-11 hours) reaction (AAC (FEV_1)) .

In order to investigate the relationship between the magnitude of the allergic reaction in the skin and the lower airways before immunotherapy, asthmatic reactions before

immunotherapy were also expressed as PD_{20} allergen for the early response and PD_{20} allergen for the late response (13).

Skin tests

Intradermal challenges (30 BU/ml, 0.03 ml) were performed at the back of the patients with a standardised HDM extract (ALK Benelux, SQ-503). The early response was scored as the wheal diameter 15 minutes after challenge. Late responses were measured 6 hours after challenge: indurations were determined according to a procedure suggested by Sokal (14). The reaction on histamine and phosphate buffer diluent served as positive and negative controls.

Study design

Subjects were admitted to the hospital for 3 successive days. Seventy-two hours before admission histamine challenge was performed to determine the PC_{20} histamine. Medication was withheld before the study period: Inhaled steroids and sodium cromoglycate were stopped one week before the study; theophyllines, oral β_2-adrenergic drugs and antihistamines were stopped 48 hours before the study; inhaled β_2-adrenergic drugs 12 hours before the tests. On the first admission day control solution was inhaled four times at 15-minute intervals. FEV_1 was measured immediately and 15 minutes after each inhalation. After the fourth inhalation, FEV_1 was recorded at 10-minute intervals for the first hour and was followed every hour until 11 hours after the last inhalation. Intradermal challenge with HDM was performed; reactions were measured 15 minutes and 6 hours after challenge . Skin challenge with histamine and phosphate were also performed. The second day subjects underwent the allergen challenge with HDM. Spirometry was performed as on the first day.
The same test protocol was repeated after one year treatment with immunotherapy (see below).

Immunotherapy protocol

Immunotherapy was performed with a standardised aluminium-absorbed depot extract (SQ 503, ALK Benelux). Subcutaneous injections were given weekly with increasing doses. When the highest tolerated dose was reached, the interval between the injections was extended till 4 weeks. Before every injection the clinical status of the patient was evaluated. After the injection patients stayed in the hospital under supervision for at least 30 minutes.

Data analysis
Comparisons were made with Wilcoxon signed rank test (WSR). Spearman's rank correlation test was used for correlation studies. P values less than 0.05 were considered significant. Multiple regression analysis was used to predict the effect of immunotherapy on allergic reactions in the airways from effects on allergic skin reactions and PC_{20} histamine.

12.4 Results
Early and late allergic reaction in the lower airways
Figure 1 shows the effect of immunotherapy on the early and late bronchial response to allergen. Before immunotherapy allergen challenge with housedustmite resulted in an early response in all patients (mean value of the area above the FEV_1 curve (AAC (FEV_1) (0.25-1 hour)): 23 +/- 6 %.hour). A detectable late reaction was present in 9 of the 10 patients. The mean value of the AAC (FEV_1) (3-11 hour) in all patients was 145 +/- 77 %.hour). The PD_{20} allergen for early and late reactions were respectively 4.04 +/- 0.44 BU/ml and 3.84 +/- 0.87 BU/ml (log transformed data).
After immunotherapy an early reaction was detectable in 3 patients; in all patients the AAC (FEV_1) was 8 +/- 11 %.hour. A late reaction was detectable in 5 patients; in all patients the AAC (FEV_1) was 43 +/- 62 %.hour. Immunotherapy resulted in a significant decrease in early allergic reaction (WSR, p=0.01) and in late allergic reaction (WSR, p=0.03) (Figure 2).

Early and late allergic reaction in the skin
Before immunotherapy the mean diameter of the early reaction was 19 +/- 3 mm; the diameter of the late reaction was 20 +/- 4 mm. After immunotherapy the mean diameter of the early reaction was 16 +/- 3 mm and the diameter of the late reaction was 13 +/- 2 mm. Immunotherapy resulted in a significant decrease in late reaction diameter (WSR, p=0.005), but not in early reaction diameter (WSR, p=0.08)(Figure 3).

Bronchial responsiveness
The effect of immunotherapy on PC_{20} histamine was evaluated in 10 patients. Before immunotherapy the mean PC_{20} histamine was 3.23 +/- 2.18 mg/ml. After 1 year of immunotherapy this value was 4.58 +/- 1.21 mg/ml. Immunotherapy resulted in a small, but statistically significant increase in PC_{20} histamine (WSR, log transformed data $p< 0.05$: Figure 4).

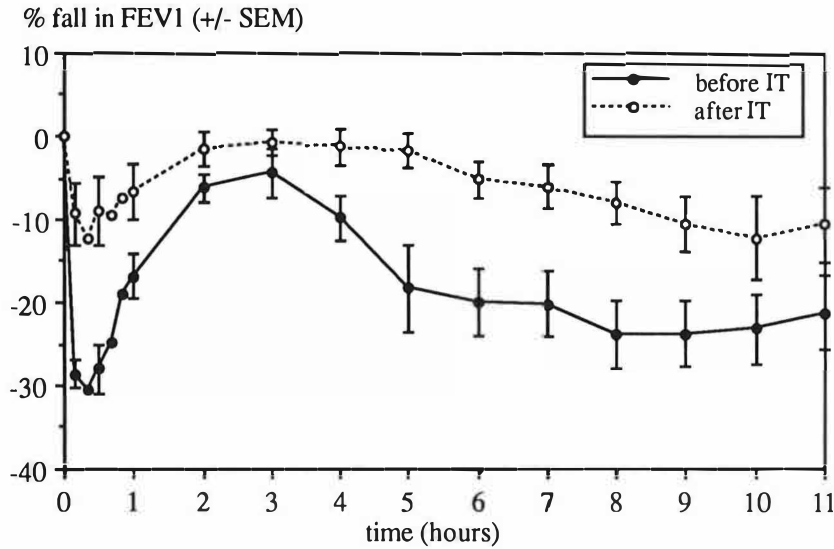

Figure 1: The effect of immunotherapy on early and late allergic reactions in the airways (N=10) .

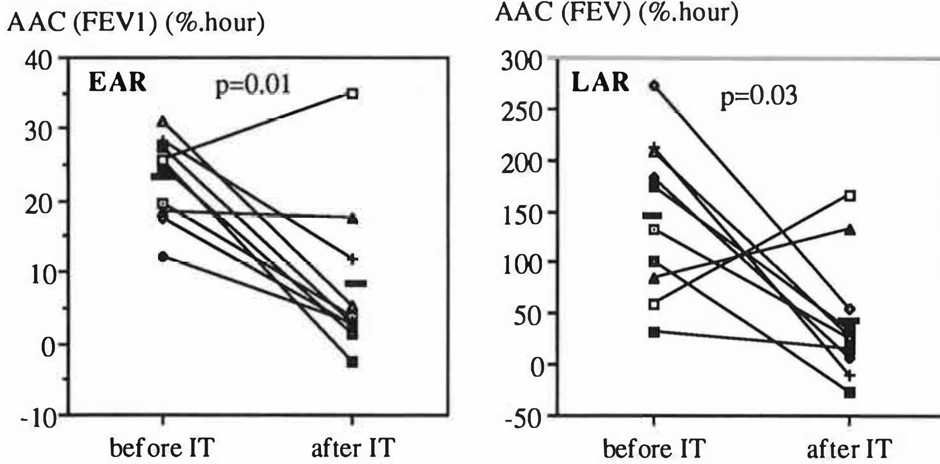

Figure 2: The effect of immunotherapy on early (EAR) and late (LAR) allergic reactions in the airways, expressed as area above the FEV_1 curve (AAC (FEV_1)) (N=10).

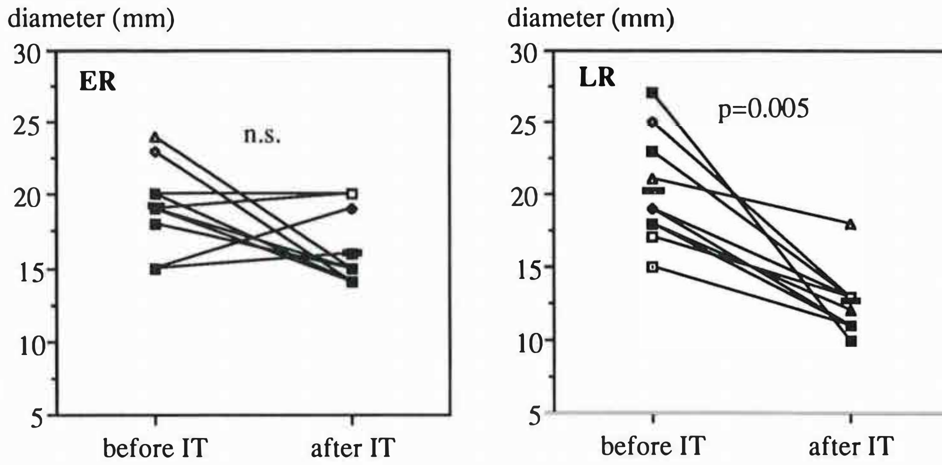

Figure 3: The effect of immunotherapy on early (A) and late (B) allergic reactions in the skin (N=10).

