Topical Corticosteroids in Allergic Rhinitis

Effects on Nasal Inflammatory Cells and Mucosa

Lokale Corticosteroiden bij Allergische Rhinitis

Effecten op Ontstekingscellen en Neusslijmvlies

Dit proefschrift is tot stand gekomen binnen de afdelingen KNO-heelkunde van het ziekenhuis Leyenburg, Den Haag en van het Academische Ziekenhuis Dijkzigt, Rotterdam, en de afdeling Pathologie van het Slotervaart ziekenhuis, Amsterdam

Het onderzoek en het drukken van dit proefschrift werd mogelijk gemaakt door financiële steun van GlaxoWellcome BV, Smith & Nephew Nederland BV en ASTRA Pharmaceutica BV.

Cover : Anonymus
Printing : Haveka B.V., Alblasserdam

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Proefschrift

Ter verkrijging van de graad van Doctor aan de
Erasmus Universitieit Rotterdam op gezag van de
Rector Magnificus
Prof. Dr. P.W.C. Akkermans M.A.
en volgens het besluit van het College van Promoties
De openbare verdediging zal plaatsvinden op
Vrijdag 23 oktober 1998 om 13.45 uur

door

Adriaan Frans Holm

geboren te Delfzijl

PROMOTIECOMMISSIE

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Chapter 1

GENERAL INTRODUCTION

Epidemiology

1.1.1 DEFINITION

In 1873, Blackley was the first to establish that pollen played a role in the causation of hay fever or "summer catarrh"(1). Von Pirquet introduced in 1906 the term allergy (2). He discovered that under certain conditions, patients, instead of developing immunity, demonstrated an increased reaction to repeated exposure with foreign substances. Nowadays, allergy has been defined as "untoward physiologic events mediated by a variety of different immunologic reactions" (3). This definition implies the acceptance of three criteria necessary for the definite diagnosis of an allergic state: (1) identification of the allergen, (2) establishment of a causal relationship between exposure to the antigen and occurrence of the disease, and (3) demonstration of an immunologic mechanism involved in the illness. Allergy is sometimes confused with atopy and the two are sometimes used as synonyms. Atopy refers to a hereditary predisposition to produce the antibody immunoglobulin E (IgE). The most important atopic diseases are atopic dermatitis, allergic rhinitis and allergic asthma.

1.1.2 TYPES OF RHINITIS

Allergic rhinitis is an IgE-mediated disease, in which symptoms are the result of aeroallergen exposure. The patient may complain of seasonal or perennial symptoms, although the latter may show seasonal exacerbations.

Seasonal allergic rhinitis Seasonal allergic rhinitis in The Netherlands is mostly due to grass pollen with symptoms presenting in May, June and July, whilst earlier symptoms may be due to tree pollen allergy. The main symptoms are itching; sneezing, watery rhinorrhea often associated with nasal congestion. Allergic rhinitis may be accompanied by itching in the throat, eyes and ears, epiphora and oedema around the eyes. Allergic rhinitis is at best a nuisance and at worst can be incapacitating. It may be complicated by headache, fatigue and lack of concentration(4).

Perennial allergic rhinitis

Perennial allergic rhinitis may be more difficult to diagnose, particularly if the patient presents with secondary symptoms as sinusitis and "a permanent cold". The symptoms of perennial allergic rhinitis differ from those of seasonal allergic rhinitis; nasal blockage often dominates and eye itching is rarely a problem. Reasons for the difference in symptoms between seasonal and perennial allergic rhinitis are unknown. On account of the obstructive nature of the disease, arising from vascular engorgement and mucosal oedema, and the associated involvement of the mucosa of the sinuses, headaches, facial pain and posterior nasal discharge, and loss of smell and taste may dominate the clinical picture. In addition, the severity of the disease may be more pronounced in individuals with an underlying structural nasal deformity. The most common cause in Western Europe to account for perennial allergic symptoms is the house dust mite (*Dermatophagoides pteronyssinus)*. Mites feed of dried skin debris and accumulates in mattresses, box springs, feather pillows, carpeting, stuffed animals and clothing. Mites represent a significant indoor burden because of our typical airtight homes with central heating that continue to circulate the allergen through the indoor air. Mites have a rather specific growth season requirements, which are dependent on an optimal temperature (16-27^{0}C) and humidity (55-85%)(5). The environment in which the patient sleeps is conducive to mite survival, as increased body temperature and perspiration create an appropriate microenvironment for their survival within the mattress and pillow. Other common perennial causes are pets, particularly cats, dogs and horses.

Non-allergic, non-infectious rhinitis

If patient experiences clinical symptoms as in allergic rhinitis, but no evidence of allergy can be found and there is no infectious rhinitis, the diagnosis non-allergic, non-infectious rhinitis is made. Perennial non-allergic, non-infectious rhinitis is often called "vasomotor rhinitis". However, there is no evidence for the existence of a "vasomotor" pathogenesis (6).

The diagnosis of inhalant allergy can usually be agreed on when history, physical examination and skin test/RAST results are combined.

1.1.3 PREVALENCE OF ALLERGIC RHINITIS

Relatively little is known about the prevalence or age distribution of allergic rhinitis. One of the two measures of prevalence is normally quoted: either the lifetime (cumulative) prevalence, or

the period prevalence over a recent interval of 1 or more years. Various factors, e.g. selection of the studied population, spontaneous resolution of symptoms, criteria for the diagnosis, are of importance and can influence the data. Our knowledge of the epidemiology of rhinitis is mostly derived from patients specifically seeking consultation for that affliction. However, community-based studies show that patient's consultation patterns for rhinitis vary considerably according to the severity of the disease, accessibility of health care and the perceived benefits of medical treatment. In a London-based study of adults with rhinitis, it was shown that only 62% had ever consulted a physician for their nasal symptoms(7). Moreover, the literature is not always clear on what form of rhinitis is investigated. Much of the available data relate to seasonal allergic rhinitis (hay fever), and caution is warranted before extrapolating epidemiological data of hayfever to other forms of rhinitis. Only one community-based study has reported on the epidemiology of both seasonal and perennial rhinitis (8). In this London-based study of 5349 adults aged 16-65 years, the prevalence of all forms of rhinitis (allergic and non-allergic) was 24%: 3% seasonal of whom 78% were atopic as indicated by skin-prick testing; 21% perennial of whom 60% were atopic. Whether this balance applies elsewhere is uncertain. A positive skin test to aeroallergens occurs in 20-30% of the total population (9). However, not everyone with a positive allergy test is likely to have clinical symptoms. This factor is also contributing to the varying prevalence rates encountered in the literature. Development of allergic rhinitis symptoms depends on exposure to allergen, age of the patient and genetic factors. Hay fever usually develops in childhood or adolescence, remains stationary for 2-3 decades, after which symptoms improve considerably in middle age and disappear in old age(10). No comparable data have been reported for the natural course of perennial allergic rhinitis.

There is much interest and controversy about whether allergic diseases, including rhinitis, are on the increase. Several studies have been published finding an increase in prevalence of allergic rhinitis in recent decades (11-15). Perhaps the best data supporting an increase in prevalence relate to medical examinations of 18-year old Swedish army recruits performed in 1971 and 1981(14). A current tendency to allergic rhinitis was diagnosed in 4.4% of 55,393 conscripts in 1971 and 8.4% of 57,150 conscripts in 1981. In contrast, the Australian National Health Survey found no appreciable change in the prevalence of self-reported hay fever from 1977-1978 to 1989-1990 (16).

1.2 Nasal airways

1.2.1 ANATOMY AND PHYSIOLOGY OF THE NOSE

The framework of the external nose is composed of the bony pyramid, the nasal bones and frontal process of the maxillary bone, and the cartilaginous pyramid, the triangular and the alar cartilage (upper resp. lower laterals). The lower laterals surround the nasal vestibule, which is lined with skin containing sebaceous and sweat glands and hairs. The nasal vestibules are trumpet-shaped orifices and each narrows from around 90mm^2 to a slit of 30 mm^2, which is the narrowest point of the nasal airway. This slit is formed by a ridge, which contains the caudal margin of the upper lateral cartilage. This area is called the nasal valve or ostium internum and is located between the vestibule and main nasal cavity(17). The nasal cavity extends from the ostium internum anteriorly to the choanae posteriorly and is lined with nasal mucosa. The nasal cavity is divided into two separate airways by the nasal septum. The lateral wall of the nasal cavity contains three turbinates or conchae, the inferior, middle and superior.

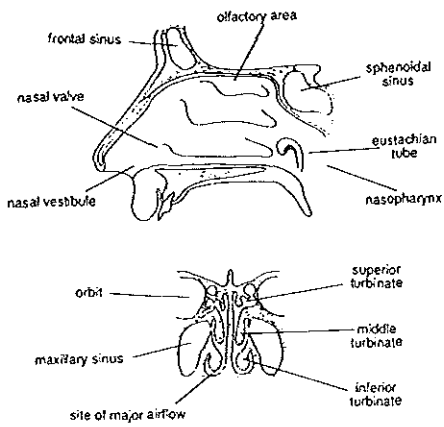

Figure 1. Diagrammatic representation of the structure of the nasal cavity with a view of the lateral wall of the nasal cavity and a cross-section through the middle of the nasal cavity.

Physiological functions of the nose are respiration and olfaction. Important respiratory functions are nasal resistance, heat exchange, humidification, and filtration of the inhaled air. Nasal resistance to airflow is primarily determined by two elements: the bony and cartilaginous

pyramid, and the state of congestion of the venous sinusoids in the mucosa of the inferior turbinate and anterior nasal septum. The level of sympathetic vasoconstrictor tone to the nasal blood vessels determines the state of congestion of the venous sinusoids. In rhinitis the state of congestion of the venous sinusoids is increased, probably due to increased nasal blood flow, leading to nasal obstruction. The changes in vascular activity are cyclical and occur between every 4 and 12 hours; they are constant for each person. This has been called the nasal cycle. By changing nasal resistance the airflow in each nasal cavity is modified.

The nose acts as an air-conditioning system. It is a very efficient organ to warm, humidify and filter the inspired air. In temperate climates, the temperature in the nasopharynx varies by 2-3^{0}C between inspiration and expiration, and the temperature of the expired air on expiration is the core temperature. In order to act as an efficient air-conditioner, the nose must offer a considerable resistance to airflow and, when nasal breathing, the nose contributes up to two thirds of the total respiratory resistance(18) and, a turbulent airflow is needed(19). This turbulence is accomplished by rapid changes in airflow direction and velocity as the air passes the nasal valve. At the junction of the nasal valve and main nasal cavity, the airway abruptly expands from 30 mm^2 to around 130 mm^2 and at this point the airstream bends through nearly 90^0. The main airstream is directed around the inferior turbinate, with the major airflow travelling close to the floor of the nasal cavity. With normal nasal breathing, relatively little airflow is directed upwards towards the middle and superior turbinates (20). One of the functions of the nose is to remove particles from the inspired air in order to protect the lower airway. The nose is able to filter out particles as small as 30 micrometer. This includes most pollen particles, which are among the smallest particles deposited, and it accounts for the fact that the nose is the commonest site of hay fever. Turbulence encountered in the airflow will increase the deposition of particles.

1.2.2 HISTOLOGY OF THE NASAL MUCOSA

The nasal cavity is lined with ciliated columnar epithelium with or without goblet cells and/or pseudostratified cuboidal epithelium, and a specialised olfactory epithelium in the olfactory region. The epithelial lining of the nose is directly exposed to the external environment and, as well as acting as an air conditioner, the surface also forms the first line of defence against toxic and infectious agents in the inspired air. The inspired air is a source of trauma to the mucosa, which may result in patches of squamous epithelium in the anterior areas of the nose, which are directly exposed to unconditioned air.

The epithelial cell types are basal cells, goblet cells, columnar cells (ciliated and non-ciliated), and migratory cells: lymphocytes, dendritic cells, macrophages, neutrophils, mast cells and eosinophils. The epithelium rests on a continuous basement membrane (21). Between the basement membrane and the underlying supportive bone is the lamina propria. The lamina propria is typically composed of a cell rich subepithelial layer with most of the mucous glands, and a deeper, collagen rich, cell poor layer lying on the supportive bone. The normal lamina propria contains several cell populations, such as: lymphocytes, dendritic cells, monocytes and macrophages, eosinophils, mast cells and neutrophils. The lamina propria further contains several blood vessels, which can be divided in resistance and capacitance vessels.

1.3 Pathophysiology of allergic rhinitis

1.3.1 REACTIONS OF NASAL MUCOSA AFTER ALLERGEN EXPOSURE

Allergen exposure leads to a cascade of immunological events in IgE-mediated allergic rhinitis. IgE-dependent activation of mast cells results in release of preformed, granule-derived mediators (e.g. histamine and tryptase) and newly-formed, membrane-derived mediators (e.g.leukotrienes D_4, C_4, and prostaglandin D_2). These mediators cause vasodilatation and an increase in vascular permeability, resulting in nasal blockage. Increased glandular secretion results in mucous rhinorrhea. Stimulation of afferent nerves may provoke itching and sneezing(4).

Most studies concerning the pathophysiology of allergic rhinitis have been performed in patients with seasonal allergic rhinitis. In seasonal allergic rhinitis the effect of allergen exposure can be investigated e.g. during natural pollen season or by artificial nasal allergen provocation performed outside the pollen season. In most studies investigating the pathophysiology of allergic rhinitis nasal allergen challenge was used. The advantages of artificial challenge are the well-controlled conditions such as allergen dose, and the precise begin and end of the challenge period. One should bear in mind, however, that in non-natural challenge studies high concentrations of allergens are administered to elicit a clear nasal response. This, in contrast to low and variable amounts of allergen inhaled by 15,000 breaths a day during natural allergen exposure(9).

The IgE-mediated allergic response after artificial allergen challenge may be characterised by two distinct phases; an early or immediate phase developing within 10 minutes after allergen exposure and a late phase occurring 3-4 hours later with a maximum at 6-8 hours after allergen exposure (22). The early phase response is characterised by immediate symptoms as itching, sneezing and rhinorrhea occurring within minutes. These symptoms are the result of

activation of mast cells and basophils by bridging of two IgE molecules on the surface of these mast cells and basophils by the allergen and subsequent mediator release.

Blackley first reported the late phase response (LPR) in 1873, who described the recurrence of symptoms several hours after the introduction of grass pollen in his own nose (1). A century later, Dolovich et al. demonstrated an early and late phase reaction occurring after skin testing. Furthermore, he found that both the early and late phase reactions were IgE-dependent (23). The LPR in the nose is less severe than the early response and more variable in time (9). The LPR is mainly characterised by nasal obstruction. In the LPR several inflammatory cells (Langerhans cells, CD4+ T cells, eosinophils, and neutrophils) are attracted to the site of allergen exposure as a result of mediator release(24-28). Increased levels of mast cell mediators(29) and influx of inflammatory and antigen presenting cells(28, 30, 31) have been found after repetitive allergen challenges. This inflammation contributes to the increased hyperresponsiveness of the nose after repeated allergen exposure. Connell has described this phenomenon as nasal priming(32).

1.3.2 CELLULAR ASPECTS OF ALLERGIC RHINITIS

1.3.2.1 *Mast cells*

Mast cells form a heterogeneous group as assessed by morphological, cytochemical and functional criteria. The pluripotent haemopoietic growth factors, GM-CSF and IL-3 influence stem cell differentiation into mast cell progenitors. Human mast cells can be divided into three sub-populations; one characterised by the presence of tryptase (MC_T), the second by the presence of tryptase and chymase (MC_{TC})(33), and the third by the presence of chymase only (MC_C(34)). More than 95% of the epithelial mast cells and 40% of the subepithelial mast cells in allergic nasal mucosa are of the MC_T-subtype(35). Mast cells have high affinity IgE-receptors on their surface, which can bind IgE. After cross-linking of two IgE molecules with allergen, degranulation follows. Mast cells secrete several mediators after degranulation: histamine, proteoglycanes, tryptase and chymase, arachidonic acid metabolites such as prostaglandine D2, leukotrienes B4, C4, D4, E4, platelet-activating factor PAF, and cytokines, such as interleukin (IL)-3, IL-4, IL-5, IL-6, granulocyte macrophage-colony stimulating factor (GM-CSF), tumour necrosis factor (TNF)-alfa(9, 36-38). Recent report suggests that mast cells can also provide support for IgE isotype switching(39). Mast cells are important in the pathogenesis of allergic rhinitis. The number of mast cells is increased in

allergic rhinitis, and levels of mast cell mediators in nasal lavage fluids are elevated after allergen challenge(30, 40-46).

1.3.2.2 *Eosinophils*

Tissue eosinophilia is characteristic of human atopic allergic inflammation, although the mechanisms are still partly unknown. Eosinophils are bone marrow derived granulocytes. The eosinophilopoiesis is unfenced by GM-CSF, IL-3 and IL-5(47-49).

Eosinphils have several surface receptors and proteins, and are sources of cytokines and appear to store many, if not all, of these cytokines in cytoplasmatic specific granules. Eosinophil products are summarised in table 1. The human eosinophils are reviewed by Weller(50).

The number of nasal mucosal eosinophils is increased in allergic rhinitis compared with controls(26, 30, 31). An eosinophil infiltration has been identified in nasal secretions as early as 30 min. after nasal allergen challenge and has been shown to persist as long as 48 hours (25, 31, 51).

Table 1. Human eosinophil lipid and protein products

Lipid products	Granule Cationic Proteins	Chemokines	Cytokines
LTC_4	MBP(core)	RANTES	TGF-α, TGF-ß
Lipoxinen	ECP(matrix)	MIP-1α	IL-1α
PGE_2	EDN(matrix)	IL-8	IL-3, IL-5
TxB_2	EPO(matrix)		GM-CSF
PAF			IL-2
			IL-4
			IL-6
			IL-10
			IL-16
			IFN-gamma
			TNF-α

1.3.2.3 *Lymphocytes*

Lymphocytes can be divided into three lineages on the basis of expression of cell surface markers: T-lymphocytes, B-lymphocytes and natural killer (NK)-lymphocytes. B-cells can differentiate into plasma cells after interaction with antigen, and subsequently secrete large amounts of immunoglobulin subclasses, including IgE. T-cells are further subdivided into T-helper (CD4+) cells and cytotoxic T-cells (CD8+). After activation T-cells express IL-2 receptors (CD25) on their surface. T-cells play a pivotal role in control of IgE synthesis(52). After activation T-cells secrete several pro-inflammatory cytokines (Table 2). In murines two functional subclasses of T-helper cells have been described on the basis of the cytokine profile: T-helper 1 cells (T_{H1}) producing IL-2 and IFN-gamma, and support cell-mediated immunity, and T-helper 2 cells (T_{H2}) producing IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, TNF-alpha and provide support to B-cells, encouraging the production of IgG1 and IgE(53). In man, also T-helper cells with a type 2 cytokine secretion profile have been isolated from atopic donors(54-56). *In vitro*, T_{H2}-cells support isotype switching to IgE while their production of IL-5, IL-3 and GM-CSF facilitates the recruitment and activation of eosinophils(57). Recent reports suggest that the route of antigen presentation and the type of antigen-presenting cell are the critical factors in determining the eventual phenotype of the CD4+ T cell(58, 59). In allergic rhinitis increased numbers of T-helper and activated T cells have been found in nasal mucosa after allergen challenge(26, 60).

Table 2

Cytokines and their major function in allergic rhinitis

Cytokine	cell source in allergic inflammation	Suspected cell target in allergic inflammation
IL-2	T-cell	T-cells, B-cells (proliferation, activation, differentiation)
IL-3	T-cells, eosinophils	mast cells, eosinophils (colony stimulating factor)
IL-4	T-cells, mast cells, eosinophils	B-cells (IgE isotype switch), endothelium (adhesion molecules up-regulation)
IL-5	T-cells mast cells, eosinophils	Eosinophils (chemotaxis)
IL-6	mast cells	T-, B-, cells (proliferation, differentiation) and fibroblasts
IL-8	mast cells, epithelial cells, eosinophils	Lymphocytes, neutrophils, basophils, eosinophils (chemotaxis)

IL-10	T-cells, monocytes, macrophages	Macrophages, T-cells (inhibitor of IFN-γ functions)
IL-13	T-cells, mast cells, eosinophils	B-cells (IgE isotype switch), endothelium (adhesion molecules up-regulation)
IFN-γ	T-cells NK cells	Macrophages (activation), IL-4 antagonist (IgE isotype switch)
RANTES	macrophages, eosinophils, T-cells and mast cells	Eosinophils and monocytes (chemotaxis)
TNF-α	mast cells	Fibroblast, endothelium (production of other cytokines and adhesion molecules (ELAM-1, ICAM-1))

1.3.2.4 *Antigen presenting cells*

A number of distinct cell types, including dendritic cells (DC), macrophages, Langerhans cells, B-lymphocytes, and epithelial cells are potentially capable of presenting antigen to T-lymphocytes. Their effectiveness as antigen presenting cell (APC) differs markedly.

DC progenitors are seeded through the blood into non-lymphoid tissues, where they develop to a stage referred to as immature DCs. These immature DCs are characterised by a high capability for antigen capture and processing, but low T cell stimulatory capability. Inflammatory mediators promote DC maturation and migration out of non-lymphoid tissues into the blood or afferent lymph. These mature DCs have lost the ability to capture antigen and have acquired an increased capacity to stimulate T cells. Recent animal experiments have suggested that resident pulmonary alveolar macrophages actively suppress the APC function of lung DC, and therefore may play an important role in local immunoregulation (61).

Langerhans cells (LC) are dendritic, bone marrow derived cells belonging to the macrophage/monocyte cell range(62, 63). It is believed that a subpopulation of dendritic cells acquires ultrastructural features as Birbeck granules and CD1a expression, characteristic of epidermal LC(64). In the nasal epithelium most cells with dendritic morphology contained Birbeck granules and were identified as LC. In the lamina propria only part of the cells with dendritic morphology contained Birbeck granules(65). Langerhans cells bear the antigen CD1, CD4, and have Fc-receptors for IgG, IgE and C3b(66, 67).

Other DC may be precursors of LC or LC migrated from the epithelium and matured into DC, losing their Birbeck granules and CD1a positivity (65, 68). Patients with allergic rhinitis have

more LC in the nasal mucosa compared to non-allergic controls(69). During the grass-pollen season significantly more LC were seen in nasal epithelium than before and after the season(70). After allergen provocation in winter an increase was seen in number of nasal mucosal LC of patients with a seasonal allergic rhinitis (28).

The antigen-presenting capacity of LC is much greater than that of blood-derived antigen-presenting cells, like monocytes(71-73). The LC is able to present antigen to T cells in allergic reactions(74-76). Antigen-induced activation of naïve T helper cells requires antigen presentation by physical contact with an antigen-presenting cell. Epidermal Langerhans cells have been shown *in vitro* to selectively support T_{H2} cell production(77). After antigen binding in the skin, LC probably move to the dermis and leave it as veiled cells via the lymphatics, finally becoming interdigitating cells which present antigen to T cells in the paracortical region of the lymph node. In asthma it has been suggested that dendritic cells (DC) play a prime role in inducing cytokine production by the local activation of T cells (78, 79). The site of presentation to T cells in nasal inflammation is still unknown. In the lamina propria of patients with allergic rhinitis sometimes large clusters of T lymphocytes and LC are seen, suggesting local activation of T cells(28).

1.3.3 MECHANISM OF THE INFLAMMATORY RESPONSE

In the initial phase, allergen interacts with sensitised mast cells to release mediators, which induce the symptoms of rhinitis. The release of TNF-α from mast cells, as has been demonstrated during the immediate allergic reaction in the nose(80), and IL-4 promote upregulation of several adhesion molecules which will result eventually in firmer leukocyte-endothelial adherence and allow leukocyte transendothelial migration under chemotactic stimuli(36, 81). The role of adhesion molecules is reviewed by Canonica et al.(82). The co-release of IL-5 will promote eosinophil differentiation, production and chemotaxis(50, 83). Uptake of allergen by IgE positive Langerhans cells in nasal epithelium leads to allergen presentation to T-lymphocytes with their consequent activation and elaboration of cytokines(84, 85). Cytokine release from T cells thus contributes to the amplification and maintenance of airway inflammation with mast cell mediator cytokine release initiating the process. The identification of mast cells, T cells and eosinophils as sources of cytokines may suggest an autoregulatory function of these cells through the generation and release of pro-inflammatory cytokines, resulting in the persistence of allergic inflammation. Apart from cytokines, which tend to have highly specific receptors, we now know there are several

chemokines whose receptors show a considerable degree of cross-reactivity(86). Chemokines also contribute to neutrophil, T cell, mast cell and eosinophil chemotaxis and activation(87-91).

1.4 Treatment of allergic rhinitis with emphasis on corticosteroids

1.4.1 MANAGEMENT OF ALLERGIC RHINITIS

The management of allergic rhinitis can be divided in three therapy regimens: a) **allergen avoidance**, b) **pharmacotherapy**, and c) **immunotherapy**. In most patients allergen avoidance will not result in complete relief of symptoms. There is a residue of symptoms requiring medical management. The use of various therapies is based on the understanding of the mechanisms of symptom development. Treatment can be directed towards antagonism of the endorgan effects of the mediators, either through specific receptor antagonism, i.e. histamine receptor H_1-antihistamines, or through functional antagonism, i.e. an α-agonist vasoconstrictor, or can be directed against differing aspects of cell infiltration and cell activation, i.e. corticosteroids(92). Antihistamines are effective in reducing symptoms as nasal itching, sneezing and watery rhinorrhea, but have little objective effect on nasal blockage. Antihistamines are usually taken orally which has the advantage of reducing systemic symptoms such as conjunctivitis and urticaria. Nowadays, topical intranasal glucocorticosteroids have become the first-line therapy for the treatment of allergic rhinitis. Steroids not only reduce nasal obstruction, but also nasal discharge and sneezing. In comparative studies they have proved more effective in symptomatic control of allergic rhinitis than sodium cromoglycate(93) and antihistamines(94-96). In severe cases of allergic rhinitis short courses of systemic corticosteroids can be of use, but should only be used with caution and when there are no contraindications.

Many patients with perennial allergic rhinitis use intranasal steroids continuously during several months and sometimes years to reduce their symptoms. Intranasal corticosteroids are considered safe regarding their effect on nasal mucosa after long-term use. Earlier studies investigating the safety of intranasal steroid sprays like Beclomethasone Dipropionate, Budesonide, and Flunisolide showed no evidence of mucosal damage and systemic side-effects (97-100). No data of Fluticasone Propionate Aqueous Nasal Spray (FPANS) concerning the adverse effects on nasal mucosa after long-term use are yet available. In

previous studies in which either long-term or high-dose treatments of FPANS were used no evidence of systemic side effects were found(101-103).

1.4.2 MOLECULAR MECHANISMS OF CORTICOSTEROID ACTION

Glucocorticosteroids exert their effects by binding to a single glucocorticoid receptor (GR), which is predominantly localised to the cytoplasm of target cells, and only on binding of the glucocorticoid does it move into the nuclear compartment (104, 105). GR is expressed in high density in airway epithelium and endothelium of bronchial vessels(106). There is a single class of GR with no evidence for subtypes of differing affinity in different tissues. The inactivated GR is bound to a protein complex that includes two subunits of the heat shock protein (hsp) 90(107). Once the steroid molecule binds to GR, hsp 90 dissociates, thus allowing the nuclear localisation of the activated GR-steroid complex and its binding to DNA (104, 105).

Steroids produce their effect on responsive cells by activating GR to regulate the transcription of certain target genes(108). The number of genes directly regulated by GR in a given cell is not certain. Activated GR binds to glucocorticoid response elements (GREs), and these interaction results in either induction (+GREs) or repression (nGREs) of steroid sensitive genes, thus changing the rate of transcription. In this way several aspects of the inflammatory process are inhibited (109). Steroids inhibit the transcription of several cytokines that are relevant in the inflammatory process, including IL-1, granulocyte-macrophage colony stimulating factor (GM-CSF), IL-3, IL-4, IL-5, IL-6 and IL-8 (110). T-lymphocyte activation is inhibited by steroids via inhibition of the activation of activator protein-1 (AP-1). Activation of AP-1 leads to the induction of a number of target genes such as IL-2, IL-2 receptor and T-cell proliferation(111). Steroids also increase the synthesis of lipocortin-1, which has an inhibitory effect on phospholipase A_2, and therefore may inhibit the production of lipid mediators such as leukotrienes, prostaglandin's, and platelet-activating factor(112). Steroids may also activate endonucleases that are involved in programmed cell death or apoptosis. This may be relevant to the action of steroids on eosinophil and mast cell survival(113).

References

1. Blackley C. Experimental researches on the cause and nature of catarrhus aestivus(hay fever or hay asthma): Balliere, Tindall and Cox, London; 1873.

2. Pirquet Cv. Allergie. Munch Med Wochenschr 1906;53:1457-58.
3. Middleton E, jr, Reed CE, Ellis EF. Allergy, principles and practice. In: Middleton E, Reed CE, Ellis EF, editors. Allergy, principles and practice. 2nd ed. ed. St. Louis: The Mosby Company; 1983. p. XXI-XXII.
4. Group IRMW. International Consensus Report on the Diagnosis and Management of Rhinitis. Allergy 1994;49(19(suppl)):1-34.
5. Platts-Mills TAE, Solomon WR. Aerobiology and inhalant allergens. In: Middleton, Reed, Ellis, Adkinson, Yuninger, Busse, editors. Allergy, Principles and Practice. 4th ed; 1993. p. 469-528.
6. Mygind M, Naclerio RM. Definition, classification, terminology. In: Mygind N, Naclerio RM, editors. Allergic and non-allergic rhinitis. Clinical aspects. 1st ed. ed. Copenhagen: Munksgaard; 1993. p. 11-14.
7. Sibbald B, Rink E. Labelling of rhinitis and hayfever by doctors. Thorax 1991;46(5):378-81.
8. Sibbald B, Rink E. Epidemiology of seasonal and perennial rhinitis: clinical presentation and medical history. Thorax 1991;46(12):895-901.
9. Mygind N, Dahl R, Pedersen S, Thestrup-Pedersen K. Basic Mechanisms. In: Essential Allergy. 2nd ed. Oxford: Blackwell Science; 1996. p. 3-60.
10. Pedersen PA, Weeke ER. Allergic rhinitis in Danish general practice. Prevalence and consultation rates. Allergy 1981;36(6):375-9.
11. Hagy GW, Settipane GA. Bronchial asthma, allergic rhinitis, and allergy skin tests among college students. J Allergy 1969;44(6):323-32.
12. Ninan TK, Russell G. Respiratory symptoms and atopy in Aberdeen schoolchildren: evidence from two surveys 25 years apart [published erratum appears in BMJ 1992 May 2;304(6835):1157] [see comments]. Bmj 1992;304(6831):873-5.
13. Wuthrich B. Epidemiology of the allergic diseases: are they really on the increase? Int Arch Allergy Appl Immunol 1989;1:3-10.
14. Aberg N. Asthma and allergic rhinitis in Swedish conscripts. Clinical & Experimental Allergy 1989;19(1):59-63.
15. Aberg N, Hesselmar B, Aberg B, Eriksson B. Increase of asthma, allergic rhinitis and eczema in Swedish schoolchildren between 1979 and 1991 [see comments]. Clin Exp Allergy 1995;25(9):815-9.
16. Statistics ABo. National Health Survey. Asthma and other respiratory conditions, Australia. Commonwealth of Australia 1991(Catalogue No. 4373.0 Canberra).
17. Cole P. Upper respiratory airflow. In: Press EB, editor. The Nose, Upper Airway Physiology and the Atmospheric Environment. Amsterdam: Elsevier Biomedical Press; 1982. p. 163-189.
18. Ferris BG, Mead J, Opie H. Partitioning of respiratory flow resistance in man. J Appl Physiol 1964;19:653-658.
19. Cole P. Modification of inspired air. In: Mathew OP, Sant-'Ambrogio G, editors. Lung biology in health and disease. New York: Marcel Dekker; 1988. p. 415-45.
20. Eccles R. Nasal Airways. In: Busse WW, Holgate ST, editors. Asthma and Rhinitis. Boston: Blackwell Scientific Publications; 1995. p. 73-79.

21. Mygind N, Pedersen M, Nielsen MH. Morphology of the upper airway epithelium. In: Proctor DF, Andersen I, editors. The Nose, Upper Airway Physiology and the Atmospheric Environment. Amsterdam: Elsevier Biomedical Press; 1982. p. 71-97.

22. Gleich GJ. The late phase of the immunoglobulin E-mediated reaction: a link between anaphylaxis and common allergic disease?. [Review]. J Allergy Clin Immunol 1982;70(3):160-9.

23. Dolovich J, Hargreave FE, Chalmers R, Shier KJ, Gauldie J, Bienenstock J. Late cutaneous allergic responses in isolated IgE-dependent reactions. J Allergy Clin Immunol 1973;52(1):38-46.

24. Naclerio RM, Kagey SA, Lichtenstein LM, Togias AG, Iliopoulos O, Pipkorn U, et al. Observations on nasal late phase reactions. [Review]. Immunological Investigations 1987;16(8):649-85.

25. Bascom R, Pipkorn U, Lichtenstein LM, Naclerio RM. The influx of inflammatory cells into nasal washings during the late response to antigen challenge. Effect of systemic steroid pretreatment. Am Rev Respir Dis 1988;138(2):406-12.

26. Varney VA, Jacobson MR, Sudderick RM, Robinson DS, Irani AM, Schwartz LB, et al. Immunohistology of the nasal mucosa following allergen-induced rhinitis. Identification of activated T lymphocytes, eosinophils, and neutrophils. American Review of Respiratory Disease 1992;146(1):170-6.

27. Bascom R, Pipkorn U, Proud D, Dunnette S, Gleich GJ, Lichtenstein LM, et al. Major basic protein and eosinophil-derived neurotoxin concentrations in nasal-lavage fluid after antigen challenge: effect of systemic corticosteroids and relationship to eosinophil influx. Journal of Allergy & Clinical Immunology 1989;84(3):338-46.

28. Godthelp T, Fokkens WJ, Kleinjan A, Holm AF, Mulder PGH, Prens EP, et al. Antigen presenting cells in the nasal mucosa of patients with allergic rhinitis during allergen provocation. Clin Exp Allergy 1996;26:677-688.

29. Wachs M, Proud D, Lichtenstein LM, Kagey-Sobotka A, Norman PS, Naclerio RM. Observations on the pathogenesis of nasal priming. J Allergy Clin Immunol 1989;84(4 Pt 1):492-501.

30. Bentley AM, Jacobson MR, Cumberworth V, Barkans JR, Moqbel R, Schwartz LB, et al. Immunohistology of the nasal mucosa in seasonal allergic rhinitis: increases in activated eosinophils and epithelial mast cells. J Allergy Clin Immunol 1992;89(4):877-83.

