IMMUNOLOGICAL ASPECTS OF NASAL POLYPS

A.E. STOOP

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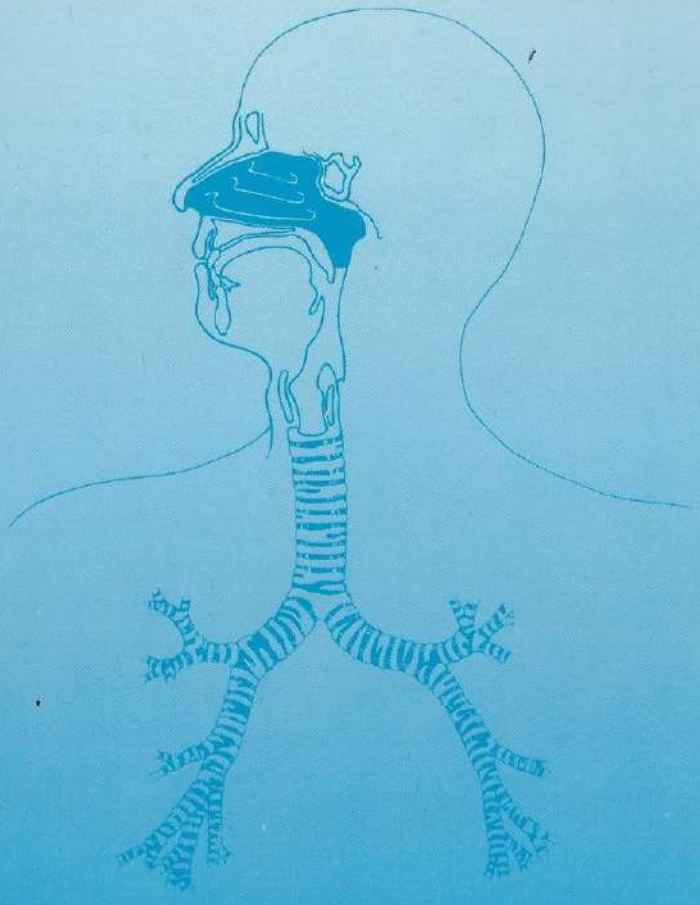

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The investigations described in this thesis were performed at the department of Otorhinolaryngology/ Head and Neck Surgery (Chairmain prof.dr. G.B.Snow), the department of Cell Biology, Devision of Histology (Chairman prof.dr. T. Sminia) and the department of Clinical Chemistry (dr. G.J. van Kamp) of the Free University Hospital in Amserdam, and were sponsered by a grant of the Dutch Asthma Foundation (Nederlands Astma Fonds).

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IMMUNOLOGICAL ASPECTS OF NASAL POLYPS

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Harada T, Hamaguchi Y, Sakakura Y, Miyoshi Y. Circadian variation of secretory IgA in nasal secretions from normal subjects. Acta Otolaryngol. 1984; 97:359-62.

Hobday JD, Cake M, Turner KJ. A comparison of the immunoglobulins IgA, IgG and IgE in nasal secretions from normal and asthmatic children. Clin. exp. Immunol. 1971; 9:577-83.

Holmes MJ, Callow KA, Parry HF. An improved method for recovery of secretory immunoglobulins and lymphocytes fronm the nasal mucosa. J. Immunol. Meth. 1987; 98:183-87.

Holt JJ, Kern EB. A new method of collecting nasal secre tions. Otolaryngol. Head & Neck Surg. 1986; 94:403-04.

Mygind M, Weeke B, Ullman S. Quantitative determination of immunoglobulins in nasal secretion. Int. Archs Allergy appl. Immunol. 1975; 49:99-107.

Mygind M, Thomsen J. Diurnal variation of nasal protein concentration. Acta Otolaryngol. 1976; 82:219-21.

Mygind M, Wihl JA. Concentration of immunoglobulins in nasal secretion from children with recurrent infections in the upper airways. Acta Otolaryngol. 1976; 82:216-18.

Salvaggio J, Lopez M, Arquembourg P, Waldman R, Sly M. Salivary, nasal wash, and sputum IgA concentrations in atopic and nonatopic individuals. J. Allergy Clin. Immunol. 1973; 51:335-43.

Sasaki Y, Araki A, Koga K. The mast cell and eosinophil in nasal secretion. Ann. Allergy 1977; 39:106-09.

Van Kamp GJ, Wolters ECh. CSF-IgM measurement in neurovenereological disease. Clin. Chim. Acta 1989; 183:295-300.

Chapter 6B

IMMUNOGLOBULIN CONCENTRATIONS IN NASAL SECRETIONS OF PATIENTS WITH NASAL POLYPS.

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Referent	:	prof.dr. P. van Cauwenberge

Aan Catherine,
Johan en Caroline,

mijn ouders et mes beaux parents

CONTENTS

"Il est des parfums comme des chairs d'enfants,
Doux comme les hautbois, verts comme les prairies,
- Et d'autres, corrompus, riches et triomphants,
Ayant l'expansion des choses infinies,
Comme l'ambre, le musc, le benjoin et l'encens,
Qui chantent les transport de l'esprit et des sens"

Charles Baudelaire

Chapter 1

INTRODUCTION

THE NOSE

Anatomy

The nose consists of the nostrils and the nasal cavities which are bisected from front to back by the nasal septum (Fig. 1 and 2). The lenght of the internal nose is 10-12 cm from the tip of the nose to the pharyngeal wall. Due to the prominence of the turbinates from the lateral wall, each of the two nasal cavities is a narrow slit, only 2-4 mm wide. The nasal valve (internal ostium), which is the narrowest part of the whole airway, separates the nostril region from the nasal cavities. The complex anatomy, together with the fact that the nose accounts for nearly 50% of the total airway resistence (Proctor 1976), indicates that it is more than a simple conductive airway.

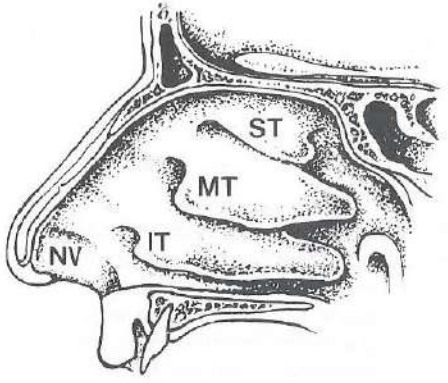

Fig. 1.
Lateral wall of the nasal cavity. NV is the nasal vestibule. The openings of the paranasal sinuses are under the middle (MT) and superior (ST) turbinates. IT is inferior turbinate.