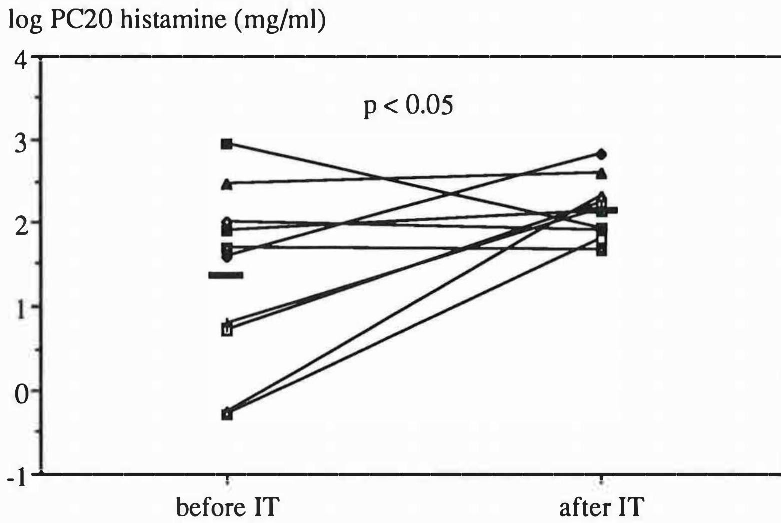

Figure 4: The effect of immunotherapy on the PC_{20} histamine (log-transformed data)(N=10)

Correlations

When comparing the severity of the early allergic reactions in the airways (expressed as PD_{20} allergen) and the skin, no significant correlation was found (r=-0.61; p=0.07). This was also true when the severity of late responses in airways (expressed as PD_{20} allergen) and skin were compared (r=-0.67; p=0.06). A significant correlation was found between the severity of the early reaction in the airways and the late skin response (r=-0.76; p=0.02)(Table 2A).

No significant correlation was found between the effect of immunotherapy on the early reaction in airways (expressed as area above the FEV_1 curve) compared to the skin (Spearman, r=0.38; p= 0.26). This was also true for the late reaction (Spearman, r=0.34; p=0.31)(Table 2B). When the 2 patients who did not show a reduction in early and late asthmatic responses (Figure 2) were excluded in the analysis, there was still no significant correlation for early (r=0.37; p=0.33) and late (r=0.14; p=0.71) reactions. Multiple regression with the allergic reaction in the airways as dependent variable and the allergic skin reaction and the PC_{20} histamine as independent variables resulted in a p-value of 0.42 for the early allergic reaction and a p-value of 0.41 for the late response.

Table 2:
A: Correlations between the severity of early and late allergic reactions in the airways (expressed as PD_{20} allergen) and skin (mm)
B: Correlations between the effect of immunotherapy on early and late allergic reactions in the airways (expressed as area above the FEV_1 curve) and skin (mm)

A

		AIRWAYS	
		ER	LR
SKIN	ER	R=-0.61 p=0.07	R=-0.39 P=0.27
	LR	R=-0.76 p=0.02	R=-0.67 p=0.06

B

		AIRWAYS	
		ER	LR
SKIN	ER	R=0.38 p=0.26	R=0.43 p=0.19
	LR	R=0.22 p=0.51	R=0.34 p=0.31

12.5 Discussion

In the present study immunotherapy resulted in a significant decrease in early and late allergic reaction in the lower airways compared to before treatment. These findings are in accordance with previous studies (4-6, 15). In addition we found a small, but significant increase in PC_{20} histamine, indicating a decrease in histamine-induced airway responsiveness. Beneficial effects of immunotherapy on non-specific airway responsiveness has been described earlier (16). A reduction in late skin reaction after immunotherapy, as we found in the present study, has also been described by several authors (8-12). Although some authors report on associations between reduction of late skin response and clinical improvement (10, 12), there are until now no data on correlations of the effect of immunotherapy on allergic bronchial responses in comparison with skin responses.

In the present study we investigated the relationship between the effect of immunotherapy on allergic reactions in the airways and skin. Although we found that immunotherapy was effective in reducing allergic reactions in both airways and skin, there was no significant correlation between the effect in both organs. This lack of correlation suggests a limited role for the skin in monitoring the effect of immunotherapy in the airways.

A possible explanation for the low correlation between the effect of immunotherapy on allergic reactions in the airways in comparison with the skin is that in the airways the response to allergen is co-determined by the degree of non-specific airway responsiveness, which in turn can be influenced by immunotherapy (16).Therefore we also measured the airway responsiveness to histamine before and after immunotherapy. However it was not possible to predict the effect of immunotherapy on allergic reactions in the airways from effects on allergic skin reactions corrected for effects on PC_{20} histamine.

Apart from non-specific stimuli, the airways are constantly exposed to specific stimuli, such as housedustmite, probably resulting in an individual degree of inflammation. This in contrast to the non-disrupted skin. Therefore the baseline condition in the airways before experimental allergen challenge is different from the situation in the intact skin and this difference in baseline condition could be one of the factors that might explain the different effect of allergen immunotherapy in both organs.

In the present study we also investigated the relationship between the severity of allergic reactions in airways and skin before immunotherapy. We expressed early and late airway reactions as PD_{20} allergen, since in the bronchial challenge tests different allergen doses were used, dependent on the reaction of the patient, while in the skin

all patients received the same amount of allergen. Thus the bronchial response was calculated corrected for differences in allergen dose (PD_{20}). Although both early and late reactions in the airways compared to the skin seems to be related (r=-0.61; r=-0.67 respectively) there was no significant correlation. Only the correlation between early reaction in the airways and late skin reaction was significant.

Mosbech et al (7) studied correlations between early phase allergic reactions in different organs in 50 housedustmite allergic patients with asthma. In this study the allergen sensitivity of the skin and the mucosa of the lower airways, nose and conjunctiva were only weekly correlated. In Mosbech's study the bronchial response was expressed as the allergen dose that elicited a positive response, and not as PD_{20} allergen. The used parameter does not give information about the strength of the reaction. Boulet et al (17) studied the relationship between late allergic reactions in skin and airways. Although the authors observe a correspondence between the tendency to develop late allergic skin- and late airway responses, there are no data on correlations between the magnitude of these reactions. In a study of Cockcroft et al (18) the PD_{20} allergen for the early response was predicted from PC_{20} histamine and skin sensitivity; also in this study there are no data on correlations between bronchial- and skin reactivity to allergen.

In conclusion: Immunotherapy resulted in a significant decrease in early and late allergic reaction in the airways and in a significant decrease in late skin reaction. There was no significant correlation between the effect of immunotherapy on allergic reactions in both organs. The effect of immunotherapy on allergic reactions in the airways could also not be predicted from effects on allergic skin reactions corrected for effects on PC_{20} histamine.

12.6 Acknowledgement

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Chapter 13

Can skin testing predict the effect of allergen immunotherapy on allergic reactions in the nose?

Interrelationship between allergic reactions in nose and skin.

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13.1 Abstract
Objective: The aim of the present study was to investigate correlations between the effect of immunotherapy on allergic reactions in the nose and the skin.
Methods: Eleven grasspollen allergic patients with seasonal rhinitis were studied. Repeated nasal challenges were performed three times with an interval of 48 hours. Reactions were registered until 10 hours after challenge, using whole body plethysmography. Repeated intradermal challenges with grasspollen were also performed three times with the same interval; early and late reactions were recorded. After one year of treatment with systemic immunotherapy using a standardised grasspollen extract, the same challenge tests were repeated.
Results: One year treatment with immunotherapy resulted in a significant decrease in early and late allergic reactions in nose and skin. No significant correlations were found between the effect of immunotherapy on allergic reactions in the nose and the skin for early and late responses. When using the model of repeated allergen challenge, the effect of immunotherapy on the late phase response pattern to repeated allergen challenge in the skin was significantly correlated with the effect of immunotherapy on early and late phase response pattern after repeated allergen challenge in the nose.
Conclusion: Immunotherapy is successful in reducing early and late allergic reactions in nose and skin, however the effects in both organs are not significantly correlated. The late skin response to repeated allergen challenge may be a useful parameter in evaluating the effect of immunotherapy in the nose.

13.2 Introduction
Allergen immunotherapy has proven to be clinically effective in patients with allergic rhinitis caused by grass-, ragweed- and tree pollen (1-9). Recent double-blind placebo-controlled studies have also shown efficacy in perennial rhinitis (10-12). Evidence of anti-inflammatory properties of immunotherapy has been proven by a reduction of the allergen-induced late phase reaction in the lower airways (13-15) and skin (8, 16-19). Because in the nose late phase responses after allergen challenge are difficult to quantify, most immunotherapy studies in allergic rhinitis patients have focused on effects on the late allergic reaction in the skin. The magnitude of allergic reactions in nose and skin are only weekly correlated (20, 21). This does, however, not imply that the effect of immunotherapy on allergic reactions in both organs is not correlated. At this moment there are no data whether a immunotherapy-induced

reduction in late phase skin response has a predictive value for the effect of immunotherapy on allergic reactions in the nose.
In the present study the correlations between the effect of immunotherapy on allergic reactions in nose and skin were investigated using 2 different challenge models. Firstly we studied the effect of immunotherapy on allergic reactions in nose and skin after a single allergen challenge; in addition we used a model of repeated allergen challenge, because this model might better reflect the chronic nature of allergen exposure (22)(chapter 9). The effect of immunotherapy on the response pattern to repeated challenge in the nose and skin was compared. For the quantification of allergic reactions in the nose we used a recently developed, non-invasive and sensitive method (23).