31. Godthelp T, Holm AF, Fokkens WJ, Doornenbal P, Mulder PG, Hoefsmit EC, et al. Dynamics of nasal eosinophils in response to a nonnatural allergen challenge in patients with allergic rhinitis and control subjects: a biopsy and brush study. J Allergy Clin Immunol 1996;97(3):800-11.

32. Connell JT. Quantitative intranasal pollen challenge. II. Effect of daily pollen challenge, environmental pollen exposure, and placebo challenge on the nasal membrane. J Allergy 1968;41(3):123-39.

33. Irani AM, Schwartz LB. Mast cell heterogeneity. [Review]. Clin Exp Allergy 1989;19(2):143-55.

34. Kleinjan A, Godthelp T, Blom H, Fokkens W, J,. Fixation with Carnoy's fluid reduces the number of chymase-positive mast cells: not all chymase-positive mast cells are also positive for tryptase. Allergy 1996;51:614-620.

35. Juliusson S, Aldenborg F, Enerback L. Proteinase content of mast cells of nasal mucosa; effects of natural allergen exposure and of local corticosteroid treatment. Allergy 1995;50(1):15-22.

36. Bradding P, Feather IH, Wilson S, Bardin PG, Heusser CH, Holgate ST, et al. Immunolocalization of cytokines in the nasal mucosa of normal and perennial rhinitic subjects. The mast cell as a source of IL-4, IL-5, and IL-6 in human allergic mucosal inflammation. Journal of Immunology 1993;151(7):3853-65.

37. Burd PR, Rogers HW, Gordon JR, Martin CA, Jayaraman S, Wilson SD, et al. Interleukin 3-dependent and -independent mast cells stimulated with IgE and antigen express multiple cytokines. J Exp Med 1989;170(1):245-57.

38. Peebles RS, Jr., Togias A. Role of mast cells and basophils in rhinitis. Chem Immunol 1995;62:60-83.

39. Gauchat JF, Henchoz S, Mazzei G, Aubry JP, Brunner T, Blasey H, et al. Induction of human IgE synthesis in B cells by mast cells and basophils. Nature 1993;365(6444):340-3.

40. Fokkens WJ, Godthelp T, Holm AF, Blom H, Mulder PG, Vroom TM, et al. Dynamics of mast cells in the nasal mucosa of patients with allergic rhinitis and non-allergic controls: a biopsy study. Clin Exp Allergy 1992;22(7):701-10.

41. Enerback L, Karlsson G, Pipkorn U. Nasal mast cell response to natural allergen exposure. Int Arch Allergy Appl Immunol 1989;88(1-2):209-11.

42. Castells M, Schwartz LB. Tryptase levels in nasal-lavage fluid as an indicator of the immediate allergic response. J Allergy Clin Immunol 1988;82(3 Pt 1):348-55.

43. Borres MP, Irander K, Bjorksten B. Metachromatic cells in nasal mucosa after allergen challenge. Allergy 1990;45(2):98-103.

44. Okuda M, Ohtsuka H, Kawabori S. Basophil leukocytes and mast cells in the nose. Eur J Respir Dis Suppl 1983;128(Pt 1):7-15.

45. Otsuka H, Dolovich J, Befus AD, Telizyn S, Bienenstock J, Denburg JA. Basophilic cell progenitors, nasal metachromatic cells, and peripheral blood basophils in ragweed-allergic patients. Journal of Allergy & Clinical Immunology 1986;78(2):365-71.

46. Slater A, Smallman LA, Drake-Lee AB. Increase in epithelial mast cell numbers in the nasal mucosa of patients with perennial allergic rhinitis. J. Laryngol. Otol. 1996;110:929-933.

47. Clutterbuck EJ, Hirst EM, Sanderson CJ. Human interleukin-5 (IL-5) regulates the production of eosinophils in human bone marrow cultures: comparison and interaction with IL-1, IL-3, IL-6, and GMCSF. Blood 1989;73(6):1504-12.

48. Owen W, Jr., Rothenberg ME, Silberstein DS, Gasson JC, Stevens RL, Austen KF, et al. Regulation of human eosinophil viability, density, and function by granulocyte/macrophage colony-stimulating factor in the presence of 3T3 fibroblasts. J Exp Med 1987;166(1):129-41.

49. Lopez AF, Sanderson CJ, Gamble JR, Campbell HD, Young IG, Vadas MA. Recombinant human interleukin 5 is a selective activator of human eosinophil function. J Exp Med 1988;167(1):219-24.

50. Weller PF. Human eosinophils. [Review] [8 refs]. J Allergy Clin Immunol 1997;100(3):283-7.

51. Pelikan Z, Pelikan-Filipek M. Cytologic changes in the nasal secretions during the immediate nasal response [published erratum appears in J Allergy Clin Immunol 1989 May;83(5):870]. J Allergy Clin Immunol 1988;82(6):1103-12.

52. Vercelli D, Jabara HH, Arai K, Geha RS. Induction of human IgE synthesis requires interleukin 4 and T/B cell interactions involving the T cell receptor/CD3 complex and MHC class II antigens. J Exp Med 1989;169(4):1295-307.

53. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol 1986;136(7):2348-57.

54. Wierenga EA, Snoek M, de Groot C, Chretien I, Bos JD, Jansen HM, et al. Evidence for compartmentalization of functional subsets of CD2+ T lymphocytes in atopic patients. J Immunol 1990;144(12):4651-6.

55. Robinson DS, Hamid Q, Ying S, Tsicopoulos A, Barkans J, Bentley AM, et al. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. N Engl J Med 1992;326(5):298-304.

56. Corrigan CJ, Hamid Q, North J, Barkans J, Moqbel R, Durham S, et al. Peripheral blood CD4 but not CD8 t-lymphocytes in patients with exacerbation of asthma transcribe and translate messenger RNA encoding cytokines which prolong eosinophil survival in the context of a Th2-type pattern: effect of glucocorticoid therapy. Am J Respir Cell Mol Biol 1995;12(5):567-78.

57. Ricci M, Rossi O, Bertoni M, Matucci A. The importance of Th2-like cells in the pathogenesis of airway allergic inflammation. Clin Exp Allergy 1993;23:360-369.

58. Secrist H, DeKruyff RH, Umetsu DT. Interleukin 4 production by CD4+ T cells from allergic individuals is modulated by antigen concentration and antigen-presenting cell type. J Exp Med 1995;181(3):1081-9.

59. van der Pouw-Kraan T, de Jong R, Aarden L. Development of human Th1 and Th2 cytokine responses: the cytokine production profile of T cells is dictated by the primary in vitro stimulus. Eur J Immunol 1993;23(1):1-5.

60. Calderon MA, Lozewicz S, Prior A, Jordan S, Trigg CJ, Davies RJ. Lymphocyte infiltration and thickness of the nasal mucous membrane in perennial and seasonal allergic rhinitis. Journal of Allergy & Clinical Immunology 1994;93(3):635-43.

61. Holt PG, Oliver J, Bilyk N, McMenamin C, McMenamin PG, Kraal G, et al. Downregulation of the antigen presenting cell function(s) of pulmonary dendritic cells in vivo by resident alveolar macrophages. J Exp Med 1993;177(2):397-407.

62. Katz SI, Tamaki K, Sachs DH. Epidermal Langerhans cells are derived from cells originating in bone marrow. Nature 1979;282(5736):324-6.

63. Volc-Platzer B, Stingl G, Wolff K, Hinterberg W, Schnedl W. Cytogenetic identification of allogeneic epidermal Langerhans cells in a bone-marrow-graft recipient [letter]. N Engl J Med 1984;310(17):1123-4.

64. Nestle FO, Nickoloff BJ. Dermal dendritic cells are important members of the skin immune system. Adv Exp Med Biol 1995;378:111-6.

65. Fokkens WJ, Broekhuis-Fluitsma DM, Rijntjes E, Vroom TM, Hoefsmit EC. Langerhans cells in nasal mucosa of patients with grass pollen allergy. Immunobiology 1991;182(2):135-42.

66. Stingl G, Wolff-Schreiner EC, Pichler WJ, Gschnait F, Knapp W, Wolff K. Epidermal Langerhans cells bear Fc and C3 receptors. Nature 1977;268(5617):245-6.

67. Bieber T, de la Salle H, de la Salle C, Hanau D, Wollenberg A. Expression of the high-affinity receptor for IgE (Fc epsilon RI) on human Langerhans cells: the END of a dogma. J Invest Dermatol 1992;99(5):10.

68. Schuler G, Steinman RM. Murine epidermal Langerhans cells mature into potent immunostimulatory dendritic cells in vitro. J Exp Med 1985;161:526-46.

69. Fokkens WJ, Vroom TM, Rijntjes E, Mulder PG. CD-1 (T6), HLA-DR-expressing cells, presumably Langerhans cells, in nasal mucosa. Allergy 1989;44(3):167-72.

70. Fokkens WJ, Rijntjes E, Vroom TM, Mulder PGH. Fluctuation of the number of CD-1(T6)-positive dendritic cells, presumably Langerhans cells, in the nasal mucosa of patients with an isolated grass-pollen allergy before, during and after the grass-pollen season. J Allergy Clin Immunol 1989;84:39-43.

71. Bjercke S, Elgo J, Braathen L, Thorsby E. Enriched epidermal Langerhans cells are potent antigen-presenting cells for T cells. J Invest Dermatol 1984;83(4):286-9.

72. Bjercke S, Gaudernack G, Braathen LR. Enriched Langerhans cells express more HLA-DR determinants than blood-derived adherent cells (monocytes and dendritic cells). Scand J Immunol 1985;21(5):489-92.

73. Rasanen L, Lehto M, Jansen C, Reunala T, Leinikki P. Human epidermal Langerhans cells and peripheral blood monocytes. Accessory cell function, autoactivating and alloactivating capacity and ETAF/IL-1 production. Scand J Immunol 1986;24(5):503-8.

74. Shelley WB, Juhlin L. Langerhans cells form a reticuloepithelial trap for external contact antigens. Nature 1976;261(5555):46-7.

75. Silberberg I, Baer RL, Rosenthal SA. The role of Langerhans cells in allergic contact hypersensitivity. A review of findings in man and guinea pigs. [Review]. J Invest Dermatol 1976;66(4):210-7.

76. Stingl G, Katz SI, Clement L, Green I, Shevach EM. Immunologic functions of Ia-bearing epidermal Langerhans cells. J Immunol 1978;121(5):2005-13.

77. Enk AH, Angeloni VL, Udey MC, Katz SI. Inhibition of Langerhans cell antigen-presenting function by IL-10. A role for IL-10 in induction of tolerance. J Immunol 1993;151(5):2390-8.

78. Robinson DS, Hamid Q, Jacobson M, Ying S, Kay AB, Durham SR. Evidence for Th2-type T helper cell control of allergic disease in vivo. [Review]. Springer Semin Immunopathol 1993;15(1):17-27.

79. Hamid Q, Azzawi M, Ying S, Moqbel R, Wardlaw AJ, Corrigan CJ, et al. Expression of mRNA for interleukin-5 in mucosal bronchial biopsies from asthma. J Clin Invest 1991;87(5):1541-6.

80. Bradding P, Mediwake R, Feather IH, Madden J, Church MK, Holgate ST, et al. TNF alpha is localized to nasal mucosal mast cells and is released in acute allergic rhinitis. Clin Exp Allergy 1995;25(5):406-15.

81. Montefort S, Holgate ST, Howarth PH. Leucocyte-endothelial adhesion molecules and their role in bronchial asthma and allergic rhinitis. [Review]. European Respiratory Journal 1993;6(7):1044-54.

82. Canonica GW, Ciprandi G, Buscaglia S, Pesce G, Bagnasco M. Adhesion molecules of allergic inflammation: recent insights into their functional roles. [Review] [59 refs]. Allergy 1994;49(3):135-41.

83. Terada N, Konno A, Tada H, Shirotori K, Ishikawa K, Togawa K. The effect of recombinant human interleukin-5 on eosinophil accumulation and degranulation in human nasal mucosa. J Allergy Clin Immunol 1992;90(2):160-8.

84. Fokkens WJ, Bruijnzeel-Koomen CA, Vroom TM, Rijntjes E, Hoefsmit EC, Mudde GC, et al. The Langerhans cell: an underestimated cell in atopic disease. [Review]. Clin Exp Allergy 1990;20(6):627-38.

85. Durham SR, Ying S, Varney VA, Jacobson MR, Sudderick RM, Mackay IS, et al. Cytokine messenger RNA expression for IL-3, IL-4, IL-5, and granulocyte/macrophage-colony-stimulating factor in the nasal mucosa after local allergen provocation: relationship to tissue eosinophilia. J Immunol 1992;148(8):2390-4.

86. Editorial. Cytokines, chemokines, T cells and allergy. Clin Exp Allergy 1996;26:2-4.

87. Kunkel SL, Standiford T, Kasahara K, Strieter RM. Interleukin-8 (IL-8): the major neutrophil chemotactic factor in the lung. Exp Lung Res 1991;17(1):17-23.

88. Warringa RA, Koenderman L, Kok PT, Kreukniet J, Bruijnzeel PL. Modulation and induction of eosinophil chemotaxis by granulocyte-macrophage colony-stimulating factor and interleukin-3. Blood 1991;77(12):2694-700.

89. Warringa RA, Schweizer RC, Maikoe T, Kuijper PH, Bruijnzeel PL, Koendermann L. Modulation of eosinophil chemotaxis by interleukin-5. Am J Respir Cell Mol Biol 1992;7(6):631-6.

90. Alam R, Stafford S, Forsythe P, Harrison R, Faubion D, Lett-Brown MA, et al. RANTES is a chemotactic and activating factor for human eosinophils. J Immunol 1993;150(8 Pt 1):3442-8.

91. Schall TJ, Bacon K, Toy KJ, Goeddel DV. Selective attraction of monocytes and T lymphocytes of the memory phenotype by cytokine RANTES. Nature 1990;347(6294):669-71.

92. Howarth PH. The medical treatment of chronic rhinitis. In: Busse WW, Holgate ST, editors. Asthma and Rhinitis. 1st ed. Boston: Blackwell Scientific Publications; 1995. p. 1415-1428.

93. Welsh PW, Stricker WE, Chu CP, Naessens JM, Reese ME, Reed CE, et al. Efficacy of beclomethasone nasal solution, flunisolide, and cromolyn in relieving symptoms of ragweed allergy. Mayo Clin Proc 1987;62(2):125-34.

94. Juniper EF, Kline PA, Hargreave FE, Dolovich J. Comparison of beclomethasone dipropionate aqueous nasal spray, astemizole, and the combination in the prophylactic treatment of ragweed pollen-induced rhinoconjunctivitis. J Allergy Clin Immunol 1989;83:627-633.

95. Charpin D, Vervloet D. Treating seasonal rhinitis: antihistamines or intranasal corticosteroids? Eur Respir Rev 1994;4:256-9.

96. Beswick KBJ, Kenyon GS, Cherry JR. A comparative study of Beclomethasone Dipropionate aqueous nasal spray with terfenadine tablets in seasonal allergic rhinitis. Curr Med Res Opin 1985;9:560-567.

97. Pipkorn U, Pukander J, Suonpaa J, Makinen J, Lindqvist N. Long-term safety of budesonide nasal aerosol: a 5.5-year follow-up study. Clinical Allergy 1988;18(3):253-9.

98. Brown HM, Storey G, Jackson FA. Beclomethasone dipropionate aerosol in the treatment of perennial and seasonal rhintis: a review of five years' experience. Br J clin Pharmac 1977;4:283S-286S.

99. Norman PS, Turkeltaub PC. Long-term treatment of perennial rhinitis with intranasal flunisolide spray. In: XIth international congress of Allergology and Clinical Immunology; 1982; 1982.

100. Poynter D. Beclomethasone dipropionate aerosol and nasal mucosa. Br J clin Pharmac 1977;4:295S-301S.

101. As van A, Bronsky EA, Grossman J, Meltzer EO, Ratner P, Reed C. Dose tolerance study of Fluticasone propionate aqueous nasal spray in patients with seasonal allergic rhinitis. Ann Allergy 1991;76:156-162.

102. Haye R, Gomez EG. A multicentre study to assess long-term use of fluticasone propionate aqueous nasal spray in comparison with beclomethasone dipropionate aqueous nasal spray in the treatment of perennial rhinitis. Rhinology 1993;31(4):169-74.

103. Meltzer EO, Orgel HA, Bronsky EA, Furukawa CT, Grossman J, LaForce CF, et al. A dose-ranging study of fluticasone propionate aqueous nasal spray for seasonal allergic rhinitis assessed by symptoms, rhinomanometry, and nasal cytology. Journal of Allergy & Clinical Immunology 1990;86(2):221-30.

104. Picard D, Yamamoto KR. Two signals mediate hormone-dependent nuclear localization of the glucocorticoid receptor. Embo J 1987;6(11):3333-40.

105. Wikstrom AC, Bakke O, Okret S, Bronnegard M, Gustafsson JA. Intracellular localization of the glucocorticoid receptor: evidence for cytoplasmic and nuclear localization. Endocrinology 1987;120(4):1232-42.

106. Adcock IM, Gilbey T, Gelder CM. Glucocorticoid receptor localization in normal and asthmatic lung. Am J Respir Crit Care Med 1996;154:771-782.

107. Bresnick EH, Dalman FC, Sanchez ER, Pratt WB. Evidence that the 90-kDa heat shock protein is necessary for the steroid binding conformation of the L cell glucocorticoid receptor. J Biol Chem 1989;264(9):4992-7.

108. Beato M. Gene regulation by steroid hormones. [Review]. Cell 1989;56(3):335-44.

109. Chung KF, Adcock IM, Barnes PJ. Actions of glucocorticoids on gene transcription: relevance to inflammatory lung disease. Thorax 1993.

110. Guyre PM, Girard MT, Morganelli PM, Manganiello PD. Glucocorticoid effects on the production and actions of immune cytokines. J Steroid Biochem 1988;30(1-6):89-93.

111. Crabtree GR. Contingent genetic regulatory events in T lymphocyte activation. Science 1989;243(4889):355-61.

112. Goulding NJ, Godolphin JL, Sharland PR, Peers SH, Sampson M, Maddison PJ, et al. Anti-inflammatory lipocortin I production by peripheral blood leucocytes in response to hydrocortisone. Lancet 1990;335(8703):1416-8.

113. Barnes PJ, Pedersen S. Efficacy and safety of inhaled corticosteroids in asthma. Am Rev Respir Dis 1993;148(4(suppl)):1-26.

Chapter 2.

Aims of the study

As mentioned in the introduction topical intranasal corticosteroids have become the first-line therapy for the treatment of allergic rhinitis. However, there is no data on mucosal changes after long-term treatment. The mode of action of topical corticosteroids, especially on nasal inflammatory cells, is still not completely elucidated. In addition, as several inflammatory cells produce cytokines, the effect of corticosteroid treatment on cytokine mRNA production needs to be further investigated. This study focuses on the effects of Fluticasone Propionate Aqueous Nasal Spray (FPANS) which is nowadays widely used for the treatment of all forms of allergic rhinitis.

In this thesis we tried to find the answers to the following questions:

1. Is long-term intranasal Fluticasone Propionate treatment in patients with perennial allergic rhinitis safe. Does long-term treatment lead to mucosal damage or atrophy?
2. Is long-term intranasal Fluticasone Propionate treatment in patients with perennial allergic rhinitis effective in reducing nasal symptoms.
3. How does intranasal Fluticasone Propionate treatment affect nasal inflammatory cells. Two models were chosen: a) during natural allergen exposure in patients with perennial allergic rhinitis, and b) a provocation model with patients with seasonal allergic rhinitis.
4. How does intranasal Fluticasone Propionate treatment affect the presence of cytokine mRNA in nasal mucosa. We used a nasal provocation model with patients with seasonal allergic rhinitis.

Chapter 3

A one-year placebo-controlled study of intranasal Fluticasone Propionate Aqueous Nasal Spray in patients with perennial allergic rhinitis: a safety and biopsy study

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Clin. Otolaryngol. 1998, 23,69-71.

Introduction

Topical intranasal glucocorticosteroids are very effective in the treatment of patients with allergic and perennial rhinitis (1-3). Many patients use intranasal steroids continuously during several months and sometimes years to relieve their symptoms. Earlier studies investigating the safety of intranasal steroid sprays like beclomethasone dipropionate, budesonide and flunisolide showed no evidence of mucosal damage and systemic side-effects (4-7).

Recently, a new and potent corticosteroid, Fluticasone Propionate Aquous Nasal Spray (FPANS), has become available for the treatment of allergic rhinitis. Studies in human volunteers have demonstrated a skin vasoconstrictor activity approximately twice that of beclomethasone dipropionate (8). Systemic bioavailability of FPANS is extremely low because of a combination of poor gastrointestinal absorption and extensive first-pass metabolism (9). A dose-tolerance study involving more than 400 patients with seasonal rhinitis receiving up to 1600 mcg/day indicated that no measure of hypothalamo-pituitary-adrenocortical axis (HPA) function was affected nor did routine laboratory tests reveal any treatment-related effects (10).

The present study was designed to investigate the long-term safety of FPANS, with regard to adverse events, tolerability and nasal mucosa, and efficacy in a one year randomised, placebo-controlled, double-blind study in patients with a perennial allergic rhinitis.

Material and methods

STUDY DESIGN

The study was designed as a single centre, double-blind, randomised, placebo-controlled, parallel-group study. Patient numbers were allocated sequentially to the patients. Patients were allocated to receive one of the two treatments in randomly permuted blocks of four patients. There was a run-in period on placebo of four weeks followed by FPANS (100 mcg b.i.d.) or placebo for 12 months. Patients were supplied with terfenadine tablets (60 mg) as rescue medication. Throughout the study, no concurrent medication for rhinitis, including sodium cromoglycate, vasoconstrictor sprays or tablets and other antihistamines, was permitted. Patients visited the clinic 12 times during the study period at 4-6 week intervals. The symptoms of nasal

blockage, nasal discharge, sneezing, nasal itching, and eye irritation were assessed by the investigator at each clinic visit and also by the patient in a diary card for a ten day period before each visit to the clinic. Both assessments used a four-point scale. At visits 3-11 patients were asked if they had used their nasal sprays regularly. Patients who had taken their medication without fail or missed only a few doses were classed as compliant. Two patients in the FPANS group between visit 3 and 4 and one patient in the FPANS group between visit 5 and 6 were classed as non-compliant.

At each clinic visit the nasal mucosa was inspected with regard to the grade of mucosal congestion, secretion, polyps, crusting, bleeding, and candidiasis. Furthermore nostril patency was determined.

A venous blood sample was taken from all patients at the beginning and the end of the trial period for determination of routine clinical chemistry and haematology parameters. Urine samples were collected and tested for the presence of blood, proteine, and glucose by the dipstick method. In addition, a 9 am plasma cortisol level was taken and in five patients a ACTH stimulation test was performed at the beginning and the end of the trial period. The values for each parameter were compared with the normal range of the laboratory where the analysis was performed.

All patients who entered the treatment phase and any patient who was withdrawn were included in the safety evaluation. In this study major adverse events were defined as: 1.death, 2.life-threatening events, 3.events which were disabling or incapacitating, 4.events which required hospitalisation, 5.clinical or laboratory events which led to withdrawal of the drug, 6.any congenital abnormality or cancer or drug overdose. All other events were considered to be minor adverse events.

The study was approved by the local Medical-Ethical Committee.

PATIENTS

Forty-two patients entered the comparative treatment period. The patients (mean age 28 yr, 28 ♂, 14 ♀) had had perennial allergic rhinitis for at least one year. Patients experienced symptoms throughout the year. The diagnosis perennial allergic rhinitis was confirmed by a skin prick test of at least 3^+ and a radio-allergosorbent test (RAST) score of at least 3^+ - 5^+) for house dust mite allergen (HDM). Patients were excluded if they had serious or unstable disease, infection of the upper and lower respiratory tract, structural abnormalities or had undergone nasal surgery less than six weeks prior to the study, or if patients were taking concurrent medication such as oral or

inhaled steroids, intranasal sodium cromoglycate or intranasal sympaticomimetic therapy. Female patients were excluded if they were pregnant or lactating. From all patients written informed consent was obtained before entering the study.

NASAL BIOPSIES AND STAINING PROCEDURE

Biopsies of nasal mucosa were taken after the run-in period and after 12 months use of the trial drug. Nasal biopsies were taken from the lower edge of the inferior turbinate using a Gerritsma forceps and processed as described elsewhere (11). From each specimen serial 6 µm-thick sections of nasal mucosa were cut on a Reichert-Jung 2800 Frigocut cryostat and transferred to gelatine-coated microscope slides. All sections were stained with Haematoxylin and Eosin, Giemsa, and von Gieson.

The features considered were: the appearance of the epithelial layer, the degree of cellular infiltration, the extent to which the sinusoids were dilated and the degree of tissue edema. These features were semi-quantitatively scored on a four-point scale. The specimens were classified into those showing a distinct overall improvement when the post-treatment specimen was compared to the pre-treatment specimen, those in which no change was detected, and those in which a deterioration was observed. The histological specimens were examined blind by the first author and confirmed by a second author(T.M.V.) who also examined the specimens blind. There was a good inter observer agreement.

STATISTICAL ANALYSIS

Symptom scores and rescue medication usage were analysed using the Van Elteren rank sum method (i.e. a stratified Wilcoxon rank sum test) adjusting for baseline. Total symptoms scores were analysed with a repeated measurement analysis of variance. Mucosal changes were analysed using a Fisher exact test. A p-value lower than 0.05 was considered statistically significant.

Results

Of the 42 patients entering the comparative treatment period 21 were randomly allocated to the FPANS-treated group and 21 to the placebo-treated group. Four patients receiving FPANS and

nine patients receiving placebo were withdrawn during the treatment period. Reasons for withdrawal are listed in table 1. This resulted in 29 evaluable patients.

Table 1

Reasons for withdrawal after start of comparative treatment period.

	FPANS	Placebo
Number of patients	21	21
Adverse event	1	0
Did not wish to continue	0	2
Failure to return	1	4
Ineffectiveness of medication	0	3
Moved from area	1	0
Work commitments	1	1
Total number of patients	4	9#

A patient may have more than one reason for withdrawal

NASAL BIOPSIES

Nasal biopsies from the inferior turbinate from 28 patients (16 FPANS, 12 placebo) with evaluable pre-treatment and post-treatment nasal mucosa specimens have been examined . The sections of nasal mucosa had an average surface area of 3 mm^2 and usually showed a lining of ciliated columnar epithelium with or without goblet cells and/or partially stratified cuboidal epithelium. The lamina propria usually consisted of a sub-epithelial cell-rich layer with most of the mucous glands and a deeper collagenous cell-poor layer onto the bone. All sections were deep enough to assess both layers. One patient had a vasovagal collaps at the moment the local anaesthesia was given, which prevented harvesting of the specimen.

Many specimens showed marked inflammatory changes, especially in the tissue immediately below the basement membrane. In the FPANS group an improvement was observed in epithelial quality, oedema and cellular infiltration. Table 2 shows the results of the evaluation of the nasal mucosa. Basement membrane thickness was slightly increased. In the placebo group the changes were less than in the FPANS group. However, basement membrane thickening occurred less after placebo treatment. However, none of the differences were statistically significant.

When comparing each individual patient before and after treatment, in eight out of 16 patients of the FPANS group and in seven out of 12 patients of the placebo group evidence of improvement was seen; less cellular infiltration, thinner basement membrane and intact epithelium. In seven patient specimens of the FPANS group no change was observed, versus one patient of the placebo group. In one patient in the FPANS group versus four patients of the placebo group a worsening as observed consisting of more cellular infiltration, more oedema and metaplasia of the epithelium. None of these differences were statistically significant.

No deleterious changes consequent on therapy were noted; no thinning of the epithelium was apparent and no suggestion of any specific effect on collagen synthesis was seen. There were no obvious effects on the connective tissue surrounding the blood vessels.

LABORATORY EVALUATIONS

Nine patients developed changes in laboratory data from baseline and they were equally distributed between the two treatment groups. There were no urine abnormalities. None of the laboratory abnormalities were considered to be related to the study drug. No unexpected changes or notable differences between treatment groups were seen for any of the parameters studied.

Plasma cortisol levels and the ACTH stimulation test did not show any evidence of suppression of the HPA axis related to FPANS after 12 months of therapy(table 3).

Table 2. Mean nasal mucosal evaluation comparing biopsy specimens before and after one-year treatment with Fluticasone Propionate (FPANS) 100 mcg b.i.d. A four-point scale was used (0-3). No statistical differences were found.

	FPANS		placebo	
	Before	after	before	after
Epithelial damage	1	0.7	1.2	1.1
Oedema	0.8	0.7	0.8	0.8
Thickened basement membrane	0.6	0.8	0.9	0.7
Cellular infiltration	1.7	1.5	2.1	2

Table 3. Nine a.m. cortisol blood level (nmol/l) after 12 months of therapy, before and after ACTH stimulation test.

	FPANS	placebo
before ACTH stimulation	434	408
after ACTH stimulation	816	688

SAFETY EVALUATIONS

No major adverse event was reported in this study. In total, 13 patients(62%) receiving FPANS and 12 patients(57%) receiving placebo reported minor adverse events. The most commonly reported adverse events in the FPANS group were headache(5), bronchitis(3), epistaxis(3) and upper respiratory tract infection(3). All these adverse events, except epistaxis, were reported by a similar number of patients in the placebo group. Hoarseness was noted in one patient who was treated with FPANS. One patient, using FPANS, withdrew due to an adverse event. This patient was known to develop mental depression after systemic corticosteroid usage, and again became depressed after using the study drug.

NASAL ASSESSMENT

Nasal assessments were carried out at each clinic visit. Table 4 shows the assessments at baseline and after one year of treatment for all patients who completed the study. After 12 months of treatment no changes in grade of mucosal congestion, nasal discharge, polyps, crusting, nasal patency and bleeding was found. None of the patients had evidence of candidiasis in the nose or throat

Table 4. Nasal assessment of percentage of patients at baseline and after one year of treatment.(FP=FPANS, Plac.=placebo)

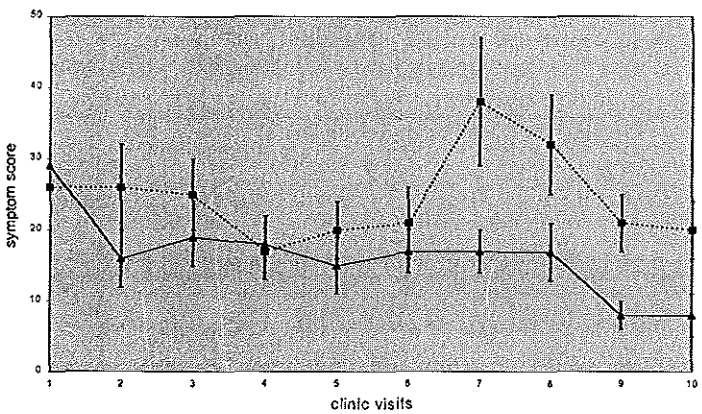
	baseline		after one year of treatment	
	FP	Plac.	FP	Plac.
mucosal swelling				
yes	23	62	11	37
evidence of crusting				
yes	8	0	14	8
evidence of bleeding				
yes	0	0	5	0
nasal polyps				
yes	0	0	0	0

NASAL SYMPTOMS

During the run-in period nasal symptoms did not differ significantly between the two groups. The total score of nasal symptoms is a sum score obtained from the diary card by double summation: over seven consecutive days before a clinic visit and over the various symptoms. This showed a reduction in total nasal symptom score in the FPANS treated group after six weeks treatment, with a further reduction after eight months treatment (fig.1). The course of total symptom score in the FPANS group showed a significantly stronger decline compared to the course of the placebo group ($p=0.009$). The symptom scores for sneezing and nasal itching were significantly better for the FPANS group than for the placebo-treated group after one year of treatment ($p<0.05$). No significant changes for nasal obstruction and nasal discharge were

observed. There was a trend for higher use of rescue medication in the placebo-treated group.

Figure 1. Total symptom score of FPANS- and placebo-treated groups. A significant stronger decline in total symptom score was seen in the FPANS group compared to the placebo group ($p=0.009$). SEM is indicated by the vertical lines. (■, placebo; ▲, FPANS)



Discussion

This primary aim of this study was to investigate the safety of FPANS (200mcg b.i.d.) when used for periods up to one year in the treatment of perennial allergic rhinitis. An obvious problem

to overcome was to keep the patient group, especially the placebo group, intact. This was anticipated by selecting a group of patients with a limited average of nasal symptomology, and by giving each patient terfenadine (60 mg) as rescue medication, with a maximum of two tablets each day. Nevertheless nine out of 21 patients in the placebo group did not complete the study versus four out of 21 patients in the FPANS group. Reasons for withdrawal in the placebo group were mainly ineffectiveness of study medication and failure to return, which probably also reflects ineffectiveness of study medication.

Intranasal corticosteroids are considered safe regarding their effect on nasal mucosa after long-term use (4-7) . This is, to our knowledge, the first placebo-controlled, long-term study in which nasal biopsies were taken to assess the safety of FPANS regarding nasal mucosa. Although Fluticasone Propionate is a potent local corticosteroid, no deleterious effects of FPANS on nasal mucosa was observed after a year of daily usage. In the present study the nasal mucosa, with regard to cellular infiltration, oedema, basement membrane thickness and epithelial changes, improved in either patient group after one year treatment in half of the patients. Only in the number of unchanged and worsened nasal mucosa specimens differences were noted between the two treatment groups, favouring the FPANS group. On the whole, 15 out of 16 patients in the FPANS group showed an improvement or, at least, no change of the mucosal specimens, versus eight out of 12 in the placebo group. The fact that active treatment as well as placebo treatment resulted in an improvement of the nasal mucosa in half of the patients may also be attributed to natural fluctuations of the disease. It is also conceivable that the daily spraying results in a minor nasal washing with beneficiary results on the quality of the nasal mucosa.

Treatment with FPANS at a dose of 100 mcg b.i.d. was well tolerated. No differences in nasal assessments and reported adverse events were found between the FPANS group and the placebo group after one year treatment. The reported incidence of epistaxis was higher in the FPANS group, but with nasal assessment after one year no difference in nasal bleeding was observed. The cause of epistaxis is not clear. Local irritation associated with nasal sprays will not differ between the FPANS and the placebo users. Therefore the occurrence of epistaxis must probably be attributed to the steroid itself, although biopsy study of the nasal mucosa showed no histological damage or atrophy.

No changes were found in HPA axis measurements, analysis of routine biochemistry, haematology and urine, probably due to the low bioavailability of FPANS. The safety evaluations in this placebo-controlled study confirm the results from previous studies in which either long-term or high-dose treatments were used (10, 12, 13).