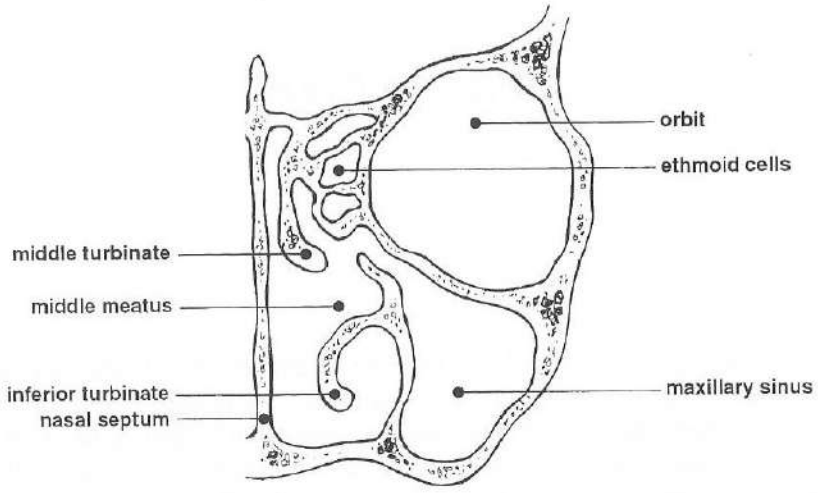

Fig. 2.
Coronal section through the nasal cavities at the level of the anterior ethmoid.

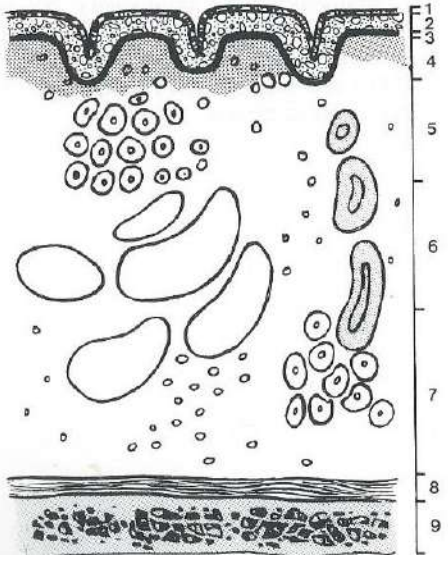

Fig. 3.
The nasal mucosa and its different layers: 1, mucus layer; 2, ciliated pseudostratified columnar epithelium; 3, basement membrane; 4-7 lamina propria with lymphoid and nonlymphoid cells, mucous glands, cavernous sinusoids; 8, 9, periosteum and bone/ cartilage.

The nasal mucosa

The nasal mucosa consists of epithelium usually covered with mucus, the basement membrane and the lamina propria. The basement membrane separates the epithelium from the lamina propria (Fig. 3). The vestibulum of the nose and the first millimeters of the nasal cavity are lined with stratified squamous epithelium. The rest of the nasal cavity is covered mainly with ciliated columnar epithelium and/ or partially stratified cuboidal epithelium (Boysen 1982).

The nasal respiratory epithelium consists of ciliated- and non-ciliated epithelial cells, basal cells and goblet cells. Moreover, migratory cells like lymphocytes, eosinophils, neutrophils, mast cells and HLA-DR+ cells (Langerhans cells and dendritic cells) can be detected between the epithelial cells.

The lamina propria is part of the nasal mucosa situated between the basement membrane and the underlying supportive tissue (cartilage or bone). Normally the lamina propria is composed of a cell-rich, collagen- poor, subepithelial layer in wich most of the mucus glands are found. The deeper layer, which is resting on the skeleton, is cell poor and collagen rich. In the lamina propria lymphocytes, plasma cells, eosinophils, mast cells, neutrophils, Langerhans cells and dendritic cells are found.

Functional aspects

The anatomy of the nose is important for its functions: warming up, humidification and filtration of inhaled air, in order to protect the delicate structures of the lower respiratory tract. Another function of the nose is the sense of smell which is vital in many animals and in humans significant for enjoyment of eating and drinking. The inability to breath through the nose is uncomfortable especially in subjects with asthma and hyperreactive airways during exposure to environmental challenges. Maintenance of nasal breathing is important, because it can partially prevent exercise-induced bronchoconstriction (Griffen et al. 1982).

Relation between upper- and lower respiratory tract

Because the upper- and lower respiratory tract is a continuum, it is possible that nasal pathology, or alterations in nasal physiology, could adversely influence lower airway performance. In situations where coexisting pathogologies exist in the nose and intrathoracic airways, deterioration in one organ may result in a decrease of function in the other. These considerations gave rise to the

hypothesis that nasal disease can be involved in the pathogenesis of lower airway disease, like asthma. Indeed, an association of nasal and paranasal sinus diseases with bronchial asthma has been described (Slavin 1982, Rachelefsky et al. 1984, Mings et al. 1988). However, the mechanisms involved (parasympathetic reflex?, blockade of β adrenoreceptors?) are still elusive.

Hosemann et al. (1990) found an improvement in the lungfunction and/ or in the use of less lungmedication in 77% of the patients with asthma who underwent endoscopic sinus surgery for chronic sinusitis. Slavin et al. (1983) described a subjective improvement of the lungperformance in 66% of patients with asthma after removal of nasal polyps, but this was not confirmed with objective lungfunction tests. To obtain more precise data, concerning the relationship between the upper and lower respiratoty tract, we compared clinical data and lungfunction tests in patients with nasal polyps before and after treatment with surgery and topical steroids (budesonide, 400 µg daily).

NASAL POLYPS

General aspects

Nasal polyps are glinstening, pale gray, smooth, soft, semitranslucent and freely movable tumors, attached by a pedicle. They arise from the nasal mucosa of the middle turbinates and the ostia of the ethmoid and maxillary sinusus (Larssen & Tos 1991). They seldom arise from the nasal septum or the inferior turbinates. There is no explanation for this striking difference in localisation. The polyps can extend to the nasal cavity filling up the nose, which causes nasal obstruction, reduction of smell and chronic sinusitis.

Multiple nasal polyps are frequently found and they occur most often bilaterally. In this thesis we only examined bilateral benign nasal polyps. When occurring unilaterally, other tumors, like inverted papilloma, have to be excluded by histological examination.

Incidence

The overall frequency of nasal polyps is 4.2% (Settipane & Chafee 1977). The incidence of nasal polyposis increases with age. They are most frequently found in patients in their third or fourth decade. The ratio man : woman = 3:1 (Friedman & Osborn 1982). The incidence of nasal polyps in children is extremely low, about 0.1% (Lanoff et al. 1973, Settipane & Chafee 1977). In children with cystic fibrosis the incidence is 6.7% (Settipane & Chafee 1977) to 29% (Schramm & Effron 1980), whereas in asthmatic children the incidence is 1.8% (Settipane & Chafee 1977). The frequency of nasal polyps in adult

asthmatics (10-50 year) is 7%. In patients with aspirin intolerance, Chafee & Settipane (1974) found nasal polyps in 36%, whereas other authors reported a frequency of 60-100% (Ogino & Harada 1986, Caplin et al. 1971, Spector et al. 1979).