13.3 Patients and methods

Patients

Eleven atopic, non-smoking grasspollen allergic patients (4 male, 7 female, age 21-55 years) participated in the study. Eight of the 11 patients also had asthma. The clinical data are presented in Table 1. All patients had positive skin tests (≥ ++) and/or elevated specific IgE (Pharmacia Cap > 0.7 PRU/ml) to grasspollen. The patients were tested outside the pollen season. None of the patients gave a history of respiratory tract infections in the 6 weeks prior to the study. All patients gave informed consent. The study was approved by the medical ethics committee of Asthmacentre Heideheuvel, The Netherlands.

Study design

Allergen challenges in nose and skin were performed before and after 1 year of grasspollen immunotherapy. Subjects visited the hospital for 4 separate days. Medication for the treatment of allergic rhinitis was stopped before the tests: Nasal steroids and cromoglicate were stopped 2 weeks before the tests; antihistamines were stopped 48 hours before the tests. None of the patients received oral steroids or immunotherapy for grasspollen. Inhalation medication for the treatment of asthma was continued.
During the first study day nasal resistance was recorded 10 minutes after the inhalation of control solution and after that until 10 hours after the inhalation using whole bodyplethysmography (see below).

Table 1: Clinical data of the patients

patient	age	sex	allergy	asthma	smokers	medication rhinitis	Skin test ER	Skin test LR
1	36	F	GP	-	-	-	25	23
2	23	M	GP	-	-	-	22	18
3	21	F	GP	+	-	S, A	14	10
4	34	F	GP	+	-	-	23	18
5	23	M	GP	+	-	-	14	23
6	23	F	GP	+	-	-	14	16
7	21	F	GP	+	-	S,A	21	27
8	42	F	GP	+	-	S,A	11	17
9	55	M	GP	-	-	S,A	14	18
10	34	F	GP	+	-	-	17	14
11	23	M	GP	+	-	S	12	0

GP: grasspollen; S: nasal steroids; A: antihistamines
ER: early reaction; LR: late reaction

On the second study day nasal challenge with grasspollen was performed and nasal resistance was recorded until 10 hours after challenge. Intradermal challenge with grasspollen was performed; reactions were measured 15 minutes and 6 hours after challenge. Skin tests with histamine and phosphate buffer diluent were also performed. On the third study day (48 hours after the second day) nasal challenge with grasspollen was repeated, using the same dose of allergen as was used for the first challenge. Intradermal challenge with grasspollen was repeated on the same site as the previous challenge. On the fourth study day (48 hours after the third day) nasal challenge with grasspollen was again repeated, using the same dose of allergen; nasal resistance was followed until 10 hours after challenge. Intradermal challenge with grasspollen was repeated on the same site as the previous challenges; reactions were measured 15 minutes and 6 hours after challenge. The reaction to repeated allergen challenge in nose and skin were expressed as the difference between the reaction after challenge 3 and challenge 1 (RAC value). The same test protocol was repeated after 1 year of immunotherapy.

Nasal challenge

Allergen solutions were prepared from stock solutions of mixed grasspollen, diluted in PBS with 0.03% human serum albumine and containing 0.0125% benzalkonium

chloride (SQ 293, ALK Benelux), to produce a range of tenfold increasing concentrations from 100 to 10.000 BU/ml. The allergen solutions were administered with a pump spray delivering a volume of 0.102 ml (ALK Benelux). Both nostrils were challenged. Before allergen challenge, patients waited 15 minutes to allow the nasal mucosa to become acclimatised. Increasing doses of allergen were given at 15 minutes intervals; no further dose was given when the nasal resistance, measured with whole body plethysmography (see below), had doubled. After the final dose nasal resistance was measured 15 minutes and thereafter every hour until 10 hours after challenge. On a control day nasal resistance was recorded until 10 hours after the inhalation of a control solution, a phosphate buffer solution with 0.03% human serum albumine and containing 0.0125% benzalkonium chloride.
Allergen-induced changes in nasal resistance were expressed as the percentage change compared to before. The early nasal response was expressed as the mean % change in $Rn_{(insp)}$ measured in a time period (0.25-2 hours after challenge). The late response was expressed as the mean % change in $Rn_{(insp)}$ measured in a time period (4-10 hours after challenge).

Whole body plethysmography
Whole body plethysmography is a non-invasive technique to determine the airway resistance. In a previous article we described this technique for the detection of early and late reactions in the nose after allergen challenge (23). In short, for the measurement of nasal resistance the mouthpiece, normally connected with the pneumotachometer, was replaced by a solid nasal mask (nasal CPAP mask). The mask was supported by both hands of the patients, keeping the mask fixed on the face of the patients without leakage of air, and preventing the patients to blow up the cheeks. The patients were instructed to breath quietly through the nose, keeping the mouth closed. Nasal flow through both nostrils was recorded. Nasal resistance (Rn) in both nostrils was then calculated from the nasal flow (V'n) and the alveolar pressure (Pa) according to the formula Rn=Pa/V'n. Because in the nose the inspiration phase is most sensitive for detection of nasal obstruction to allergen challenge, we used the inspiratory nasal resistance ($Rn_{(insp)}$) for the monitoring of the allergic reaction. The initial value of $Rn_{(insp)}$ on the control day and on the allergen challenge days were considered as 0%. The other values of $Rn_{(insp)}$ were expressed as percentage changes compared to the initial value.

Skin challenges
Intradermal challenges (30 BU/ml, 0.03 ml) were performed at the back of the patients with a standardised grasspollen extract (ALK Benelux, SQ-293). The early response was scored as the wheal diameter 15 minutes after challenge. Late responses were measured 6 hours after challenge: indurations were determined according to a procedure suggested by Sokal (24). The reaction on histamine and phosphate buffer diluent served as positive and negative controls.

Immunotherapy protocol
Immunotherapy was performed with a standardised aluminium-absorbed depot extract (SQ 293, ALK Benelux). Subcutaneous injections were given weekly with increasing doses. When the highest tolerated dose was reached, the interval between the injections was extended till 4 weeks. Before every injection the clinical status of the patient was evaluated. After the injection patients stayed in the hospital under supervision for at least 30 minutes.

Data analysis
Comparisons were made with the Wilcoxon Signed Rank test (WSR). Spearman's rank correlation test was used for correlation studies. P values less than 0.05 were considered significant.

13.4 Results

The effect of immunotherapy on early and late reactions in the nose
Figure 1 shows the effect of immunotherapy on the early and late allergic reaction in the nose after allergen challenge. Before immunotherapy the mean percentage change in $Rn_{(insp)}$ during early response (0.25-2 hours) was 164+/- 123%; after immunotherapy 60 +/- 105%. Immunotherapy in 11 patients resulted in a significant decrease in early response ($p < 0.05$). The mean percentage change in $Rn_{(insp)}$ during late response was 28 +/- 31 % before immunotherapy and 3 +/- 12 % after immunotherapy. There was also a significant decrease in late response (4-10 hours) ($p < 0.05$). On the control day the $Rn_{(insp)}$ showed minor variation; no effect of challenge with control solution on $Rn_{(insp)}$ was found. The mean percentage change in $Rn_{(insp)}$ during "early and late phase period" on the control day were calculated; these values were -4 +/- 15% and -1 +/- 12% respectively.

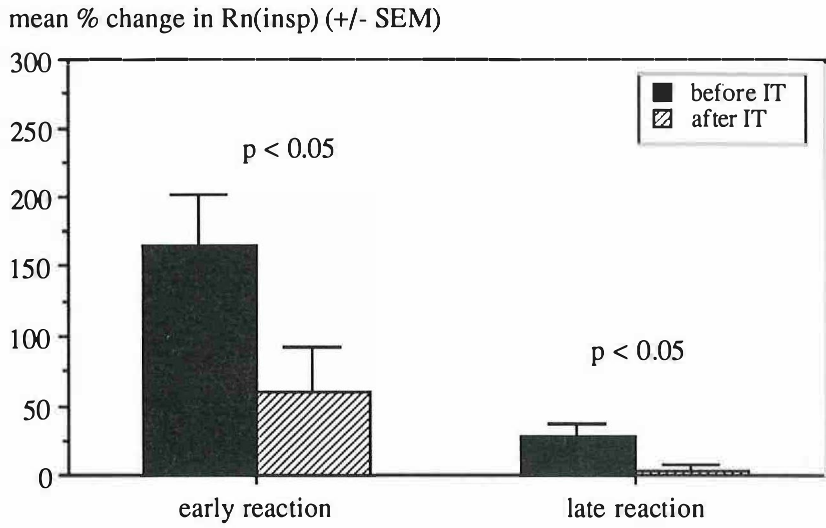

Figure 1: The effect of immunotherapy on early and late allergic reaction in the nose (N=11).