FPANS has been proven effective in other studies concerning either seasonal or perennial allergic rhinitis, reducing nasal obstruction, sneezing and discharge (10, 12, 13). Our results show that after one year treatment sneezing, itching and total symptom score was significantly reduced in the FPANS group, with no significant change for nasal obstruction and discharge. There are several explanations for the fact that we did not find significant improvement in all nasal symptoms. The symptom score we used with a four point scale may not be sensitive enough to detect subtle changes. Also, compared to other studies (10, 12, 13), the total number of included patients was small. Moreover, the median symptom score in our patient group was not high when they were selected, therefore the feasible improvement is small.

Surprisingly, after an initial decrease in total symptom score a further decrease was seen after eight months' therapy in the FPANS treated group. These findings suggest that the maximum efficacy of topical intranasal steroids is reached after long-term treatment, and thus suggests longer usage before treatment is stopped because of presumed inefficacy.

In conclusion, FPANS is a well tolerated, safe treatment for patients with a perennial allergic rhinitis with no evidence of mucosal damage after long-term use.

References

1. Juniper EF, Kline PA, Hargreave FE, Dolovich J. Comparison of beclomethasone dipropionate aqueous nasal spray, astemizole, and the combination in the prophylactic treatment of ragweed pollen-induced rhinoconjunctivitis. J Allergy Clin Immunol 1989;83:627-633.

2. Norman PS, Creticos PS, Tobey R, et al. Budesonide in grass pollen rhinitis. Ann Allergy 1992;69:309-316.

3. Incaudo G, Schatz M, Yamamoto F, et al. Intranasal flunisolide in the treatment of perennial rhinitis:correlation with immunological parameters. J Allergy Clin Immunol 1980;65:41-49.

4. Pipkorn U, Pukander J, Suonpaa J, Makinen J, Lindqvist N. Long-term safety of budesonide nasal aerosol: a 5.5-year follow-up study. Clinical Allergy 1988;18(3):253-9.

5. Brown HM, Storey G, Jackson FA. Beclomethasone dipropionate aerosol in the treatment of perennial and seasonal rhintis: a review of five years' experience. Br J clin Pharmac 1977;4:283S-286S.

6. Turkeltaub PC, Norman PS, Johnson JD, Crepea S. Treatment of seasonal and perennial rhinitis with intranasal flunisolide. Allergy 1982;37(5):303-11.

7. Poynter D. Beclomethasone dipropionate aerosol and nasal mucosa. Br J clin Pharmac 1977;4:295S-301S.

8. Phillips GH. Structure-activity relationships of topically active steroids: the selection of fluticasone propionate. Respiratory Medicine 1990;84(suppl A):19-23.

9. Harding SM. The human pharmacology of fluticasone propionate. Respir Med 1990;84:25-9.

10. As van A, Bronsky EA, Grossman J, Meltzer EO, Ratner P, Reed C. Dose tolerance study ofFluticasone propionate aqueous nasal spray in patients with seasonal allergic rhinitis. Ann Allergy 1991;76:156-162.

11. Fokkens WJ, Rijntjes E, Vroom TM, Gerritsma V. A mild biopsy method to obtain high-quality specimens of nasal mucosa. Rhinology 1988;26(4):293-295.

12. Haye R, Gomez EG. A multicentre study to assess long-term use of fluticasone propionate aqueous nasal spray in comparison with beclomethasone dipropionate aqueous nasal spray in the treatment of perennial rhinitis. Rhinology 1993;31(4):169-74.

13. Meltzer EO, Orgel HA, Bronsky EA, et al. A dose-ranging study of fluticasone propionate aqueous nasal spray for seasonal allergic rhinitis assessed by symptoms, rhinomanometry, and nasal cytology. Journal of Allergy & Clinical Immunology 1990;86(2):221-30.

Chapter 4

The effect of 3 months' nasal steroid spray on nasal T cells and Langerhans cells in patients suffering from allergic rhinitis

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Allergy, 1995, 50, 204-209

Introduction

Topical corticosteroids are of proven efficacy in allergic rhinitis and have been shown to inhibit both the immediate and late response to allergen (1-5). Topical steroids prevent the accumulation of mast cells in the nasal mucosa, as well as the degranulation of mast cells and reduce the levels of histamine, TAME-esterase activity, and kinins in the early, late and rechallenge allergic reactions in nasal secretions (4-7). Corticosteroids have also been found to inhibit the migration of neutrophils and eosinophils (8,9). Treatment with the corticosteroid nasal spray Budesonide significantly reduces the number of eosinophils in nasal secretion of patients with perennial rhinitis (10). The operation of these drugs, however, remains uncertain.

In the skin, topically applied corticosteroids cause a decreased expression of MHC class II antigens (HLA-DR) on Langerhans cells (11). Also a subsequent reduction has been reported of the number of Langerhans cells in the epidermis (12). Moreover, topical corticosteroid therapy was claimed to decrease Langerhans cell-dependent T cell activation, which could not be restored by exogenous interleukin-1 (13). These findings in the skin suggest that topical steroid therapy suppress antigen presentation by a direct effect on the antigen presenting function of Langerhans cells.

In earlier studies, we identified Langerhans cells in the nasal mucosa (14). The incidence of Langerhans cells was found to increase during allergen provocation (15,16). Recently, activated T cells, expressing interleukin (IL) 2 receptors (CD25+) on the cell surface, were identified in nasal mucosa of patients with allergic rhinitis, suggesting a role for these cells in nasal allergy (17,18).

To investigate whether the number of Langerhans cells, total T cells (CD3+) and T cell subsets (T-helper, CD4+ and T-suppresor, CD8+) in the nasal mucosa is influenced by corticosteroids, the effect of Fluticasone propionate aqueous nasal spray (FPANS), a new corticosteroid spray, was studied in the nasal mucosa of 22 patients with perennial allergic rhinitis.

Material and methods

PATIENTS AND CONTROLS

Twenty-two patients participated in the study. The patients (mean age 26 yr., 15 ♂, 7 ♀) had had perennial allergic rhinitis for at least one year. The diagnosis perennial allergic rhinitis was confirmed by a radio-allergosorbent test (RAST) score of at least 3^+ (3^+ - 5^+) for house dust mite allergen (HDM) and sometimes other allergens like dander or pollen.

NASAL BIOPSIES

The study was conducted in a double blind manner with patients randomised into two groups. There was a run-in period on placebo of four weeks followed by Fluticasone propionate or placebo for 12 weeks. Biopsies of nasal mucosa were taken after the run-in period and after three months use of the trial drug. Nasal biopts were taken from the lower edge of the inferior turbinate using a Gerritsma forceps and processed as described elsewhere (19).

NASAL SYMPTOM SCORE

The symptoms of nasal blockage, nasal discharge, sneezing, and nasal itching were assessed by the investigator at each clinic visit and also by the patient in a diary card for a ten day period before each visit to the clinic. Both assessments used a four-point scale.

STAINING PROCEDURE

From each specimen serial 6 µm-thick sections of nasal mucosa were cut on a Reichert-Jung 2800 Frigocut cryostat and transferred to gelatine-coated microscope slides. The sections were stained according to a previously described immuno-alkaline phosphatase (AP) method (Fokkens et al., 1989a) using the monoclonal antibodies 66IIC7 (anti-CD1a on Langerhans cells)(Sanbio Monosan), anti-HLA-DR for dendritic cells/Langerhans cells and lymphocytes (Central Laboratory of the Red Cross Blood Transfusion Service, Amsterdam), anti-CD4 (T-helper cell) (Central Laboratory of the Red Cross Blood Transfusion Service, Amsterdam), anti-CD3 (total T cell number)(Becton Dickinson, Mountain View, Ca, USA), anti-CD25 (anti-IL2 receptor on activated T cells) (Becton Dickinson, Mountain View, Ca, USA), and anti-CD8(T-suppressor cell) (Monosan, Sanbio, Uden, the Netherlands).

CELL QUANTIFICATION

The surface area of two sections in total and of the epithelium and lamina propria separately, was estimated by means of the image analysis system Videoplan Kontron 2.1. Cells binding the monoclonal antibody used, had bright red surface membranes, red stained cytoplasm or both, depending on the cell type and/or monoclonal antibody evaluated. Cells were counted if they stained red and contained a nucleus. When a group of positive cells lay very close together or overlapped each other, only the definitely positive cells were counted.

The number of Langerhans cells and T cells were counted separately in the epithelium and the lamina propria of two sections at a magnification of 250x. The number of cells/mm^2 section area was calculated in epithelium and lamina propria.

The number of HLA-DR^+ cells could not be determined reliably because many positive cells were in close proximity and/or separate dendritic cells could not be readily distinguished. The number of HLA-DR^+ cells was assessed semi-quantitatively on a score from 0 to 4 with steps of 0.5, representing a range from practically no HLA-DR^+ cells to large numbers of HLA-DR^+ cells. Moreover, based on morphology and localisation, semi-quantitative assessment was made of the number of HLA-DR^+ Dendritic Cells (DC)/ macrophages, lymphocytes and epithelial cells, also on a 0 to 4 scale. Only cells clearly resembling lymphocytes (small, round, practically no cytoplasm) were judged to be lymphocytes. The rest of the migratory cells were judged to be DC/macrophages. HLA-DR^+ epithelial cells were easily recognised by morphology and location. Control sections treated with phosphate-buffered saline or an irrelevant monoclonal antibody were negative.

STATISTICAL ANALYSIS

Since the frequency distribution of the number of $CD1a^+$ cells and T cells per mm^2 in epithelium and lamina propria was not symmetrical and the variances were unequal, the two-tailed Mann-Whitney rank sum test of the differences was used. Differences in the score of HLA-DR^+ cells were analysed with the sign test. A p-value < 0.05 was considered to indicate a significant difference between groups.

Results

The sections of nasal mucosa had an average surface area of 3 mm^2 and usually showed a lining of ciliated columnar epithelium with or without goblet cells and/or partially stratified cuboidal epithelium. The epithelium could not be evaluated in 1 patient who was excluded from the study. The lamina propria usually consisted of a sub-epithelial cell-rich layer with most of the mucous glands and a deeper collagenous cell-poor layer onto the bone. All sections were deep enough to assess both layers.

After three months of therapy no histological changes of nasal mucosa were observed in either patient group.

$CD1a^+$ cells were found in the epithelium and predominantly in the sub-epithelial layer, in and around the glandular tissue of the lamina propria. All sections showed $CD1a^+$ dendritic cells in the epithelium and in the lamina propria before the start of the trial. After three months of therapy, the number of $CD1a^+$ cells in epithelium and lamina propria (Fig.1) in the group receiving FPANS was significantly lower than in the placebo group (epithelium $p<0.01$, lamina propria $p<0.01$).

$HLA\text{-}DR^+$ cells were found in the epithelium and predominantly in the subepithelial layer of the lamina propria. The $HLA\text{-}DR^+$ migratory cells showed mostly dendritic and sometimes lymphocytic morphology. Epithelial cells in the epithelium and/or epithelial cells in the mucous glands were positive in 13 out of 44 sections evaluated. Occasionally clusters (100-1000 cells) of $HLA\text{-}DR^+$ cells (dendritic and lymphocytic morphology) were seen in the lamina propria, mainly in the sub-epithelial layer.

Before the trial, no significant difference was found in the total score of $HLA\text{-}DR^+$ cells, or the score of $HLA\text{-}DR^+$ cells with dendritic or lymphoid morphology between the placebo group and the group about to receive FPANS. However, the score of $HLA\text{-}DR^+$ cells differed significantly between the patients. After three months of therapy, the group receiving FPANS showed a significant decrease in the score of the $HLA\text{-}DR^+$ cells with a dendritic morphology (epithelium $p<0.01$, lamina propria $p<0.01$), the score of the $HLA\text{-}DR^+$ cells with a lymphocytic morphology (epithelium $p<0.05$, lamina propria $p<0.005$) and the total number of $HLA\text{-}DR^+$ cells in epithelium ($p<0.001$) and lamina propria ($p<0.001$), whereas the placebo group did not do so (Fig 1).

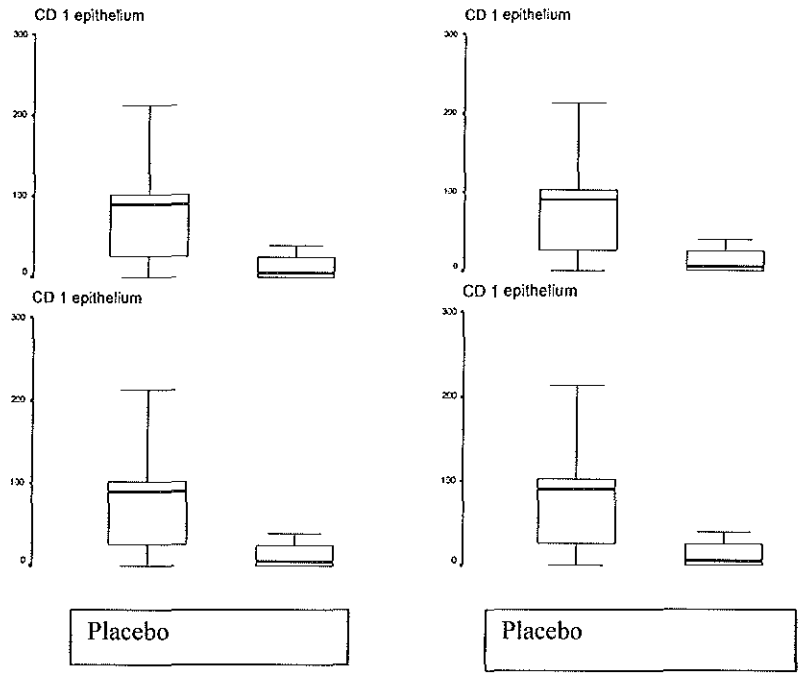

Figure 1. Number of CD1 and HLA-Dr positive cells in nasal epithelium and lamina propria of patients with perennial allergic rhinitis after 3 months of either Fluticasone Propionate Aqueous Nasal Spray (FPANS) or placebo therapy. Significant lower cell counts for CD1 and HLA-Dr positive cells were observed in the FPANS group compared with placebo

T cells were mainly observed in the lower and middle layer of the epithelium. In the lamina propria most T cells were seen in the subepithelial layer and between the glands. No significant change in number of total T cells, T cell subsets and activated T cells was found after corticosteroid therapy in nasal mucosa (Fig. 2,3). In nasal epithelium of patients treated with FPANS a clear downward trend of both CD3+ and CD8+ T cells was observed, although no significant difference with placebo-treated patients was found ($p=0.06$ resp. $p=0.07$).

Symptom score as assessed by the dairy card did not show a significant difference in total symptoms score or separate symptom score between the FPANS-treated group and the placebo-treated group during the treatment period (data not shown).

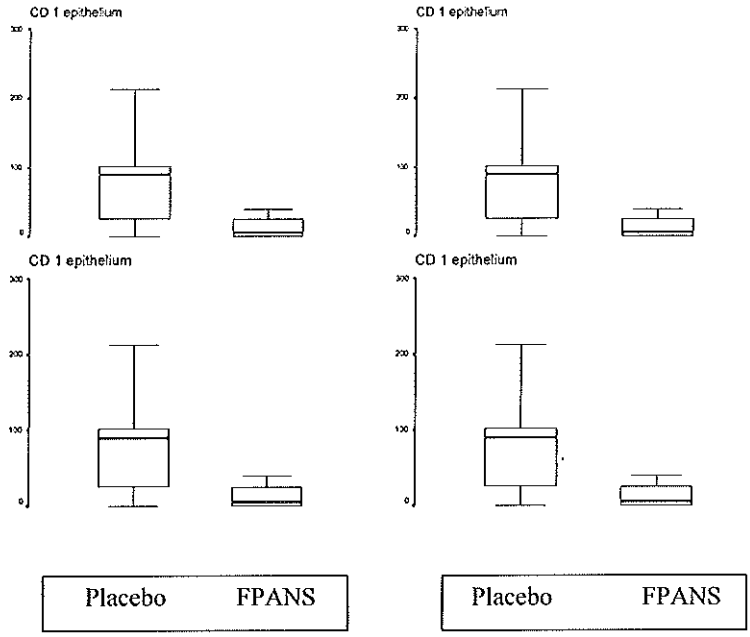

Figure 2. Number of T cells and subsets in nasal epithelium of patients with perennial allergic rhinitis after 3 months of either Fluticasone Propionate Aqueous Nasal Spray (FPANS) or placebo therapy. No significant difference in cell numbers were seen.

Discussion

In this study the influence of a new nasal corticosteroid, i.e. Fluticasone propionate aqueous nasal spray, on nasal Langerhans cells, T cells and HLA-Dr+ cells was investigated.

Nasal corticosteroid therapy has become an established procedure in allergic rhinitis. The exact working mode, however, is unclear. In studies on biopts of nasal mucosa, contrary to the skin, so far no significant structural changes have been found following local corticosteroid therapy (20,21).

In the skin topical corticosteroids reduce the number of Langerhans cells and the expression of HLA-DR on these cells. Possibly due to the decreased HLA-DR expression, the antigen

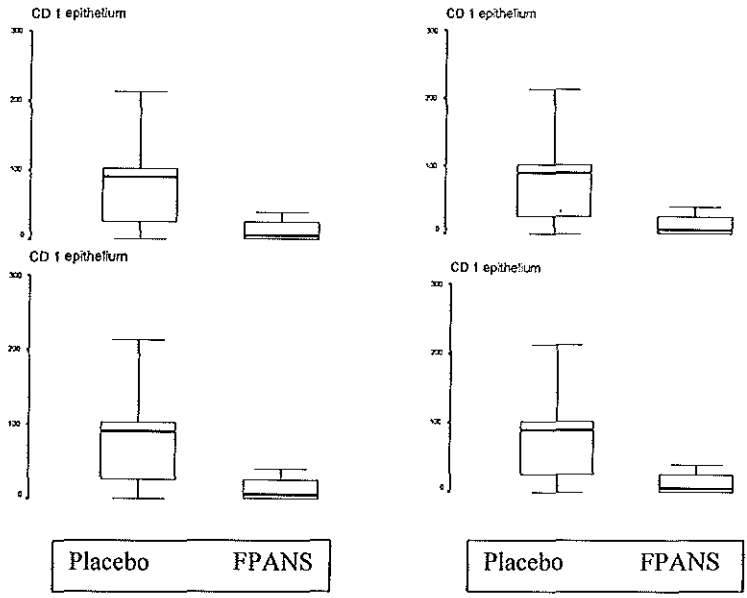

Figure 3. Number of T cells and subsets in lamina propria of patients with perennial allergic rhinitis after 3 months of either Fluticasone Propionate Aqueous Nasal Spray (FPANS) or placebo therapy. No significant difference in cell numbers were seen

presenting capacity of Langerhans cells was reduced in mice, and possibly also in man (13,22). These findings suggest that topical steroid therapy suppress antigen presentation by a direct effect on the antigen presenting function of Langerhans cells.

In this study we evaluated the number of $CD1a^+$ cells and $HLA\text{-}DR^+$ cells in the nasal mucosa of patients with perennial allergic rhinitis before and 3 months after FPANS therapy.

The score of $HLA\text{-}DR^+$ cells decreased significantly during therapy. In the skin of the guinea pig, Belsito et al. described a loss of Ia (MHC class II) but no irreversible structural changes during glucocorticosteroid treatment (23). Also in the human skin selective reduction in the expression of HLA-DR was found, but not of CD1a (11,12). These results suggest a change in cell surface markers rather than loss of the whole cell. However, studies in the human skin also show a decrease of $CD1a^+$ cells following more prolonged (a few days) glucocorticosteroid treatment. In literature no EM studies of human skin could be found to definitely prove that not only the expression of CD1a decreases, but the number of $CD1a^+$ cells as well.

In this study we found a considerable decrease in numbers of $CD1a^+$ cells in the epithelium during FPANS therapy (median numbers from 127 to 5). This decrease is much larger than the decreases reported in the skin. Possible explanations are that the glucocorticosteroid therapy is more effective in the nose, probably due to better penetration or that the new FPANS is a stronger glucocorticosteroid. In the skin, the decrease in the number of $CD1a^+$ cells was dependent on the potency of the topical corticosteroid used (24).

In vitro, corticosteroids have been known to reduce T cell responses to mitogen (25). Interleukin-2 receptor expression does not seem to be affected after corticosteroid therapy (22). During the late phase response a reduction of the influx of mononuclear cells in the nose after pre-treatment with Flunisolide was seen (27). However, no data are available concerning the dynamics of T cells in nasal mucosa in the continuous allergic reaction. In our study no significant change in number of T cells, T cell subsets and activated (IL-2R+) T cells in nasal mucosa was found after three months of corticosteroid therapy, which apparently corresponds with the in vitro findings.

The efficacy of FPANS has been proven in the treatment of allergic rhinitis (3). In our study no significant reduction in symptom score was found. The patients in this study were selected on having a RAST score of at least 3^+. Some patients had serious, while others had only mild nasal complaints. This, in combination with a relatively small patient group (22), probably explains why FPANS failed to reduce the symptoms significantly in this study.

In conclusion, the present study shows a decrease in $CD1a^+$ cells and $HLA\text{-}DR^+$ cells, but no significant change in T cell and activated T cell numbers, in nasal mucosa of patients with perennial allergic rhinitis during FPANS therapy. This finding suggests a decrease in antigen presentation. The subsequent decrease in T cells stimulation, without a corresponding decrease in IL-2 receptor expression, may result in a reduction of the reactions that are dependent on T cell derived mediators. Further study is necessary in order to determine whether Langerhans cells disappear from the nasal mucosa during FPANS therapy or that these findings are a result of a change in cell surface markers. Also the effect of FPANS therapy on antigen presentation of Langerhans cells and T cell function has yet to be evaluated.

References

1. Mygind N. Local effect of intranasal Beclomethasone Dipropionate aerosol in hay fever. Br Med J 1973:4:464-466.
2. Pipkorn U, Norman PS, Middleton Jr E. Topical Steroids. In: Mygind N, Weeke ER, Eds. Allergic and vasomotor rhinitis: Clinical aspects. Copenhagen: Munksgaard, 1985:165-170.
3. Meltzer EO, Orgel HA, Bronsky EA. A dose-ranging study of Fluticasone propionate aqueous nasal spray for seasonal allergic rhinitis assessed by symptoms, rhinomanometry, and nasal cytology. J Allergy Clin Immunol 1990:86:221-230.
4. Okuda M, Sakaguchi K, Ohtsuka H. Intranasal Beclomethasone: Mode of action in nasal allergy. Ann Allergy 1983:50:116-120.
5. Pipkorn U, Proud D, Lichtenstein LM, Kagey-Sobotka A, Norman PS, Naclerio RM. Inhibition of mediator release in allergic rhinitis by pretreatment with topical glucocorticosteroids. New Engl J Med 1987:316:1506-1510.
6. Gomez E, Claque JE, Garland D, Davies RJ. Effect of topical corticosteroids on seasonally induced increases in nasal mast cells. Br Med J 1988:296:1572-1573.
7. Elwany S, Talaat M, Talaat M, Helmy A. Allergic nasal mucosa following topical treatment with beclomethasone dipropionate (Bdp) aerosol, an electronmicroscopic study. J Laryngol Otol 1983:97:165-176.
8. Hoover RL, Karnovsky MJ, Austen KF, Corey EJ, Lewis RA. Leukotriene B4 actions on endothelium mediates augmented neutrophil/endothelial adhesion. Proc Nat Acad Sci 1984:81:2191-2193.
9. Altman LC, Hill JS, Hairfield WM, Mullarkey MF. Effects of corticosteroids on eosinophil chemotaxis and adherence. J Clin Invest 1981:67:28-36.
10. Wihl JA. Topical corticosteroids and nasal reactivity. Eur J Respir Dis 1984:129:625-629.
11. Berman B, France D, Martinelli GP, Hass A. Modulation of expression of epidermal Langerhans cell p roperties following in situ exposure to glucocorticosteroids. J Invest Dermatol 1982:80:168-171.
12. Pakes WL, Muller HK, Schwarz MA, Marks R. Langerhans cells - a reduction in numbers and their reappearance following steroid and cytotoxic therapy in humans. Clin Exp Dermatol 1986:11:450-459.
13. Ashworth J, Booker J, Breatnach SM. Effects of topical corticosteroid therapy on Langerhans cell antigen presenting functioning human skin. Br J Dermatol 1988:118:457-470.
14 Fokkens WJ, Vroom ThM, Rijntjes E, Mulder PGH. CD-1(T6), HLA-DR-expressing cells, presumably Langerhans cells, in nasal mucosa. Allergy 1989:44:852-857.
15. Fokkens WJ, Rijntjes E, Vroom ThM, Mulder PGH. Fluctuation of the number of CD-1(T6)-positive dendritic cells, presumably Langerhans Cells, in the nasal mucosa of patients with an isolated grass-pollen allergy before, during and after the grass-pollen season. J Allergy Clin Immunol 1989:84:39-43.
16. Fokkens WJ, Godthelp T, Holm AF, ThM Vroom, Mulder PHG, Rijntjes E. Dynamics of nasal Langerhans cells in patients with allergic rhinitis and non-allergic controls during allergen provocation, a biopsy study. Submitted for publication.

17. Varney,VA, Jacobson,MR, Suderick,RM, et al.
Immunohistology of the nasal mucosa following allergen-induced rhinitis
Am Rev Resp Dis 1992:146:170-176

18 Holm,AF, Godthelp,T, Fokkens,WJ, Mulder, PGH, Blom,H, Rijntjes,E
T cell activation in patients with an allergic rhinitis during allergen provocation ; a biopsy study
Submitted for publication

19. Fokkens,WJ, Rijntjes,E, Vroom,ThM, Gerritsma,V, 1988
A mild biopsy method to obtain high-quality specimens of nasal mucosa. Rhinology 1988:26(4):293-295

20. Mygind N, Sörensen H, Pedersen CB. The nasal mucosa during long term treatment with beclomethasone dipropionate. Acta Otolaryngol 1978:85:437-443.

21. Knight A, Kolin A. Long term efficacy and safety of beclomethasone dipropionate aerosol in perennial rhinitis. Ann Allergy 1983:50:81-84.

22. Aberer W, Stingl L, Pogantsch S, Stingl G. Effect of glucocorticoids on epidermal cell-induced immune responses. J Immunol 1984:133:792-797.

23. Belsito DV, Flotte TJ, Lim HW, Baer RL, Thorbeck GZJ, Gigli I. Effect of glucocorticosteroids on epidermal Langerhans cells. J Exp Med 1982:155:291-302.

24. Ashworth J. Potency classification of topical corticosteroids: modern perspectives. Acta Dermatol Venerol 1989:69 suppl 151:20-25.

25. Larsson,E-L;Cyclosporin A and dexamethasone suppress T cell responses by selectively acting at dictinct sites of the triggering process.J Immunol 1980:124:2828-2833

26. Reed,JC, Abidi,AH, Alpers,JD, Hoover,RG, Robb, RJ, Nowell,PC, Effect of cyclosporine A and dexamethasone on interleukin 2 receptor gene expression
J Immunology:1986: 137-150

27 Naclerio, RM
The pathophysiology of allergic rhinitis; impact of therapeutic intervention
J. Allergy Clin Immunol 1988:82:927-934

Chapter 5

Long-term effects of corticosteroid nasal spray on nasal inflammatory cells in patients with perennial allergic

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Accepted Clin Exp Allergy, 1998

Introduction

Allergic rhinitis is associated with the local accumulation of Langerhans cells, T cells and activated T cells, eosinophils and neutrophils in the nasal mucosa after allergen challenge (1-4).

Topical intranasal glucocorticosteroids are very effective in the treatment of patients with allergic and perennial rhinitis. Corticosteroids reduce the influx of eosinophils, neutrophils and mononuclear cells in nasal secretions in allergic rhinitis (5-7). Topical corticosteroids are usually effective after one or two weeks. Andersson et al. even found that short (one day) pre-treatment with topical steroids abolished the allergen-induced increase in nasal hyperresponsiveness (5).

It is, however, not clear whether topical corticosteroids have the maximum effect on either symptoms or nasal mucosal inflammatory cells after this short period of one to two weeks, or whether efficacy increases after prolonged treatment, with concomitant alterations in inflammatory cell numbers. Few studies have looked at the influence of prolonged treatment with topical corticosteroids on inflammatory cells in the nasal mucosa (8-10). In a previous study we reported a significant decrement in the number of Langerhans cells in the nasal mucosa and a trend of decreasing numbers of T cells in the nasal epithelium after three months of Fluticasone Propionate Aqueous Nasal Spray (FPANS) treatment (9). No data is yet available concerning the long-term effect of topical intranasal steroids on the cellular response in the nasal mucosa during natural allergen exposure.

The present study was designed to investigate the long-term effect of FPANS with reference to the nasal mucosa and inflammatory cells, efficacy, adverse events, and tolerability in a one-year, randomised, placebo-controlled, double-blind study in patients with perennial allergic rhinitis. This paper presents the effects of FPANS on inflammatory cells. Data about safety (adverse events) and tolerability has been published separately. FPANS proved to be a well-tolerated safe treatment for patients with perennial allergic rhinitis. Furthermore, no deleterious changes were observed consequent to therapy in nasal mucosal biopsies after one year of treatment (11).

Material and methods

STUDY DESIGN

This study was designed as a single-centre, double-blind, randomised, placebo-controlled, parallel-group study. There was a run-in period on placebo of four weeks followed by FPANS (100 µg b.i.d.) or placebo for 52 weeks. Patients were supplied with terfenadine tablets (60 mg) as rescue medication. Throughout the study, no concurrent medication was permitted for rhinitis, including sodium cromoglycate, vasoconstrictor sprays or tablets and other antihistamines. Patients visited the clinic 11 times during the study period at 4-6 week intervals. This made it possible to monitor the patients as well as possible without making the burden of the visits to the clinic excessive. The symptoms of nasal blockage, nasal discharge, sneezing, and nasal itching were assessed by the investigator at each clinic visit and also by the patient in a diary card for a ten-day period prior to each visit to the clinic. Both assessments used a four-point scale (0=no symptoms, 1=mild, 2=moderate, 3=severe).

The local Medical Ethics Committee approved the study.

PATIENTS

Forty-two patients entered the comparative treatment period. The patients (mean age 28 years, 28 male, and 14 female) had had perennial allergic rhinitis for at least one year. Patients experienced symptoms throughout the year. A skin prick test and a radio-allergosorbent test (RAST) confirmed the diagnosis of perennial allergic rhinitis A minimum of a 3+ positive reaction to house dust mite allergen (HDM) was required in the skin prick test. If there were more positive reactions, the reaction to HDM had to be the most positive reaction. Table 1 shows which allergens patients were sensitised to. Patients were excluded if they had serious or unstable disease, recurrent or chronic infection of the upper and lower respiratory tract or structural abnormalities. They were also excluded if they had undergone nasal surgery less than six weeks prior to the study or if they were taking concurrent medication such as oral or inhaled steroids, intranasal sodium cromoglycate or intranasal sympaticomimetic therapy. Female patients were excluded if they were pregnant or lactating. Written informed consent was from all patients prior to entry to the study.

Table 1. Results of the skin-prick test for the intent-to-treat placebo and FPANS groups ($ minimum reaction 3+)

positive skin-prick test results	Placebo	FPANS
house dust mite$	21	21
grass pollen	7	9
tree pollen	2	1
cat	8	8
dog	5	7
horse	2	2
rabbit	4	1
parakeet	1	0
guinea pig	3	5

NASAL BIOPSIES AND STAINING PROCEDURE

Biopsies of nasal mucosa were taken after the run-in period, after three months and after 12 months use of the trial drug. Patients were instructed not to take Terfenadine 48 hours before their visit to the clinic in order to minimise the effect on nasal mucosa. Nasal biopsies were taken from the lower edge of the inferior turbinate using a Gerritsma forceps and processed as described elsewhere (12). From each specimen, serial 6 µm-thick sections of nasal mucosa were cut on a Reichert-Jung 2800 Frigocut cryostat and transferred to gelatine-coated microscope slides. The sections were stained for cell surface and intracellular markers using a previously-described alkaline phosphatase anti-alkaline phosphatase method (13). Langerhans cells were stained with the monoclonal antibody 66IIC7 (CD1a, Sanbio, Monosan, Uden, the Netherlands), T cells and subsets with anti-CD4 (Central Laboratory of the Red Cross Blood Transfusion Service (CLB), Amsterdam, the Netherlands), anti-CD3 and anti-CD25 (Becton Dickinson, Mountain View, Ca, USA) and anti-CD8 (Monosan, Sanbio, Uden, the Netherlands). Macrophages were stained with anti-CD68, monocytes with anti-CD14, (Mon.1, Central Laboratory of the Red Cross Blood Transfusion Service (CLB), Amsterdam, the Netherlands), eosinophils with anti-BMK13, and activated ECP-secreting eosinophils with anti-EG2. Mast cells were stained with the mAb anti-tryptase (G3 4ìg/ml) and with anti-chymase biotin (B7 3.3ìg/ml, Chemicon, Brunschwig, Amsterdam, the Netherlands).

CELL QUANTIFICATION

Estimates were made of the total surface area of two sections and of the epithelium and lamina propria separately using the Videoplan Kontron 2.1 image analysis system. Cells binding a monoclonal antibody had bright red surface membranes, red stained cytoplasm or both, depending on the cell type and/or monoclonal antibody evaluated. Cells were counted if they stained red and contained a nucleus. When groups of positive cells lay very close together or overlapped each other, only the definitely positive cells were counted. The cell numbers in epithelium and lamina propria were divided by the area and expressed as number of cells per mm^2. The number of CD14 positive cells was assessed semi-quantitatively using a four-point scale (0-3: epithelium:0 = 0-5 cells per view, magnification 20X, 1 = 5-15 cells, 2 = 15–20 cells, 3 = more than 20 cells; lamina propria: 0 = 0-10 cells, 1 = 10-30 cells, 2 = 30-60 cells, 3 = more than 60 cells).

STATISTICAL ANALYSIS

Since the frequency distribution of the number of $CD1a^+$ cells, T cells, CD68+ cells, mast cells and eosinophils per mm^2 in the epithelium and lamina propria was not symmetrical and the variances were unequal, the two-tailed Mann-Whitney rank sum test of the differences was used. Differences in the score of CD14 positive cells were analysed with the sign test. The total score of nasal symptoms was obtained from the diary card. It covered seven consecutive days before a clinic visit and the various symptoms. Total symptom scores were summarised per patient as average change from baseline during weeks 4 to 52. A comparison was then made between the two treatment groups using a t-test. A repeated measures analysis of variance was conducted to test for a difference between the two treatment groups in the trend over time for the total symptom scores, taking all ten repeated measurements into account (weeks 0 to 52). The common intercept used for the two treatment groups was the mean baseline level (week 0). Separate symptom scores were analysed using the Van Elteren rank sum method (i.e. a stratified Wilcoxon rank sum test), adjusting for baseline. A p-value < 0.05 was considered to indicate a significant difference between the groups.