Etiology

Historically the existence of nasal polyps dates as far as 1000 B.C. (Vancil 1969). Despite much research through the centuries the etiology of nasal polyps is still unknown.

Association with systemic diseases

In the past much attention has been payed to **IgE-mediated allergy** as a causal factor for nasal polyps. Bunnag et al. (1983) suggested that allergy is a constant feature in nasal polyposis, because of the occurrence of positive skin tests in combination with elevated allergen-specific IgE in nasal polyp fluid. However, Perkins et al. (1989) found no evidence for a cause-and-effect relationship between nasal polyps and an IgE-mediated allergy by determination of allergen-specific IgE in nasal secretions and nasal polyps, skin tests, serum RAST and clinical history of allergy. Moreover, in patients with a proved allergy (skintests/ RAST) the incidence of nasal polyps is low (0,5% reported by Caplin et al. 1971, 3% by Sakaguchi et al. 1986) and nasal polyps occur significantly more frequent in patients with asthma and negative skin tests than in patients with asthma and positive skin tests (12,5% versus 5,0%, Settipane & Chafee 1977). Furthermore, nasal polyps are rare in children and adolescents with atopic dermatitis, hay fever and allergic asthma. Finally, Delaney (1976) noted that the prevalence of positive skin tests in patients with nasal polyps is similar to the prevalence in patients with the same respiratory complaints without nasal polyps. Although it can be concluded that an IgE mediated allergy is not a causal factor for nasal polyps, patients with an IgE-mediaded allergy have a significantly higher recurrence rate of nasal polyps after endoscopic surgical removal than patients without an IgE-mediated allergy (50% versus 25% respectively, Vleming et al. 1991).

Asthma, **aspirin intolerance** and nasal polyps form the triad of aspirin induced asthma (AIA). Aspirin or aspirin-like anti-inflammatory drugs inhibit the conversion of arachidonic acid into prostaglandines and divert more arachidonic acid into the lipoxygenase pathway, causing an increase of 15-HETE and leukotriene synthesis (Jung et al. 1987, Fig. 4). In inflammatory reactions of the respiratory tract these mediators modulate the vascular permeability, chemotaxis of inflammatory cells and airway mucus secretion (Samuelsson 1983). These processes stimulate inflammatory reactions and may explain the

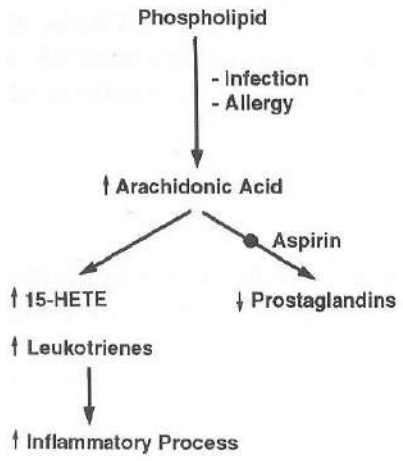

Fig. 4.
Possible role of the arachidonic acid metabolites in the inflammatory process that may cause nasal polyps in patients with an aspirin intolerance (15 HETE= hydroxyeicosatetraenoic acid, Jung et al. 1987).

high incidence of nasal polyps among patients with an aspirin intolerance (36%, Settipane 1987).

Patients with **cystic fibrosis** also have a high incidence of nasal polyps (20%, Settipane 1987). This disease is characterized by generalized dysfunction of all exocrine glands, including those which secrete mucus. The abnormal mucus secretion results in a reduction of the mucociliary clearance and causes chronic infections in the respiratory tract which may lead to the formation of nasal polyps. Also **primary ciliary dyskinesia** is associated with the occurrence of nasal polyps. In this disease the impaired movements of the cilia give a reduction of mucociliary clearance in the respiratory tract (van der Baan 1985) which evokes chronic infections.

Local factors

Although the aforementioned systemic diseases cause similar inflammatory reactions in the mucosa of the upper and lower respiratory tract, this leads to the formation of nasal polyps in the mucosa of the ethmoid region and the middle turbinates only. Apparently there are local factors which contribute to the growth of nasal polyps at specific sites in the nasal mucosa. It is not clear wich factors are of importance: aerodynamic conditions, hemodynamic factors, frequent contact of the mucosa with infected material or changes in local immunity. Because little is known about the formation of nasal polyps with respect to the distribution of immunologically active cells in the nasal mucosa and nasal polyps, we focussed on these aspects in this thesis.

Pathogenesis

The formation of nasal polyps has been referred to as adenomatosis (Billroth 1855), as inflammatory hyperplasia of the mucosa (Zuckerkandl 1882), or a myxomatous degeneration c.q. fibromatosis. According to Hajek (1896) the formation of polyps starts with submucosal edema and with an increase of glandular contents , while Yonge (1907) ascribed the formation of nasal polyps to cystic degeneration of glandular ducts causing an obstruction of blood vessels of the nasal mucosa followed by edema. Krajina (1963) believed that inflammatory infiltrations of the nasal mucosa during chronic infections and allergic episodes, combined with a localized increase of nasal glands, both causing bulging of the mucosa, are main factors in the pathogenesis of nasal polyps. According to the theory of Tos & Mogensen (1977) the reduced air flow through the upper part of an allergic and chronically infected nose is the main etiological factor of nasal polyps. Infiltration and edema of the nasal mucosa result in the rupture of the epithelium followed by the appearence of granulation tissue which gradually becomes lined with pseudostratified columnar epithelium (Fig. 5).

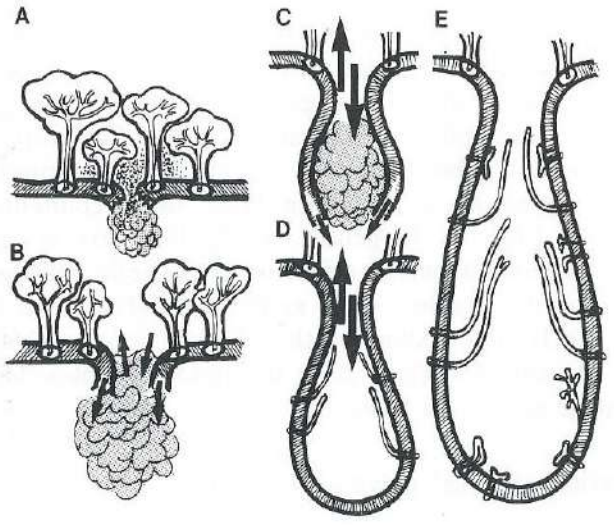

Fig. 5.
Schematic presentation on polyp formation: A, infiltrate and oedema of the nasal mucosa, rupture of the epithelium and formation of granulation; B and C, epithelisation of the granulation tissue and formation of the vascular stalk (VS); D, formation of glands; E, passive elongation of the long tubular glands (Tos & Mogensen 1977).