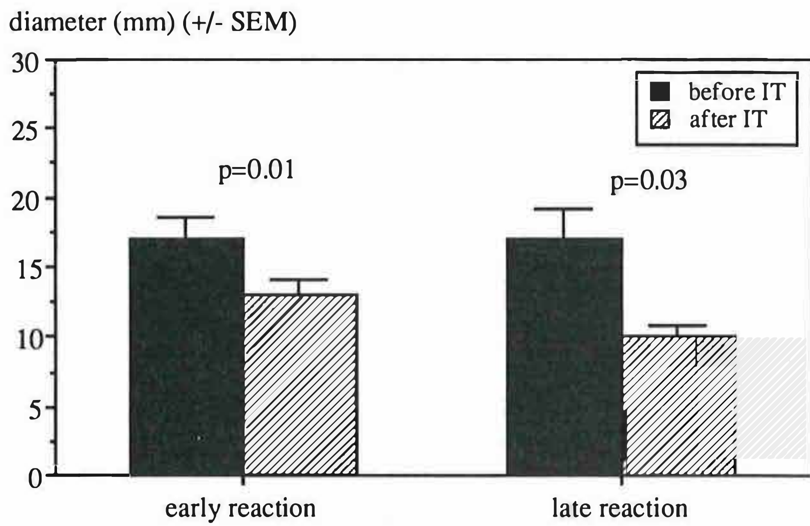

Figure 2: The effect of immunotherapy on early and late allergic reactions in the skin (N=11).

The effect of immunotherapy on early and late allergic reactions in the skin
Before immunotherapy intradermal skin challenge resulted in an early reaction with a mean diameter in 11 patients of 17 +/- 5 mm and a late reaction with a mean diameter of 17 +/- 7 mm. After immunotherapy the mean diameter of the early response was 13 +/- 3 mm; for the late response 10 +/- 3 mm. Immunotherapy resulted in a significant decrease in early and late allergic reaction in the skin (p=0.01, p=0.03 respectively) (Figure 2).

Correlation between nose and skin reactions
There was no significant correlation between the effect of immunotherapy on the early reaction in the nose and on the early skin reaction (r=0.046 ;p= 0.88). Although there was a stronger relationship between the effect of immunotherapy on late nasal response and late skin response, the correlation was not significant (r=0.56; p=0.08)(Figure 3).

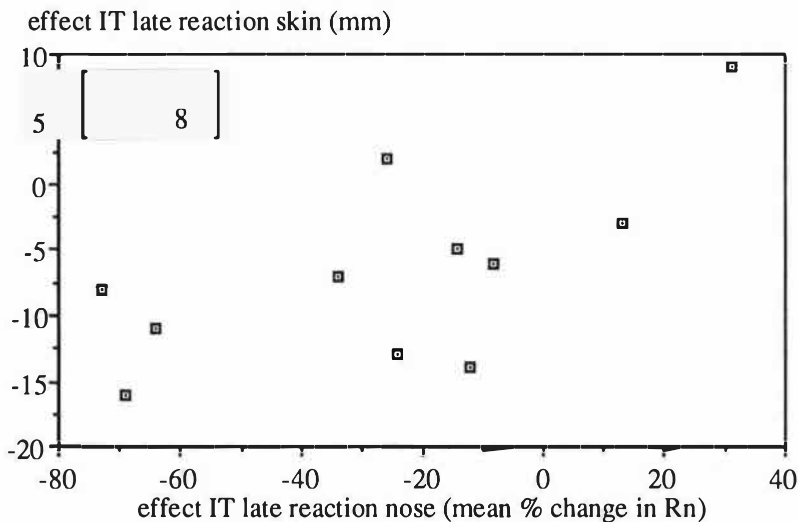

Figure 3: Correlation between the effect of immunotherapy on the late allergic reaction in the nose and the skin after single allergen challenge (N=11)

The effect of immunotherapy on allergic reactions after repeated allergen challenge in the nose

Repeated allergen challenge was performed in 10 of the 11 patients. Because one patient showed a very strong response to the first challenge, no further challenges were performed. Repeated allergen challenge before immunotherapy did not result in significant changes in early and late response. The response to repeated allergen challenge was expressed as RAC value, which is the difference between the size of reaction 3 and reaction 1. Before immunotherapy the mean RAC value for the early response 4 +/- 185 % ; for the late response -8 +/- 42% (Table 2).

After immunotherapy repeated allergen challenge did not result in significant changes in early and late response. The mean RAC value for the early response was -23 +/- 75 % ; for the late response -5 +/- 9 %. Immunotherapy did not result in significant changes in RAC values for early and late response ($p=0.80$; $p=0.76$ respectively)(Table 2).

The effect of immunotherapy on allergic reactions after repeated allergen challenge in the skin

Before immunotherapy repeated allergen challenge did not result in significant changes in early and late skin response. The mean RAC value for the early response was -3 +/- 5 mm. For the late response a mean RACs value of -4 +/- 10 mm was found (Table 2).

After immunotherapy a significant decrease in late skin reaction after challenge 3 compared to challenge 1 was found for the late response ($p=0.007$). This was not true for the early response. Repeated allergen challenge resulted in a mean RAC value of -2 +/- 3 mm for the early reaction and a RAC value of -7 +/- 4 mm for the late reaction. Immunotherapy did not result in significant changes in RAC values for early and late skin response ($p=0.48$; $p=0.26$ respectively)(Table 2).

Correlation between nose and skin reactions after repeated allergen challenge

Before immunotherapy a significant correlation was found between the effect of repeated allergen challenge on the late reaction in the nose and in the skin ($r=0.81$; $p=0.02$). This was not true for the early response ($r=-0.14$; $p=0.69$).

The effects of immunotherapy on the late phase response pattern after repeated allergen challenge (RAC value) in the nose and the skin were significantly correlated ($r=0.69$; $p=0.04$) (Figure 4; Table 3). The effect of immunotherapy on the late phase response pattern after repeated allergen challenge in the skin was also significantly

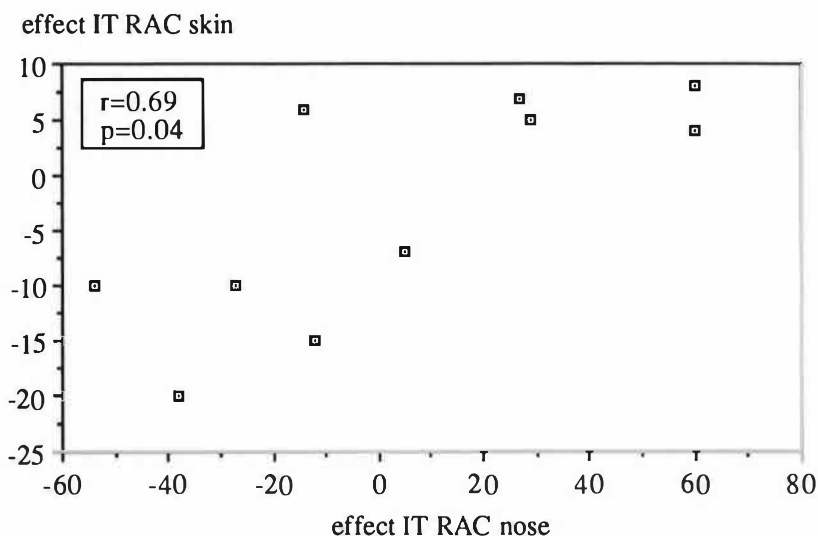

Figure 4: Correlations between the effect of immunotherapy on the response pattern of the late allergic reaction in the nose and the skin after repeated allergen challenge (N=10)

correlated with the effect of immunotherapy on the early phase response pattern after repeated allergen challenge in the nose (r=0.72; p=0.03) (Table 3).
The effect of immunotherapy on the early phase response pattern after repeated allergen challenge in the skin was not significantly correlated with early or late phase response pattern in the nose (r=0.15; p= 0.65 and r=-0.24; p=0.47 respectively) (Table 3).

13.5 Discussion

In the present study immunotherapy resulted in a significant decrease in early and late allergic reactions in the nose and in the skin after allergen challenge. A significant decrease in late skin response after immunotherapy is found by several authors, while the results concerning effect of immunotherapy on the early response are controversial (8, 16-19, 25). In the nose several authors have described a significant reduction of the early response (4, 5, 9, 26), and a limited number of studies report on a reduction in late allergic reaction after immunotherapy compared to before or to a placebo group (19, 27).

Table 2: RAC* values of nose and skin before and after immunotherapy

NOSE	EARLY REACTION			LATE REACTION		
patient	before IT	after IT	effect IT	before IT	after IT	effect IT
1	-176	87	263	21	7	-14
2	457	-65	-522	44	-10	-54
3	81	1	-80	25	-2	-27
4	60	2	-58	-40	-11	29
5	-119	-7	112	-27	0	27
6	-127	-13	114	-66	-6	60
7	53	-208	-261	-11	-23	-12
8	-151	-23	128	-69	-9	60
9	-43	-17	26	-5	0	5
10	6	17	11	45	7	-38
mean	4	-23	-27 (ns)	-8	-5	4 (ns)
SD	185	75	-109	42	9	-33

SKIN	EARLY REACTION			LATE REACTION		
patient	before IT	after IT	effect IT	before IT	after IT	effect IT
1	-9	-2	7	-16	-10	6
2	-12	-1	11	5	-5	-10
3	-1	-4	-3	5	-5	-10
4	-9	-7	2	-18	-13	5
5	-4	0	4	-16	-9	7
6	1	0	-1	-5	-1	4
7	4	-5	-9	2	-13	-15
8	-1	-3	-2	-17	-9	8
9	-4	0	4	-1	-8	-7
10	6	5	-1	20	0	-20
			0			
mean	-3	-2	1 (ns)	-4	-7	-3 (ns)
SD	5	3	-3	10	4	-6

* RAC value: reaction to challenge 3 - reaction to challenge 1
ns: not significant

Table 3:
A: Correlations between the effect of immunotherapy on early and late alleric reactions in nose and skin
B: Correlations between the effect of immunotherapy on the response to repeated allergen challenge in nose and skin

A

		NOSE	
		ER	LR
SKIN	ER	R=0.05 p=0.88	R=-0.10 P=0.75
	LR	R=0.35 p=0.27	R=0.56 p=0.08

B

		NOSE	
		ER	LR
SKIN	ER	R=0.15 p=0.65	R=-0.24 p=0.47
	LR	*R=0.72 p=0.03*	*R=0.69 p=0.04*

The primary aim of the present study was to investigate the correlation between the effect of systemic allergen immunotherapy on allergic reactions in nose and skin. The allergic responses in both skin and nose were found to be decreased after immunotherapy, however we failed to show a significant correlation between the effects on the nose and the skin for early and late allergic responses in this group of 11 patients. Although a reduction of late skin response is sometimes associated with clinical improvement in patients suffering from allergic rhinitis (8, 17), there are no data on correlations between effects of immunotherapy on allergic reactions in skin and nose. In a study of Iliopoulos (19) the effect of immunotherapy on allergic reactions in the nose and the skin was studied at the same time, however there are no data on correlations between these parameters.