Results

Of the 42 patients entering the comparative treatment period, 21 were randomly allocated to the FPANS group and 21 to the placebo group. Four patients receiving FPANS and 9 patients receiving the placebo were withdrawn during the treatment period (table 2). In four patients, there was no evaluable mucosa. Of the patients included in the analysis, sixteen were treated with FPANS and nine with the placebo.

Table 2

Reasons for withdrawal after start of comparative treatment period.

Reason	FPANS	placebo
adverse event	1	0
did not wish to continue	0	2
failure to return	1	4
ineffectiveness of medication	0	3
moved from area	1	0
work commitments	1	1
total	4	9#

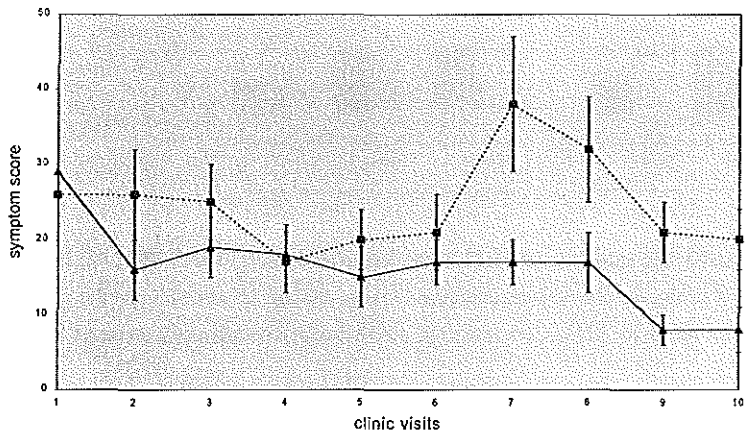
A patient may have more than one reason for withdrawal

NASAL SYMPTOMS

During the run-in period, nasal symptoms did not differ significantly between the two groups. The total symptom score showed a reduction in the FPANS group after four weeks of treatment (first visit after commencing treatment), with a further reduction after 46 weeks of treatment (fig.1). Between week 4 and week 52, the mean change from baseline in the average total symptom score was significantly higher in the FPANS group than in the placebo group ($p=0.018$). A repeated measures analysis of variance showed, in the FPANS group, a downward linear trend over time in the total symptom score which was significantly greater

than in the placebo group (p=0.009). The symptom scores for sneezing and nasal itching were significantly better for the FPANS group than for the placebo group after one year of treatment ($p<0.05$). No significant changes for nasal obstruction and nasal discharge were observed. There was no statistical difference in the use of Terfenadine between the two treatment groups during the whole treatment period. No correlation was found between symptoms and cell parameters.

Figure 1. Total symptom score of FPANS- and placebo-treated groups. A significant stronger decline in total symptom score was seen in the FPANS group compared to the placebo group (p=0.009). SEM is indicated by the vertical lines. (■, placebo; ▲, FPANS)



LYMPHOCYTES

T cells were mainly observed in the lower and middle layer of the epithelium. In the lamina propria, most T cells were seen in the subepithelial layer. In the nasal epithelium of patients treated with FPANS, a clear downward trend was seen after three months in the numbers of CD3 and CD8 positive cells. After one year of treatment, significantly lower numbers of CD3+, CD4+ and CD8+ positive T cells were seen in the active treatment group compared to placebo ($p<0.05$, $p<0.05$, $p<0.01$, respectively)(figure 2). There was no difference between the two treatment groups during therapy in the number of CD25+ cells. After three months and one year of treatment, no differences were seen in the total number of T cells and T cell subsets in the lamina propria between the FPANS and placebo groups (figure 3).

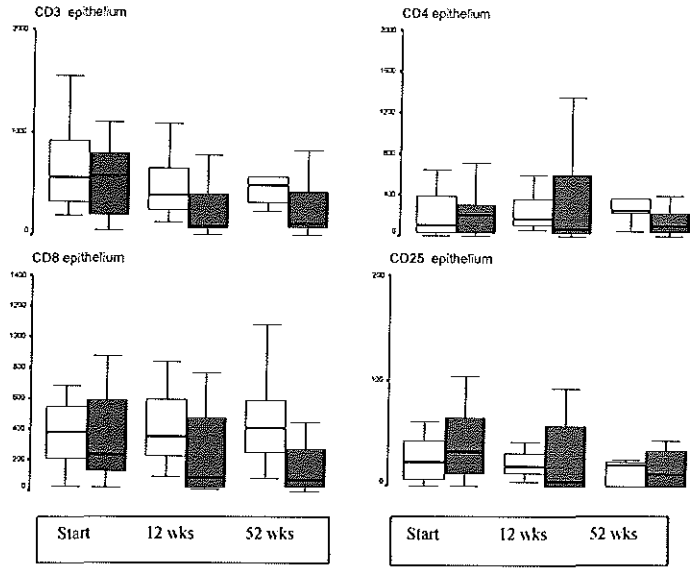


Figure 2.

The number of T cells and T cell subsets (cells/mm^2) in the epithelium in the FPANS (■) and placebo (□) groups during one year of treatment. The median, interquartile range and the 2.5 and 97.5 centiles are shown. Significant differences are found for CD3, CD4 and CD8 positive cells between the two treatment groups ($p<0.05$).

LANGERHANS CELLS, MACROPHAGES and MONOCYTES

CD1a$^+$ cells were found in the epithelium, predominantly in the sub-epithelial layer. In all sections, there were CD1a$^+$ dendritic cells in the epithelium and in the lamina propria before the start of the trial. After three months and one year of treatment, the number of epithelial CD1a+ cells in the FPANS group was significantly lower ($p<0.01$) than in the placebo group. After three months of FPANS treatment, significantly lower numbers of CD1+ cells ($p<0.01$) were seen in the lamina propria, but not after one year of treatment. The number of macrophages and monocytes did not change significantly during treatment (figure 4).

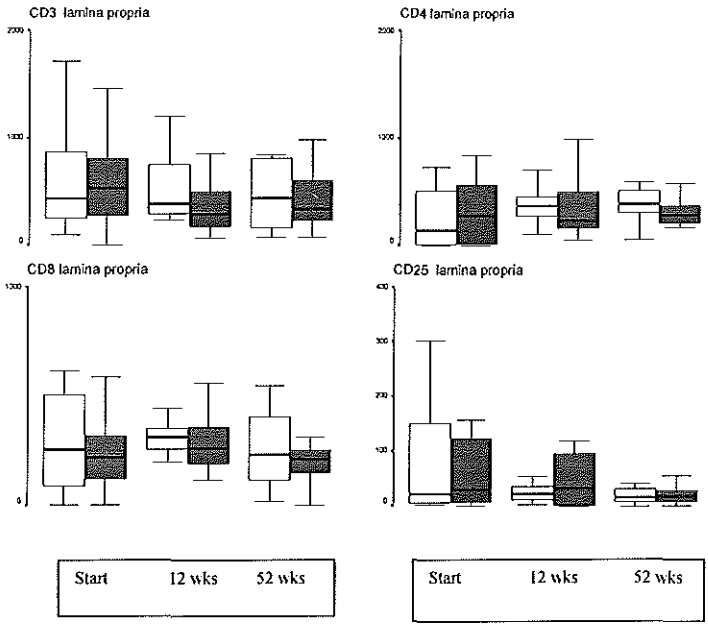


Figure 3.

The number of T cells and T cell subsets (cells/mm^2) in the lamina propria in the FPANS (■) and placebo (□) groups during one year of treatment. No significant changes were found during one year of treatment.

EOSINOPHILS

After three months and twelve months, the total number of eosinophils was significantly reduced in both the epithelium and lamina propria ($p<0.05$) in the FPANS group compared to the placebo group. The number of EG2+ cells in the lamina propria in the FPANS group was significantly reduced ($p<0.05$) after three and twelve months. In the epithelium, there was a downward trend after three months and twelve months ($p=0.06$)(figure5). There was no difference in the ratio of EG2/bmk13 positive cells between the treatment groups after three and twelve months.

MAST CELLS

Most of the biopsy specimens showed no tryptase or chymase positive cells in the epithelium. No significant differences in epithelial tryptase and chymase positive cells were observed between the two treatment groups after three and twelve months. Tryptase and chymase

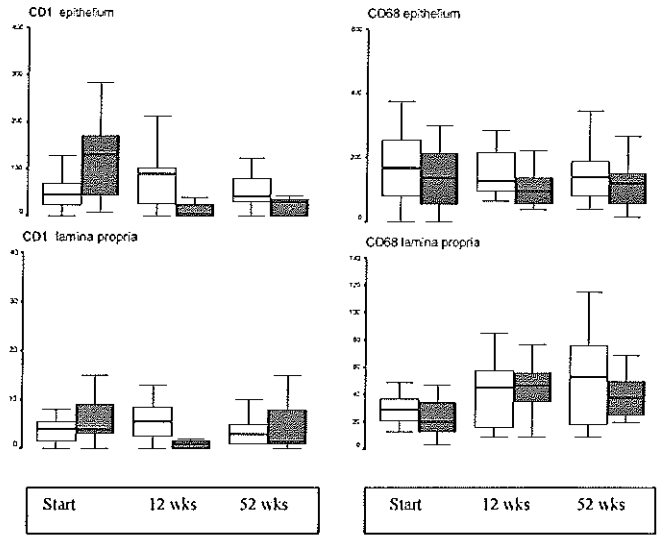


Figure 4.

The number of CD1 and CD68 positive cells in the nasal mucosa in the FPANS (■) and placebo (□) groups during one year of treatment. The median, interquartile range and the 2.5 and 97.5 centiles are shown. Differences are indicated between the two treatment groups (* = $p<0.05$, ** = $p<0.01$).

positive cells were found throughout the lamina propria. Both tryptase and chymase positive cells were significantly reduced in the FPANS group compared to the placebo group ($p<0.05$) after three and twelve months (figure 6).

Discussion

In the present study we confirmed our previous finding of a reduction in the number of Langerhans cells in nasal mucosa after three months of steroid treatment in patients with perennial allergic rhinitis (9). Moreover, we found a significant decrease in the number of T cells and T cell subsets in the nasal epithelium after one year of steroid treatment. To our knowledge, no data is available about the dynamics of T cells in the continuous allergic reaction during corticosteroid treatment. A few studies have addressed the long-term use of intranasal steroids, but they did not investigate nasal mucosal inflammatory cell changes (14-16). These studies investigated the effect of topical steroids on nasal mucosa integrity. Others

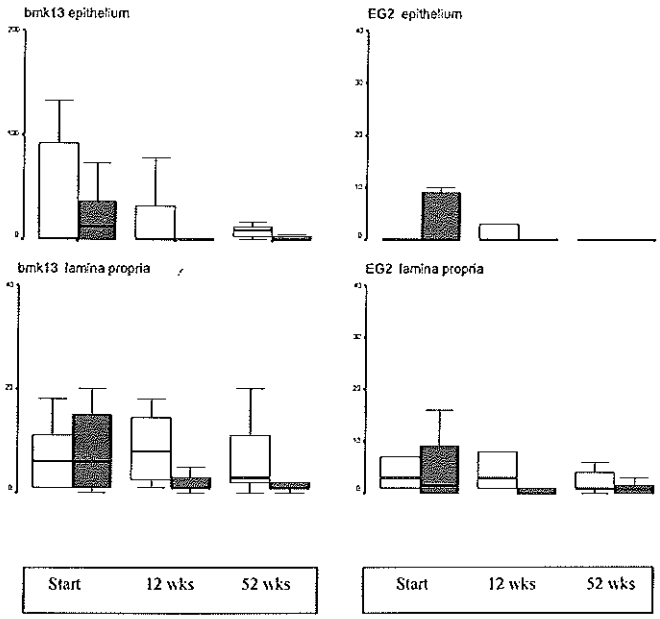


Figure 5.

The number of eosinophils in nasal mucosa in the FPANS (■) and placebo (□) groups during one year of treatment. The median, interquartile range and the 2.5 and 97.5 centiles are shown. Differences are indicated between the two treatment groups (* = $p<0.05$).

have presented data on the effect of topical intranasal steroid therapy on nasal mucosal inflammatory cells, but only after a short treatment period and after artificial allergen provocation (17-19). Rak et al. have investigated the effect of prolonged treatment (six weeks) with a topical corticosteroid on cellular infiltration in the nasal mucosa after allergen challenge (20). Twenty-four hours after allergen challenge, they found a reduction in the number of nasal mucosal T cells after treatment with FPANS. This reduction was more pronounced in the epithelium than in the lamina propria. This result is consistent with our findings. Our study found no significant changes in T cells in the lamina propria after steroid treatment. This difference with the finding of Rak et al. can probably be explained by the higher allergen dose which was used in their study for local provocation, with a more marked increase in both early and late phase response compared with natural allergen exposure in our study. Unlike Rak et al., we were unable to find a reduction in the numbers of IL-2 receptor (CD25) positive T cells.

In vitro, corticosteroids have been known to reduce T cell responses to mitogen (21).

In vitro, also, interleukin-2 synthesis is reduced by glucocorticosteroids with an inhibition of T lymphocyte proliferation as a result (22). In addition, glucocorticosteroids inhibited IL-2 receptor expression by T cells (23, 24). Our findings that CD4 positive, CD8 positive and CD3 positive lymphocyte numbers were reduced in the epithelium of the nasal mucosa therefore correspond to the in vitro findings. However, our findings of unchanged numbers of CD25 positive cells (activated T cells) in the nasal mucosa is in contrast to what we had expected based on the in vitro results(23, 24) and the study of Rak et al.(20).

An interesting finding is the significant reduction in the number of T cells after one year of treatment, something which was not seen after three months of steroid treatment, although a trend was noticed (9). This finding may be explained by the fact that the three-month and one-year biopsies were taken in different seasons. However, patients entered the study over a period of six months. Seasonal influences are therefore not likely to affect to any major extent mean nasal mucosal cell counts.

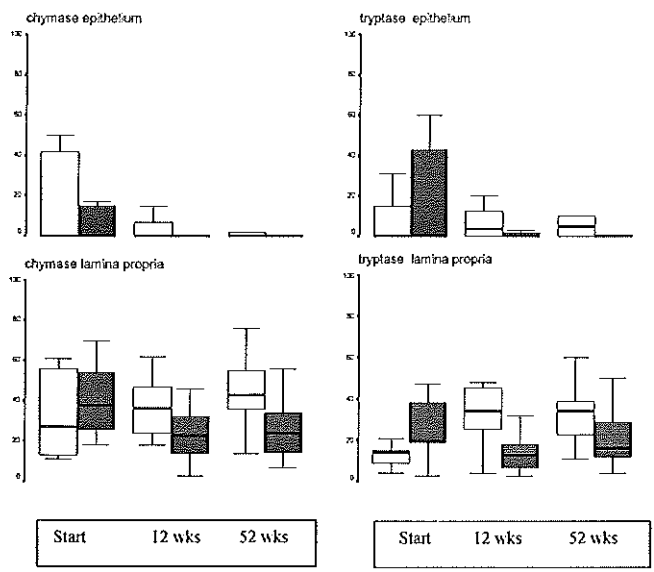


Figure 6. The number of mast cells in nasal mucosa in the FPANS (■) and placebo (□) groups during one year of treatment. The median, interquartile range and the 2.5 and 97.5 centiles are shown. Differences are indicated between the two treatment groups (* = $p<0.05$, ** = $p<0.01$).

Langerhans cells are known to be potent antigen-presenting cells (APC) and their numbers increase in nasal mucosa during allergen provocation (1, 25). The number of Langerhans cells in the epithelium and lamina propria after three months was significantly lower in the FPANS group compared to the placebo group. After 12 months, however, a significant reduction in the number of Langerhans cells was seen only in the epithelium in the FPANS group compared to the placebo group. The reason for this finding is unclear. It does not seem to reflect a transient effect of corticosteroids, since it is not seen with other cell types. In addition, a transient effect of corticosteroids is not reflected in symptomatology.

The effectiveness of FPANS in reducing the symptoms of seasonal and perennial rhinitis has been well documented (6, 26, 27). Topical nasal steroids are potent drugs for the relief of nasal symptoms in allergic rhinitis. Efficacy is also confirmed with this study as the total nasal symptom score improves significantly after twelve months of FPANS treatment compared with placebo treatment. Looking at separate nasal symptoms, however, we could not find any significant decrease in nasal obstruction and rhinorrhea was seen throughout the one-year treatment. There may be several explanations for this finding. The symptom score we used with a four-point scale may not be sensitive enough to detect changes. Moreover, the median symptom score in our patient group was not high when they were selected. The potential for improvement is therefore small. In addition, compared to other studies, the total number of included patients was small.

The eosinophil is considered important in the pathophysiology of allergic rhinitis (17, 28). Lozewicz et al. found that pre-treatment with steroids inhibits the activation of infiltrating eosinophils in the nasal mucosa, with no change in the numbers of total numbers of eosinophils (19). Others found a reduction of eosinophils in nasal washings and nasal cytograms, but did not use monoclonal antibodies to stain eosinophils and activated eosinophils (6, 17, 29). Bende at al. found, in patients treated for between three and 36 months with Beclomethasone diproprionate and non-treated controls for non-allergic perennial rhinitis, no differences in eosinophils, leukocytes and general inflammation in nasal mucosa (30). In the present study we found a reduction in both total number of eosinophils, and in the number of activated eosinophils in the nasal mucosa. This concurs with the findings of others who used monoclonal antibodies for staining (10, 20), indicating not only an inhibition of eosinophil activation, but also an inhibition of infiltration of eosinophils in the nasal mucosa. We did not find a difference between the ratios of EG2/bmk13 positive cells in nasal mucosa

in the two treatment groups. However, if the number of activated eosinophils and the number of total eosinophils are equally reduced in the actively-treated group, the ratio of these two cells would not change. This may explain why we did not find a significant difference in the ratio of EG2/bmk13 positive cells after three and twelve months.

In the present study, a significant reduction was observed in the number of MC_T and MC_C in the FPANS group. This reduction in MC_T was also found by Juliusson et al. after steroid treatment (31).

Macrophages have been claimed to be involved in the inflammatory process of allergic rhinitis (32). However, with Juliusson, we found no change in the number of macrophages and monocytes after steroid treatment.

In summary, long-term topical nasal steroid therapy results in a reduction of nasal inflammatory cells compared to placebo treatment. From this study, it is clear that corticosteroid treatment reduces several cells involved in the inflammatory cascade. Furthermore, this study suggests that the maximum effect of topical steroid treatment is reached after prolonged treatment.

References

1. Fokkens WJ, Rijntjes E, Vroom TM, Mulder PGH. Fluctuation of the number of CD-1(T6)-positive dendritic cells, presumably Langerhans cells, in the nasal mucosa of patients with an isolated grass-pollen allergy before, during and after the grass-pollen season. J Allergy Clin Immunol 1989;84:39-43.
2. Calderon MA, Lozewicz S, Prior A, Jordan S, Trigg CJ, Davies RJ. Lymphocyte infiltration and thickness of the nasal mucous membrane in perennial and seasonal allergic rhinitis. Journal of Allergy & Clinical Immunology 1994;93(3):635-43.
3. Varney VA, Jacobson MR, Sudderick RM, Robinson DS, Irani AM, Schwartz LB, et al. Immunohistology of the nasal mucosa following allergen-induced rhinitis. Identification of activated T lymphocytes, eosinophils, and neutrophils. American Review of Respiratory Disease 1992;146(1):170-6.
4. Godthelp T, Holm AF, Fokkens WJ, Doornenbal P, Mulder PG, Hoefsmit EC, et al. Dynamics of nasal eosinophils in response to a nonnatural allergen challenge in patients with allergic rhinitis and control subjects: a biopsy and brush study. J Allergy Clin Immunol 1996;97(3):800-11.
5. Andersson M, Andersson P, Pipkorn U. Topical glucocorticosteroids and allergen-induced increase in nasal reactivity: relationship between treatment time and inhibitory effect. J Allergy Clin Immunol 1988;82:1019-1026.

6. Meltzer EO, Orgel HA, Bronsky EA, Furukawa CT, Grossman J, LaForce CF, et al. A dose-ranging study of fluticasone propionate aqueous nasal spray for seasonal allergic rhinitis assessed by symptoms, rhinomanometry, and nasal cytology. Journal of Allergy & Clinical Immunology 1990;86(2):221-30.
7. Naclerio RM. The pathophysiology of allergic rhinitis: impact of therapeutic intervention. J Allergy Clin Immunol 1988;82:927-934.
8. Orgel HA, Meltzer EO, Bierman CW, Bronsky E, Connell JT, Lieberman PL, et al. Intranasal fluocortin butyl in patients with perennial rhinitis: a 12-month efficacy and safety study including nasal biopsy. Journal of Allergy & Clinical Immunology 1991;88(2):257-64.
9. Holm AF, Fokkens WJ, Godthelp T, Mulder PGH, Vroom TM, Rijntjes E. The effect of three months nasal steroid therapy on nasal T cells and Langerhans cells in patients suffering from allergic rhinitis. Allergy 1995;50:204-209.
10. Godthelp T, Holm AF, Blom H, Klein-Jan A, Rijntjes E, Fokkens WJ. The effect of fluticasone propionate aqueous nasal spray on nasal mucosal inflammation in perennial allergic rhinitis. Allergy 1995;50(23 Suppl):21-4.
11. Holm AF, Fokkens WJ, Godthelp T, Mulder PGH, Vroom TM, Rijntjes E. A one-year placebo-controlled study of intranasal Fluticasone Propionate Aqueous Nasal Spray in patients with perennial allergic rhinitis: a safety and biopsy study. Clin Otolaryngology 1998;23:69-73.
12. Fokkens WJ, Rijntjes E, Vroom TM, Gerritsma V. A mild biopsy method to obtain high-quality specimens of nasal mucosa. Rhinology 1988;26(4):293-295.
13. Cantani A, Paganelli R, Meglio P, Ferrara M, Fameles BE, Businco L. Effect of flunisolide on nasal eosinophils and IgE, and symptom score in children with allergic rhinitis. Journal of Investigative Allergology & Clinical Immunology 1992;2(4):181-6.
14. Pipkorn U, Pukander J, Suonpaa J, Makinen J, Lindqvist N. Long-term safety of budesonide nasal aerosol: a 5.5-year follow-up study. Clinical Allergy 1988;18(3):253-9.
15. Brown HM, Storey G, Jackson FA. Beclomethasone dipropionate aerosol in the treatment of perennial and seasonal rhintis: a review of five years' experience. Br J clin Pharmac 1977;4:283S-286S.
16. Norman PS, Turkeltaub PC. Long-term treatment of perennial rhinitis with intranasal flunisolide spray. in: XIth international congress of Allergology and Clinical Immunology; 1982; 1982.
17. Bascom R, Pipkorn U, Lichtenstein LM, Naclerio RM. The influx of inflammatory cells into nasal washings during the late response to antigen challenge. Effect of systemic steroid pretreatment. Am Rev Respir Dis 1988;138(2):406-12.
18. Bisgaard H, Gronborg H, Mygind N, Dahl R, Lindquist N, Venge P. Allergen-induced increase of eosinophil cationic protein in nasal lavage fluid: effect of the glucocorticoid budesonide. J Allergy Clin Immunol 1990;85:891-895.
19. Lozewicz S, Wang J, Duddle J, Thomas K, chalstrey S, Reilly G, et al. Topical glucocorticoids inhibit activation by allergen in the upper respiratory tract. J Allergy Clin Immunol 1992;89:951-957.
20. Rak S, Jacobson MR, Sudderick RM, Masuyama K, Juliusson S, Kay AB, et al. Influence of prolonged treatment with topical corticosteroid (fluticasone propionate) on early and late phase nasal responses

and cellular infiltration in the nasal mucosa after allergen challenge. Clin Exp Allergy 1994;24(10):930-9.

21. Larsson E-L. Cyclosporin A and dexamethasone suppress T cell responses by selectively acting at distinct site of the triggering process. J Immunol 1980;124:2828-2833.

22. Haczku A, Alexander A, Brown P, Assoufi B, Li B, Kay AB, et al. The effect of dexamethasone, cyclosporin, and rapamycine on T-lymphocyte proliferation in vitro: comparison of cells from patients with glucocorticoid-sensitive and glucocorticoid-resistant chronic asthma. J Allergy Clin Immunol 1994;93:510-519.

23. Batuman OA, Ferrero AP, Diaz A, Berger B, Pomerantz RJ. Glucocorticoid-mediated inhibition of interleukin-2 receptor alpha and -beta subunit expression by human T cells. Immunopharmacology 1994;27(1):43-55.

24. Briggs WA, Gao ZH, Xing JJ, Gimenez LF, Samaniego MD, Scheel PJ, et al. Suppression of lymphocyte interleukin-2 receptor expression by glucocorticoids, cyclosporine, or both. J Clin Pharmacol 1996;36(10):931-7.

25. Fokkens WJ, Bruijnzeel-Koomen CA, Vroom TM, Rijntjes E, Hoefsmit EC, Mudde GC, et al. The Langerhans cell: an underestimated cell in atopic disease. [Review]. Clin Exp Allergy 1990;20(6):627-38.

26. As van A, Bronsky EA, Grossman J, Meltzer EO, Ratner P, Reed C. Dose tolerance study of Fluticasone propionate aqueous nasal spray in patients with seasonal allergic rhinitis. Ann Allergy 1991;76:156-162.

27. Haye R, Gomez EG. A multicentre study to assess long-term use of fluticasone propionate aqueous nasal spray in comparison with beclomethasone dipropionate aqueous nasal spray in the treatment of perennial rhinitis. Rhinology 1993;31(4):169-74.

28. Viegas M, Gomez E, Brooks J, Gatland D, Davies RJ. Effect of the pollen season on nasal mast cells. Br Med J 1987;294(6569):414.

29. Bascom R, Wachs M, Naclerio RM, Pipkorn U, Galli SJ, Lichtenstein LM. Basophil influx occurs after nasal antigen challenge: effects of topical corticosteroid pretreatment. J Allergy Clin Immunol 1988;81(3):580-9.

30. Bende M, Mark J. Long-term effects of topical corticosteroids in the nose. Journal of Laryngology & Otology 1992;106(9):810-2.

31. Juliusson S, Aldenborg F, Enerback L. Proteinase content of mast cells of nasal mucosa; effects of natural allergen exposure and of local corticosteroid treatment. Allergy 1995;50(1):15-22.

32. Juliusson S, Bachert C, Klementsson H, Karlsson G, Pipkorn U. Macrophages on the nasal mucosal surface in provoked and naturally occurring allergic rhinitis. Acta Oto Laryngologica 1991;111(5):946-53.

Chapter 6

Pretreatment with topical corticosteroid nasal spray: effects on inflammatory cells before and after allergen challenge

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Submitted for publication

Introduction

The mucosal inflammation in allergic rhinitis is characterized by the presence of tissue infiltration of Langerhans cells, T cells and activated T cells, mast cells, eosinophils and neutrophils in the nasal mucosa after allergen challenge (1-6). Topical steroids are effective in the clinical management of allergic rhinitis and inhibit both early and late phase responses after allergen challenge. Topical corticosteroids reduce the influx of eosinophils, neutrophils, Langerhans cells and mononuclear cells in nasal secretions and nasal biopsies in allergic rhinitis (7-10). Usually, in allergen provocation studies investigating the effect of corticosteroids on nasal mucosal inflammatory cells, baseline nasal biopsies are taken before the treatment period and a second biopsy at some moment (maximum 24 hours) after nasal allergen provocation (11, 12). Lozewicz et al. investigated the effect of FPANS on eosinophils in nasal mucosal biopsies taken at several time points between 0 and 8 hours (13). There, however, remains a shortage of knowledge concerning treatment effects on unchallenged nasal mucosa of allergic rhinitis patients, and how treatment interferes with events occurring directly, and after an extended period after allergen provocation. With a repeated biopsy method we tried to understand better the pathophysiological aspects occurring before and after allergen provocation with corticosteroid protection. Topical corticosteroids are usually effective in reducing nasal symptoms after one or two week's usage. Andersson et al. found that even a short (one day) pre-treatment with topical steroids abolished the allergen-induced increase in nasal hyperresponsiveness (7). Allergic patients usually start taking their medication at the presentation of the first symptoms, but there are clues that it is advisable to start the steroid treatment before the grass-pollen season begins(14). In this study we have examined the influence of six week pretreatment with a topical corticosteroid Fluticasone Propionate Aqueous Nasal Spray (FPANS) on nasal mucosal inflammatory cells during allergen provocation.

Material and methods

PATIENTS

Nineteen patients, 11 female, 8 male, between 18-51 years old participated in this study. They all had a history of a seasonal allergic rhinitis for grass pollen for at least two years. None of the patients had a history of an allergy to other common allergens like house dust mite, cat and dog. This was confirmed by a positive skin prick test (≥3+) with Alutard Soluprick

extract of 1 SQ/ml and no extensive skin prick reactions (≤1+) to other relevant allergens. The patients were not taking medication at the time of the study and suffered no other nasal complaints. They received no immunotherapy in the three years before this study. No abnormalities were seen at ENT-examination. There were no significant differences between the FPANS and placebo groups concerning severity or duration of allergic rhinitis. There were no significant differences in demographic characteristics.

All patients gave their written informed consent and the medical ethical committee of the hospital approved the study.

STUDY DESIGN

This double blind, randomized, placebo-controlled, parallel-group study was conducted during autumn of 1995, out of the hay fever season. After randomization 10 patients used Fluticasone Propionate Aqueous Nasal Spray (FPANS) 200 µg twice daily and nine patients used placebo for six weeks. After this treatment period, provocation took place and the use of FPANS or placebo was stopped. Nasal mucosa biopsies were taken from the inferior turbinate using a Gerritsma forceps(15). Each patient underwent five nasal biopsies: 1.at baseline, 2.after 4 weeks of treatment (two weeks before provocation), 3.one hour after provocation with grass pollen, 4.twenty-four hours after provocation and 5.one week after provocation (figure 1).

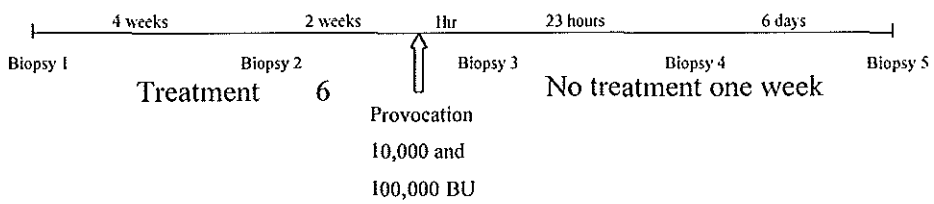

Figure 1. Schematic chart of the study design. After 6 weeks of FPANS or placebo treatment an allergen challenge was performed. Each patient underwent five nasal biopsies: 1.at baseline, 2.after 4 weeks of treatment (two weeks before provocation), 3.one hour after provocation with grass pollen, 4.twenty-four hours after provocation and 5.one week after provocation

PROVOCATION

The patient acclimatized to the room for 15 minutes. After that, PBS (phosphate buffered saline), an inert waterish solution was administered, to rule out aspecific hyperreactivity. After ten minutes, patients were provoked in each nostril with 50 µl of an aqueous nasal spray containing grass pollen 10,000 BU (ALK, Groningen, The Netherlands) and ten minutes later, again, with 100,000 BU (ALK, Groningen, The Netherlands). After both provocations, nasal symptoms (nasal blockage, sneezing, itching and rhinorrhea) were recorded on a four point (0-3) scale. Patients were asked to rate their symptoms 3, 6, 9, 12 and 24 hours after provocation .

NASAL BIOPSIES

Five nasal biopsies were obtained from each patient. Local anesthesia was achieved by applying a cotton-wool carrier with 50-100 mg cocaine and 3 drops of adrenaline (1:1000) under the inferior turbinate without touching the biopsy site for approximately ten minutes. Biopsies of nasal mucosa were taken from the lower edge of the inferior turbinate, about 2-cm posterior to the edge, using a Gerritsma forceps with a cup diameter of 2.5 mm. The biopsy specimens were embedded in Tissue-Tek II O.C.T. compound in a gelatin capsule and immediately frozen.

From each specimen serial 6 µm-thick sections of nasal mucosa were cut on a Reichert-Jung 2800 Frigocut cryostat and transferred to gelatin-coated microscope slides. The sections were stained for cell surface and intracellular markers according to a previously described supersensitive immunoalkaline method (16). Langerhans cells were stained with the monoclonal antibody 66IIC7 (CD1a, Sanbio, Monosan, Uden, The Netherlands), T cells and subsets with anti-CD4 (Central Laboratory of the Red Cross Blood Transfusion Service (CLB), Amsterdam, The Netherlands), anti-CD3 (Becton Dickinson, Mountain View, Ca, USA), anti CD8 (Monosan, Sanbio, Uden, the Netherlands). Macrophages were stained with anti-CD68, eosinophils with anti-BMK13. Mast cells were stained with the mAb anti-tryptase (G3 4ìg/ml) and with anti-chymase biotin (B7 3.3ìg/ml, Chemicon, Brunschwig, Amsterdam, the Netherlands).

CELL QUANTIFICATION

The surface area of two sections in total and of the epithelium and lamina propria separately, was estimated by means of the image analysis system Videoplan Kontron 2.1. Cells, binding

the monoclonal antibody used, had bright red surface membranes, red stained cytoplasm or both, depending on the cell type and/or monoclonal antibody evaluated. Cells were counted if they stained red and contained a nucleus. When groups of positive cells lay very close together or overlapped each other, only the definitely positive cells were counted. The cell numbers in epithelium and lamina propria were divided by the area and expressed as number of cells per mm^2.

STATISTICAL ANALYSIS

The ANOVA repeated measurement was used for statistical analysis of changes before treatment (biopsy 1) and after allergen provocation (biopsies 3,4 and 5) between the groups. If significant differences were found using this ANOVA, the Mann-Whitney U-test was used to analyze the differences in cell numbers at each biopsy moment, and in severity of symptoms between groups. A p-value < 0.05 was considered to indicate a significant difference. Correlations were performed using Spearman's rank method. Because of multiple testing a p-value < 0.01 was considered to indicate a significant difference.

Results

CLINICAL RESPONSE TO GRASS POLLEN

Nasal challenge with grass pollen resulted in a significant increase in immediate and late nasal symptoms in both groups with increases in sneezes, itching, nasal blockage and rhinorrhea. A significant reduction in early phase obstruction (p=0.006), itching (p=0.015), and late phase obstruction (p=0.024), rhinorrea (p=0.04) was found in the FPANS treated group compared with the placebo group.