HISTOLOGY OF NASAL POLYPS

General features

The stroma of nasal polyps is very edematous and is infiltrated with variable numbers of inflammatory cells. Mucous glands in nasal polyps are few and denervated (Tos & Mogensen 1977, Cauna et al. 1972). Bende & Flisberg (1985) showed that the mean blood flow in polyps is only 38% of the normal mucosal blood flow.

Kakoi & Hiraida (1987) classified nasal polyps histologically into three types: (1) edematous type (60 %) in which the interstitium is composed mainly of edematous tissue which contains some mucous glands without cyst formation, (2) glandular and cystic type (27 %) in which mucous glands and/ or cystic formations are the most prominent structures, (3) fibrous type (13 %) in which proliferation of fibroblasts and collagen fibers are most prominent.

Epithelium

The surface epithelium of nasal polyps is usually of the respiratory type and consists of ciliated columnar epithelium and goblet cells; occasionally squamous metaplasia occurs (Drake-Lee et al. 1984). Epithelial damage is commonly seen in nasal polyps (Wladislavosky-Wasserman et al. 1982). In larger nasal polyps the epithelium consists of hypertrophic cylindrical cells with a marked intercellular distention, and it is characterized by total loss of cilia. These changes are considered to be a result of rupture of the original epithelium and subsequent formation of a new, one layer thick epithelium to line the granulation tissue (Tos & Mogensen 1977, Paludetti et al. 1983). Since compressive forces can act on the epithelium, its cells may become flattened and loose their specific features, such as the cilia. (Friedman & Osborn 1982, Paludetti et al. 1983). The extent of the damage is variable, ranging from relatively little damage (less than 20%) to almost complete loss of functional epithelium (damage more than 85%).

Cellular infiltration of nasal polyps

The edematous stroma of nasal polyps contains inflammatory cells like HLA-DR+ cells, lymphocytes, eosinophilic granulocytes, mast cells and neutrophilic granulocytes. The action and interaction of these cells and their mediators, which may sustain the inflammatory process, is very complex and only partially understood. The most important cells in nasal polyps will be discussed.

HLA-DR+ cells

HLA-DR molecules can be found on epithelial cells (Selby et al. 1983, Lindhahl et al. 1985), dendritic and Langerhans cells (Fokkens et al. 1989), macrophages (Hirschberg et al. 1976) and on activated T cells (Metzgar et al. 1979). HLA-DR+ dedritic cells, Langerhans cells and macrophages play a role in processing, transport and presentation of antigens (Brandtzaeg 1984, Fokkens et al. 1989). HLA-DR+ epithelial cells are also considered as antigen presenting cells (Brandtzaeg 1984). Chronic inflammatory diseases in the mucosa of the upper respiratory tract seem to be correlated with an increased expression of HLA-DR, since HLA-DR expression on glandular epithelial cells in nasal mucosa of patients with nasal complaints occurs almost twice as often as in the nasal mucosa of healthy controls (Hameleers et al. 1990).

Lymphocytes

T lymphocytes

Most studies on lymphocytes in the upper respiratory tract concern the tonsils (Brandtzaeg 1984). Only Winther et al. (1987) described the T lymphocyte distribution in the inferior turbinates of normal human nasal mucosa. In the maxillary mucosa of patients with chronic sinusitis infiltration of lymphocytes is one of the characteristic findings (Nishimoto et al. 1988).

T lymphocytes and their subsets are regulatory and effector cells in the inflammatory response (Ernst et al. 1987). T cell factors (interleukin 3, interleukin 5 and GM-CSF) initiate and support the proliferation, differentiation and activation of eosinophils (Miller & McGarry 1976, Vadas et al. 1983, Enokihara et al. 1985, Sanderson et al. 1985, Metcalf et al. 1986, Campbell et al. 1987, Owen et al. 1987, Fujisawa et al. 1989). Moreover, interleukin 3, 4 and 5 are important growth and activation factors for mast cells and basophilic granulocytes (Ihle et al. 1983, Hamaguchi et al. 1987, Miyajima et al. 1988). Finally, interleukin 4 and 5 enhance the synthesis of IgE by B lymphocytes (Defrance et al. 1987), whereas this effect is inhibited by INF-γ which is also produced by T lymphocytes (Coffman & Carty 1986, Romagnani et al 1989).

CD4+ (helper/ inducer) T cells and CD8+ (suppressor/ cytotoxic) T cells are two lymphocyte subsets which are considered to have a helper and suppressor function respectively, but the precise role of CD4+ and CD8+ cells is still a matter of controversy (Takada & Engleman 1987, Schrezenmeier & Fleischer 1988). CD4+ T cells may exercise a cytotoxic function (Rotteveel et al. 1988) and CD8+ T cells with cytotoxic or contrasuppressor activity have been described (Green et al. 1982, Lehner et al. 1985, Lee et al. 1988).

Changes in ratios of CD4+:CD8+ cells are possibly related to inflammatory processes in the respiratory mucosa. In the nasal mucosa of the inferior turbinates of healthy controls Winther et al. (1987) found more Anti-Leu-3a+ (helper/ inducer) cells than Anti-Leu-2a+ (suppressor/ cytotoxic) cells, whereas in inflamed maxillary sinus mucosa more CD8+ (suppressor/ cytotoxic) than CD4+ (helper/ inducer) cells are found (Nishimoto et al. 1988). Moreover, Gonzales et al. (1987) demonstrated an increase of CD8+ cells and a decrease of CD4+ cells in broncho-alveolar-lavages (BAL) of asthmatic patients after allergen provocation. A selective mobilisation of CD8+ cells into the respiratory mucosa of these patients is suggested.

Because nasal polyps are probably the result of chronic inflammation and T lymphocytes play an important regulatory role in (local) inflammatory processes we studied the local distribution of T cell subsets in the nasal mucosa and polyps of patients with nasal polyposis.

B lymphocytes and plasma cells

T lymphocytes and macrophages, Langerhans cells and dendritic cells which process and present antigens, may stimulate B lymphocytes. These activated B cells proliferate and differentiate into antigen specific memory cells and plasmablasts and further develop into mature plasma cells which produce immunoglobulins.