Low correlations between the magnitude of allergic reactions in the skin and the nose has been reported by Mosbech et al(21) and Richerson et al (20). Also in the lower airways allergic reactions are weekly correlated with allergic skin reactions (21, 28, 29). The different expression of allergic reactions in nose and skin can partly be explained by differences in primarily effector tissue and local allergen handling. Another explanation for the different expression of allergic nose- and skin reactions might be that the baseline condition in the nose is different from the situation in the non-disrupted skin. In patients suffering from allergic rhinitis the nasal mucosa is affected as a result of allergic and non-specific external stimuli, while the skin is in a

resting state. This difference in baseline condition may also explain the lack of correlation between the effect of immunotherapy on nose and skin.
In an attempt to better reflect the chronic character of natural airway exposure to allergens, we used a test model of repeated allergen challenge. For the late reaction, the response pattern to repeated allergen challenge showed a significant correlation between nose and skin, suggesting that the response to repeated allergen challenge is a better reflection of the individual patient's allergen handling than single allergen challenge. The effect of immunotherapy on the late phase response pattern in the skin after repeated allergen challenge was significantly correlated with the effect of immunotherapy on the early and late phase response pattern in the nose after repeated allergen challenge. This finding suggests that the effect of immunotherapy on the response to repeated allergen exposure in the nose can be predicted by its effect on the late phase response pattern in the skin after repeated allergen challenge.
In conclusion: Immunotherapy is successful in reducing early and late allergic reactions in both the nose and the skin, however the effects on both organs are not significantly correlated.
The late skin response to repeated allergen challenge may be a useful parameter in evaluating the effect of immunotherapy in the nose.
Further studies are needed to investigate a possible role for repeated allergen challenge in the skin in evaluating the effect of immunotherapy.

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Chapter 14

General discussion

14.1 Introduction

The purpose of this thesis was to investigate the dynamics of the allergic reaction in the airways and skin. To this purpose we used 2 intervention methods: Repeated allergen challenge and systemic allergen immunotherapy. The following research questions were central in this thesis:

1- Dynamics of the allergic reaction:

a) What is the effect of repeated allergen challenge on allergic reactions in the nose and skin in individual patients?

b) What is the effect of immunotherapy on the response pattern to repeated allergen challenge?

c) Does repeated allergen challenge provide information on the regulation of the allergic reaction?

2- Can the allergic skin reaction be used as a model for allergic reactions in the airways in the evaluation of immunotherapy.

In order to address these questions we felt that is was necessary to optimise the documentation of allergen-induced allergic reactions in the airways and nose.

14.2 Documentation of allergen-induced allergic reactions in the nose and lower airways

Allergen challenge tests in shock organs, such as nose and lower airways, are a useful tool in research and are often used in studies on drug efficacy. It is very important that the challenge tests are adequately performed and controlled. In chapter 5 we described the importance of a control day before bronchial allergen challenge in order to registrate individual diurnal variation in FEV_1 value. Circadian rhythms in lungfunction may be related to circadian rhythms in histamine excretion and cortisol levels(1, 2). It is also possible that changes in FEV_1 value occurs as a result of allergen avoidance (3) when patients stay in hospital during the tests. When FEV_1 values after allergen challenge are not corrected for the patients' diurnal variation in FEV_1 value, false positive or false negative late phase responses may be registered. Although less is known about diurnal variation in nasal patency, a control day before nasal allergen challenge is also recommendable in order to registrate individual variation in day rhythm and because of a possible effect of allergen avoidance on nasal patency.

In the literature different parameters are used to define an allergic reaction. The maximal change in lungfunction or nasal airways resistance is often used to document the allergic response. The disadvantage of this definition is that it is based

on one single value. Especially for the late response it seems better to use a definition based on more time points, such as the mean percentage change in lungfunction or nasal resistance during a certain time period or as an area above/under the curve. In addition it is important to define the time period, in which the late phase response is registered. In the literature frequently follow-up periods of 8 hours or less are reported. In chapter 5 we found an increasing strength in late bronchial response when the definition was based on a longer measuring time. When the registration period was shortened from 10 to 8 or 6 hours the number of patients with a late phase response was 20, 18 and 12 respectively. Also in the nose we found that the changes in nasal resistance during the late phase response were most pronounced 8-10 hours after challenge (chapter 6). Therefore we recommend a measuring time of at least 10 hours after challenge in the lower airways and nose for a proper registration and definition of the late allergic response.
A definition of the allergic reaction which includes the provocative allergen dose, is the PD_{15} or PD_{20} allergen. This definition should be used when 2 challenges with different allergen doses are compared.
When comparing late phase allergic responses in lower airways and nose, the physiological changes in the lower airways are far more pronounced than in the nose, a difference which can be explained by the contribution of the smooth muscle component in the lower airways. The difficult registration of the subtle changes in nasal patency during late phase responses result in a large variation in reported numbers of late phase responders in the literature (3-75%). As nasal patency is easily influenced by mechanical factors and nasal cycle (4), it is preferable to use a registration method that does not disturb the external nasal anatomy and which is not influenced by nasal cycle. Although the method we used in our nose studies (chapter 6) fulfils these criteria, it is important to bear in mind that only one component of the allergic reaction in the nose is documented. Other components, such as number of sneezes, amount of secretion and itch were not included in this study.

14.3 What is the effect of repeated allergen challenge on allergic reactions in the nose and skin in individual patients and what is the effect of immunotherapy on the response pattern to repeated allergen challenge?

Traditionally, repeated or chronic allergen exposition in the nose is being associated with an increase in airway reactivity, so called priming effect (5, 6). However when critically looking at the different studies that use repeated allergen challenges, the literature is not conclusive. Whether or not priming occurs depends on various

factors. One of these factors is the way in which the response is registered. When reactions to repeated allergen challenges are registered using symptom scores, the number of sneezes were significantly increased after repeated allergen challenges (7). When the effect of repeated allergen challenge is monitored by measuring nasal obstruction most studies (8, 9), including our own study (chapter 10), fail to demonstrate priming. The strongest evidence for priming comes from observations on changes in mediator levels and inflammatory cell numbers in nasal lavages and nasal brushes after repeated allergen challenge. However it is possible that these changes not always coincide with physiological or clinical changes. In chapter 8 and chapter 10 no priming for early and late allergic reactions were observed in lower airways and nose when the reactions were documented with physiological methods. These findings are in accordance with other studies that use the physiological registration methods.

The difference in response pattern to repeated allergen challenge possibly needs a more individual approach. In chapter 10 a significant correlation between late phase response patterns to repeated allergen challenge in nose and skin were found. Therefore it seems that the individual response patterns to repeated allergen challenge for the late reaction are not just a coincidence or a regression to the mean. In some patients the allergic response seems to be self-limiting, because repeated allergen exposure leads to diminution (down-regulation) of the late reponse. Down-regulation of the late skin response after repeated allergen challenge was observed in nearly all patients who were treated with allergen immunotherapy during 1 year (chapter 11 and 13). In addition the change in late phase response pattern to repeated allergen challenge after immunotherapy coincided with an increased percentage of CD8+ T cells in skin biopsies taken 24 hours after the first challenge (chapter 11). These CD8+ T cells, possibly a subset of cells bearing the $\gamma\delta$–receptor, might have a potential role in the suppression of the subsequent allergic reactions by production of cytokines, such as IFNγ and IL-2 (11-13). The increases in mRNA for INFγ and IL-2, which was observed in skin biopsies after immunotherapy (14) might also be derived from CD8+ cells. However it seems that some patients, in our study especially grass pollen allergic patients, have a natural potential to downregulate their late response after repeated allergen exposition. The inverse correlation between the response to repeated allergen challenge and the reaction to the first challenge suggests that a rather strong reaction is necessary to induce down-regulation. From literature it seems that repeated high local exposition (10) or exposition to increasing amount to

allergen (10, 15-19) can lead to down-regulation, while repeated low exposition (20) or decreasing amounts of allergen (5) may result in priming.
Whether or not priming occurs may also depend on the type of allergen involved. Repeated allergen challenge with housedustmite (chapter 9 and 11) more often results in an increase in late skin reaction than repeated grasspollen challenges (chapter 10).
In conclusion, repeated allergen challenge in lower airways, nose and skin can lead to priming as well as to down-regulation of allergic reactions. The response pattern (especially late response) may depend on patients characteristics, the level of allergen exposition and the type of allergen. Treatment with increasing allergen doses (allergen immunotherapy) can induce a down-regulating effect on the late response to repeated allergen challenge (Figure 1).