IMMUNOHISTOCHEMISTRY

Effect of treatment (before provocation)

At baseline there were no significant differences in number of cells between the two treatment groups, therefore the randomization of the two groups was correct. After four weeks of treatment but before allergen provocation in epithelium fewer Langerhans cells (p=0.0014), CD68+ cells (p=0.04), BMK13+ cells (p=0.007), CD3+ cells (p=0.006), CD4+ cells (p=0.007), CD8+ cells (p=0.0006), chymase positive cells (p=0.01), and tryptase positive cells (p=0.03) were counted in the FPANS group compared to the placebo group. In the lamina propria fewer Langerhans cells (p=0.03) and BMK13+ cells (p=0.003) were found in

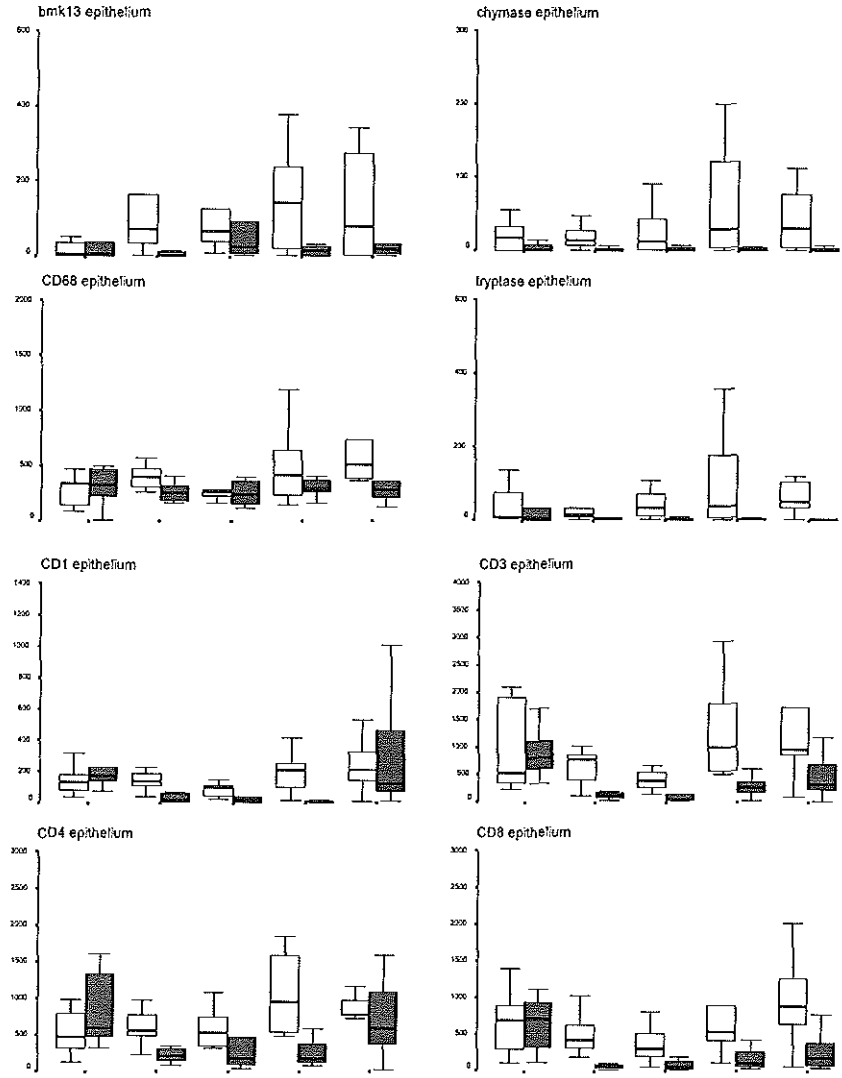

Figure 2. Cell counts per mm² in the nasal epithelium at five moments during the study period (■ FPANS; □ placebo) Results expressed as median, interquartile ranges and the 2.5 and 97.5 centiles.

the FPANS group. Results of nasal epithelium and lamina propria are depicted separately in fig.2, resp. 3.

Changes after allergen provocation

Cell influx in nasal mucosa after allergen provocation was significantly inhibited in the FPANS group compared to the placebo group for epithelial Langerhans cells ($p<0.001$),

chymase positive cells (p=0.036), macrophages (p=0.005), CD3+(p=0.0001) and CD8+ cells (p=0.0001), and lamina propria eosinophils (p=0.012), chymase positive cells (p=0.04), Langerhans cells (p=0.0001), macrophages (p=0.034), CD3+ cells (p=0.0001), CD8+ cells(p=0.042) and CD4+ cells(p=0.04). Because of the effect of corticosteroid treatment on cell numbers before allergen provocation (biopsy 2), medication related differences between the two treatment groups were analyzed. This depicts only the effect of treatment on cell numbers after allergen provocation and does not take into account changes that occur before allergen provocation. Significant medication related differences between the two treatment groups were found in eosinophils (p=0.007), chymase positive cells (p=0.001), tryptase positive cells (p=0.0001), Langerhans cells (p=0.0001), CD3+ cells (p=0.0001), CD4+ cells (p=0-.0001), and CD8+ cells (p=0.0001), and chymase positive cells (p=0.001), tryptase positive cells (p=<0.0001), Langerhans cells (p=0.04), and CD8+ cells (p=0.0001).

Associations were sought between the number of cells in nasal mucosa and the size of the early and late phase responses. Because of the repeated biopsies it is possible to relate cell numbers before allergen provocation with the severity of the nasal complaints after allergen provocation, and early and late phase responses with cell numbers after ½ and 24 hours. Results are shown in table 1 for the placebo treated group and in table 2 for the FPANS treated group.

Table 1. Correlation between nasal symptoms and cell numbers in the placebo treated group

early phase sneezing	IgE lam.prop. ½hr	r=-0.80, p=0.009
	eosinophil epithelium½h	r=-0.82, p=0.007
	chymase lam.prop. ½hr	r=-0.80, p=0.009
	IgE epithelium 24hr	r=-0.87, p=0.005
sneezing 12hr after provocation	tryptase lamina propria biopsy 2	r=0.96, p=0.003

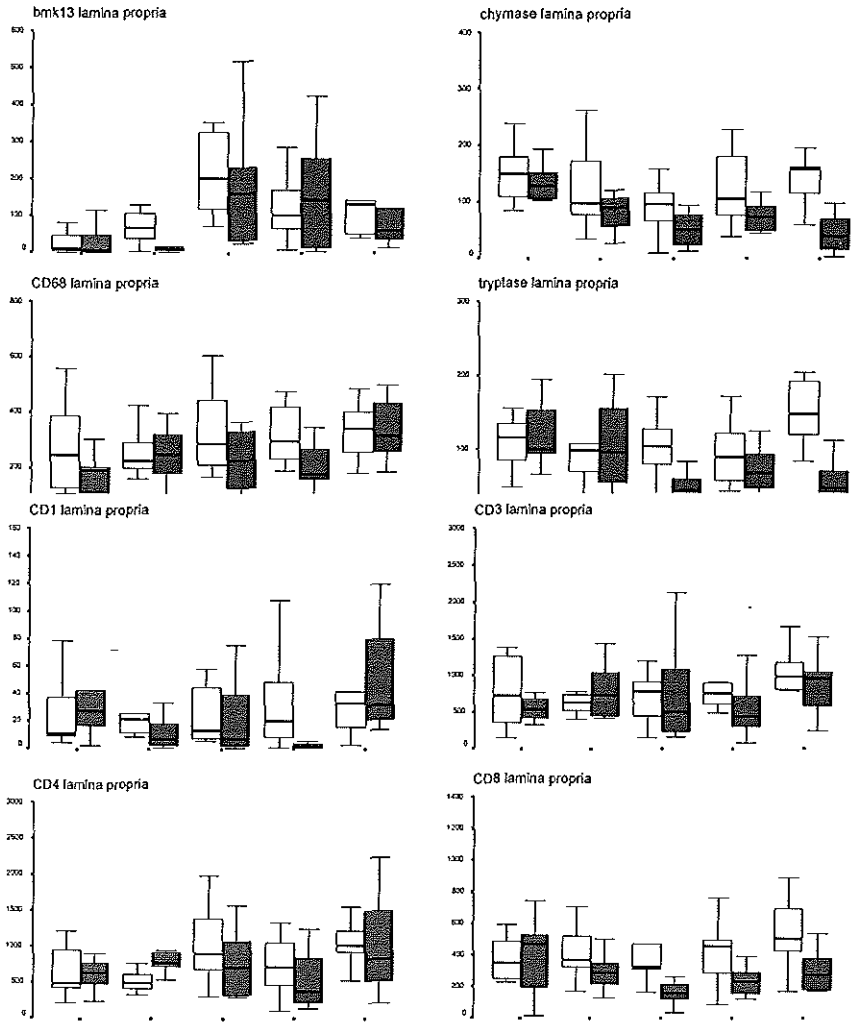

Figure 3. Cell counts per mm² lamina propria at five moments during the study period (■ FPANS; □ placebo) Results expressed as median, interquartile ranges and the 2.5 and 97.5 centiles.

Table 2. Correlation between nasal symptoms and number of cells for the FPANS treated group.

eosinophil epithelium biopsy 3	Rhinorrea 9hr	r=-0.84, p=0.005
	Rhinorrea 12hr	r=-0.87, p=0.005
chymase epithelium biopsy 4	duration symptoms	r=0.88, p=0.002

Discussion

In this study we were able to obtain five nasal mucosa biopsies in each patient within a period of seven weeks, with tree biopsies in a period of approximately 24 hours. Although very demanding for the patients, the procedure was well tolerated and not considered unethical by the investigators and the local Medical-Ethical Committee. With this study design it was possible not only to investigate the early and late phase reaction, but also to study the events which occur after that period. In addition, the effect of pretreatment with a topical corticosteroid on unchallenged allergic nasal mucosa was investigated. Our results show that four weeks corticosteroid treatment without allergen challenge results in a decrease in number of epithelial CD3+ cells, CD4+ cells, CD8+ cells, tryptase+ cells, chymase+ cells, Langerhans cells, macrophages and eosinophils, and a decrease of eosinophils and Langerhans cells in lamina propria. It is interesting to note that even in unchallenged nasal mucosa of asymptomatic patients inflammatory cells decrease in number in nasal epithelium after corticosteroid treatment. In nasal mucosa biopsies and nasal washings of unchallenged patients with seasonal allergic rhinitis, no changes in mast cell and eosinophil numbers were found after one or two week topical or systemic corticosteroid pretreatment (13, 17, 18). Contrary, Konno et al. found a significant reduction of eosinophils and basophils in nasal lavages after two weeks of FPANS pretreatment without allergen provocation(19). It is possible that the duration of pretreatment with topical steroids is of importance for the effects on cell numbers in epithelium. We treated the patients for four weeks with FPANS before taking nasal mucosa biopsies, in contrast to Lozewicz et al.(13) who pretreated their patients for two weeks and Bascom et al. (17) who pretreated their patients for one week with topical corticosteroids. An alternative explanation may be a persistent immunological activation in the allergic nasal mucosa, as a result of air pollution and exposure to bacterial and viral
antigens. Juliusson et al. found more intraepithelial mast cells in the pre-seasonal nasal mucosa of asymptomatic atopic patients compared to normal non-allergic controls, which also may indicate immunological activation(11). This persistent immunological activation is attenuated by local corticosteroid treatment. Interestingly, also in patients with nonallergic, noninfectious perennial rhinitis a significant decrease in nasal mucosa inflammatory cells after treatment with topical corticosteroids was found (16). This decrease was more pronounced in nasal epithelium and after a longer treatment period (4 versus 8 weeks).
This pretreatment effect of FPANS with lowered numbers of several cells hampers interpretation of the changes that occur after allergen challenge. Mast cells disappear from the

epithelium after active treatment and remain absent despite allergen provocation, indicating good sensitivity of mast cells to steroid treatment irrespective of mucosal condition. In the placebo treated group the numbers of epithelial chymase positive cells and lamina propria tryptase positive cells increased significantly. Eosinophils already increase in the placebo pre-treated group in unchallenged nasal mucosa, with a further increase after allergen provocation. With only two biopsy moments, one baseline before treatment and one after provocation, it is not possible to distinguish between changes in cell counts due to allergen provocation, treatment or other stimuli as e.g. placebo spray.

Epithelial T cells and Langerhans cells showed a clear response to corticosteroid treatment with significant decreases after the beginning of the active treatment, even without allergen challenge. In a previous study we have demonstrated in perennial allergic rhinitis that treatment with topical corticosteroids reduces the nasal mucosal Langerhans cells (10).

In this study we were able to monitor the allergic response in nasal mucosa up to one week after single allergen provocation. In the placebo group eosinophils in lamina propria, and epithelial macrophages rose significantly during the whole observation period ($p=0.015$, resp.$p=0.003$). Furthermore an increasing trend was noted in the number of epithelial CD3+($p=0.1$) and tryptase positive cells ($p=0.07$) in the placebo group. This suggests that one single provocation can trigger the allergic response for several days. Mechanisms responsible for this sustained reaction are probably the released T-helper-2-cytokines (20 , 21, 22).

In this study, cell numbers at three biopsy moments (2,3,4) with nasal symptoms at several time points were compared, giving us a better insight in the pathophysiological events occurring in the nasal mucosa. Between early phase sneezing and IgE+ cells in epithelium and lamina propria, lamina propria chymase+ cells, epithelial eosinophils after ½ hour a strong, but inverse correlation was observed in the placebo group. A possible explanation may be that with patients experiencing more symptoms, effector cells move faster from the lamina propria to epithelium and then into secretion, compared to patients who have only mild symptoms. This seems to be corroborated by the findings of Pipkorn et al. who found in nasal lavages a significant correlation between eosinophil numbers and the degree of symptoms(23). Without a similar cell influx into the mucosa from the blood vessels, this results in decreasing numbers of resident mucosal cells. Other investigators, however, could not detect a correlation between the symptoms during the acute response to allergen challenge and the subsequent influx of total cells or individual cell types during the late response to antigen challenge(18, 24). A very strong correlation was found between the number of lamina propria tryptase positive before

allergen provocation (biopsy 2) and sneezing 12 hours after allergen provocation (r=0.96, p=0.003), suggesting that the number of mast cells present in nasal mucosa before allergen provocation dictates part of the late phase response. This is in contrast with the findings of Naclerio et al. (25) who found that basophils, and not mast cells are partly responsible for the late phase response.

The susceptibility of the several inflammatory cells to corticosteroids and the duration of the response to corticosteroids after allergen provocation differ. Mast cell, IgE positive cell and T cell numbers decrease and remain low after allergen provocation for one week even after having stopped the active treatment, Langerhans cells decrease with corticosteroid treatment and remain low despite allergen provocation but return to baseline levels after one week. Eosinophils are very sensitive to corticosteroid treatment, and are found to be reduced in the nasal mucosa after local corticosteroid treatment during both artificial and natural allergen challenge (12, 26-28). However, in the present study with a large allergen stimulus a significant increase in nasal mucosal eosinophils in the following 24 hours was found despite treatment with FPANS 400 μg. This finding suggests a decreased sensitivity of eosinophils to corticosteroids. On the other hand, perhaps the stimulus given with the allergen provocation over-ride the effect of corticosteroids on cells.

After 24 hours after allergen provocation patients in either treatment group experienced symptoms with no difference in clinical symptoms between the two treatment groups, apart from sneezing which was lower in the FPANS group. Sneezing is a result of mast cell degranulation. In the FPANS group no mast cells were seen in the nasal epithelium 24 hours after provocation, suggesting a low to zero mast cell mediator release in this group. Despite a six-week active corticosteroid treatment, symptomatology 24 hours after a single allergen p-rovocation is the same as in the placebo group. These findings suggest that the duration of the effects of the local corticosteroid is shorter than the duration of the allergic response. To corroborate this hypothesis, a second allergen provocation after 24 hours would have resulted in the comparable response in either treatment group. This, however, was not undertaken.

A clinical consequence can be the importance of daily use of topical corticosteroids. If the medication is not properly used, symptoms may not be well controlled. This has also been found by Juniper et al. during the grass-pollen season with patients with seasonal allergic rhinitis (29). In her study Juniper et al. describe that a subgroup of patients using Beclomethasone Dipropionate on a "as needed" base experienced significantly more symptoms than the patient group using the medication on a daily base. Furthermore, from this

study we can not substantiate the need to commence with topical corticosteroid treatment six weeks prior to the start of the pollen season.

References

1. Fokkens WJ, Rijntjes E, Vroom TM, Mulder PGH. Fluctuation of the number of CD-1(T6)-positive dendritic cells, presumably Langerhans cells, in the nasal mucosa of patients with an isolated grass-pollen allergy before, during and after the grass-pollen season. J Allergy Clin Immunol 1989;84:39-43.
2. Calderon MA, Lozewicz S, Prior A, Jordan S, Trigg CJ, Davies RJ. Lymphocyte infiltration and thickness of the nasal mucous membrane in perennial and seasonal allergic rhinitis. Journal of Allergy & Clinical Immunology 1994;93(3):635-43.
3. Varney VA, Jacobson MR, Sudderick RM, Robinson DS, Irani AM, Schwartz LB, et al. Immunohistology of the nasal mucosa following allergen-induced rhinitis. Identification of activated T lymphocytes, eosinophils, and neutrophils. American Review of Respiratory Disease 1992;146(1):170-6.
4. Godthelp T, Holm AF, Fokkens WJ, Doornenbal P, Mulder PG, Hoefsmit EC, et al. Dynamics of nasal eosinophils in response to a nonnatural allergen challenge in patients with allergic rhinitis and control subjects: a biopsy and brush study. J Allergy Clin Immunol 1996;97(3):800-11.
5. Bentley AM, Jacobson MR, Cumberworth V, Barkans JR, Moqbel R, Schwartz LB, et al. Immunohistology of the nasal mucosa in seasonal allergic rhinitis: increases in activated eosinophils and epithelial mast cells. J Allergy Clin Immunol 1992;89(4):877-83.
6. Slater A, Smallman LA, Drake-Lee AB. Increase in epithelial mast cell numbers in the nasal mucosa of patients with perennial allergic rhinitis. J. Laryngol. Otol. 1996;110:929-933.
7. Andersson M, Andersson P, Pipkorn U. Topical glucocorticosteroids and allergen-induced increase in nasal reactivity: relationship between treatment time and inhibitory effect. J Allergy Clin Immunol 1988;82:1019-1026.
8. Meltzer EO, Orgel HA, Bronsky EA, Furukawa CT, Grossman J, LaForce CF, et al. A dose-ranging study of fluticasone propionate aqueous nasal spray for seasonal allergic rhinitis assessed by symptoms, rhinomanometry, and nasal cytology. Journal of Allergy & Clinical Immunology 1990;86(2):221-30.
9. Naclerio RM. The pathophysiology of allergic rhinitis: impact of therapeutic intervention. J Allergy Clin Immunol 1988;82:927-934.
10. Holm AF, Fokkens WJ, Godthelp T, Mulder PGH, Vroom TM, Rijntjes E. The effect of three months nasal steroid therapy on nasal T cells and Langerhans cells in patients suffering from allergic rhinitis. Allergy 1995;50:204-209.
11. Juliusson S, Aldenborg F, Enerback L. Proteinase content of mast cells of nasal mucosa; effects of natural allergen exposure and of local corticosteroid treatment. Allergy 1995;50(1):15-22.
12. Rak S, Jacobson MR, Sudderick RM, Masuyama K, Juliusson S, Kay AB, et al. Influence of prolonged treatment with topical corticosteroid (fluticasone propionate) on early and late phase nasal responses and cellular infiltration in the nasal mucosa after allergen challenge. Clin Exp Allergy 1994;24(10):930-9.

13. Lozewicz S, Wang J, Duddle J, Thomas K, chalstrey S, Reilly G, et al. Topical glucocorticoids inhibit activation by allergen in the upper respiratory tract. J Allergy Clin Immunol 1992;89:951-957.
14. Bousquet J, Chanal I, Alquie MC, Charpin D, Didier A, Germouty J, et al. Prevention of pollen rhinitis symptoms: comparison of fluticasone propionate aqueous nasal spray and disodium cromoglycate aqueous nasal spray. A multicenter, double-blind, double-dummy, parallel-group study. Allergy 1993;48(5):327-33.
15. Fokkens WJ, Rijntjes E, Vroom TM, Gerritsma V. A mild biopsy method to obtain high-quality specimens of nasal mucosa. Rhinology 1988;26(4):293-295.
16. Blom H, Godthelp T, Fokkens W, KleiJan A, Mulder P, Rijntjes E. The effect of nasal steroid aqueous spray on nasal complaint scores and cellular infiltrates in the nasal mucosa of patients with nonallergic, noninfectious perennial rhinitis. J Allergy Clin Immunol 1997;100:739-47.
17. Bascom R, Wachs M, Naclerio RM, Pipkorn U, Galli SJ, Lichtenstein LM. Basophil influx occurs after nasal antigen challenge: effects of topical corticosteroid pretreatment. J Allergy Clin Immunol 1988;81(3):580-9.
18. Bascom R, Pipkorn U, Lichtenstein LM, Naclerio RM. The influx of inflammatory cells into nasal washings during the late response to antigen challenge. Effect of systemic steroid pretreatment. Am Rev Respir Dis 1988;138(2):406-12.
19. Konno A, Yamakoshi T, Terada N, Fujita Y. Mode of action of a topical steroid on immediate phase reaction after antigen challenge and nonspecific nasal hyperreactivity in nasal allergy. International Archives of Allergy & Immunology 1994;103(1):79-87.
20. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol 1986;136(7):2348-57.
21. Wierenga EA, Snoek M, de Groot C, Chretien I, Bos JD, Jansen HM, et al. Evidence for compartmentalization of functional subsets of CD2+ T lymphocytes in atopic patients. J Immunol 1990;144(12):4651-6.
22. Bradding P, Feather IH, Wilson S, Bardin PG, Heusser CH, Holgate ST, et al. Immunolocalization of cytokines in the nasal mucosa of normal and perennial rhinitic subjects. The mast cell as a source of IL-4, IL-5, and IL-6 in human allergic mucosal inflammation. Journal of Immunology 1993;151(7):3853-65.
23. Pipkorn U, Karlsson G, Enerback L. Nasal mucosal response to repeated challenges with pollen allergen. Am Rev Respir Dis 1989;140(3):729-36.
24. Durham SR, Ying S, Varney VA, Jacobson MR, Sudderick RM, Mackay IS, et al. Cytokine messenger RNA expression for IL-3, IL-4, IL-5, and granulocyte/macrophage-colony-stimulating factor in the nasal mucosa after local allergen provocation: relationship to tissue eosinophilia. J Immunol 1992;148(8):2390-4.
25. Naclerio RM, Proud D, Togias AG, Adkinson N, Jr., Meyers DA, Kagey-Sobotka A, et al. Inflammatory mediators in late antigen-induced rhinitis. N Engl J Med 1985;313(2):65-70.
26. Orgel HA, Meltzer EO, Bierman CW, Bronsky E, Connell JT, Lieberman PL, et al. Intranasal fluocortin butyl in patients with perennial rhinitis: a 12-month efficacy and safety study including nasal biopsy. Journal of Allergy & Clinical Immunology 1991;88(2):257-64.

27. Masuyama K, Jacobson MR, Rak S, Meng Q, Sudderick RM, Kay AB, et al. Topical glucocorticosteroid (fluticasone propionate) inhibits cells expressing cytokine mRNA for interleukin-4 in the nasal mucosa in allergen-induced rhinitis. Immunology 1994;82(2):192-9.

28. Holm AF, Fokkens WJ, Godthelp T, Mulder PGH, Vroom TM, Rijntjes E. A one-year placebo-controlled study of intranasal Fluticasone Propionate Aqueous Nasal Spray in patients with perennial allergic rhinitis: a safety and biopsy study. Clin Otolaryngology 1998;23:69-73.

29. Juniper EF, Guyatt GH, Archer B, Ferrie PJ. Aqueous beclomethasone dipropionate in the treatment of ragweed pollen-induced rhinitis: further exploration of "as needed" use. J Allergy Clin Immunol 1993;92(1 Pt 1):66-72.

Chapter 7

Intranasal fluticasone propionate treatment inhibits cytokine mRNA expression before and after a single nasal allergen provocation.

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Submitted for publication.

Introduction

Topical corticosteroids relieve allergic symptoms by reducing the numbers of inflammatory cells and chemical mediators (1,2). Allergic patients usually start taking their medication at the presentation of the first symptoms, but there are indications that it is advisable to start taking corticosteroids before the grass pollen season begins (3-5). Allergen challenge results in mast cell degranulation, migration of mast cells to the surface of the upper airway mucosa (6), and an influx of inflammatory cells (5,7-9). This allergic mucosal inflammation is regulated by the local production and release of a number of cytokines (10-13). A major role was originally assigned to the cytokines produced by Th2 lymphocytes, such as IL-3, IL-4, IL-5, IL-10 and IL-13 (10,12,14-19). In addition to the well-known Th2 cytokines, chemokines (Il-8 and RANTES) were found to play a role in allergic inflammation (table I) (11,13,20,21). Accumulating data now points to a large network of interacting cytokines produced by a number of cells that regulate mucosal allergic inflammation (22).

A number of studies have been performed which assess the effect of local corticosteroid therapy on cytokines at the mRNA and protein levels in the airway mucosa and in lavages.

Allergen challenge studies have found reductions in mRNA and protein for IL-4 and IL-13 after steroid treatment (12,18,19,23-25). No steroid therapy effect was observed for IL-5 and IFN-γ in the nasal mucosa after allergen provocation (12,18,19,23-25). However, studies in the lung and in *in vitro* experiments showed that corticosteroids inhibit IL-5 and IFN-γ mRNA expression and protein production (26-30). There were no differences in IL-6 on the protein level (protein positive cells) in the upper airways between preseason and in season for allergic patients with or without corticosteroid treatment (12). However, Weido et al observed, in nasal lavage fluids, IL-6 protein levels during the late-phase reaction which was inhibited by pretreatment with FPANS (31). Local corticosteroid treatment was found to inhibit allergen challenge-dependent secretion of IL-8 and RANTES protein in the nasal lavage (11,31).

To our knowledge, no data is available in vivo about the dynamics of cells expressing IL-6, IL-8, IL-10, RANTES and TNF-α in the upper airway mucosa after allergen provocation. For other cytokines, details of time-related changes after allergen provocation and corticosteroid pretreatment are incomplete.

On the basis of earlier studies performed by our group, we hypothesised that local corticosteroid treatment reduces the number of inflammatory cells irrespective of the presence

of symptomatology (32). In this out-of-season biopsy study, we investigated the effect of pretreatment with a local corticosteroid, Fluticasone Propionate Aqueous Nasal Spray (FPANS), on a single provocation with grass pollen in in-patients with an isolated grass-pollen allergy. Biopsies were taken before and during treatment, before provocation and at several points after the provocation. Eosinophils and mRNA positive cells (*in situ* hybridisation) for IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IFN-γ, RANTES and TNF-α were determined in mucosal biopsies. Furthermore, correlations will be evaluated between the cells and nasal symptoms and also between eosinophils (as a marker of allergic inflammation) and cytokines.

Methods

PATIENTS

Nineteen patients with a median age of 22 (range 18-51) participated in this study (11 female and 8 male). They all had a history of seasonal allergic rhinitis for grass pollen of at least two years. Allergy to grass pollen was confirmed by a positive skin prick test (3+) with Alutard Soluprick extract (1 SQ/ml), with no skin prick reactions to other relevant allergens. All patients were symptom-free at the start of the study. None of the patients used any medication during the study or had undergone immunotherapy in the three years before this study. No relevant abnormalities were found in ENT examination at the start of the study. All patients gave their written informed consent and the medical ethics committee approved the study.

STUDY DESIGN

This double-blind, randomised, placebo-controlled, parallel-group study was conducted between October and December 1995, well outside the hay fever season. After randomisation, 10 patients (6 female and 4 male) aged 21 (18-51) used 200μg Fluticasone Propionate Aqueous Nasal Spray (FPANS) twice daily and 9 patients (5 female and 4 male) aged 23 (19-31) used a placebo for six weeks. After this treatment period, provocation took place and the use of FPANS or placebo was stopped. Biopsies were taken from the inferior turbinate using a Gerritsma forceps (33). Each patient underwent five nasal biopsies: the first at baseline, the second after 4 weeks of treatment (before provocation), the third one hour after provocation with grass pollen, the fourth 24 hours after provocation and the fifth one week after provocation (Table I).

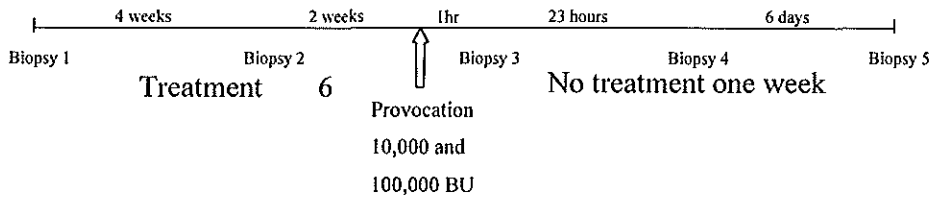


Table I

Study design - time schedule

PROVOCATION

The patients acclimatised to the room for at least 15 minutes. After that, PBS (phosphate buffered saline), an inert water-rich solution, was administered to rule out non-specific hyper-reactivity. After ten minutes, patients were provoked with 50 µl of an aqueous nasal spray containing grass pollen (10,000 BU/ml, ALK, Groningen, Netherlands) and another ten minutes later with 100,000 BU/ml (ALK, Groningen, Netherlands). After both provocations, symptoms like sneezing, rhinorrhea, itching and nasal blockage were recorded on a four-point (0-3) scale. Patients were also asked to rate their symptoms during the day following provocation after 3, 6, 9, 12 and 24 hours.

IMMUNOHISTOCHEMICAL STAINING OF EOSINOPHILS

Eosinophil staining was performed in an Alkaline Phosphatase procedure using monoclonal antibody against Major Basic Protein (BMK 13) (Sanbio, Uden, Netherlands) as previously described by Godthelp et al (34).

DIGOXIGENIN PROBE MANUFACTURED BY PCR

The digoxigenin probe was manufactured by PCR using a modification of the method described by Klein et al (35,36). Total RNA was isolated from stimulated (Con A or LPS) blood mononuclear cells or in vivo allergen-stimulated nasal mucosa by the phenol extraction method, a modification of the protocol described by Chomczynski and Sacchi (37). 1 µg RNA was then reverse-transcribed (Rt) to cDNA (Rev. Trans.AMV Boehringer Mannheim Biochemica 109 118) The Rt mix, a total volume of 20 µl, contains 50mM Tris-HCL pH8.3,

10 mM MgCl2, 50 mM KCl, 1mM DTT (dithiotreitol), 1mM EDTA, 10µg/ml BSA (Boehringer Mannheim 711 454), dNTP mix of 1mM (Pharmacia 27-2094-01), oligo (dT)12-18 10µg/ml (Pharmacia 27-7858-01), hexanucleotides pd (N)6 2.5 OD/ml (Pharmacia 27-2166) and spermidine-HCl 1mM. This mixture was incubated at 42°C for 60 min. After this reaction the final volume was topped up to a total volume of 200 µl with milliQ.

10 µl of the cDNA was subjected directly to PCR in the presence of a master mix containing PCR buffer, MgCL2 (1.5mM), KCl (50 mM) TRIS-HCl (10 mM pH8.3) gelatine (0.1%) AmpliTaqpolymerase (Ampli Taq, Perkin Elmer Cetus, Norwalk CT) 1 Unit, 0.025 mM dNTP and 1µl from each cytokine primer with a total volume of 50 µl (Table III).

The reaction tubes were placed for 3 minutes in the PCR block (MJ Research), which was preheated at 94°C (to avoid cold oligodeoxyribonuclear fusion). After preparing the PCR mixture on ice, PCR was carried out for 35 cycles under the following conditions: denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds and extension at 72°C for 1 min. Final-cycle

extension was at 72°C for 10 min.

The specificity of amplification was checked by assessing whether a fragment of the expected size had been obtained by gel (1.5 %) electrophoresis or by southern blot hybridization with an internal 32P-gamma ATP (Amersham) labelled probe. The membranes were exposed to Kodak XAR films.

PCR reaction products were run on gel, isolated from gel and cleaned up with the Wizard DNA Clean-Up System (Promega). The isolated DNA was used in a repeated PCR with the same cytokine specific primers under the following conditions: 5 µl of isolated PCR product, 10 µl 10x Taq polymerase buffer, 3 µl 10 OD/ml sense primer and 3 µl 10 OD/ml antisense primer (Table III), 5 U Taq polymerase (Ampli Taq, Perkin Elmer Cetus, Norwalk CT) and 5 µl dNTP (dATP, dCTP, dGTP 2mM each, 1.3mM dTTP and 0.7mM DIG-11-dUTP Boehringer Mannheim) in a total volume of 100 µl. The PCR protocol started with an adaptation of the hot start to avoid cold oligodeoxyribonuclear fusion. Subsequently, 30 sec at 94°C, 45 sec at 55°C, 1.5 min. at 72°C during 200 cycles. The resulting dig labelled DNA (probe) was controlled on 1.5% agarose gel.

Table II

Cytokines and their major function in allergic rhinitis

Cytokine	cell source in allergic inflammation[58]	Suspected cell target in allergic inflammation[58]
IL-2	T-cell	T-cells, B-cells (proliferation, activation, differentiation)
IL-3	T-cells, eosinophils	mast cells, eosinophils (colony stimulating factor)
IL-4	T-cells, mast cells, eosinophils	B-cells (IgE isotype switch), endothelium (adhesion molecules up-regulation)
IL-5	T-cells mast cells, eosinophils	Eosinophils (chemotaxis)
IL-6	mast cells	T-, B-, cells (proliferation, differentiation) and fibroblasts
IL-8	mast cells, epithelial cells, eosinophils	Lymphocytes, neutrophils, basophils, eosinophils (chemotaxis)
IL-10	T-cells, monocytes, macrophages	Macrophages, T-cells (inhibitor of IFN-U functions)
IL-13	T-cells, mast cells, eosinophils	B-cells (IgE isotype switch), endothelium (adhesion molecules up-regulation)
IFN-γ	T-cells NK cells	Macrophages (activation), IL-4 antagonist (IgE isotype switch)
RANTES	macrophages, eosinophils, T-cells and mast cells	Eosinophils and monocytes (chemotaxis)
TNF-α	mast cells	Fibroblast, endothelium (production of other cytokines and adhesion molecules (ELAM-1, ICAM-1))

The specificity of amplification was checked by assessing whether a fragment of the expected size (approximately 25% larger in bp than normal PCR product) had been obtained.