Kakoi et al. (1987) found plasma cells in 91% of the edematous type polyps, in 94% of the glandular and cystic type polyps and in 64% of the fibrous type polyps. Especially in the edematous- and the glandular/ cystic type polyps, IgG+, IgA+, and IgE+ plasma cells were frequently observed in the loose stroma immediately beneath the mucosal epithelium and around the glands. Takasaka et al. (1986) found IgE+ plasma cells in polyps of patients with an IgE mediated allergy and patients with an aspirin-intolerance, but very few in polyps of chronically infected patients; IgA+ plasma cells were found in polyps of patients with an IgE-mediated allergy, an aspirine-intolerance and in chronic infection; IgG plasma cells in half of the polyps in IgE-mediated allergy and chronic infections, but not in aspirine-intolerance. These authors suggest that IgA- and IgG producing plasma cells in polyps play a role in mast cell degranulation, probably mediated by an immune-complex mediated mechanism.

Small et al. (1985) demonstrated IgE+ plasma cells in 73% of all polyps, whereas Whiteside et al. (1975) found IgE producing plasma cells only in nasal polyps of patients with an IgE-mediated hypersensitivity. The latter authors concluded that these polyps probably had a different etiology from polyps of non-allergic persons.

Eosinophilic granulocytes

In 65,2% (Ogawa et al. 1986) to more than 90% (Friedman & Osborn 1982) of nasal polyps infiltration of eosinophilic granulocytes is found. They are usually localized around the vessels and/or glands and immediately beneath the basement membrane. Kakoi et al. (1987) observed eosinophils in 73% of the edematous type polyps, in 52% of the glandular and cystic type and in 13% of the fibrous type. In patients with aspirin intolerance nasal polyps contain many eosinophilic cells, together with a striking eosinophilia in the peripheral blood (Ogino et al. 1986). In nasal polyps of patients with cystic fibrosis (CF) eosinophils may be present, although fewer than in polyps of non-CF patients (Sörensen et al. 1977).

Because the infiltration of eosinophils is a striking feature in many nasal polyps it is likely that these cells play a role in the pathogenesis of nasal polyps. This is stressed by the fact that patients with eosinophilic non-allergic rhinitis (ENR) are characterized by their high prevalence of nasal polyps (Settipane & Chafee 1977, Mullarky 1988). Furthermore, in patients with recurrent polyposis, Krajina et al. (1987) found an eosinophilic infiltration in 74% of the polyps, in contrast to non-recurrent nasal polyps in which eosinophils were found in only 44%.

In the past eosinophilia in tissues has been considered as an indication for an IgE-mediated allergy (Baumgarten et al. 1980). However, in only 25-30% of patients with eosinophil-infiltrated nasal polyps an IgE-mediated rhinopathy could be demonstrated by medical history, skin tests and/ or RAST (Mygind et al. 1975, John & Merret 1979, Ogawa et al. 1986). Moreover, eosinophils possess, apart from low-affinity receptors for IgE, also receptors for IgG, IgA , IgM, complement C3 and platelet activating factor (PAF) (Capron et al. 1984, 1985, 1989, Dent et al. 1989, Fukuda et al. 1987, Ukena et al. 1989). This is an indication that eosinophils can be stimulated in the absence of IgE. The aforementioned data argue against an IgE-mediated allergy as characteristic for eosinophilic infiltration in nasal polyps.

Because eosinophils probably play an important role in the development of nasal polyps we will discuss these cells in detail.

Eosinophil differentiation and migrating factors

The migration and infiltration of eosinophils is under control of chemotactic substances such as platelet activating factor (PAF), eosinophilic chemotactic factor (ECF), leukotriene B_4 (LTB_4), histamine, hydroxyeicosatetraeonic-acid (15 HETE) and complement C5 secreted by mast cells, T lymphocytes, macrophages and eosinophils (Ogawa et al. 1981, Kroegel et al. 1981, Arnoux et al. 1982, Nagy et al. 1982, Sigal et al. 1987, Tamura et al. 1987, Venge et al. 1987,

Wardlaw & Kay 1987, Rotenberg et al. 1988, Wayoff & Moneret 1988). These factors contribute to the accumulation of eosinophils in nasal polyps. Moreover, Ogawa et al. (1981) demonstrated that eosinophils can generate from complement C5a a chemotactic product which selectively attracts eosinophils. In vitro, PAF and C5a probably have the strongest chemotactic activity on eosinophils (Tumaru et al. 1987, Nagy et al. 1982, Sigal et al. 1987, Wardlaw et al. 1986). Furthermore, at higher concentrations LTB_4 and PAF induce mediator release of eosinophils (Lewis et al. 1981, Wardlaw et al. 1986, Bruijnzeel et al. 1986).

Inflammatory products of eosinophils

When stimulated, eosinophils produce inflammatory products, like platelet-activating factor (PAF), leukotriene-C4 (LTC_4) and oxygen-derived radicals (Venge et al. 1987). LTC_4 possesses potent vasoconstricting and bronchospastic activity (Weiss et al. 1982, Soter et al. 1983). PAF increases vascular permeability, causes further influx of eosinophils (Denjean et al. 1984) and augments the adherence of eosinophils on endothelial cells. Moreover, PAF stimulates the migration of eosinophils through the respiratory epithelium (Capron et al. 1984, 1988, Little & Casale 1990, Wardlaw et al. 1988) and may transform eosinophils into an activated stage by an increased release of LTC_4, superoxide and major basic protein (MBP) from human eosinophils (Shaw et al. 1985, Henocq 1988, Bruijnzeel et al. 1989). It is demonstrated that eosinophils from asthmatic patients have increased ability to release LTC_4 (Taniguchi et al. 1985, Schauer et al. 1989), indicating that eosinophils from these patients are probably activated.

The **cytoplasmic granules** in eosinophils contain toxic proteins like major basic protein (MBP) (Gleich et al. 1976), eosinophil cationic protein (ECP) (Olsson et al. 1977) and eosinophil peroxidase (EPO) (Egensten et al. 1986). When released, these (preformed) products cause an inflammatory process in the respiratory mucosa, with exfoliation of epithelial cells and impairment of ciliary function of mammalian and human respiratory epithelium (Frigas et al. 1980, Hastie et al. 1987, Gleich et al. 1988, Harlin et al. 1988, Motojima et al. 1987, 1989, Hisamatsu et al. 1990).

MBP is quantitatively predominant (more than 50%) in the granules and is revealed by electron microscopy as a crystalloid core (Weller 1984). Harlin et al. (1988) found a striking association between the presence of extracellular depositions of MBP and damage of the sinus mucosa. Furthermore, Hisamatsu et al. (1990) demonstrated that the nasal mucosa of allergic patients is damaged by concentrations of MBP that have no effect on mucosal specimens from normal subjects.

Besides exfoliation of epithelial cells and impairment of ciliary function, ECP might exert proliferatory effects on lymphocytes because an enhancement of suppressor cell activity was found after stimulation of lymphocytes with ECP (Peterson et al. 1986).