14.4 Does repeated allergen challenge provide additional information on the regulation of the allergic reaction ?

The allergic reaction after experimental allergen challenge results from mast cell degranulation after allergen binding to IgE on mast cells. The response to repeated allergen challenge may give more information on the patient's handling of chronic allergen exposition. Repeated allergen challenge in individual patients showed a large variation, varying from a strongly enhanced response to a complete extinction of the response (chapter 10). Chronic or repeated allergen exposition may result in an allergen-specific immune response, involving allergen-specific T-cell activation. Different subsets of T-cells can exert either activating or suppressive effects on inflammatory cells involved in subsequent allergic reactions. CD8+ T-cells might exert suppressive effects, because they are associated with a subsequent down-regulation of the late skin response after repeated allergen challenge (chapter 11)(Figure 1).
In conclusion, in contrast to a single allergen challenge, the response to repeated allergen challenge gives information about the allergen- specific immune response which is in turn responsible for the regulation of the allergic reaction.

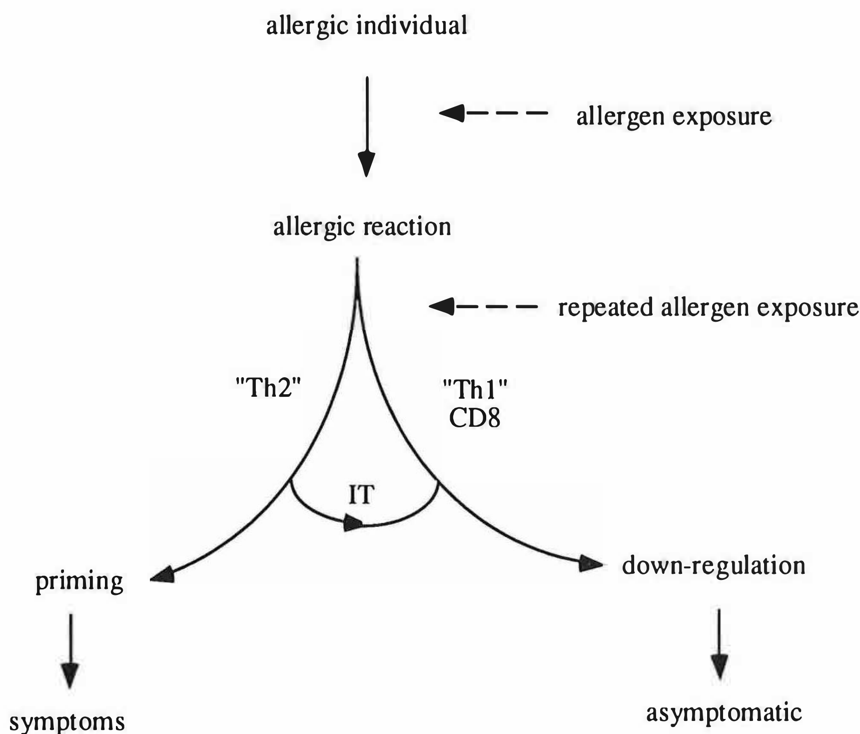

Figure 1: Different effects of repeated allergen challenge on the allergic reaction

14.5 Can the allergic skin reaction be used as a model for allergic reactions in the airways in the evaluation of immunotherapy.

Skin reactions after intradermal allergen challenge can be used to detect allergic sensitisation, to study inflammatory processes and to evaluate the effect of systemic treatment. When using the allergic skin reaction as a model for allergic reactions in other organs, it is important to bear in mind that the expression of allergic reactions in the different tissues is dependent on systemic factors, such as the presence of IgE, but also on local tissue factors. It is therefore that a positive skin test does not always predict allergic symptoms in shock organs (asymptomatic sensibilisation) and that allergic reactions in skin and airways may not correlate (21, 22). When the microscopic changes after allergen challenge are compared in lower airways, nose and skin only minor differences are found (chapter 2.4 and 2.6). The different macroscopic expression of allergic reactions in the lower airways, nose and skin seem to result from differences in "translation" of the microscopic processes into a measurable macroscopic reactions (see chapter 2 Table 2). The first step in this

"translation process" is the effector tissue. Inflammatory cell-derived products exert their effects on effector tissues, such as vasculature, secretory glands and airway smooth muscle. The relative contribution of these components in the target organs is different; the primarily effector tissue in the lower airways is the smooth muscle component, while in the nose and skin it is the vasculature that is responsible for the expression of the allergic response. In the nose and lower airways secretory glands are also involved in the expression of the allergic reaction, in contrast to the skin. The second step in the "translation process" from the microscopic changes during allergic reactions into measurable macroscopic changes is the manner in which responses are registered. In the skin reactions are measured directly as visible wheal diameters, while in the airways the reaction is often registered indirectly by measuring airway narrowing as a result of processes in the airway wall.

The fact that the magnitude of allergic reactions in the airways and skin in patient groups do not correlate, does not imply a lack of correlation between the effect of immunotherapy on the airways and skin. In several studies, including our own studies, systemic allergen immunotherapy is associated with a reduction in late phase allergic response in skin (23-27), lower airways (28-30) and nose (31, 32), suggesting an overall anti-inflammatory effect. Especially correlations between the effect of immunotherapy on late skin- and airway reactions are of interest when monitoring the anti-inflammatory effect of immunotherapy at distance from the shock organ. However when comparing the effect of immunotherapy on late phase reactions in skin and airways, we found no significant correlations. The weak correlation between the effect of immunotherapy on lower airways and skin might be explained by additional effects of immunotherapy on bronchial reactivity to non-specific stimuli, as has been demonstrated in our own study and in literature (33). However when the effect of immunotherapy on late skin reaction was corrected for the effect on bronchial reactivity to histamine, the correlation between effects on late airway reactions and late skin reactions did not improve. This finding suggests that direct effects of immunotherapy on bronchial reactivity are not responsible for the low correlation between the effect of immunotherapy on late phase reactions in skin and lower airways. Another possible reason for the low correlation between the effect of IT on airways and skin is the difference in baseline condition. In all the patients the skin was in a "resting state", while the airways were constantly exposed to allergen possibly resulting in a individual level of inflammation. Especially in the lower airway study, where patients with housedustmite allergy were investigated, this difference in baseline condition might explain the low correlation between effects on skin and

lower airways. In the nose study, grass pollen allergic patients were tested outside the season. Although the nose might not be in resting state because of non-specific stimuli and allergen exposition to other allergens, the allergic specific baseline condition in skin and nose was not different. This might explain the higher correlation between immunotherapy effects on skin and nose compared to skin and lower airways.
A significant correlation between the effect of immunotherapy on nose and skin was found when changes in late allergic response patterns to repeated allergen challenges were compared (chapter 13). This repeated challenge model was used in the evaluation of immunotherapy, because the response to repeated allergen challenge might be a better reflection of the individual patients' allergen handling than the response to a single allergen challenge (chapter 10). Repeated allergen challenge in the lower airways was not performed for safety reasons; therefore we could not study the correlation of immunotherapy effects on late phase response patterns to repeated allergen challenge in lower airways and skin.
In conclusion, when using allergic skin reactions as a model for allergic reactions in the airways in the evaluation of allergen immunotherapy, the late phase response pattern to repeated allergen challenge seems to provide the best achievable information.

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Chapter 15

Summary and conclusions

In this thesis a number of studies are described concerning the dynamics of the allergic reaction in airways and skin. Two intervention methods were used: Repeated allergen challenge and systemic allergen immunotherapy. The following research questions are central in this thesis:

1- Dynamics of the allergic reaction:

a) What is the effect of repeated allergen challenge on allergic reactions in the nose and skin in individual patients?

b) What is the effect of immunotherapy on the response pattern to repeated allergen challenge?

c) Does repeated allergen challenge provide information on the regulation of the allergic reaction?

2- Can the allergic skin reaction be used as a model for allergic reactions in the airways in the evaluation of immunotherapy.

The patients who were investigated in the different studies were allergic and suffered from asthma and/or rhinitis. Definitions and prevalences of allergy (1.1), asthma (1.2) and rhinitis(1.3) in the Netherlands are described in chapter 1. In addition a table concerning the classification of the severity of astma is added.

In chapter 2 different aspects of the allergic reaction are described. Chapter 2.1 deals with the physiological responses to allergen challenge in the lower airways and nose. Experimental allergen challenge in the lower airways (2.1.1) frequently results in dual responses. The fall in FEV_1 value is a sensitive method to measure early and late allergic reactions. In the nose (2.1.2), the large variety in investigation methods, used for the documentation of allergic responses, results in a large variation in reported numbers of late phase reactions. The subtle changes in nasal patency during late phase responses can only be detected with very sensitive methods.