IN SITU HYBRIDIZATION

All reactions were carried out with RNase free materials and solutions except for the RNase treatment of the negative controls. Each tissue specimen was cut into serial 6 μm-thick sections on a Reichert-Jung 2800e frigocut cryostat and transferred to poly-L-lysine (Sigma)-

coated microscope slides, dried and stored at -80°C. The slides were used within 3 months of storage, heated to room temperature, dried and fixed in buffered 4% formalin for 15 min. The slides were rinsed twice with PBS for 5 min. Proteinase K 1 µg/ml (Boehringer Mannheim) treatment was applied for 5 min at 37°C, followed by washing with PBS and fixing in buffered 4% formalin for 5 min in order to stop the proteinase K reaction. There then followed two more rinses in PBS for 5 min., permeabilisation with 0.01% triton-X100 and a repeat washing with PBS. This was followed by dehydration by incubation with increasing amounts of ethanol (70% (2 min.), 96% (2 min.), and 100 % (5 min.)) air drying. The mixture for hybridization was pipetted onto ice and contained 30 µl 100% deionized formamide, 20 µl 20x SSC, 40 µl denatured salmon sperm DNA (10 µg/ml TE; Boehringer Mannheim), 1 µl t-RNA (100 mg/ml) and 9 µl diluted probe. The hybridization mix was incubated at 100°C for 1 min. to make the DNA single stranded and cooled immediately
on ice for 5 min. The sections were incubated with the hybridization mix at 42°C for 1 hr and then (67) for 16 hr (overnight) at 37°C in a humidity room. After hybridization, the cells were washed with 30% formamide/2x SSC at room temperature, 30% formamide/0.2x SSC at 42°C for 15 min.
and washed with PBS for 5 min. The slides were then placed in a semi-automatic stainer (Sequenza, Shandon). Following this, the sections were incubated for 10 min with BSA 0.5-1% in

PBS, incubated with normal sheep serum (CLB, Netherlands) for 10 min. and subsequently incubated with 1:500 Alkaline Phosphate-conjugated-sheep-digoxigenin F (ab)2 fragments, diluted in PBS BSA 0.5-1% and Normal Human Serum (10% NHS). They were rinsed again with PBS for 5 min, rinsed with TRIS buffer (0.1 M pH 9.5) for 5 min and incubated with NBT BCIP substrate (Sigma). The incubation time is probe-dependent. Substrate development was checked with the microscope. Finally, the sections were rinsed in distilled water and mounted in glycerin-gelatine. Optimal probe concentration was obtained by dilution titration.

CONTROLLING THE METHOD

The controls used were those previously described by Bloch et al. (38)

To control the mRNA in situ hybridization, cryostat sections were incubated with RNase A (Boehringer Mannheim). This resulted in a significant signal reduction. Hybridization without

a probe or unrelated probes gave no signal. Different cytokine probes resulted in different staining patterns (35,36).

Table III

Primer sequence for each cytokine used for making dig dUTP DNA probes.

Spec	Sequence (5'- 3') primers	fragment (Bp)
IL-2 sense	AAGAATCCCAAACTCACCAGGATGC (exon 2)	200 Bp
IL-2 αsense	CCCTTTAGTTCCAGAACTATTACGT (exon 3-4) (59)	
IL-3 sense	GCCTTTGCTGGACTTCAACA (exon 1-2)	194 Bp
IL-3 αsense	TTGGATGTCGCGTGGGTGCG (exon 4-5) (60)	
IL-4 sense	ACTCTGTGCACCGAGTTGACCGTAA (exon 2)	300 Bp
IL-4 αsense	TCTCATGATCGTCTTTAGCCTTTCC (exon 4) (57)	
IL-5 sense	AGCCAATGAGACTCTGAGGA (exon 1-2)	319 Bp
IL-5 αsense	GGGAATAGGCACACTGGAGAGTCAA (exon 4) (61)	
IL-6 sense	ATGAACTCCTTCTCCACAAGC (exon 1)	610 Bp
IL-6 αsense	TGGACTGCAGGAACTCCTT (exon 5) (62)	
IL-8 sense	CTGTGTGAAGGTGCAGTTTTGCC (exon 1-2)	237 Bp
IL-8 αsense	CTCAGCCCTCTTCAAAAACTTCTCC (exon 3-4) (63)	
IL-10 sense	ATGCCCCAAGCTGAGAACCAAGACCCA (exon ?)	352 Bp
IL-10 αsense	TCTCAAGGGGCTGGGTCAGCTATCCCA (exon ?) (64)	
IL-13 sense	CCCAGAACCAGAAGGCTCCGC (exon 1-2)	185 Bp
IL-13 αsense	GCTGGAAAACTGCCCAGCTGAG (exon 3-4) (65)	
IFN-γ sense	TTTAATGCAGGTCATTCAGATG (exon 1-2)	388 Bp
IFN-γ αsense	CAGGGATGCTTCTTCGACCTCGAAAC (exon 4) (57)	
Rantes sense	CGCTGTCATCCTCATTGCTA (exon ?)	197 Bp
Rantes αsense	CACACACTTGGCGGTTCTT (exon ?) (66)	
TNF-α sense	AGAGGGAAGAGTTCCCCAGGGAC (exon 1-2)	443 Bp
TNF-α αsense	TGAGTCGGTCACCCTTCTCCAG (exon 4)	

QUANTIFICATION

The biopsies were coded and two sections of each biopsy were counted in a blinded fashion at a magnification of 400 x. The surface area of the epithelium and the lamina propria of two sections were determined by computer image analysis (Kontrons Image Analysis System Videoplan). The cell numbers for eosinophils in the epithelium and lamina propria and the number for mRNA positive cells only in the lamina propria were calculated per square millimetre of the section area.

STATISTICAL ANALYSIS (SPSS 7.5 FOR WINDOWS 95 AND BMDP5V FOR DOS)

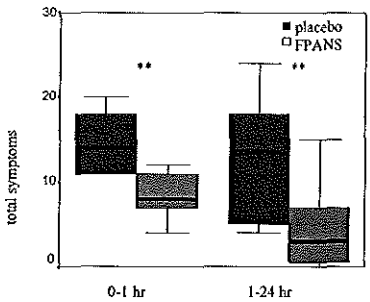
The ANOVA repeat measurement was used for statistical analysis of treatment effects between the groups. If significant differences were found using ANOVA, further comparisons were made using the Mann-Whitney U-test per time-point. A p-value < 0.05 was considered to indicate a significant difference between groups. The Wilcoxon signed rank test was used for a comparison between baseline and one week after provocation.

Correlation coefficients were obtained using Spearman's rank method for cells from each biopsy, for symptoms (baseline, after four weeks treatment, early phase G2 (10,000 BU provocation) and late phase 3 - 24 hours after provocation) correlated with cells (biopsies baseline, after four weeks treatment, one hour and 24 hours after provocation). Correlation coefficients were obtained for each biopsy. Because of multiple testing a p-value of < 0.01 was considered to indicate a significant correlation.

RESULTS

No symptoms were observed before provocation or provocation with saline. Nasal provocation with allergen resulted in a significant increase in immediate (placebo (9 out of 9), FPANS (10 out of 10)) and late nasal symptoms (placebo (9 out of 9), FPANS (7 out of 10)) in both groups with increases in sneezes, itching, nasal blockage and rhinorrhea. A significant reduction in total symptomatology (in the early phase ($p=0.01$) and in the late phase ($p=0.009$)) was found in the FPANS group compared with the placebo group (see figure 1).

Figure 1. Total symptom score after allergen provocation. In the first hour and during the following day total symptoms were significantly reduced in the FPANS treated group compared to placebo(in the early phase (p=0.01) and in the late phase (p=0.009).



Significantly less symptoms were observed in the FPANS group than in the placebo group after provocation with 1,000 BU (G1) for itching (p=0.005), nasal blockage (p=0.004), after provocation with 10,000 BU (G2), itching (p=0.02), nasal blockage (p=0.004) during 1-3 hours for rhinorrhea (p=0.04), during 3-6 hours for nasal blockage (p=0.05), during 6-9 hours for nasal blockage (p=0.02) and during 12-24 hours for sneezing (p=0.04).

MICROSCOPIC EVALUATION

The cryostat sections of the nasal mucosa had an average surface area of 2 mm^2 and an intact epithelium. Staining with mAb for eosinophil Major Basic Protein (BMK-13) resulted in easy identification with the red cytoplasmic granules and dark violet nucleus. Eosinophil numbers were determined in the lamina propria. The mRNA in situ hybridization staining pattern for cytokines varied from a dark purple circle to a large dark purple dot. Messenger RNA positive cells were located in the inflammatory cell infiltrate in the lamina propria. The majority of cells showing hybridization signals for cytokines were present in the sub-epithelial layer. The number of positive cells was only determined in the lamina propria because the number of positive cells in the epithelium was too small for statistical analysis. Figure 2 shows representative sections from nasal mucosal biopsies expressing mRNA for IL-3(a), IL-5(b), IL-8,(c) and IFN-γ(d).

EFFECT OF PRETREATMENT (BEFORE PROVOCATION FIG.3)

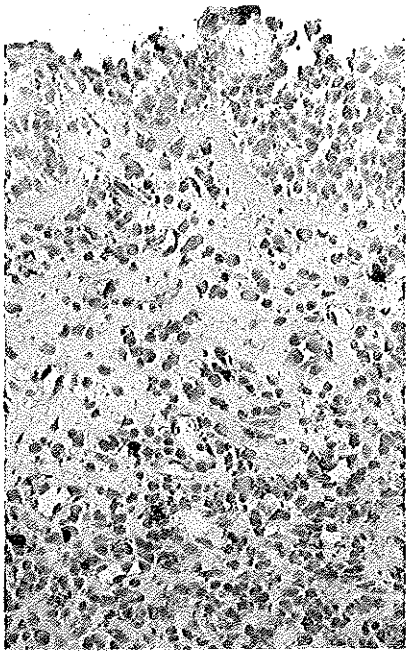
The number of eosinophils showed no significant difference between the groups at baseline. After 4 weeks of pretreatment, before allergen provocation, a significant lower number of eosinophils was observed in the epithelium ($p= 0.002$) and in the lamina propria ($p=0.004$) of the FPANS group compared to the placebo group.

At baseline, no significant differences were found in the number of cytokine mRNA positive cells between the two groups. After 4 weeks of pretreatment, a significantly lower number of IL-5 and IL-6 mRNA positive cells were found in the FPANS group compared with the placebo group ($p=0.03$ and $p=0.05$).

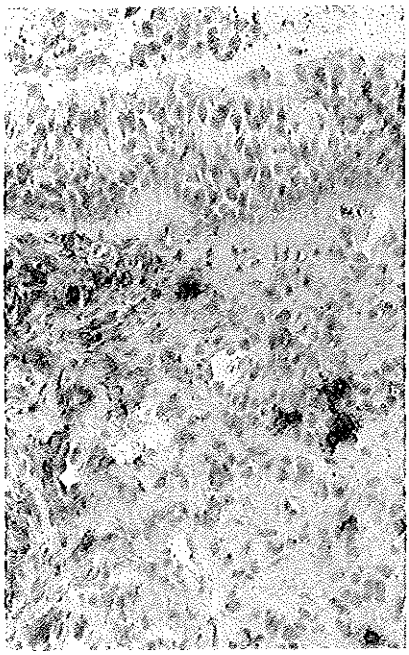
Figure 2 a-d

Photomicrographs showing representative examples of in situ hybridization of nasal biopsies of allergic rhinitis patients following allergen challenge, using digoxigenin-labeled DNA probes coding for IL-3 (a), IL-5 (b), IL-8 (c) and IFN-γ (d). Messenger RNA positive cells exhibited a dark purple staining and give optimal cellular and sub-cellular resolution, nuclear staining with gill's heamatoxilin(overleaf).

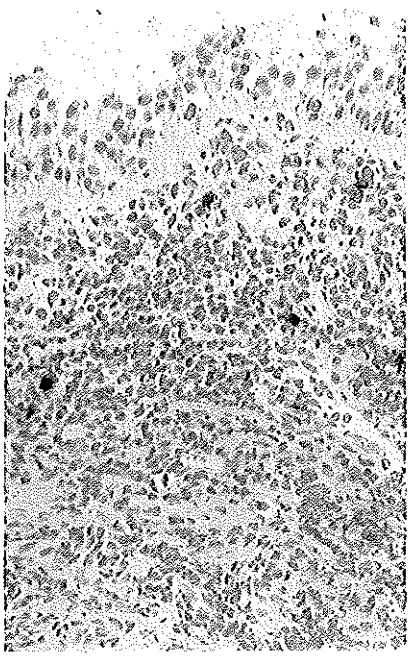
a



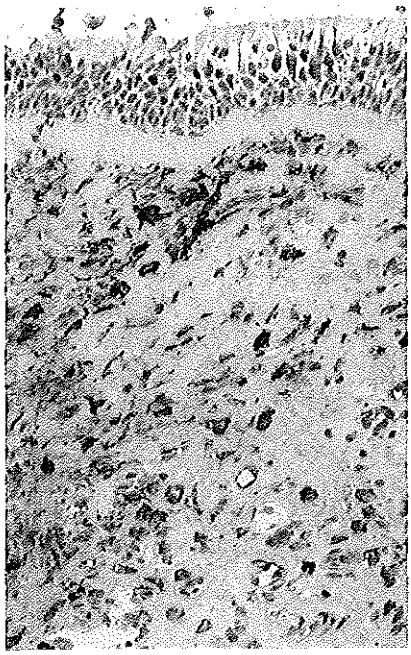
b



c



d



TREATMENT EFFECTS AFTER ALLERGEN PROVOCATION (FIG. 3)

During the whole study period, fewer eosinophils were present in the epithelium ($p<0.0001$) of the FPANS group compared to the placebo group. The same trend was found in the lamina propria ($p=0.07$).

The numbers of IL-3 mRNA positive cells showed a significant ($p=0.02$) treatment effect during provocation. The rise in IL-3 positive cells was lower in numbers and shorter in time in the FPANS group than in the placebo group after allergen provocation. This result lasted until a week after provocation ($p=0.0006$).

For the whole study period, significantly ($p<0.0001$) fewer IL-5 mRNA positive cells in the FPANS group were found compared to the placebo group. Lower IL-5 mRNA positive cell numbers were observed in the FPANS group compared to the placebo group 24 hours and one week ($p=0.01$and $p=0.04$) after allergen provocation.

IL-6 mRNA positive cells were significantly lower in numbers in the FPANS group compared to the placebo group ($p=0.03$) for the whole study period.

Fewer IFN-γ mRNA positive cells ($p=0.0001$) were observed in the FPANS group than in the placebo group during the whole study period. Numbers of IFN-γ mRNA positive cells were lower in the FPANS group than in the placebo group at one hour ($p=0.02$), 24 hours ($p=0.01$) and one week ($p=0.009$) after provocation. Less RANTES mRNA positive cells were present in the FPANS group than in the placebo group during the whole study period ($p=0.01$). At one hour after provocation, significantly fewer RANTES mRNA positive cells were observed in the FPANS group compared to the placebo group ($p=0.05$). Numbers of TNF-α mRNA positive cells were found to be lower in the FPANS group than in placebo group ($p=0.001$) for the whole study period.

Cell numbers for IL-2, IL-4, IL-8, IL-10 mRNA positive cells showed no significant treatment-dependent differences between the groups.

Figure 3 a-m

Box plots showing the number of mRNA positive cells in nasal mucosa biopsies. Nasal mucosa biopsies were taken **before** and 4 weeks after starting treatment (**dur. tr.**), **1 hour** after provocation, **24 hours** after provocation and **1 week** after provocation. Allergen challenge (**ch**) with 1,000 and 10,000 Bu grass pollen. **Treatment** with placebo n=9 (grey bar) and FPANS n=10 (white bar) * $p<0.05$ ** $p<0.01$ (Mann Whitney U test).(Overleaf)

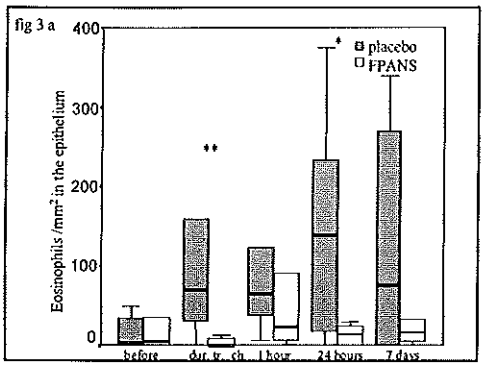
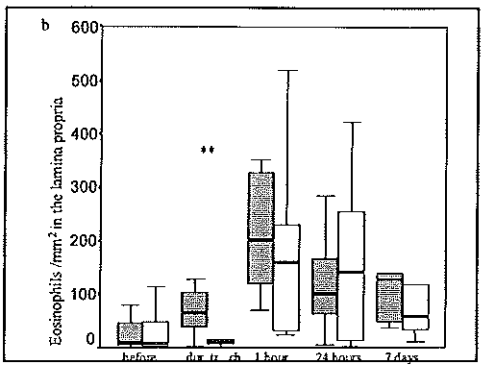
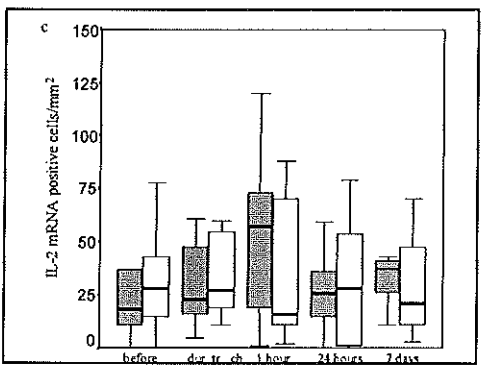
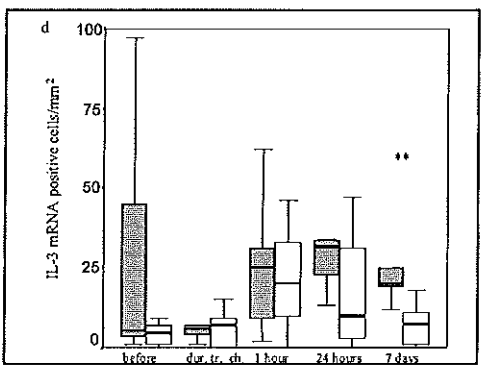
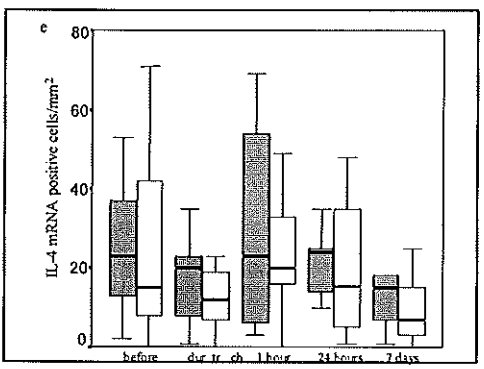


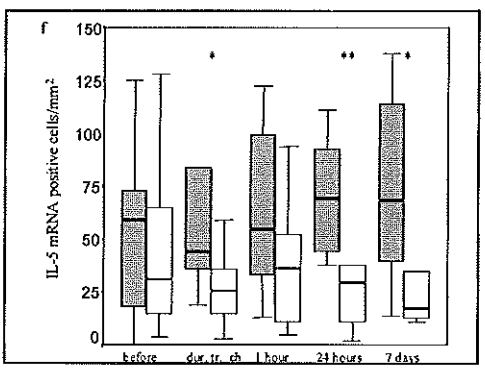
fig 3 a
placebo
PANS
Eosinophils /mm² in the epithelium
400
300
200
100
0
*
**
before
dur. tr.
ch.
1 hour
24 hours
7 days

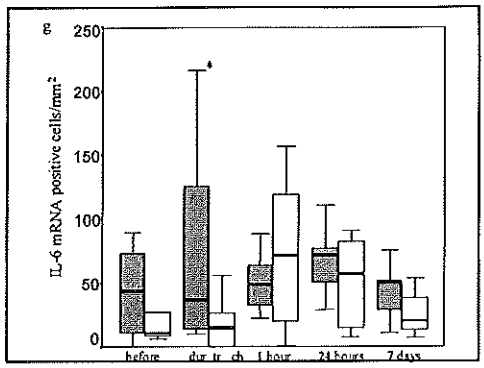

b
Eosinophils /mm² in the lamina propria
600
500
400
300
200
100
0
**
before
dur. tr.
ch.
1 hour
24 hours
7 days

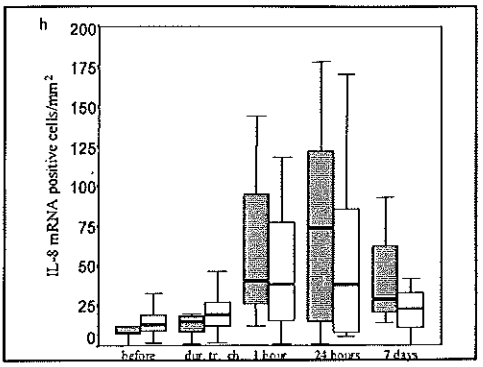

c
IL-2 mRNA positive cells/mm²
150
125
100
75
50
25
0
before
dur. tr.
ch.
1 hour
24 hours
7 days

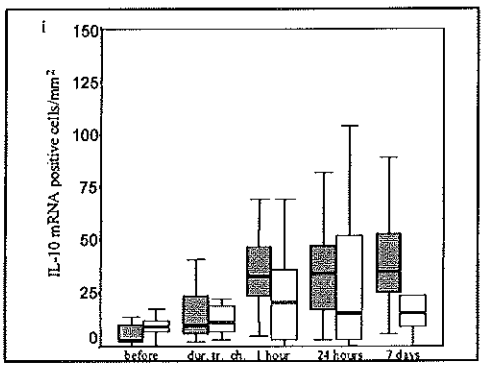

d
IL-3 mRNA positive cells/mm²
100
75
50
25
0
**
before
dur. tr.
ch.
1 hour
24 hours
7 days

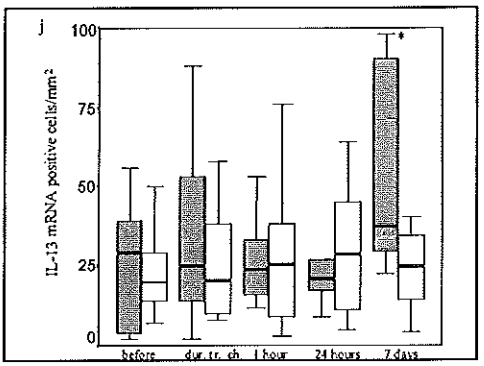

e
IL-4 mRNA positive cells/mm²
80
60
40
20
0
before
dur. tr.
ch.
1 hour
24 hours
7 days

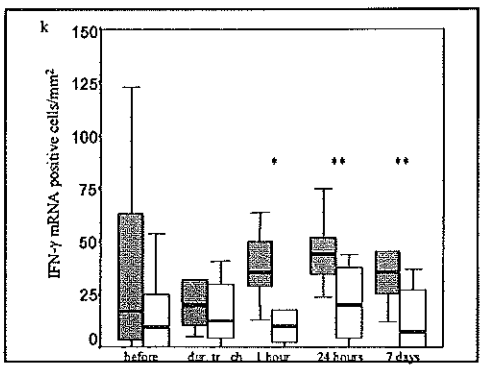

f
IL-5 mRNA positive cells/mm²
150
125
100
75
50
25
0
*
**
*
before
dur. tr.
ch.
1 hour
24 hours
7 days

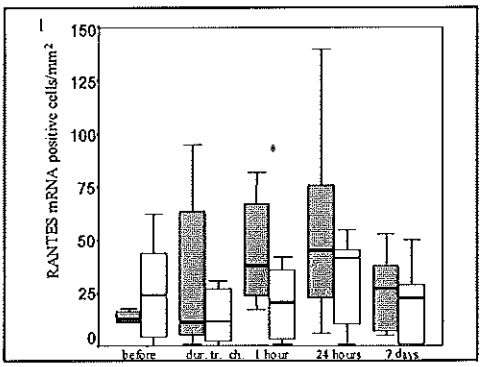

g
IL-6 mRNA positive cells/mm²
250
200
150
100
50
0
before
dur. tr. ch.
1 hour
24 hours
7 days


h
IL-8 mRNA positive cells/mm²
200
175
150
125
100
75
50
25
0
before
dur. tr. ch.
1 hour
24 hours
7 days


i
IL-10 mRNA positive cells/mm²
150
125
100
75
50
25
0
before
dur. tr. ch.
1 hour
24 hours
7 days


j
IL-13 mRNA positive cells/mm²
100
75
50
25
0
before
dur. tr. ch.
1 hour
24 hours
7 days


k
IFN-γ mRNA positive cells/mm²
150
125
100
75
50
25
0
before
dur. tr. ch.
1 hour
24 hours
7 days


l
RANTES mRNA positive cells/mm²
150
125
100
75
50
25
0
before
dur. tr. ch.
1 hour
24 hours
7 days

COMPARING BASELINE AND ONE WEEK AFTER PROVOCATION.

In the placebo group, higher mRNA positive cell numbers were observed for IL-10 (comparing baseline with one week after provocation: p=0.04) and IL-13 (0.04) one week after allergen provocation. By contrast, the mRNA positive cell numbers for all measured cytokines in the FPANS group dropped back to baseline level within one week after allergen provocation.

CELLULAR RELATIONSHIP TO NASAL SYMPTOMS

Significant, but weak, correlations were found between numbers of eosinophils in biopsy sections and nasal symptom score for blockage, itching, discharge, sneezing and total symptom score (see Table IV). Also correlations were observed between IL-3 mRNA and Il-8 mRNA expressing cells in biopsy sections and nasal symptom score for blockage, discharge, sneezing and total symptom score. Moreover, correlations were observed between IL-6 mRNA expressing cells and itching, discharge and total symptom score.

Table IV

Correlations between numbers of eosinophils, IL-3, IL-6 and Il-8 mRNA in biopsy sections and nasal symptom score for blockage, itching, discharge, sneezing and total symptom score (for r- (regression) and p-values (n.s.= not sigifiacant)).

cell type	symptom correlations (regression value and (p-value))				
	Blockage	discharge	itching	Sneezing	total
Eosinophil	0.323 (0.005)	0.382 (0.001)	0.405 (0.001)	0.475 (0.001)	0.435 (0.001)
IL-3	n.s.	n.s.	n.s.	n.s.	n.s.
IL-6	n.s.	0.317 (0.006)	n.s.	n.s.	0.301 (0.009)
IL-8	0.320 (0.006)	0.354 (0.002)	n.s.	n.s.	0.366 (0.001)

CYTOKINE mRNA RELATIONSHIP TO TISSUE EOSINOPHILIA

Before allergen provocation, no significant correlations were observed between cells expressing mRNA for cytokines and eosinophils. In the early and late phase, significant

correlations were observed between the number of eosinophils and the number of cells expressing mRNA for IL-3, IL-4, IL-5, IL-10, and IL-13. Moreover, this was also the case in early phase for IL-2, IL-8, RANTES, TNF-α and IFN-γ mRNA positive cell numbers. As late as one week after the allergen provocation, correlations were found between eosinophils and IL-5, IL-10, RANTES, TNFα and IFN-γ mRNA positive cell numbers (for r- (regression) and p-values, see table V).

Table V

Correlations between the number of eosinophils compared with the number of cells expressing positive hybridization signals at each biopsy moment (for r- (regression) and p-values).(n.s.= not significant)

	Eosinophils at baseline	Eosinophils before provocation after pretreatment	Eosinophils 1hr after ch.	Eosinophils 24 hr after ch.	Eosinophils 1 week after ch.
IL-2	n.s.	n.s.	n.s.	n.s.	n.s.
IL-3	n.s.	n.s.	0.74 (0.0001)	0.59 (0.009)	n.s.
IL-4	n.s.	n.s.	0.61 (0.006)	n.s.	n.s.
IL-5	n.s.	n.s.	n.s.	0.63 (0.004)	0.59 (0.008)
IL-6	n.s.	n.s.	n.s.	n.s.	n.s.
IL-8	n.s.	n.s.	0.75 (0.0001)	n.s.	n.s.
IL-10	n.s.	n.s.	n.s.	0.70 (0.001)	n.s.
IL-13	n.s.	n.s.	0.66 (0.002)	0.62 (0.005)	n.s.
IFN-γ	n.s.	n.s.	n.s.	n.s.	0.68 (0.001)
RANTES	n.s.	n.s.	0.63 (0.004)	n.s.	n.s.
TNF-α	n.s.	n.s.	0.69 (0.001)	n.s.	0.68 (0.001)

Discussion

In this study, we investigated the effect on cytokine mRNA expressing cells in nasal mucosal biopsies of corticosteroid pretreatment at baseline (before treatment) and after 4 weeks of treatment before provocation and one hour, 24 hours and one week after a single provocation with grass pollen and compared these sets of data to tissue eosinophilia and symptomatology.
FPANS treatment was effective in reducing spontaneous expression of the Th2 cytokines IL-5 and IL-6 before allergen provocation. After allergen provocation, lower cell numbers for IL-3, IL-5, IL-6, IL-13, IFN-γ, RANTES and TNF-α mRNA were found in the FPANS pretreatment group compared with the placebo group. In the placebo group, as late as one week after allergen provocation, higher mRNA positive cell numbers for IL-10 and IL-13 mRNA were observed compared to baseline cell numbers. By contrast, in the FPANS group, all cells investigated dropped back to baseline level within one week after allergen provocation. The effect of pretreatment with FPANS on mRNA cytokine expression can still be found one week after a single allergen provocation. These findings further stress the usefulness of pretreatment of allergic patients before the season.
Local corticosteroid treatment is effective in reducing cytokines which have a Glucocorticoid Response Element (GRE), irrespective of their importance in the allergic inflammation. The presence or absence of GRE in the promoter region of cytokines could explain why corticosteroid treatment has a direct effect e.g. on IL-5 expression or why it cannot have any direct effect but only an indirect effect e.g. on IL-2 expression (39).
Allergen provocation induces nasal symptoms and tissue eosinophilia, which can be used as a marker for allergic inflammation (34,40,41). In this study, the number of eosinophils correlated with nasal symptomatology, as found by Pipkorn et al. (42) but in contrast with the finding of Durham using disk provocation (10). It is probable that the provocation of the whole nasal mucosa by spray results in a more general mucosal response and symptomatology than the filter provocation method. Corticosteroid treatment reduces the number of eosinophils, as shown in this provocation study and other studies (2,43).
IL-2, a Th1 cytokine, has proliferating, activating and differentiating functions on T-cells and B-cells. No IL-2 mRNA effects were observed, this Th1 cytokine proved not to be involved in allergic inflammation during allergen provocation and no effect of corticosteroid treatment was observed. These findings confirm other reports that did not find changes in IL-2 (on the

protein and mRNA levels) in the nasal mucosa (18,44), in lung (45,46) and in skin (47) after allergen challenge. The lack of corticosteroid effect is consistent with the reports of Barnes, which showed that IL-2 has no GRE in its promoter region (39).

IL-4 and IL-13 have similar functions in B-cell isotype switching to IgE and also have a pivotal role in the up-regulation of adhesion molecules on endothelial cells (48). Data on IL-4 and IL-13 are conflicting. In this steroid pretreatment study, no changes were observed in IL-4 mRNA positive cells. In a previous repeated allergen provocation study, however, we did find a significant reduction in IL-4 mRNA in the FPANS group using Rt PCR (1). Masuyama et al found a significant difference in IL-4 expressing mRNA 24 hours after allergen challenge in the FPANS group compared to the placebo group (18). Bradding also showed FPANS to be effective in suppressing IL-4 protein. However, he found no up-regulation in the hay fever season compared to pre-season (12).

One important explanation for the different results obtained from these studies is the method of allergen provocation. Filter disk provocation (18) and repeated allergen provocation (1) result in an increase in cytokine expression for IL-4 mRNA. These two methods result in a stronger stimulus compared to a single allergen challenge by spray as performed in the present study and natural provocation in a grass pollen season (12) in which no IL-4 protein increase could be observed.

A significant increase in the number of IL-13 mRNA positive cells was found in the placebo group during allergen provocation, but the treatment effects did not reach significance. Ghaffer was able, in his study with filter disk provocation, to show a significant reduction in the rise in IL-13 mRNA and IL-13 protein positive cells 24 hours after allergen challenge in the FPANS group (19).

IL-3 and IL-5, Th2 cytokines that are produced by T-cells and eosinophils, stimulate, for example, the chemotaxis of eosinophils. The number of IL-3 mRNA positive cells was found to be increased after allergen provocation. Corticosteroid pretreatment reduces this increase. Steroid therapy in asthmatic patients was also found to be associated with a reduction in the percentages of T-cells expressing IL-3 mRNA and releasing IL-3 protein (49). In this study, a significant reduction of spontaneously transcription levels of IL-5 was found in the FPANS group compared to the placebo group during pretreatment. After allergen provocation, a significantly lower number of IL-5 mRNA was found in the FPANS pretreatment group than the control group. This confirms previous studies performed in our group showing a reduction of IL-5 mRNA transcripts using RtPCR during repeated allergen provocation (1). In addition,

Garrelds described a reduction in IL-5 protein in nasal lavage after local steroid treatment during the late phase allergic reaction (50). Moreover, Corrigan and colleagues found glucocorticoid therapy in asthmatics to be associated with a reduction IL-5 mRNA and protein (49). In vitro data also confirms the suppression of IL-5 by steroids (28,51). These findings are in contrast with studies which failed to find a significant reduction after FPANS treatment in IL-5 mRNA positive cells in a filter disk allergen provocation stud y (18) or Il-5 protein positive cells in a seasonal study (12). However, in the last study, the dosage used was only 200 μg FPANS.

The chemokines, RANTES and IL-8, are pro-inflammatory cytokines which are important in, for example, eosinophil chemotaxis. The correlation between IL-8 and nasal symptoms found in this study confirms data from Douglass et al. who observed an increase in rhinitis symptomatology after IL-8 challenge in the nasal mucosa (52). Pretreatment followed by allergen provocation (in the present study) failed to show a reduction in IL-8 mRNA expressing cells. However, Sim's group found an inhibition of secretion of IL-8 and RANTES protein in nasal lavage after allergen challenge (11,31). In this study also, FPANS treatment significantly reduced the rise in RANTES mRNA expressing cells during allergen provocation. The presence of GRE in the promoter region of RANTES and the absence in the promoter region of IL-8 explains why corticosteroid treatment has a significant effect on RANTES but cannot have a direct effect on IL-8 (39). It is probable that these differences are more pronounced on the mRNA level than on the protein level.