Extracellular release of EPO following allergen provocation was described by Watanabe et al. (1977). EPO (supplemented by H_2O_2 and halide) induces mast cell degranulation and histamine release (Henderson et al. 1980a). Moreover, EPO binds to mast cell granules and the EPO-mast cell granule complex catalyses iodination of proteins and killing of microorganisms (Henderson et al. 1980b).

Normo- and hypodense eosinophils

Eosinophils exist in two forms: normodense and hypodense. Electron microscopic studies on hypodense eosinophils are contradictory. Some authors suggest that hypodensity is the result of degranulation and vacuolisation (Prin et al. 1983, Masuyama et al. 1988), whereas other authors explain the hypodensity as an increase in number of the smaller granules, which suggest formation of new granules (Henderson et al. 1985, Tai et al. 1985, Kauffman et al. 1987, Peters et al. 1988, Shult et al. 1988, Kloprogge et al. 1989).

Electron microscopic studies of eosinophils obtained from nasal secretions of patients with allergic rhinitis showed that these eosinophils were hypodense in contrast to blood eosinophils (Masuyama et al. 1988). Hypodense eosinophils are probably metabolically and functionally distinct from normodense eosinophils. Ogasawara et al. (1988) demonstrated that hypodense eosinophils in nasal secretions from patients have an increased oxidative metabolism which may imply an active stage of these eosinophils. Compared to normodense eosinophils, hypodense eosinophils express more receptors for IgE ($Fc\varepsilon RII$ with low affinity, IgG ($Fc\gamma RII$) and complement (Winqvist et al. 1982, Capron et al. 1984, Walsh et al. 1989) and produce larger amounts of EPO, LTC_4 and PAF (Tai & Spry 1981, Lee et al. 1982, Jouvin et al. 1984, Kajita et al. 1985, Khalife et al. 1986, Fitzharris et al. 1987, Kauffman et al. 1987).

Because hypodensity and activation stage of eosinophils in nasal polyps and nasal mucosa may provide information as to the development of nasal polyps, we further studied these aspects.

Mast cells

The role of mast cells in the pathogenesis of nasal polyps is not entirely clear, but degranulation suggesting an ongoing release of mediators has been demonstrated (Drake-Lee et al. 1984, Takasaka et al. 1986). Mast cells produce histamine, chemotactic factors such as mastcell derived eosinophilic chemotactic factor of anaphylaxis (ECF-A), cell activating factors i.e. arachidonic acid

metabolites (Prostaglandine D_2, Leukotriene B_4, Leukotriene C_4) and Platelet Activating Factor (PAF) (Holgate et al. 1988, Galli & Lichtenstein 1988). Prostaglandine D2 is a potent vasodilatator and increases vascular permeability (Beasly et al. 1988).

In patients with an IgE-mediated rhinitis mast cells migrate into the surface epithelium of the nasal mucosa where they degranulate (Enerback et al. 1986). However, in patients with nasal polyps (degranulated) mast cells are predominantly found in the pedicle and deeper stroma of the polyps and in the submucosa (Samter et al. 1961, Cauna et al. 1972, Bumsted et al. 1977, Sasaki et al. 1986). If inhaled allergens react with specific IgE antibodies on the mast cells within nasal polyps, more degranulation can be expected of the mast cells in the superficial tissue than in the deep stroma. Ruhno et al. (1990) demonstrated that the number of mast cells in the epithelium of nasal polyps and the adjacent mucosa was elevated compared to normal nasal epithelium but the increased number did not depend upon an IgE-mediated allergy. These data suggest that there are other mechanisms of mast cell activation besides IgE-mediated allergy.

The consequence of mast cell degranulation is initiation of a mast cell-granulocyte-cytokine-cascade, in which mast cell derived cytokines contribute to an influx of eosinophils which in turn provide additional cytokine activities in the progression of the inflammatory process and, thereby, to the possible development of nasal polyps.

Neutrophils

Neutrophilic leukocytes are found in small numbers in nasal polyps. Because neutrophils are especially associated with acute inflammatory processes and nasal polyps are more associated with chronic inflammation, we will not further discuss these cells.

NASAL SECRETIONS

General aspects

The nasal secretions form a viscous layer, overlying the respiratory mucosa, in which antigens can be trapped.

The secretory layer lining the mucosal surfaces in the respiratory tract contains proteins such as lactoferrin which inhibit bacterial growth and sIgA (secretory immunoglobulin A) which protects the respiratory mucosa against invasion of microorganisms by aggregation of microorganisms (Arnold et al. 1976). Antigens which are trapped in the mucous blanket are cleared by the mucociliary transport system. The aforementioned mechanisms form the *"first line of defense"* in the respiratory mucosa.

When the antigen load on the mucous membrane is heavy and persistent, or when the "first line of defense" is weak (e.g. cystic fibrosis, primary ciliary dyskinesia, IgA-deficiency), an influx of foreign material may occur. Underneath the epithelium IgA- but also IgG- producing cells are found (Nakashima & Hamashima 1980, Brandtzaeg 1985, Brandtzaeg & Bjerke 1989). The local production of IgA and IgG in combination with exudation of serum IgG and complement factors form the *"second line of defense"* (Korsrud & Brandtzaeg 1983).

Immunoglobulins in nasal polyps and nasal secretions

Immune and inflammatory reactions in the upper respiratory tract are reflected in the presence of inflammatory cells in the nasal mucosa as well as in protein and immunoglobulin composition of polyp fluid and nasal secretions.

In polyp fluid the concentrations of IgE, IgA, IgG and IgM are higher than expected from passive filtration only, suggesting local production of these immunoglobulins (Chandra & Abrol 1974, Waller et al. 1976).

Local production of IgE in the nasal mucosa and nasal polyps has been suggested by several authors. Pulido et al. (1983) demonstrated an increase of IgE in nasal secretions from subjects with bilateral polyps as compared to healthy subjects. In the fluid of nasal polyps and in nasal secretions of patients with nasal polyps a positive RAST was found, whereas these patients had negative skin tests and a negative IgE serum RAST (Small et al. 1985, Jones et al. 1987). Although these data suggest local production of IgE in the nasal mucosa and in nasal polyps, other authors demonstrated that mast cells can carry IgE into the nasal secretion by accumulation of IgE antibodies on their surfaces and migration into the epithelium in grass pollen allergic patients within the pollen season (Enerback et al. 1986, Ganzer & Bachert 1988). They concluded that there

was no local IgE production in the nasal mucosa of these patients.

In nasal secretions of healthy individuals sIgA is the major immunoglobulin. It accounts for 50% of the total protein content and is synthetized locally by plasma cells around the seromucous glands (Johansson & Deuschl 1976, Brandtzaeg 1983). IgA is linked with the secretory component (SC) on the membrane of the glandular epithelial cells and sIgA is then actively transported through the epithelium into the nasal secretions. Nakashima et al. (1980) found that the sIgA secretory activity of glands in nasal mucosa is well retained in severe inflammation, whereas it is impaired in dilated glandular ductules within nasal polyps.