Chapter 2.2 deals with the immunohistology of the allergic reaction in the airways. Cells, which are involved in the effector phase and the regulation of the allergic reaction are described separately (2.2.2). Mast cells are important effector cells in the early phase reaction through the release of various mediators and can play a role in initiating inflammatory responses by the release of cytokines. Late phase inflammatory reactions are caused by products derived from inflammatory cells, such as eosinophils, neutrophils and basophils. Different subsets of T-lymphocytes play an important role in regulating allergic inflammation. Antigen presenting cells are necessary for the initiation of allergen specific T-cell responses and can direct the immune response.

In chapter 2.3 a short overview of neuroregulation of the nose and bronchi is given. Sympathetic and parasympathetic components of the nervous system control airway vasculature, secretory glands and airway smooth muscle.
Differences between allergic manifestations in nose and bronchi are described in chapter 2.4. The different manifestation of allergic reactions in nose and lower airways can probably be explained by a different "translation" from inflammatory processes into physiological changes and symptoms.
In chapter 2.5 macroscopy and immunohistology of the early and late allergic reaction in the skin after intradermal challenge is described. Allergic inflammation is caused by products derived from infiltrating effector cells such as eosinophils, basophils and neutrophils. In addition, T-lymphocytes have regulating effects on the inflammatory response .
When comparing allergic reactions in the airways and the skin (chapter 2.6), the microscopic changes during the late allergic skin reaction after intradermal challenge seem to resemble the microscopic changes during allergen-induced inflammatory responses in the airways. However the macroscopic expression of allergic reactions in airways and skin is quite different.
Chapter 3 deals with the dynamics of the allergic reaction. Effects of repeated or chronic allergen exposition in nose (3.2.1), lower airways (3.2.2) and skin (3.2.3) are described. In chapter 3.3 a short overview of systemic allergen immunotherapy is given.
In order to address the research questions the documentation of allergen-induced reactions in the airways and nose was optimised. In chapter 5 technical and methodological aspects of bronchial challenge tests with inhalant allergens are described. FEV_1 values after allergen challenge should be corrected for diurnal variation and a follow-up period after allergen challenge of at least 10 hours is necessary. In chapter 6 a new, non-invasive, technique was developed to register allergen-induced early and late reactions in the nose. In addition criteria were suggested for the definition of early and late phase nasal reactions.
In chapter 7 the effect of Cetirizine was studied on early and late asthmatic reactions after experimental allergen challenge in 16 housedustmite allergic asthmatic patients. In addition effects on blood ECP levels and PC_{20} methacholine were studied. Eighteen days of treatment with Cetirizine did not significantly reduce the intensity of the early and late asthmatic response. There was also no significant effect on the blood ECP levels and PC_{20} methacholine.

In chapter 8 the effect of allergen challenge on airway responsiveness to histamine was investigated in 21 atopic asthmatic patients. Depending on baseline PC_{20} histamine, a significant decrease in PC_{20} histamine was only found in patients with relatively high baseline PC_{20} histamine. A significant inverse correlation was found between baseline PC_{20} and allergen-induced change in PC_{20} histamine. In addition the effect of repeated allergen challenge on early and late asthmatic rations and on airway responsiveness was studied in 8 patients. Repeated allergen challenge with an interval of 48 hours did not result in significant changes in early and late asthmatic reactions. The first allergen challenge resulted in a significant decrease in PC_{20} histamine; no further decrease in mean PC_{20} histamine was seen after the second allergen challenge.
In chapter 9 repeated intradermal challenge with housedustmite resulted in a significant increase in late phase skin reactions at 6, 24 and 48 hours in 13 housedustmite allergic patients. CD4+ T-lymphocytes and eosinophils were increased up to 96 hours after challenge.
In chapter 11 repeated intradermal challenges with housedustmite were performed before and after 1 year of immunotherapy with a standardised housedustmite extract (SQ 503) in 10 patients, allergic to housedustmite. Immunotherapy resulted in a significant decrease in late skin reaction; in addition repeated allergen challenge after immunotherapy resulted in a further significant decrease in late reaction diameter, when challenge 3 was compared to challenge 1. In the skin biopsies, taken 24 hours after the first challenge, a significant increase in the percentage of CD8+ and CD25+ cells was found after immunotherapy compared to before, while there was no significant change in mean overall mononuclear cell density.
Repeated allergen challenges with grasspollen in the nose and skin of 11 grasspollen allergic patients did not result in significant overall changes in early and late allergic responses in both organs (chapter 10). Large individual differences in response patterns were found. A significant correlation was found between the effect of repeated allergen challenge on the late response in the nose and the skin, suggesting that the late response pattern to repeated allergen challenge may be a characteristic of the individual patient.
In chapter 12 the relationship between the effect of immunotherapy with a standardised housedustmite extract (SQ 503) on allergic reactions in the airways compared to the skin was investigated in 10 housedustmite allergic patients with asthma. Immunotherapy resulted in a significant decrease in early and late allergic reaction in the airways and in late skin reaction. The effect of immunotherapy on

allergic reactions in the airways could not be predicted from effects on early or late skin reactions or from effects on skin reactions, corrected for effects on PC_{20} histamine.
Correlations between the effect of immunotherapy on allergic reactions in the nose and the skin were investigated in chapter 13. Repeated nasal challenges and intradermal challenges with grasspollen were performed in 11 grasspollen allergic patients before and after 1 year of immunotherapy with a standardised grasspollen extract (SQ 293). Immunotherapy resulted in a significant decrease in early and late allergic reactions in nose and skin. No significant correlations were found between the effect of immunotherapy on allergic reactions in the nose and the skin for early and late responses. When using the model of repeated allergen challenge, the effect of immunotherapy on the late phase response pattern to repeated allergen challenge in the skin was significantly correlated with the effect of immunotherapy on early and late phase response pattern after repeated allergen challenge in the nose.

Conclusions

1) Dynamics of the allergic reaction
- Repeated allergen challenge in nose and skin in individual patients can lead to priming as well as to downregulation of the allergic reaction. The late phase response pattern to repeated allergen challenge seems to be a characteristic of the individual patient.
- Repeated allergen challenge in the skin after immunotherapy results in a significant decrease in late phase diameter. This response pattern can possibly be explained by suppressive actions of CD8+ T-cells.

2) The allergic skin reaction as a model for allergic reactions in the airways in the evaluation of immunotherapy
- The effect of immunotherapy on allergic reactions in skin and airways is not significantly correlated.
- The effect of immunotherapy on late phase response patterns to repeated allergen challenge in nose and skin is significantly correlated.
- When allergic skin reactions are used in the evaluation of the effect of allergen immunotherapy on allergic reactions in the airways, it seems better to use late phase reponse patterns to repeated allergen challenge.

Samenvatting voor niet ingewijden

In dit proefschrift worden een aantal studies beschreven welke betrekking hebben op de dynamiek van de allergische reactie in de luchtwegen en de huid. Bij een patient die allergisch is voor een bepaald allergeen, bijvoorbeeld huisstofmijt, graspollen of katten, kan contact met dat betreffende allergeen leiden tot en zogenaamde allergische reactie. Deze reactie kan optreden in verschillende organen; in dit proefschrift wordt de allergische reactie in de longen, neus en huid bestudeerd. Afhankelijk van het reagerende orgaan zal de allergische reactie zich op verschillende manieren manifesteren. In de longen leidt een allergische reactie tot benauwdheid, wat gemeten kan worden m.b.v. longfunktie onderzoek. In de neus leidt een allergische reactie tot klachten als neusverstopping, slijm productie, jeuk en niezen. De reactie in de neus kan gemeten worden door het registreren van de klachten of door het meten van de slijmproductie of de neusdoorgankelijkheid. In de huid leidt het inspuiten van allergeen (de zogenaamde huidtest) tot zwelling en jeuk. De huidreactie wordt geregistreerd door meting van de diameter van de reactie.
Naast een zogenaamde vroege allergische reactie, die optreedt ongeveer 15 minuten na allergeen toediening, treedt in vele gevallen een late allergische reactie op. Deze reactie ontstaat veel langzamer (3-10 uur na allergeen contact), maar duurt ook veel langer (soms meer dan 24 uur). Deze late allergische reactie heeft een grotere betekenis voor de kliniek omdat het een veel betere weergave is van de processen welke een rol spelen bij allergisch astma en allergische rhinitis (= neusslijmvlies ontsteking).
In dit proefschrift worden 2 interventie methoden beschreven, die de sterkte van de allergische reactie mogelijk kunnen beïnvloeden. Ten eerste wordt gebruik gemaakt van immunotherapie met allergenen (desensibilisatie). Hierbij worden injecties toegediend met opklimmende hoeveelheid allergeen, met als doel de gevoeligheid van de patient voor dat betreffende allergeen te doen verminderen. Daarnaast wordt gebruik gemaakt van herhaalde allergeenprovocaties.

De volgende onderzoeksvragen zijn opgesteld

1- Betreffende de dynamiek van de allergische reactie:

a) Wat is het effect van herhaalde allergeen provocatie op allergische reacties in de neus en de huid bij individuele patienten?

b) Wat is het effect van immunotherapie op het reactiepatroon na herhaalde provocatie?

c) Geeft herhaalde allergeen provocatie informatie over de regulatie van de allergische reactie?

2- Kan de allergische huidreactie gebruikt worden als model voor de allergische reactie in de luchtwegen bij het vervolgen van het effect van immunotherapie?