The reduction in number of TNF-α mRNA expressing cells after provocation implies that TNF-α was down-regulated in allergic inflammation, irrespective of the treatment. In the FPANS group, we found a reduction in the number of cells expressing TNF-α compared to the placebo group. Studies in nasal polyps showing that TNF-α was significantly reduced in an allergic group compared to a non-allergic group also indicate that TNF-α does not play a specific role in allergic inflammation (53). However, several sets of data indicate that TNF-α has a pivotal role in bacterial infections (54,55).

Pretreatment with FPANS resulted in a reduction in IL-6 mRNA positive cells compared to placebo. IL-6 showed no significant increase after allergen provocation in both groups, although a significant correlation was found between the symptomatology and the number of IL-6 mRNA positive cells. On the protein level (protein positive cells), no differences were observed in the upper airways after natural allergen provocation in the season (12). However,

Weido et al observed an increase in IL-6 protein levels in nasal lavage fluids during the late-phase reaction which could be inhibited by pretreatment with FPANS (31).

Numbers of IL-10 mRNA positive cells increased after provocation and were not reduced by steroid treatment. IL-10, considered to be a Th2 cytokine with anti-inflammatory and immunoregulatory properties, inhibits the release of IFN-γ (56) and generally inhibits cytokine synthesis by human monocytes (57). IFN-γ, which is considered to be a Th1 cytokine, did not increase significantly after allergen provocation. However, although the increase in IFN-γ was not significant, a significant reduction in mRNA positive cells was found during allergen provocation in the FPANS group compared to the placebo group. Other studies found no significant reduction in IFN-γ after FPANS treatment of allergic rhinitis patients (18). The decrease found in this study can be explained as an overall decrease in inflammatory cytokine production after local corticosteroid treatment without specific anti-allergic effects and may be also as a co-effect of the anti-inflammatory IL-10, numbers of which increase after local allergen provocation.

In the early phase cytokine mRNA for IL-3, IL-4, IL-8, IL-13, RANTES and TNF-α was significantly correlated with eosinophil numbers (see table V). The early phase is identified by nasal symptoms and the release of histamine and other inflammatory mediators by mast cells (58). The early phase mediators have effects on the endothelium and on the inflammatory cells present in the nasal mucosa, inflammatory cells will be generally activated (mRNA expressing) and an influx of activated cells (mRNA expressing cells) will occur. However, in the late phase 24 hours after provocation, significant correlations were only found between eosinophils and cytokine mRNA of the Th2 cytokines IL-3, IL-5, IL-10 and IL-13.

This study shows, for the first time, that pretreatment with FPANS, even in the absence of allergic inflammation and symptomatology, results in a decrease of inflammatory cells (eosinophils) and cells expressing cytokine mRNA (IL-5 and IL-6). Almost all cytokine mRNA expressing cells increase in the early phase symptomatology, but only the cytokines known to be relevant for allergic disease are correlated to eosinophils in the late phase symptomatology, again emphasising the role of these cytokines in allergic disease. Local corticosteroid treatment results in a significant decrease of a number of cytokines in the early and late phase. The effect of FPANS on eosinophils and cytokines is still visible a week after one single allergen provocation.

References

1 Fokkens, W.J., Godthelp, T., Holm, A.F., Blom, H. and KleinJan, A. 1997 Pahtophysiology of perennial allergic rhinitis. Allergy 52, 29-32.

2 Wiseman, L.R. and Benfield, P. 1997 Intranasal Fluticasone Propionate; A reappriasal of its pharmacology and clinical efficacy in the treatment of rhinitis. Drugs 53, 885-907.

3 Bousquet, J., Chanal, I., Alquie, M.C., Charpin, D., Didier, A., Germouty, J., Greillier, P., Ickovic, M.H., Maria, Y. and Montane, F. 1993 Prevention of pollen rhinitis symptoms: comparison of fluticasone propionate aqueous nasal spray and disodium cromoglycate aqueous nasal spray. A multicenter, double-blind, double-dummy, parallel-group study. Allergy 48, 327-33.

4 Dolovich, J., Hargreave, F.E., Jordana, M. and Denburg, J. 1989 Late-phase airway reaction and inflammation. J Allergy Clin Immunol 83, 521-4.

5 Bascom, R., Wachs, M., Naclerio, R.M., Pipkorn, U., Galli, S.J. and Lichtenstein, L.M. 1988 Basophil influx occurs after nasal antigen challenge: effects of topical corticosteroid pretreatment. J Allergy Clin Immunol 81, 580-9.

6 Peebles, R.J. and Togias, A. 1995 Role of mast cells and basophils in rhinitis. Review 113 refs. Chem Immunol 62, 60-83.

7 Iliopoulos, O., Proud, D., Adkinson, N.F., Jr., Norman, P.S., Kagey-Sobotka, A., Lichtenstein, L.M. and Naclerio, R.M. 1990 Relationship between the early, late, and rechallenge reaction to nasal challenge with antigen: observations on the role of inflammatory mediators and cells. J Allergy Clin Immunol 86, 851-61.

8 Fokkens, W.J., Holm, A.F., Rijntjes, E., Mulder, P.G. and Vroom, T.M. 1990 Characterization and quantification of cellular infiltrates in nasal mucosa of patients with grass pollen allergy, non-allergic patients with nasal polyps and controls. Int Arch Allergy Appl Immunol 93, 66-72.

9 Varney, V.A., Jacobson, M.R., Sudderick, R.M., Robinson, D.S., Irani, A.M., Schwartz, L.B., Mackay, I.S., Kay, A.B. and Durham, S.R. 1992 Immunohistology of the nasal mucosa following allergen-induced rhinitis. Identification of activated T lymphocytes, eosinophils, and neutrophils. Am Rev Respir Dis 146, 170-6.

10 Durham, S.R., Ying, S., Varney, V.A., Jacobson, M.R., Sudderick, R.M., Mackay, I.S., Kay, A.B. and Hamid, Q.A. 1992 Cytokine messenger RNA expression for IL-3, IL-4, IL-5, and granulocyte/macrophage-

colony-stimulating factor in the nasal mucosa after local allergen provocation: relationship to tissue eosinophilia. J Immunol 148, 2390-4.

11 Sim, T.C., Reece, L.M., Hilsmeier, K.A., Grant, J.A. and Alam, R. 1995 Secretion of chemokines and other cytokines in allergen-induced nasal responses: inhibition by topical steroid treatment. Am J Respir Crit Care Med 152, 927-33.

12 Bradding, P., Feather, I.H., Wilson, S., Holgate, S.T. and Howarth, P.H. 1995 Cytokine immunoreactivity in seasonal rhinitis: regulation by a topical corticosteroid. Am J Respir Crit Care Med 151, 1900-6.

13 Bachert, C., Wagenmann, M. and holtappels, G. 1998 Cytokines and adhesion molecules in allergic rhinitis. Am.J. Rhinology 12, 1-8.

14 Del Prete, G., Maggi, E., Parronchi, P., Chretien, I., Tiri, A., Macchia, D., Ricci, M., Banchereau, J., De Vries, J. and Romagnani, S. 1988 IL-4 is an essential factor for the IgE synthesis induced in vitro by human T cell clones and their supernatants. J Immunol 140, 4193-8.

15 Romagnani, S. 1991 Human TH1 and TH2 subsets: doubt no more. Immunol Today 12, 256-7.

16 de Vries, J.E. and Zurawski, G. 1995 Immunoregulatory properties of IL-13: its potential role in atopic disease. Int Arch Allergy Immunol 106, 175-9.

17 Hamilos, D.L., Leung, D.Y., Wood, R., Cunningham, L., Bean, D.K., Yasruel, Z., Schotman, E. and Hamid, Q. 1995 Evidence for distinct cytokine expression in allergic versus nonallergic chronic sinusitis. J Allergy Clin Immunol 96, 537-44.

18 Masuyama, K., Jacobson, M.R., Rak, S., Meng, Q., Sudderick, R.M., Kay, A.B., Lowhagen, O., Hamid, Q. and Durham, S.R. 1994 Topical glucocorticosteroid (fluticasone propionate) inhibits cells expressing cytokine mRNA for interleukin-4 in the nasal mucosa in allergen-induced rhinitis. Immunology 82, 192-9.

19 Ghaffar, O., Laberge, S., Jacobson, M.R., Lowhagen, O., Rak, S., Durham, S.R. and Hamid, Q. 1997 IL-13 mRNA and immunoreactivity in allergen-induced rhinitis: comparison with IL-4 expression and modulation by topical glucocorticoid therapy. Am J Respir Cell Mol Biol 17, 17-24.

20 Baggiolini, M. and Dahinden, C.A. 1994 CC chemokines in allergic inflammation. Immunol Today 15, 127-33.

21 Alam, R., Stafford, S., Forsythe, P., Harrison, R., Faubion, D., Lett-Brown, M.A. and Grant, J.A. 1993 RANTES is a chemotactic and activating factor for human eosinophils. J Immunol 150, 3442-8.

22 KleinJan, A., Dijkstra, M., Boks, S., Severijnen, E., Mulder, P. and Fokkens, W.J. 1998 Increase in IL-8, IL-10, IL-13 and RANTES mRNA levels (in situ hybridization) in the nasal mucosa after nasal allergen provocation. Submitted for publication .

23 Durham, S.R., Gould, H.J., Thienes, C.P., Jacobson, M.R., Masuyama, K., Rak, S., Lowhagen, O., Schotman, E., Cameron, L. and Hamid, Q.A. 1997 Expression of epsilon germ-line gene transcripts and mRNA for the epsilon heavy chain of IgE in nasal B cells and the effects of topical corticosteroid. Eur J Immunol 27, 2899-906.

24 Cameron, L.A., Durham, S.R., Jacobson, M.R., Masuyama, K., Juliusson, S., Gould, H.J., Lowhagen, O., Minshall, E.M. and Hamid, Q.A. 1998 Expression of IL-4, Cepsilon RNA, and Iepsilon RNA in the nasal mucosa of patients with seasonal rhinitis: effect of topical corticosteroids. J Allergy Clin Immunol 101, 330-6.

25 al Ghamdi, K., Ghaffar, O., Small, P., Frenkiel, S. and Hamid, Q. 1997 IL-4 and IL-13 expression in chronic sinusitis: relationship with cellular infiltrate and effect of topical corticosteroid treatment. J Otolaryngol 26, 160-6.

26 Bentley, A.M., Hamid, Q., Robinson, D.S., Schotman, E., Meng, Q., Assoufi, B., Kay, A.B. and Durham, S.R. 1996 Prednisolone treatment in asthma. Reduction in the numbers of eosinophils, T cells, tryptase-only positive mast cells, and modulation of IL-4, IL-5, and interferon-gamma cytokine gene expression within the bronchial mucosa. Am J Respir Crit Care Med 153, 551-6.

27 Sewell, W.A., Scurr, L.L., Orphanides, H., Kinder, S. and Ludowyke, R.I. 1998 Induction of interleukin-4 and interleukin-5 expression in mast cells is inhibited by glucocorticoids. Clin Diagn Lab Immunol 5, 18-23.

28 Braun, C.M., Huang, S.K., Bashian, G.G., Kagey-Sobotka, A., Lichtenstein, L.M. and Essayan, D.M. 1997 Corticosteroid modulation of human, antigen-specific Th1 and Th2 responses. J Allergy Clin Immunol 100, 400-7.

29 Mori, A., Kaminuma, O., Suko, M., Inoue, S., Ohmura, T., Hoshino, A., Asakura, Y., Miyazawa, K., Yokota, T., Okumura, Y., Ito, K. and Okudaira, H. 1997 Two distinct pathways of interleukin-5 synthesis in allergen-specific human T-cell clones are suppressed by glucocorticoids. Blood 89, 2891-900.

30 John, M., Lim, S., Seybold, J., Jose, P., Robichaud, A., B, O.C., Barnes, P.J. and Chung, K.F. 1998 Inhaled corticosteroids increase interleukin-10 but reduce macrophage inflammatory protein-1alpha, granulocyte-macrophage colony-stimulating factor, and interferon-gamma release from alveolar macrophages in asthma. Am J Respir Crit Care Med 157, 256-62.

31 Weido, A.J., Reece, L.M., Alam, R., Cook, C.K. and Sim, T.C. 1996 Intranasal fluticasone propionate inhibits recovery of chemokines and other cytokines in nasal secretions in allergen-induced rhinitis. Ann Allergy Asthma Immunol 77, 407-15.

32 Blom, H.M., Godthelp, T., Fokkens, W.J., KleinJan, A., Mulder, P.G. and Rijntjes, E. 1997 The effect of nasal steroid aqueous spray on nasal complaint scores and cellular infiltrates in the nasal mucosa of patients with nonallergic, noninfectious perennial rhinitis. J Allergy Clin Immunol 100, 739-47.

33 Fokkens, W.J., Vroom, T.M., Gerritsma, V. and Rijntjes, E. 1988 A biopsy method to obtain high quality specimens of nasal mucosa. Rhinology 26, 293-5.

34 Godthelp, T., Holm, A.F., Fokkens, W.J., Doornenbal, P., Mulder, P.G., Hoefsmit, E.C., Kleinjan, A., Prens, E.P. and Rijntjes, E. 1996 Dynamics of nasal eosinophils in response to a nonnatural allergen challenge in patients with allergic rhinitis and control subjects: a biopsy and brush study. J Allergy Clin Immunol 97, 800-11.

35 Klein, S.C., van Wichen, D.F., Golverdingen, J.G., Jacobse, K.C., Broekhuizen, R. and de Weger, R.A. 1995 An improved, sensitive, non-radioactive in situ hybridization method for the detection of cytokine mRNAs. Apmis 103, 345-53.

36 Van Hoffen, E., Van Wichen, D., Stuij, I., De Jonge, N., Klopping, C., Lahpor, J., Van Den Tweel, J., Gmelig-Meyling, F. and De Weger, R. 1996 In situ expression of cytokines in human heart allografts. Am J Pathol 149, 1991-2003.

37 Chomczynski, P. and Sacchi, N. 1987 Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162, 156-9.

38 Bloch, B. 1993 Biotinylated probes for in situ hybridization histochemistry: use for mRNA detection. J Histochem Cytochem 41, 1751-4.

39 Barnes, P.J. 1996 Mechanisms of action of glucocorticoids in asthma. Am J Respir Crit Care Med 154, S21-6; discussion S26-7.

40 Wihl, J.A., Ipsen, H., Petersen, B.N., Munch, E.P., Janniche, H. and Lowenstein, H. 1988 Immunotherapy with partially purified and standardized tree pollen extracts. II. Results of skin prick tests and nasal provocation tests from a three-year double-blind study of patients treated with pollen extracts either of birch or combinations of alder, birch and hazel. Allergy 43, 363-9.

41 Kay, A.B., Barata, L., Meng, Q., Durham, S.R. and Ying, S. 1997 Eosinophils and eosinophil-associated cytokines in allergic inflammation. Int Arch Allergy Immunol 113, 196-9.

42 Pipkorn, U., Karlsson, G. and Enerback, L. 1989 Nasal mucosal response to repeated challenges with pollen allergen. Am Rev Respir Dis 140, 729-36.

43 Fokkens, W.J., Godthelp, T., Holm, A.F. and Klein-Jan, A. 1998 Local corticosteroid treatment: the effect on cells and cytokines in nasal allergic inflammation. Am J Rhinol 12, 21-6.

44 Moss, R.B., Nagata, H., Goff, J., Okubo, K., Hausefeld, J. and Kaliner, M.A. 1997 Constitutive mRNA and immunoreactivity for IL-2 in human nasal mucosa. Clin Exp Allergy 27, 406-12.

45 Krug, N., Madden, J., Redington, A.E., Lackie, P., Djukanovic, R., Schauer, U., Holgate, S.T., Frew, A.J. and Howarth, P.H. 1996 T-cell cytokine profile evaluated at the single cell level in BAL and blood in allergic asthma see comments. Am J Respir Cell Mol Biol 14, 319-26.

46 Virchow, J.C., Jr., Walker, C., Hafner, D., Kortsik, C., Werner, P., Matthys, H. and Kroegel, C. 1995 T cells and cytokines in bronchoalveolar lavage fluid after segmental allergen provocation in atopic asthma. Am J Respir Crit Care Med 151, 960-8.

47 Kay, A.B., Ying, S., Varney, V., Gaga, M., Durham, S.R., Moqbel, R., Wardlaw, A.J. and Hamid, Q. 1991 Messenger RNA expression of the cytokine gene cluster, interleukin 3 (IL-3), IL-4, IL-5, and granulocyte/macrophage colony-stimulating factor, in allergen-induced late-phase cutaneous reactions in atopic subjects. J Exp Med 173, 775-8.

48 Jahnsen, F.L., Brandtzaeg, P., Haye, R. and Haraldsen, G. 1997 Expression of functional VCAM-1 by cultured nasal polyp-derived microvascular endothelium. Am J Pathol 150, 2113-23.

49 Corrigan, C.J., Hamid, Q., North, J., Barkans, J., Moqbel, R., Durham, S., Gemou-Engesaeth, V. and Kay, A.B. 1995 Peripheral blood CD4 but not CD8 t-lymphocytes in patients with exacerbation of asthma transcribe and translate messenger RNA encoding cytokines which prolong eosinophil survival in the context of a Th2-type pattern: effect of glucocorticoid therapy. Am J Respir Cell Mol Biol 12, 567-78.

50 Garrelds, I.M., De Graaf-in 't Veld, T., Nahori, M.A., Vargaftig, B.B., Gerth van Wijk, R. and Zijlstra, F.J. 1995 Interleukin-5 and eosinophil cationic protein in nasal lavages of rhinitis patients. Eur J Pharmacol 275, 295-300.

51 Mori, A., Suko, M., Nishizaki, Y., Kaminuma, O., Kobayashi, S., Matsuzaki, G., Yamamoto, K., Ito, K., Tsuruoka, N. and Okudaira, H. 1995 IL-5 production by CD4+ T cells of asthmatic patients is suppressed by glucocorticoids and the immunosuppressants FK506 and cyclosporin A. Int Immunol 7, 449-57.

52 Douglass, J.A., Dhami, D., Gurr, C.E., Bulpitt, M., Shute, J.K., Howarth, P.H., Lindley, I.J., Church, M.K. and Holgate, S.T. 1994 Influence of interleukin-8 challenge in the nasal mucosa in atopic and nonatopic subjects. Am J Respir Crit Care Med 150, 1108-13.

53 Hamilos, D.L., Leung, D.Y., Wood, R., Bean, D.K., Song, Y.L., Schotman, E. and Hamid, Q. 1996 Eosinophil infiltration in nonallergic chronic hyperplastic sinusitis with nasal polyposis (CHS/NP) is associated with endothelial VCAM-1 upregulation and expression of TNF-alpha. Am J Respir Cell Mol Biol 15, 443-50.

54 Fong, Y. and Lowry, S.F. 1990 Tumor necrosis factor in the pathophysiology of infection and sepsis. Clin Immunol Immunopathol 55, 157-70.

55 Cybulsky, M.I., Chan, M.K. and Movat, H.Z. 1988 Acute inflammation and microthrombosis induced by endotoxin, interleukin-1, and tumor necrosis factor and their implication in gram-negative infection. Lab Invest 58, 365-78.

56 Pene, J., Rousset, F., Briere, F., Chretien, I., Bonnefoy, J.Y., Spits, H., Yokota, T., Arai, N., Arai, K., Banchereau, J. and et al. 1988 IgE production by normal human lymphocytes is induced by interleukin 4 and suppressed by interferons gamma and alpha and prostaglandin E2. Proc Natl Acad Sci U S A 85, 6880-4.

57 de Waal Malefijt, T. (1991) Molecular aspects of human T cell activation and lymphokine production. In: pp. 201, Puglisher, Amsterdam.

58 Busse, W.W. and Holgate, S.T., eds. 1995 Asthma and Rhinitis Blackwell Scientific Publications, Boston.

59 Taniguchi, T., Matsui, H., Fujita, T., Takaoka, C., Kashima, N., Yoshimoto, R. and Hamuro, J. 1983 Structure and expression of a cloned cDNA for human interleukin-2. Nature 302, 305-10.

60 Yang, Y.C., Ciarletta, A.B., Temple, P.A., Chung, M.P., Kovacic, S., Witek-Giannotti, J.S., Leary, A.C., Kriz, R., Donahue, R.E., Wong, G.G. and et al. 1986 Human IL-3 (multi-CSF): identification by expression cloning of a novel hematopoietic growth factor related to murine IL-3. Cell 47, 3-10.

61 Tanabe, T., Konishi, M., Mizuta, T., Noma, T. and Honjo, T. 1987 Molecular cloning and structure of the human interleukin-5 gene. J Biol Chem 262, 16580-4.

62 Taga, T. and Kishimoto, T. 1995 Interleukin-6. In: Human cytokines: their role in disease and therapy (Aggarwal, B.B. and Puri, R.K., eds.), pp. 142-153, Blackwell Scientific, Cambridge.

63 Mukaida, N., Shiroo, M. and Matsushima, K. 1989 Genomic structure of the human monocyte-derived neutrophil chemotactic factor IL-8. J Immunol 143, 1366-71.

64 Vieira, P., de Waal-Malefyt, R., Dang, M.N., Johnson, K.E., Kastelein, R., Fiorentino, D.F., deVries, J.E., Roncarolo, M.G., Mosmann, T.R. and Moore, K.W. 1991 Isolation and expression of human cytokine synthesis inhibitory factor cDNA clones: homology to Epstein-Barr virus open reading frame BCRFI. Proc Natl Acad Sci U S A 88, 1172-6.

65 McKenzie, A.N., Li, X., Largaespada, D.A., Sato, A., Kaneda, A., Zurawski, S.M., Doyle, E.L., Milatovich, A., Francke, U., Copeland, N.G. and et al. 1993 Structural comparison and chromosomal localization of the human and mouse IL-13 genes. J Immunol 150, 5436-44.

66 Schall, T.J., Jongstra, J., Dyer, B.J., Jorgensen, J., Clayberger, C., Davis, M.M. and Krensky, A.M. 1988 A human T cell-specific molecule is a member of a new gene family. J Immunol 141, 1018-25.

67 Wang, A.M., Creasey, A.A., Ladner, M.B., Lin, L.S., Strickler, J., Van Arsdell, J.N., Yamamoto, R. and Mark, D.F. 1985 Molecular cloning of the complementary DNA for human tumor necrosis factor. Science 228, 149-54.

Chapter 8

General discussion

Adapted from: Wytske J. Fokkens, Tom Godthelp, Adriaan F. Holm et al. Am J Rhinol, 1998, 12, 21-26

GENERAL DISCUSSION

Allergen provocation in susceptible atopic individuals leads to allergic symptoms. The symptomatology of allergic rhinitis is currently considered to be mainly caused by accumulation and activation of infiltrating cells with concomitant release of inflammatory mediators, resulting in allergic inflammation. This allergic inflammation causes priming to allergen and hyperreactivity, resulting in an increase in symptomatology. Allergic inflammation and the effects of treatment can be studied in many ways, ranging from single provocation with a large dose of allergens (punch on the nose) usually performed in patients with a pollen allergy, to repeated daily allergen provocations with small doses of allergen to simulate a more naturally proceeding disease, to naturally occurring seasonal allergic disease, and finally to naturally occurring perennial allergic disease (1-4). Of course, studying differences and therapeutic effects in the single provocation model is considerably easier than in naturally occurring disease. However, although exaggerated provocation is a useful study tool, the findings need to be confirmed in naturally occurring disease in order to be completely reliable.

To investigate inflammatory cells in the nasal mucosa a biopsy method was chosen(5). This method has proven to be safe, tolerable and repeatable over a short period of time (one day) for a limited number of biopsies. Drawback of the nasal biopsy method is that information is gained only from a small part of nasal mucosa, and that studying functional aspects of cells is not possible. Nasal mucosal biopsy specimens can not only be used for immunohistochemical staining, but also for *polymerase chain reaction (PCR)* and *in situ* hybridisation techniques. With these techniques inflammatory cells, but also cytokine and chemokine mRNA presence can be investigated.

The nasal lavage method according to Naclerio *et al.* (6) is less uncomfortable for the patients and can be used more often compared to the biopsy method, but only small amounts of inflammatory cells shed in the nasal lumen can be detected in the retrieved fluids. Alternative ways to investigate nasal mucosa are nasal scrapings and brushes. Drawback of both methods is that information is gained only from nasal epithelium and not from the lamina propria. Moreover, nasal brushes are more unpleasant for the patient than nasal biopsies.

Since more than two decades topical corticosteroids are used for the treatment of allergic rhinitis. This study was confined to the effects of Fluticasone Propionate Aqueous Nasal

Spray (FPANS). As summarised in chapter 2 the aim of this study was to investigate the safety, efficacy and effects on nasal mucosal inflammatory cells of FPANS in perennial allergic rhinitis in a one-year placebo controlled study, and to investigate the effects of FPANS on nasal mucosal cells and cytokine mRNA in a nasal provocation study with patients with seasonal allergic rhinitis.

SAFETY OF TOPICAL FPANS THERAPY

Local side effects

There is still some concern about the risk of mucosal atrophy after long-term corticosteroid treatment, although long-term clinical experience and a number of biopsy studies did not produce any indication for such a risk in the nose (7-12).

This is the first placebo-controlled study examining in nasal mucosal biopsies the long-term effect of an intranasal corticosteroid. No evidence of mucosal damage and systemic side-effects after one-year use were observed(chapter 3). Long-term FPANS use was found to be associated with few adverse events as headache, upper and lower respiratory tract infections. The patients using placebo (chapter 3) also reported these adverse events. Few patients in both the FPANS and placebo group developed crusts on the nasal mucosa. Epistaxis was only reported by three out of 21 patients in the FPANS group, and must be considered due to the corticosteroid, although biopsy study of the nasal mucosa showed no signs of histological damage or atrophy. However, these biopsies were taken form the inferior turbinate and therefore may not be representative for the mucosa of the nasal septum. Epistaxis was never a reason to stop FPANS treatment. No signs of mucosal candidiasis in the nose or throat were found after long-term FPANS treatment.

Systemic side effects

Long-term intranasal treatment of adults with 200-400 μg/d of Beclomethasone Dipropionate, Budesonide and Flunisolide has shown that the risk of systemic adverse events is very small (13). In our study no changes were found in hypothalamo-pituitary-adrenocortical axis (HPA) measurements, analysis of routine biochemistry, haematology and urine, probably due to the low bioavailability of FPANS (chapter 3). The safety evaluations in this placebo-controlled study confirm the results from previous studies in which either long-term or high-dose

treatments were used (14-17). FPANS appeared to be a well-tolerated and safe treatment for adult patients with perennial allergic rhinitis.

Recent data indicate that children receiving an adult dose of intranasal Budesonide (200 μg twice daily during six weeks) show short term growth inhibition as measured with lower leg length (knemometry)(18), where as this did not occur when 200 or even 400 μg were given once daily (19). We are not aware of data on nasal Fluticasone Propionate and growth inhibition in children, but in asthmatic children no short-term growth inhibition was seen using inhaled Fluticasone in 200 and 400 μg daily (20). In the treatment of perennial allergic rhinitis in children of 4-11years, 100 and 200 μg FPANS once daily for a period of 12 weeks has been proven safe, with no evidence of systemic corticosteroid effect (21). It is too early to judge the clinical significance of these short terms results, but according to Mygind it seems advisable in children to use the lowest dose which can control symptoms until data on long-term effects is available (22).

EFFICACY OF FPANS THERAPY

It is generally appreciated that the treatment of perennial allergic rhinitis is more difficult compared to seasonal rhinitis (22). Reasons for this difference in efficacy may be the chronic low-grade exposure to allergens in perennial allergic rhinitis compared with the high exposure to allergens in patients with seasonal rhinitis. In addition, the more pronounced nasal obstruction in perennial allergic rhinitis results in a diminished access of the topical corticosteroid therapy to the site of inflammation(23). Not all nasal symptoms experienced by patients with perennial allergic rhinitis can be attributed to allergy. In perennial allergic rhinitis with continuous exposition to allergens, sensitivity, not only to allergens, but also to non-specific irritants as perfumes and tobacco smoke is increased. Consequently, part of the symptoms may be due to non-specific hyperreactivity (24). Even if the allergic part is diminished by corticosteroid therapy, this non-specific hyperreactivity may continue to give complaints and should be addressed by the physician as part of the treatment.

FPANS has been proven effective in previous studies concerning seasonal and perennial allergic rhinitis (14-16). In chapter 3 we found FPANS to be effective in controlling sneezing, nasal itching and total symptom score in perennial allergic rhinitis. However, in

contrast to what was expected, nasal obstruction and discharge were not significantly reduced after one-year treatment.

Reasons why we were not able to demonstrate statistically significant improvements of all nasal symptoms in our perennial patient group may be the relatively small patient group. In addition, patients experienced mild symptoms. Therefore, the potential for improvement is small. A patient selection bias may also be a contributing factor. Patients were recruited from a ENT-practice after referral by a general practitioner (GP). Most GP's initially treat allergic patients themselves, often with topical corticosteroids and refer only patients not responding to treatment. Therefore, the patient population recruited by us may be a subgroup, existing of corticosteroid non-responders. After nasal allergen provocation in seasonal allergic patients (chapter 6) FPANS was found to be effective in reducing early and late phase nasal obstruction and late phase discharge.

In perennial allergic rhinitis it is usually thought that it takes 2-4 weeks topical corticosteroid treatment to obtain maximal symptom relief (23). As reported in chapter 3, symptoms were certainly reduced after 4 week FPANS treatment, but maximal relief was obtained after 10 months of treatment. In the placebo group no change in total symptom score was observed. This finding suggests an improvement of efficacy with increasing duration of the treatment. This phenomenon was also observed in childhood asthma with Budesonide treatment, where symptoms improved during 18 months treatment and PD20 response to histamine stabilised after 22 months treatment (25). We could find no explanation for this long-term effect of corticosteroids on symptomatology.

There are different opinions concerning the moment to start with corticosteroid treatment in seasonal rhinitis. Bousquet et al. advise to start therapy before the onset of the season (26). Andersson et al. showed that already one-day pre-treatment with corticosteroids abolished the allergen-induced increase in nasal hyperresponsiveness (27). Others found in nasal allergen provocation studies that a pretreatment period of one or two weeks with a topical corticosteroid reduced nasal symptoms significantly compared with placebo (28-30). In chapter 6 we investigated whether a 6-week pre-treatment period would give complete relief of nasal symptoms after allergen challenge in patients with seasonal rhinitis. Symptoms were well controlled after allergen provocation in patients having used active pre-treatment, but were not abolished. Comparing our results with a pre-treatment period of six weeks with the results of other authors using a shorter pre-treatment period, no beneficial effect of a six week

period can be observed. In order to find the optimal pre-treatment period, further studies are necessary.

LOCAL CORTICOSTEROID TREATMENT EFFECT ON INFLAMMATORY CELLS IN NASAL MUCOSA

The effect of local corticosteroids on nasal symptoms and nasal mucosal inflammatory cells in allergic rhinitis seems dose-dependent. In chapters 3-5 a dose of 200 μg FPANS daily was used, which is considered a low dose. Effect of local corticosteroid treatment on nasal inflammatory cells will be discussed in the following paragraphs.

Mast cells

Data on the effects of allergen provocation on mast cell numbers in the nasal mucosa is contradictory and comparison with results from different authors is restricted by different immunohistochemical techniques. The same holds true for data on the effects of corticosteroid treatment on mast cells. There are few studies using monoclonal antibodies against IgE, tryptase and chymase. The data available seems to point to different reactions related to the strength of the allergen stimulus and the intensity of the therapy applied. Bradding found a reduced influx of tryptase positive cells in the epithelium, and no change in the lamina propria using Fluticasone Propionate aqueous nasal spray (FPANS) 200 μg once daily in seasonal allergic rhinitis compared to placebo (31). Juliusson described the same using Budesonide 400 μg (29). In our department, (FPANS) 200 μg daily was compared with placebo in a two-week daily threshold allergen provocation study in patients with an isolated grass-pollen allergy. A reduction in tryptase-positive cells was found in the epithelium, but not in the lamina propria (32). As described in chapter 6, using FPANS 400 μg, both in epithelium and lamina propria a significant inhibition in mast cell influx compared to placebo was seen after allergen provocation. Rak et al. did find a reduction in mast cells in the lamina propria using FPANS 200 μg, but this was found in an allergen provocation study with a large single-allergen dose (4). In chapter 5 we found in perennial allergic rhinitis, using FPANS 200μg, a significant reduction in epithelial mast cells, but not in the lamina propria. Godthelp et al. described, in a perennial allergic rhinitis study comparing FPANS 400 μg, FPANS 200 μg and placebo, a significant reduction in tryptase and chymase-positive cells in the epithelium for

both therapies compared to placebo. In the lamina propria a significant reduction was found after treatment with FPANS 400 μg, but not after treatment with FPANS 200 μg (33). From this data it is concluded that epithelial mast cells are reduced by local corticosteroid treatment. Reduction of the numbers of mast cells in the lamina propria can only be demonstrated under extreme conditions, either by using a large allergen stimulus, or high dose treatment.

Antigen- presenting cells and macrophages

Studies performed in our group in perennial allergic rhinitis and in provocation studies showed that even low dosages of local steroids result in total disappearance of CD1+ cells during disease and a virtually complete inhibition of the influx of LC during allergen provocation in the epithelium (chapter 4-6)(32, 33). Also, a significant reduction in the number of CD1+ cells and HLA-DR+ cells during disease and a significant inhibition of the influx were seen in the lamina propria of the nasal mucosa (chapter 4). As described in chapter 6, we found a significant effect inhibition of influx of macrophages in the nasal mucosa after allergen provocation in the FPANS treated group. No change in monocyte and macrophage cell numbers were seen after corticosteroid treatment in patients with perennial allergic rhinitis (chapter 5). This is in accordance with the above mentioned study by Juliusson (34).

T cells

The effect of local corticosteroid therapy on T cell numbers depends on the intensity of the allergen stimulus and the dose and duration of the therapy.

Rak et al. performed a provocation study in grass pollen allergic patients. Nasal biopsies were performed before treatment and 24 h after an allergen provocation and subsequently processed for immunohistology. Local corticosteroid treatment (high-dose) resulted in a marked reduction in T lymphocytes and CD25+ (interleukin-2 receptor bearing) cells in both the epithelium and submucosa (4). In a similar study (chapter 6), we also found T cell reduction in epithelium and lamina propria after single allergen provocation in the high-dose FPANS group. However, we did not find any differences in number of CD25+ cells.