The extremely high IgG and IgM concentrations in nasal secretions of patients with nasal polyps are probably due to leakage of plasma proteins from nasal polyp tissue (Donovan & Johansson 1970, Mygind et al. 1975, Biewenga et al. 1991). Significant correlations between the concentrations of albumin and IgA, and of albumin and IgG in nasal secretions of patients with chronic sinusitis were found by Hamaguchi et al. (1982). These correlations may reflect an increased transudation of serum protein at the inflammatory site.

Although in several studies high concentrations of immunoglobulins are found in nasal secretions of patients with nasal polyps, it is difficult to compare these studies because of differences in methods of collection and parameters analyzed.

TREATMENT OF NASAL POLYPS

General aspects

Nasal polyps are principally treated by surgery. However, the recurrence rate after this type of treatment is high. Corticosteroids are regarded to to postpone c.q. prevent recurrences of nasal polyps after surgical treatment (Virolianen & Puhakka 1980, Karlsson & Rundcrantz 1982, Drettner et al. 1982, Dingsor et al. 1985, Hartwig et al. 1988, Lildholdt et al. 1991). Corticosteroids have anti-inflammatory properties. They reduce vasopermeability, edema and the influx of inflammatory cells in the respiratory mucosa (Lungren et al. 1988, Siegel 1988) and they inhibit the release of inflammatory mediators in the upper respiratory tract (Pipkorn et al. 1987).

Systemic corticosteroids can be used as (initial) therapy for nasal polyps, but the burst should be short because corticosteroids may cause a clinically significant suppression of the pituitary-adrenal axis (Settipane 1987). Topical corticosteroids can be used for years without any side-effects (Holopainen ey al. 1982, Lindqvist et al. 1986, Pipkorn et al. 1987). Injection of steroids in the nasal turbinates and polyps is not advocated because it can result in visual loss,

probably due to emboli in the retinal vessels (Mabry 1981).

Influence of corticosteroids on humoral and cellular immunity

In nasal secretions of patients with nasal polyps the concentrations of albumin, IgG and IgE decrease during treatment with corticosteroids (Sorensen et al. 1976). Furthermore, corticosteroids inhibit the production and release of interleukin 2 and interferon-gamma (IFN-γ) from T lymphocytes (Schleimer et al. 1988) and are capable of causing a redistribution of lymphocytes from the circulation into other body compartiments (Fauci & Dale 1975). This effect is greater on T than on B lymphocytes (Parrillo & Fauci 1979). Although the aforementioned studies suggest a T cell dependent immune modulating effect of corticosteroids, so far little attention has been payed to the influence of these drugs on the local distribution of lymphocytes and their subsets in the upper respiratory tract.

Patients with eosinophilic non-allergic rhinitis (ENR) and nasal polyps respond well to topical steroid therapy (Mullarky 1988). Human eosinophils have glucocorticoid receptors (Peterson et al. 1981), but in hypodense eosinophils the expression of the corticosteroid receptors seems to be diminished (Prin 1989). Corticosteroids diminish the chemotaxis and adherence of eosinophils (Winqvist et al. 1984) and they may decrease the prolongation of survival of eosinophils, possibly resulting in a shorter exposure of tissues to eosinophils. This could be important in the control of eosinophilia associated diseases (Lamas et al. 1990, Wallen et al. 1990). Topical corticosteroids may inhibit ECP release from eosinophils because in patients with allergic rhinitis Bisgaard et al. (1990) found a late occurring increase in the ECP concentration in nasal lavage fluid after allergen provocation which could be completely inhibited by pretreatment with budesonide. On the other hand, Andersson et al. (1989) did not found a significant change in the levels of ECP in nasal lavages after treatment with topical corticosteroids in patients with allergen-induced hyperresponsiveness.

Corticosteroids seem to inhibit proliferation of mucosal mast cells (Schleimer et al. 1989). Although it is not sure if corticosteroids inhibit the release of mediators from human mast cells, Bachelet et al. (1990) demonstrated that in mast cells from guinea pigs lungs corticosteroids prevent mast cell degranulation. Furthermore, corticosteroids inhibit the release of arachidonic acid from membrane phospholipids (Vanderhoek et al. 1984). This may partly explain the harmful effect of corticosteroids in the treatment of nasal polyps. Finally, in vitro studies have demonstrated that corticosteroids inhibit release of PAF from macrophages (Tonnel et al. 1986). This can be of importance to reduce the infiltration and activation of eosinophils.

The aforementioned data demonstrate that corticosteroids can influence inflammatory processes by inhibition of the production and release of

mediators which induce local recruitment, proliferation and activation of leukocytes. Moreover, corticosteroids probably inhibit activation of inflammatory cells which are present locally. However, the precise mechanisms whereby corticosteroids modulate the immune and inflammatory response require further elucidation (Siegel 1988).

AIM OF THE STUDY

Inflammatory processes within the mucosa of the upper respiratory tract probably play a role in the development of nasal polyps. Untill now little attention has been paid to the distribution of inflammatory cells like lymphocytes and their subsets, eosinophilic granulocytes and antigen presenting cells in nasal polyps and in the (inflamed) nasal mucosa. Using monoclonal antibodies, morphologically similar cells with different cell surface proteins can be distinguished. By analysis of surface antigens, cells may also be characterized according to the state of maturation and activation. With these techniques the cell populations in the nasal mucosa and in nasal polyps were investigated. To get a better insight into inflammatory processes in the upper respiratory tract we investigated the distribution of immunologically active cells in biopsy specimens of nasal polyps and the macroscopically unaffected nasal mucosa of the middle and inferior turbinates from patients with nasal polyps and healthy subjects. The histological findings were correlated with the clinical parameters of the upper and lower respiratory tract (**chapter 2 and 3**).

Although corticosteroids may postpone c.q. prevent recurrences of nasal polyps the precise mechanisms involved are incompletely understood. Therefore we investigated cellular aspects of nasal polyps at the time of surgery and after follow-up periods of 6 months and 1 year, during which time the patients were treated with topical steroids. Moreover, clinical parameters of the upper- and lower respiratory tract were evaluated (**chapter 4**). Further characterization of eosinophilic granulocytes in nasal polyps and nasal mucosa was performed with special emphasis on eosinophil activation (**chapter 5**).