In hoofdstuk 1 worden de definities en het voorkomen van allergie (1.1), astma (1.2) en rhinitis (1.3) beschreven. Er is een tabel bijgevoegd waarin de ernst van het astma geclassificeerd wordt.
In hoofdstuk 2 worden verschillende facetten van de allergische reactie besproken. In hoofdstuk 2.1 wordt beschreven hoe de allergische reactie in de longen en neus gemeten wordt. Allergeen provocatie in de longen leidt relatief vaak tot zowel een vroege- als en late reactie. Het vervolgen van de longfunktie (één seconde waarde) is een gevoelige methode om vroege en late reacties te registreren. Voor het registeren van de allergische reactie in de neus (2.1.2) worden verschillende methoden gebruikt. Dit leidt ertoe dat het aantal gerapporteerde patienten met een late neusreactie sterk wisselt. Tijdens de late neusreactie zijn de verandering in neusweerstand zeer subtiel, waardoor ze alleen geregistreerd kunnen worden met zeer gevoelige methoden.
In hoofdstuk 2.2 worden de cellen beschreven, die een rol spelen bij de expressie en de regulatie van de allergische reactie. De vroege allergische reactie wordt voornamelijk veroorzaakt door mestcellen, terwijl late allergische reacties worden voornamelijk veroorzaakt door producten afkomstig van ontstekingscellen, zoals eosinofiele granulocyten, neutrofiele granulocyten en basofiele granulocyten. Verschillende soorten T-lymfocyten spelen een belangrijke rol bij de regulatie van de allergische reactie.
Verschillen in expressie van allergische reacties in longen en neus worden beschreven in hoofdstuk 2.4. Deze verschillen kunnen mogelijk verklaard worden door een verschil in "vertaling" van de allergische ontstekingsreactie naar waarneembare symptomen.
De allergische reactie in de huid wordt beschreven in hoofdstuk 2.5. Bij de vergelijking tussen de allergische reactie in huid en luchtwegen (hoofdstuk 2.6) lijkt

er op microscopisch niveau weinig verschil. Echter de expressie van de allergische reactie in de huid en luchtwegen in anders.
In hoofdstuk 3 wordt het effect van herhaald en chronisch allergeen contact beschreven in de neus (3.2.1), longen (3.2.2) en huid (3.2.3). Daarnaast wordt een overzicht gegeven betreffende effecten van immunotherapie (3.3).
Op antwoord te geven op de onderzoeksvragen is allereerst begonnen met het optimaliseren van de documentatie van de allergische reacties in de neus en long. In hoofdstuk 5 worden technische en methodologische aspecten van de bronchiaal provocatie test met allergenen beschreven. Longfunctie waarden na allergeen provocatie moeten gecorrigeerd worden voor het dagritme in longfunctie van de patient. Daarnaast moet de longfunctie tot minimaal 10 uur na provocatie vervolgd worden.
In hoofdstuk 6 wordt een nieuwe, weinig belastende, en gevoelige techniek beschreven voor het registreren van vroege- en late neusreacties na allergeen provocatie.
Hoofdstuk 7 beschrijft het effect van Cetirizine, een antihistaminicum, op de vroege en late allergische reactie in de luchtwegen, op de histamine drempel en op de hoeveelheid ECP (= Eosinophilic Cationic Protein, een stof afkomstig uit de eosinofiele granulocyt) in het bloed. Na 18 dagen behandeling werden er geen significante effecten gevonden op de genoemde parameters.
Het effect van allergeen provocatie op de histamine gevoeligheid van de luchtwegen bij 21 allergische patienten wordt beschreven in hoofdstuk 8. Een significante stijging van de histamine gevoeligheid werd alleen gevonden bij patienten met een relatieve lage histamine gevoeligheid voorafgaande aan de allergeen provocatie. De toename van histamine gevoeligheid was omgekeerd gecorreleerd aan de uitgangs-gevoeligheid. Daarnaast wordt in deze studie het effect van herhaalde allergeen provocatie op de vroege en late allergische reactie van de lage luchtwegen bestudeerd. Herhaalde allergeen provocatie met een interval van 48 uur resulteerde niet in significante veranderingen van vroege of late bronchiaal reactie. De histamine gevoeligheid nam toe na de eerste allergeen provocatie, maar nam niet verder toe na de tweede allergeen provocatie.
In hoofdstuk 9 wordt beschreven dat herhaalde huidprovocatie met huisstofmijt resulteerde in een significante toename van de late huidreactie, gemeten op 6, 24 en 48 uur na provocatie. Tot 96 uur na provocatie werd een toegenomen hoeveelheid ontstekingscellen (lymfocyten en eosinofiele granulocyten) gevonden in huidbiopten.

In hoofdstuk 11 worden herhaalde huidprovocaties met huisstofmijt uitgevoerd voor en na de behandeling met immunotherapie met huisstofmijt gedurende 1 jaar bij 10 huisstofmijt allergische patienten. Na immunotherapie was de late huidreactie na éénmalige provocatie significant afgenomen t.o.v. vooraf. Herhaalde huidprovocatie na immunotherapie resulteerde in een verdere significante afname van de late huidreactie. In huidbiopten, welke 24 uur na de eerste provocatie genomen waren, werd een significante toename gevonden van T-suppressor cellen (CD8+) t.o.v. vooraf.
Herhaalde provocaties met graspollen in de neus en huid van 11 graspollen allergische patienten resulteerde niet in significante veranderingen van vroege en late allergische reacties in de neus en de huid (hoofdstuk 10). Echter er waren grote individuele verschillen in reactie patronen op herhaalde provocatie. Het reactie patroon van de late reactie op herhaalde provocatie van neus en huid was significant gecorreleerd. Dit suggereert dat het reactiepatroon van de late reactie op herhaalde allergeen provocatie karakteristiek is voor de patient.
De relatie tussen het effect van immunotherapie op de allergische reacties in huid en luchtwegen werd onderzocht in hoofdstuk 12. Immunotherapie met huisstofmijt resulteerde in een significante afname van de vroege en late bronchiaal reactie en in late huidreactie. Echter het effect van immunotherapie in de luchtwegen kon niet voorspeld worden vanuit effecten op de huidreactie.
In hoofdstuk 13 wordt de relatie beschreven tussen het effect van immunotherapie op de allergische reacties in neus en huid. Herhaalde neus- en huid provocaties met graspollen werden uitgevoerd voor en na 1 jaar behandeling met graspollen immunotherapie. Immunotherapie resulteerde in een significante afname van vroege en late allergische reacties in de neus en huid, maar het effect in beide organen was niet significant gecorreleerd. Echter het effect van immunotherapie op het reactiepatroon van de late huidreactie na herhaalde provocatie correleerde significant met het effect van immunotherapie op het reactiepatroon van de vroege en late huidreactie na herhaalde neusprovocatie.

Conclusies

1) Betreffende de dynamiek van de allergische reactie:
 - Herhaalde provocatie in neus en huid kan zowel tot priming (versterking) als tot down-regulatie (uitdoving) van de allergische reactie leiden. Het type respons patroon karakteristiek lijkt voor de individuele patient.
 - Herhaalde allergeen provocatie in de huid na immunotherapie leidt tot een significante afname van de late huidreactie. Dit reactie patroon kan mogelijk verklaard worden door suppressieve eigenschappen van CD8+ T-lymfocyten.

2) Kan de allergische huidreactie gebruikt worden als model voor de allergische reactie in de luchtwegen bij het vervolgen van het effect van immunotherapie?
 - Het effect van immunotherapie op allergische reacties in huid en luchtwegen is niet significant gecorreleerd.
 - Het effect van immunotherapie op het reactiepatroon van de late allergische reactie in neus en huid is significant gecorreleerd.
 - Wanneer allergische reacties in de huid gebruikt worden bij het evalueren van het effect van immunotherapie op allergische reacties in de luchtwegen, lijkt het reactiepatroon van de late reactie op herhaalde provocatie en beter model.

Curriculum Vitae

De auteur van dit proefschrift werd geboren op 12 januari 1961 te Groningen. Het Atheneum B diploma werd behaald in 1979 op het Coornhert Lyceum te Haarlem. In 1979 werd gestart met de opleiding Fysiotherapie te Leiden na uitloten voor de studie geneeskunde. In 1983 werd het diploma Fysiotherapie behaald, waarna aansluitend gestart werd met de studie Geneeskunde aan de Rijksuniversiteit Leiden. Tijdens deze studie is werkervaring opgedaan in de Fysiotherapie. In 1987 werd het doctoraal examen behaald. Het artsexamen werd in februari 1990 (cum laude) behaald.
Vanaf maart 1990 tot en met december 1996 werd het in dit proefschrift beschreven onderzoek uitgevoerd als arts-onderzoeker binnen Astmacentrum Heideheuvel te Hilversum, in samenwerking met de afdeling Longziekten van het Academisch Medisch Centrum, de afdeling Dermatologie van het Academisch Ziekenhuis Utrecht en de afdeling Allergologie van het Academisch Ziekenhuis Groningen.
In januari 1997 is gestart met de opleiding Dermatologie en Venereologie in het Academisch Ziekenhuis Utrecht (Opleider prof. dr. W.A. van Vloten).
De auteur van dit proefschrift is in 1990 getrouwd met Herman Jaap de Bruin en heeft 2 kinderen, Roeland en Digna.