Using low-dose FPANS treatment in perennial allergic rhinitis, we found no significant differences in the number of CD3, CD4, CD8 and CD25 positive cells in epithelium and lamina propria after three months of therapy (chapter 4). However, after one year a significant decrease was found in CD3, CD4 and CD8 positive cells in the epithelium. No significant

changes were found in the CD25+ cells and no significant changes were found in the lamina propria (chapter 5). In a double blind comparison of two different doses of FPANS in the treatment of patients with perennial allergic rhinitis a significant reduction was found in CD3+ and CD4+ cells in the epithelium and the lamina propria when FPANS 400 μg was compared with a placebo (33).

Eosinophils

Studies performed in our group and by Orgel, Rak and Bradding , showed that a considerable reduction of eosinophils, activated eosinophils and eosinophilic products occurs after local corticosteroid treatment (chapter 6)(4, 11, 31, 35-37) Even when the allergen stimulus is large, as in allergen provocation studies, or when the local corticosteroid dose is relatively low, the decrease in cells is substantial. However, despite prolonged treatment with FPANS 400 μg, eosinophils increased significantly in nasal mucosa in the following 24 hours when a large allergen stimulus is used (chapter 6). With the repeated biopsy method we were able to find an increase of eosinophils in the placebo pre-treated group in unchallenged nasal mucosa. Tissue eosinophilia after nasal allergen challenge is normally thought to occur as a result of the allergen stimulus, and inhibited by topical corticosteroids. We suggest it might also occur partly due to placebo treatment itself.

These results show that the accumulation of activated eosinophils after allergen provocation is decreased by nasal steroid. This reduction in eosinophil numbers tends to be more pronounced in the epithelium than in the lamina propria. The magnitude of this difference seems to be related to the intensity of the allergen challenge.

Cytokines

Bradding et al. showed a suppression of the number of IL-4 protein positive cells in the lamina propria in a study in patients with seasonal allergic rhinitis receiving topical Fluticasone nasal spray (200 micrograms daily) or matching placebo during the pollen season. Fluticasone treatment, however, failed to influence the number of IL-5 and IL-6 protein positive cells (31). In addition, Godthelp also found a significant reduction of IL-4 protein positive cells in the epithelium and lamina propria after treatment with 400 μg FPANS in the double blind comparison of two different doses of FPANS in patients with perennial allergic rhinitis mentioned above (33). Using *in situ* hybridisation, Masuyama et al. showed a decrease in the cells expressing RNA for IL-4, but not for IL-5, after treatment with Fluticasone

aqueous nasal spray (37). In our group, inhibition of mRNA for Il-4 and IL-5 was found in a threshold provocation model using polymerase chain reaction techniques (36). Using *in situ* hybridisation, we found that FPANS treatment implemented out of season in patients with seasonal rhinitis reduced the numbers of IL-5 and IL-6 mRNA positive cells in nasal mucosa. After allergen provocation FPANS treatment inhibited the increase of IL-3, IL-5, IL-13, IFN-γ, and RANTES mRNA positive cells. We failed to find any IL-4 effects in this study (chapter 7).

Inflammatory cells, FPANS and nasal symptoms

In patients with seasonal and perennial allergic rhinitis the number of nasal mucosal inflammatory cells is increased compared to controls. Intranasal corticosteroid treatment was demonstrated to reduce these numbers(1, 28, 33, 38, 39). What is the significance of the reduction in numbers of inflammatory cells for the understanding of the efficacy of corticosteroids. In symptomatic allergic patients changes in nasal complaints are associated with corresponding changes in numbers of inflammatory cells (chapters 5 and 6). However, we, nor others, could demonstrate a statistical significant correlation between changes in cell numbers and nasal symptoms. We found that in non-symptomatic hayfever patients out of the season FPANS pre-treatment reduced the non-elevated number of mucosal Langerhans cells, T cells, eosinophils, mast cells and macrophages without any change in symptoms (chapter 6). Blom et al. showed in patients with non-allergic, non-infectious perennial rhinitis (NANIPER) a significant reduction in numbers of immunocompetent cells after treatment with FPANS, also without a change in nasal complaints (40). It seems that nasal corticosteroids reduce inflammatory cells in nasal mucosa irrespective of the underlying nasal disease or condition (allergic or non-allergic rhinitis, challenged or non-challenged). From the above it is clear that it is very difficult to relate the changes in nasal mucosa cell numbers occurring after treatment to the clinical efficacy of the corticosteroid treatment. Other factors as cell activation and cell derived mediators also should be considered (3, 35, 41). On the other hand, the corticosteroid induced decrease of cell numbers probably contributes to the efficacy of the treatment as less cells produce less mediators.

From the studies described in this thesis a difference in effect on nasal mucosal lymphocytes between perennial and seasonal allergic rhinitis of FPANS can be observed. In perennial allergic rhinitis no change in number of T cells is seen in lamina propria after FPANS treatment (chapter 4 and 5). However, after a strong allergen provocation outside the season

in patients with seasonal allergic rhinitis, FPANS significantly reduced the number of T cells in epithelium and lamina propria (chapter 6). It can be hypothesised that the antigen challenge in a perennial population is ongoing but less vigorous, therefore leading to a less prominent inflammatory infiltrate more susceptible to intranasal steroids. Nevertheless, the number of lymphocytes in the lamina propria was not inhibited by steroid treatment in the perennial patient group. Perhaps, the number of lymphocytes involved in the allergic mechanism constitutes only a small portion of total lymphocytes, hence, the lack of inhibition by corticosteroids. Further studies are necessary to explain these differences between perennial and seasonal allergic rhinitis after corticosteroid treatment.

FINAL REMARKS

Despite the current available therapeutic strategies, some patients still experience symptoms. Several pathophysiological mechanisms in allergic rhinitis are eligible for new treatment approaches, e.g. adhesion molecules, cytokines and chemokines and inflammatory mediators such as leukotrienes. Localisation and migration of immunocompetent cells into the tissues require, first, adhesion to the vascular endothelium, and next, locomotion to the target organ. In perennial allergic rhinitis increased expression of adhesion molecules (ICAM -1 and VCAM-1) on endothelial cells has been found, associated with increased numbers of lymphocytes and eosinophils(42). After allergen challenge in seasonal rhinitis, VCAM-1, but not ICAM-1, expression is increased in nasal mucosa (43, 44). Prevention of migration of inflammatory cells into the nasal mucosa may be accomplished by reducing the expression of adhesion molecules. Currently available drugs as corticosteroids and cyclosporin are known to diminish the expression of ICAM-1 (45). Monoclonal antibodies directed against adhesion molecules may eventually prove to be effective in the treatment of inflammatory diseases.
Also interesting is the possibility of regulating the T-helper cells responsible in mediating many of the effects associated with allergic reactions. If activation of these TH2 cells can be prevented the downstream effects, such as the inflammation and hypersensitivity, may be abrogated. One way of achieving this is via antigen presenting cells (APCs). For instance, interleukin (IL)-10 has been shown to inhibit T cell proliferation in response to exogenous antigens indirectly by acting on APC function. Furthermore, IL-10 treated dendritic cells

(DC) induce a state of alloantigen-specific anergy in CD4+ T cells and might thus be useful to down-regulate unwanted immune responses such as allergy (46).

Recently, leukotriene synthesis inhibitors and leukotriene receptor antagonists have become available for the treatment of allergic diseases. Leukotriene synthesis is reduced by inhibition of 5-lipoxygenase or 5-lipoxygenase activating protein (FLAP), preventing arachidonic acid to convert into leukotriene B4, C4, D4 and E4. There are so far few studies that have addressed the effects of these new drugs on allergic rhinitis. (47, 48). It seems that nasal obstruction is the symptom that is reduced most by these new drugs. Perhaps, combination therapy of leukotriene synthesis inhibitors and leukotriene receptor antagonists with other therapeutic regimens may provide patients with optimal relief of allergic symptoms.

Conclusions

1. This thesis reports the first placebo-controlled study on the effects of long-term FPANS treatment in perennial allergic rhinitis patients, on nasal symptomatology and immunohistology of the nasal mucosa
2. It was demonstrated that in a period of 12 months no mucosal damage occurred and no systemic effects were found.
3. We found that the maximal efficacy of intranasal FPANS is not reached after a treatment period of 4 weeks (as previously thought), but after a treatment period of 10 months.
4. Intranasal corticosteroid treatment results in the reduction of the number of nasal mucosal inflammatory cells of non-symptomatic and symptomatic hay fever patients. Changes in cell numbers are not accompanied by changes in nasal symptomatology.

References

1. Godthelp T, Holm AF, Fokkens WJ, Doornenbal P, Mulder PG, Hoefsmit EC, et al. Dynamics of nasal eosinophils in response to a nonnatural allergen challenge in patients with allergic rhinitis and control subjects: a biopsy and brush study. J Allergy Clin Immunol 1996;97(3):800-11.
2. Holm AF, Fokkens WJ, Godthelp T, Mulder PGH, Vroom TM, Rijntjes E. The effect of three months nasal steroid therapy on nasal T cells and Langerhans cells in patients suffering from allergic rhinitis. Allergy 1995;50:204-209.

3. Lozewicz S, Wang J, Duddle J, Thomas K, chalstrey S, Reilly G, et al. Topical glucocorticoids inhibit activation by allergen in the upper respiratory tract. J Allergy Clin Immunol 1992;89:951-957.
4. Rak S, Jacobson MR, Sudderick RM, Masuyama K, Juliusson S, Kay AB, et al. Influence of prolonged treatment with topical corticosteroid (fluticasone propionate) on early and late phase nasal responses and cellular infiltration in the nasal mucosa after allergen challenge. Clin Exp Allergy 1994;24(10):930-9.
5. Fokkens WJ, Rijntjes E, Vroom TM, Gerritsma V. A mild biopsy method to obtain high-quality specimens of nasal mucosa. Rhinology 1988;26(4):293-295.
6. Naclerio RM, Meier HL, Kagey-Sobotka A, Adkinson N, Jr., Meyers DA, Norman PS, et al. Mediator release after nasal airway challenge with allergen. Am Rev Respir Dis 1983;128(4):597-602.
7. Brown HM, Storey G, Jackson FA. Beclomethasone dipropionate aerosol in the treatment of perennial and seasonal rhintis: a review of five years' experience. Br J clin Pharmac 1977;4:283S-286S.
8. Mygind N. Mucosal changes in the airways during long-term corticosteroid treatment. Eur J Respir Dis Suppl 1982;122:229-34.
9. Pipkorn U, Pukander J, Suonpaa J, Makinen J, Lindqvist N. Long-term safety of budesonide nasal aerosol: a 5.5-year follow-up study. Clinical Allergy 1988;18(3):253-9.
10. Bende M, Mark J. Long-term effects of topical corticosteroids in the nose. Journal of Laryngology & Otology 1992;106(9):810-2.
11. Orgel HA, Meltzer EO, Bierman CW, Bronsky E, Connell JT, Lieberman PL, et al. Intranasal fluocortin butyl in patients with perennial rhinitis: a 12-month efficacy and safety study including nasal biopsy. Journal of Allergy & Clinical Immunology 1991;88(2):257-64.
12. Poynter D. Beclomethasone dipropionate aerosol and nasal mucosa. Br J clin Pharmac 1977;4:295S-301S.
13. Mygind N. Glucocorticosteroids and rhinitis. [Review]. Allergy 1993;48(7):476-90.
14. As van A, Bronsky EA, Grossman J, Meltzer EO, Ratner P, Reed C. Dose tolerance study of Fluticasone propionate aqueous nasal spray in patients with seasonal allergic rhinitis. Ann Allergy 1991;76:156-162.
15. Haye R, Gomez EG. A multicentre study to assess long-term use of fluticasone propionate aqueous nasal spray in comparison with beclomethasone dipropionate aqueous nasal spray in the treatment of perennial rhinitis. Rhinology 1993;31(4):169-74.
16. Meltzer EO, Orgel HA, Bronsky EA, Furukawa CT, Grossman J, LaForce CF, et al. A dose-ranging study of fluticasone propionate aqueous nasal spray for seasonal allergic rhinitis assessed by symptoms, rhinomanometry, and nasal cytology. Journal of Allergy & Clinical Immunology 1990;86(2):221-30.
17. Howland Wr. Fluticasone propionate: topical or systemic effects? Clin Exp Allergy 1996;3:18-22.
18. Wolthers OD, Pedersen S. Short-term growth in children with allergic rhinitis treated with oral antihistamine, depot and intranasal glucocorticosteroids [see comments]. Acta Paediatr 1993;82(8):635-40.
19. Wolthers OD, Pedersen S. Knemometric assessment of systemic activity of once daily intranasal dry-powder budesonide in children. Allergy 1994;49(2):96-9.

20. Agertoft L, Pedersen S. Short-term knemometry and urine cortisol excretion in children treated with fluticasone propionate and budesonide: a dose response study. Eur Respir J 1997;10(7):1507-12.
21. Richards DH, Milton CM. Fluticasone propionate aqueous nasal spray: a well-tolerated and effective treatment for children with perennial rhinitis. Pediatr Allergy Immunol 1996;7(1):35-43.
22. Mygind N. Glucocorticosteroids and rhinitis. [Review] [192 refs]. Allergy 1993;48(7):476-90.
23. Mygind N, Dahl R, Pedersen S, Thestrup-Pedersen K. Rhinitis. In: Mygind N, Dahl R, Pedersen S, Thestrup-Pedersen K, editors. Essential Allergy. Oxford: Blackwell Science; 1996. p. 195-251.
24. in 't Veld C, Garrelds IM. Nasal hyperreactivity and inflammation in perennial allergic rhinitis . Rotterdam: Erasmus University; 1995.
25. van Essen-Zandvliet EE, Hughes MD, Waalkens HJ, Duiverman EJ, Kerrebijn KF. Remission of childhood asthma after long-term treatment with an inhaled corticosteroid (budesonide): can it be achieved? Dutch CNSLD Study Group. Eur Respir J 1994;7(1):63-8.
26. Bousquet J, Chanal I, Alquie MC, Charpin D, Didier A, Germouty J, et al. Prevention of pollen rhinitis symptoms: comparison of fluticasone propionate aqueous nasal spray and disodium cromoglycate aqueous nasal spray. A multicenter, double-blind, double-dummy, parallel-group study. Allergy 1993;48(5):327-33.
27. Andersson M, Andersson P, Pipkorn U. Topical glucocorticosteroids and allergen-induced increase in nasal reactivity: relationship between treatment time and inhibitory effect. J Allergy Clin Immunol 1988;82:1019-1026.
28. Bascom R, Wachs M, Naclerio RM, Pipkorn U, Galli SJ, Lichtenstein LM. Basophil influx occurs after nasal antigen challenge: effects of topical corticosteroid pretreatment. J Allergy Clin Immunol 1988;81(3):580-9.
29. Juliusson S, Aldenborg F, Enerback L. Proteinase content of mast cells of nasal mucosa; effects of natural allergen exposure and of local corticosteroid treatment. Allergy 1995;50(1):15-22.
30. Gronborg H, Bisgaard H, Romeling F, Mygind N. Early and late nasal symptom response to allergen challenge. The effect of pretreatment with a glucocorticosteroid spray. Allergy 1993;48(2):87-93.
31. Bradding P, Feather IH, Wilson S, Holgate ST, Howarth PH. Cytokine immunoreactivity in seasonal rhinitis: regulation by a topical corticosteroid. Am J Respir Crit Care Med 1995;151(6):1900-6.
32. Godthelp T, Holm AF, Blom H, Klein-Jan A, Rijntjes E, Fokkens WJ. The effect of fluticasone propionate aqueous nasal spray on nasal mucosal inflammation in perennial allergic rhinitis. Allergy 1995;50(23 Suppl):21-4.
33. Godthelp T, Holm AF, Blom HM, Fokkens WJ. A double-blind comparison of two different dosages of Fluticasone Propionate Aqueous Nasal Spray in the treatment of patients with perennial allergic rhinitis - a biopsy study. Allergologie 1996;19:42.
34. Juliusson S, Bachert C, Klementsson H, Karlsson G, Pipkorn U. Macrophages on the nasal mucosal surface in provoked and naturally occurring allergic rhinitis. Acta Oto Laryngologica 1991;111(5):946-53.

35. Bisgaard H, Gronborg H, Mygind N, Dahl R, Lindquist N, Venge P. Allergen-induced increase of eosinophil cationic protein in nasal lavage fluid: effect of the glucocorticoid budesonide. J Allergy Clin Immunol 1990;85:891-895.

36. Fokkens WJ, Godthelp T, Holm AF, Blom H, Klein-Jan A. Allergic rhinitis and inflammation: the effect of nasal corticosteroid therapy. [Review] [23 refs]. Allergy 1997;52(36 Suppl):29-32.

37. Masuyama K, Jacobson MR, Rak S, Meng Q, Sudderick RM, Kay AB, et al. Topical glucocorticosteroid (fluticasone propionate) inhibits cells expressing cytokine mRNA for interleukin-4 in the nasal mucosa in allergen-induced rhinitis. Immunology 1994;82(2):192-9.

38. Meltzer EO, Orgel HA, Rogenes PR, Field EA. Nasal cytology in patients with allergic rhinitis: effects of intranasal fluticasone propionate. J Allergy Clin Immunol 1994;94(4):708-15.

39. Gomez E, Clague JE, Gatland D, Davies RJ. Effect of topical corticosteroids on seasonally induced increases in nasal mast cells. Br Med J 1988;296(6636):1572-3.

40. Blom H, Godthelp T, Fokkens W, KleiJan A, Mulder P, Rijntjes E. The effect of nasal steroid aqueous spray on nasal complaint scores and cellular infiltrates in the nasal mucosa of patients with nonallergic, noninfectious perennial rhinitis. J Allergy Clin Immunol 1997;100:739-47.

41. Pipkorn U, Proud D, Lichtenstein LM, Kagey-Sobotka A, Norman PS, Naclerio RM. Inhibition of mediator release in allergic rhinitis by pretreatment with topical glucocorticosteroids. N Engl J Med 1987;316(24):1506-10.

42. Montefort S, Feather IH, Wilson SJ, Haskard DO, Lee TH, Holgate ST, et al. The expression of leukocyte-endothelial adhesion molecules is increased in perennial allergic rhinitis. American Journal of Respiratory Cell & Molecular Biology 1992;7(4):393-8.

43. Lee BJ, Naclerio RM, Bochner BS, Taylor RM, Lim MC, Baroody FM. Nasal challenge with allergen upregulates the local expression of vascular endothelial adhesion molecules. J Allergy Clin Immunol 1994;94(6 Pt 1):1006-16.

44. Saito H, Asakura K, Kataura A. Study on the profiles of infiltrating T lymphocytes and ICAM-1 expression in allergic nasal mucosa. Acta Oto Laryngologica 1994;114(3):315-23.

45. Canonica GW, Ciprandi G, Buscaglia S, Pesce G, Bagnasco M. Adhesion molecules of allergic inflammation: recent insights into their functional roles. [Review] [59 refs]. Allergy 1994;49(3):135-41.

46. Steinbrink K, Wolfl M, Jonuleit H, Knop J, Enk AH. Induction of tolerance by IL-10-treated dendritic ells. J Immunol 1997;159(10):4772-80.

47. Knapp HR. Reduced allergen-induced nasal congestion and leukotriene synthesis with an orally active 5-lipoxygenase inhibitor [see comments]. New England Journal of Medicine 1990;323(25):1745-8.

48. Donnelly AL, Glass M, Minkwitz MC, Casale TB. The leukotriene D4-receptor antagonist, ICI 204,219, relieves symptoms of acute seasonal allergic rhinitis. Am J Respir Crit Care Med 1995;151(6):1734-9.

Summary

Topical nasal corticosteroids are frequently used in the treatment of allergic rhinitis. Nasal corticosteroids reduce most of the nasal symptoms caused by aeroallergens. Patients with perennial (allergic) rhinitis sometimes use this medication for a long period of time. The aim of this study was to investigate the effect of nasal corticosteroids on nasal mucosa and on nasal mucosal inflammatory cells of patients with perennial allergic rhinitis during long-term use, but also after artificial allergen provocation with grass-pollen in patients with seasonal allergic rhinitis.

In **chapter 1** allergy is defined, the different forms of rhinitis are described, and epidemiological aspects are discussed. An update of the pathophysiology and cellular aspects of allergic rhinitis is given. In addition, the therapy of allergic rhinitis is discussed, focussing on corticosteroids.

In **chapter 2** the aims of the study are described.

In **chapter 3** the safety and efficacy of Fluticasone Propionate (FPANS) is investigated in a double blind, placebo-controlled, randomised study during a one-year treatment period of patients with perennial allergic rhinitis. FPANS was a well-tolerated, safe treatment for patients with perennial allergic rhinitis with no evidence of deleterious effects on nasal mucosa. The efficacy of FPANS improves after long-term treatment.

In **chapter 4** the effect of 3 months' nasal steroid therapy (FPANS) on nasal T cells and Langerhans cells is investigated in nasal mucosa of patients with perennial allergic rhinitis. FPANS significantly reduced the number of Langerhans cells and HLA-Dr positive cells in nasal epithelium and lamina propria, but not of nasal T cells. These findings suggest that steroid treatment decrease antigen presentation in nasal allergy .

In **chapter 5** the effect of Fluticasone Propionate (FPANS) on nasal mucosal inflammatory cells is investigated in a double blind, placebo-controlled, randomised study during a one-year treatment period of patients with perennial allergic rhinitis. After one year of treatment, a significant decrease was seen in the epithelium in numbers of Langerhans cells, CD3+, CD4+, CD8+ cells, mast cells and eosinophils. In the lamina propria, there was a significant decrease in eosinophils. These findings show that FPANS treatment results in a decrease of nasal inflammatory cells.

In **chapter 6** the effect of six weeks pre-treatment with Fluticasone Propionate on nasal symptoms and inflammatory cell numbers after nasal allergen provocation in patients with seasonal allergic rhinitis was investigated in a double blind, placebo-controlled study.

Nasal mucosa biopsies were taken five times in every patient. A significant reduction in early and late phase symptoms was found in the FPANS treated group compared with the placebo group. After four weeks of treatment but before allergen provocation significantly fewer epithelial Langerhans cells, CD68+ cells, BMK13+ cells, CD3+ cells, CD4+ cells, CD8+ cell, chymase positive cells, and tryptase positive cells were found in the FPANS group compared to the placebo group. In the lamina propria significantly fewer Langerhans cells and BMK13+ cells were found in the FPANS group. Cell influx in nasal mucosa after allergen provocation was significantly inhibited in the FPANS group compared to the placebo group for epithelial Langerhans cells, chymase positive cells, macrophages, CD3+ and CD8+ cells, and lamina propria eosinophils, chymase positive cells, Langerhans cells, macrophages, CD3+ cells, CD8+ cells and CD4+ cells. The efficacy of even a prolonged period with corticosteroid treatment is diminished 24 hours after allergen provocation.

In **chapter 7** the effect of six weeks pre-treatment with Fluticasone Propionate on eosinophils and mRNA positive cells (*in situ* hybridisation for IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IFN-γ, RANTES and TNF-α) after nasal allergen provocation in patients with seasonal allergic rhinitis was investigated in a double blind, placebo-controlled study. Treatment with FPANS out of season resulted in a decrease in eosinophils and mRNA positive cells for IL-5 and IL-6. After allergen provocation, levels of most of the measured cytokines (IL-3, IL-5, IL-6, IL-13, IFN-γ, RANTES and TNF-α) and eosinophils were reduced using corticosteroids. The numbers of cells (eosinophils, IL-3, IL-6 and IL-8) correlated with nasal symptoms. Significant correlations in the early and late allergic phase were found between eosinophils and cytokines (IL-3, IL-4, IL-5, IL8, IL-10 and IL-13). These results indicate that pretreatment with FPANS prior to contact with grass pollen is effective in reducing the increase of cytokine mRNA positive cells in reaction to grass pollen contact.

Samenvatting

Lokale corticosteroïden worden veelvuldig gebruikt voor de behandeling van allergische rhinitis. Nasale steroïden verminderen vrijwel alle neusklachten veroorzaakt door de allergie. Bij patiënten met klachten het hele jaar door worden deze medicamenten soms langdurig gebruikt. Het doel van deze studie was het onderzoeken van het effect van lokale corticosteroïden op neusslijmvlies en op ontstekingscellen in het neusslijmvlies van patiënten met een allergische rhinitis tijdens langdurig gebruik, maar ook tijdens artificiële allergeen provocatie buiten het seizoen bij patiënten met een graspollen allergie.

In **hoofdstuk 1** wordt allergie gedefinieerd, een overzicht gegeven van de verschillende vormen van rhinitis, en de epidemiologie van allergische rhinitis besproken. De huidige kennis betreffende de pathofysiologie en cellulaire aspecten van allergische rhinitis wordt beschreven. Ten slotte wordt ingegaan op de behandeling van allergische rhinitis met nadruk op het werkingsmechanisme van corticosteroïden.

In **hoofdstuk 2** wordt het doel het onderzoek omschreven.

In **hoofdstuk 3** zijn de veiligheidsaspecten en werkzaamheid van een 1 jaar durende behandeling met Fluticasone propionate (FPANS) bij patiënten met een perenniale allergische rhinitis onderzocht in een gerandomiseerde, placebo gecontroleerde studie. Er werden geen systemische bijwerkingen gevonden na 1 jaar behandeling. Ook werden in het neusslijmvlies van deze patiënten geen nadelige invloeden van de behandeling gezien. Er waren geen aanwijzingen voor slijmvliesatrofie of bindweefsel veranderingen. De werkzaamheid van Fluticasone propionate lijkt te verbeteren na langdurige behandeling.

In **hoofdstuk 4** wordt het effect van 3 maanden behandeling met Fluticasone propionate op Langerhans en T cellen in het neusslijmvlies van patiënten met een perenniale allergische rhinitis onderzocht. Het aantal Langerhans cellen en HLA-Dr positieve cellen is na 3 maanden behandeling verminderd in de actief behandelde groep, vergeleken met de placebo behandelde groep. Er is geen significant verschil in T cellen tussen de twee behandelingsgroepen. Deze bevindingen suggereren een verminderde antigeen presentatie als gevolg van steroid behandeling.

In **hoofdstuk 5** wordt het effect van 1 jaar, placebo gecontroleerde, behandeling met Fluticasone propionate onderzocht op diverse ontstekingscellen in het neusslijmvlies van patiënten met een perenniale allergische rhinitis. Het aantal epitheliale Langerhans cellen,

CD3+, CD4+ en CD8+ cellen, mestcellen en eosinofielen in de actief behandelde groep is significant lager na 1 jaar behandeling. In de lamina propria was na 1 jaar actieve behandeling alleen het aantal eosinofielen significant lager.

In **hoofdstuk 6** wordt het effect van 6 weken voorbehandeling met FPANS op klachten en cellulaire aspecten van het neusslijmvlies geanalyseerd. In deze studie werden patiënten met een graspollen allergische rhinitis na 6 weken, placebo gecontroleerde, behandeling geprovoceerd met graspollen. Gedurende de onderzoeksfase werden 5 neusslijmvlies biopten genomen. Na actieve behandeling, maar voor allergeen provocatie, was het aantal epitheliale Langerhans cellen, mestcellen, eosinofielen, macrofagen, en T cellen significant lager dan in de placebo behandelde groep. In de lamina propria was het aantal Langerhans cellen en eosinofielen significant lager. Na allergeen provocatie was er een significante vermindering van influx in het neusslijmvlies van Langerhans cellen, mestcellen, T cellen en macrofagen in de FPANS behandelde groep in vergelijking met de placebo groep. Door de herhaalde neusslijmvlies biopten wordt een beter inzicht verkregen in de cellulaire aspecten. Deze worden besproken.

In **hoofdstuk 7** wordt het effect van 6 weken voorbehandeling met FPANS op mRNA van verschillende cytokines en chemokines in het neusslijmvlies onderzocht met behulp van *in situ* hybridisatie technieken. In deze studie werden patiënten met een graspollen allergische rhinitis na 6 weken, placebo gecontroleerde, behandeling geprovoceerd met graspollen. Gedurende de onderzoeksfase werden 5 neusslijmvlies biopten genomen. Behandeling met FPANS, voorafgaande aan allergeen provocatie, resulteerde in een verlaging van mRNA van interleukine(IL)-5 en IL-6. Na allergeen provocatie was het aantal mRNA positieve cellen voor IL-3, IL-5, IL-13, interferon(IFN)-γ, en RANTES in de FPANS groep significant lager dan in de placebo groep. Er werden significante correlaties gevonden tussen eosinofielen in het neusslijmvlies en mRNA van bijna alle geteste cytokines en chemokines.

Abbreviations

AP	Alkalisch phosphatase
APAAP	Alkalisch phosphatase anti-Alkalisch phosphatase
APC	Antigen Presenting Cell
BU	Biological Units
CD	Cluster of Differentiation
DC	Dendritic Cell
ECP	Eosinophilic Cationic Protein
EDN	Eosinophil Derived Neurotoxin
EM	Electron Microscope
EPO	Eosinophil PerOxidase
FLAP	5-lipoxygenase activating protein
FPANS	Fluticasone Propionate Aqueous Nasal Spray
GM-CSF	Granulocyte Macrophage-Colony Stimulating Factor
GP	General Practitioner
GR	Glucocorticoid Receptor
GRE	Glucocorticoid Response Element
HDM	House Dust Mite
HLA	Human Leukocyte Antigen
HPA	Hypothalamo-pituitary-adrenocorticoal axis
hr	hour
ICAM	InterCellular Adhesion Molecule
IFN-γ	Interferon-gamma
Ig	Immuneglobuline
IL	Interleukine
LC	Langerhans Cell
LPR	Late Phase Response
LTC4	Leukotriene C4
MBP	Major Basic Protein
MC	Mast Cell
MHC	Major Histocompatibility Complex
MIP	Macrophage Inflammatory Protein

NANIPER	Non-allergic, non-infectious perennial rhinitis
NHS	Normal Human Serum
PAF	Platelet-Activating Factor
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PD20	Provocative Dose causing a 20% fall in forced expiratory volume in one second
PGE2	Prostaglandine E2
RAST	Radio-Allergo-Sorbent-Test
TNF-α	Tumor Necrosis Factor –alpha
TxB2	Tromboxane B2
VCAM	Vascular Cellular Adhesion Molecule

Dankwoord

Het in dit proefschrift beschreven onderzoek werd verricht op de KNO-afdelingen van het Leyenburg Ziekenhuis te Den Haag en van het Academisch Ziekenhuis Dijkzigt Rotterdam, en de afdeling Pathologie van het Slotervaart ziekenhuis te Amsterdam.

Zonder de medewerking van veel mensen zou dit proefschrift nooit tot stand zijn gekomen. Hierdoor wil ik dan ook eenieder bedanken die hieraan heeft bijgedragen.

Een aantal personen wil ik echter in het bijzonder noemen.

Professor dr. C.D.A. Verwoerd. Beste Carel, ik wil je bijzonder bedanken voor je steun, geduld en kritische blik. Je hebt er steeds op gewezen hoe belangrijk het afronden van dit onderzoek was en mij de tijd gegund om naast de klinische werkzaamheden het onderzoek in mijn eigen tempo af te ronden.

Dr. W. J. Fokkens. Beste Wytske, jou continue betrokkenheid bij het onderzoek heeft de kwaliteit zonder twijfel verhoogd. De vele discussies met jou waren verhelderend en vaak stimulerend. Ik hoop dat we in de toekomst ook buiten de rhinologie onze samenwerking kunnen voortzetten.

De leden van de promotie commissie, prof. dr. H.J. Hoogsteden, prof. dr. P.R. Saxena en prof. dr. W.J. Mooi, wil ik bedanken voor het lezen en beoordelen van het proefschrift.

Dr. E. Rijntjes. Beste Evert, je inzet en enthousiasme bij het opstarten van het onderzoek in het Ziekenhuis Leyenburg zijn voor mij een grote stimulans geweest. Daarnaast heb je wezenlijk bijgedragen aan mijn klinische vorming tot KNO-arts.

Professor dr. Th. M. Vroom. Beste Thea, jij hebt mij veel geleerd over immunologie en immunohistochemie, hetgeen mij voortdurend van nut is geweest. Daarnaast had je een goede neus voor belangrijke immunologische ontwikkelingen op het gebied van allergische rhinitis.

Dr. P.G.H. Mulder. Beste Paul, hartelijk dank voor de statistische verwerking van de vele data. Ik heb veel van je geleerd, maar zal ook in de toekomst nog een beroep op je (moeten) blijven doen.

Dr. Ewout Baarsma en Cock Hoogerwerf bedank ik voor het enthousiasmeren van hun patiënten voor mijn onderzoek en de mogelijkheid in hun praktijk ervaring op te doen.

Alex KleinJan en Lies-Anne Severijnen wil ik bedanken voor het laboratorium werk dat ze hebben verricht.Hun werkzaamheden waren onontbeerlijk voor het onderzoek.

De medewerkers van het pathologisch laboratorium van het Slotervaart Ziekenhuis in Amsterdam, met name Ger Scholte, Marga Rijken ,Caroline Bierman en Alexander van Leeuwen, wil ik bedanken voor hun hulp bij de immunohistochemische verwerking van vele neusslijmvlies biopten in de beginperiode van mijn onderzoek.

Henk Blom. Beste Henk, is de verwarring nu compleet?

Tom Godthelp bedank ik voor de zeer goede samenwerking in de afgelopen jaren op zowel wetenschappelijk als klinisch terrein. Je specifieke immunologische kennis en je vermogen tot relativeren heb ik zeer gewaardeerd.

Alle patienten die meegewerkt hebben aan dit onderzoek wil ik bedanken. Zonder hun inzet, zelfs in het weekeinde, was het nooit gelukt.

Henriëtte.

Curriculum Vitae

Adriaan Frans Holm werd op 19 maart 1960 te Delfzijl geboren. Het diploma Atheneum B werd in 1978 behaald aan de Rijks Scholen Gemeenschap te Appingedam. Van 1980 tot 1987 studeerde hij Geneeskunde aan de Rijks Universiteit te Groningen. Van 1 juli 1988 tot 1 juni 1990 was hij werkzaam als AGNIO KNO in het Ziekenhuis Leyenburg te Den Haag. Hier werd onder supervisie van dr. E. Rijntjes begonnen met het onderzoek dat uiteindelijk resulteerde in dit proefschrift. Van 1 augustus 1990 tot 1 mei 1995 werd de opleiding tot KNO-arts gevolgd in het Academisch Ziekenhuis te Rotterdam onder leiding van Professor Verwoerd. Sinds 1 mei 1995 is hij als staflid verbonden aan de afdeling KNO-heelkunde van het AZR, met als aandachtsgebied de otologie en de schedelbasischirurgie. Hij is getrouwd met Henriëtte Robaard; zij hebben 4 kinderen: Karin, Marten, en een tweeling Robert en Willemijn.