Inflammatory reactions in the upper respiratory tract can be reflected in protein and immunoglobulin composition of nasal secretions. However, studies on the composition of nasal secretions in patients with nasal polyps are difficult to compare because of differences in collecting methods of immunoglobulins. Therefore, we developed a direct aspiration system which enabled us to measure absolute concentrations of nasal secretion immunoglobulin levels. With this system we collected secretions from healthy subjects and patients with nasal polyps before and after surgery and treatment with topical steroids and analysed them for total protein, albumin and immunoglobulins levels (**chapter 6A and 6B**).

References

Andersson M, Andersson P, Venge P, Pipkorn U. Eosinophils and eosinophil cationic protein in nasal lavages in allergen-induced hyperresponsiveness: Effects of topical glucocorticosteroid treatment. Allergy 1989; 44 5: 342-48.

Arnold RR, Mestecky J, McGhee JR. Naturally occurring secretory immunoglobulin A antibodies to Streptococces mutans in human colostrum and saliva. Infect Immun 1976; 14: 355-62.

Arnoux B, Duval D, Benveniste J. Release of platelet-activating factor from alveolair macrophages by the calcium inophore A23187. Eur J Invest 1980; 10: 437-43. Baan van der S. Primaire ciliare dyskinesie. Thesis 1985, Amsterdam.

Bachelet CM, Bernaudin JF, Fleury-Feith J. Hitological study of mast cells in the actively sesitized guinea pig lung and after challange: Effect of corticosteroid. Int arch Allergy appl Immunol 1990; 91: 171-74.

Baumgarten C, Kunkel G, Rudolph R, Staud RD, Sperner I, Gelderblom H. Histopathological examinations of nasal polyps of different etiology. Arch Otorhinolaryngol 1980; 226: 187-97.

Beasly R, Hovel C, Mani R, Robinson C, Varley J, Holgate ST. Comparitive vascular effects of histamine, prostaglandin (PGD2) and its metabolite 9 alpha, 11 beta-PGF2 in human skin. Clin Allergy 1988; 18: 619-27.

Bende M, Flisberg K. Blood flow in nasal polyps. J Laryngol Otology 1985; 99: 167-69.

Biewenga J, Stoop AE, Baker HE, Swart SJ, Nauta JJP, van Kamp GJ, van der Baan. Nasal secretions from patients with polyps and healthy individuals, collected with a new aspiration system: evaluation of total protein and immunoglobulin concentrations. Ann Clin Bioch 1991; 28: 260-66.

Billroth T. Uber den Bau der Schleimpolypen. Berlin: Georg Riemer, 1855, 1.

Bisgaard H, Gronborg H, Mygind N, Dahl R, Lindqvist N, Venge P. Allegen-induced increase of eosinophil cationic protein in nasal lavage fluid: Effect of the glucocorticoid budesonide. J allergy Clin Immunol 1990; 85: 891-95.

Boysen M. Thesurface structure of the human nasal mucosa I. Ciliated and metaplastic scanning/ transmission electron and light microscopy. Vircoows Arch B (Cell Pathol) 1982; 40: 279-94.

Brandtzaeg P. Immunohistochemical characterisation of intra cellular J chain and binding site for secretory component in human immunoglobulin-producing cells. 1983: Mol Immunol 23: 941-66.

Brandtzaeg P. (1984). Immune functions of nasal mucosa and tonsils in health and disease. In: Immunology of the lung and upper respiratory tract. Ed. J. Bienenstock. McGraw-Hill Book Company New York, pp28-95.

Brandtzaeg P. Cells producing immunoglobulins and other immune factors in human nasal mucosa. Prot Biol Fluids 1985; 32: 363-66.

Brandtzaeg P, Bjerke K. Human pweyers patches: Lympho-epithelial relationships and characteristics of immunoglobulin producing cells. Immunol Invest 1989; 18: 29-45.

Brindley IL, Sweet JM, Goetzl EJ. Stimulation of histamine release from basophils by human platelet factor 4. J Clin Invest 1983; 72: 1218-23.

Bruijnzeel PLB, Koenderman L, Kok PTM. Platelet-activating factor (PAF-acether)-induced leukotriene 4 formation and Luminol chemiluminescence by human eosinophils. Pharmacol Res Commun 1986; (Suppl) 18: 61-69.

Bumsted RM, El-Ackad T, Montgomory Smith J, Brody MJ. Biogenic amines in nasal mucosa and nasal polyps. Otorhinolaryngol 1977; 84: 729-41.

Bunnag C, Pacharee P, Vipulakom P, Siriyananda C. A study of allergic factor in nasal polyp patients. Ann Allergy 1983; 50: 126-32.

Campbell HD, Tucker WQJ, Hort Y. Molecular cloning, nucleotide sequence, and expression of the gene encoding human eosinophil differentiationfactor (interleukin 5). Proc Natl Acad Sci USA 1987; 84: 6629-33.

Caplin I, Haynes JT, Spahn J. Are nasal polyps an allergic phenomenon? Ann Allergy 1971; 29: 631-34.

Capron M, Kusnierz JP, Prin L, Spiegelberg HL, Ovlaque G, Gosset P, Tonnel AB, Capron A. Cytophilic IgE on human blood and tissue eosinophils: detection by flow microfluorometry. J Immunol 1985; 134: 3013-18.

Capron M, Spiegelberg HL, Prin L, Benrich H, Butterworth AE, Pierce RJ, Quaissi MA, Capron A. Role of IgE-receptors in effector function of human eosinophils. J Immunol 1984; 132-1: 462-68.

Capron M, Benveniste J, Braquet P, et al. Role of PAF-acether in IgE, dependent activation of eosinophils. In: Braquet P ed., The role of platelet activating factor in immune disorders. Basel, Karger, 1988: 10-17.

Capron M, Tomassini M, Torpier G, Kusnierz JP, McDonald S, Capron A. Selectivity of mediators released by eosinophils. Int Arch Allergy appl Immunol, 1989; 88: 54-58.

Cauna N, Hindover KH, Manzethi GW, Swanson EW. Fine structure of nasal polyps. Ann Otol Rhinol Laryngol 1972; 81: 41-58.

Chafee FH, Settipane GA. Aspirin intolerance: Frequency in an allergic population. J Allergy Clin Immunol 1974; 53: 193-99.

Chandra RK, Abrol BM. Immunopathology of nasal polyps. J Laryngol Otol 1974; 88: 1019-24.

Coffmann RL, Carty J. A T cell activity that enhances polyclonal IgE production and its inhibition by interferon-gamma. J Immunol 1986; 136: 949-54.

Defrance T, Aubry JP, Rousset F, et al. Human recombinant interleukin-4 induces Fce receptors (CD 23) on normal human B lymphocytes. J Exp Med 1987; 165: 1459-67.

Delaney JC. Aspirin idiosyncrasy in patients admitted for nasal polypectomy. Clin Otolaryngol 1976; 1: 27.

Denjean A, Arnoux B, Lockhart A, Masse R, Benveniste J. Modification of alveolar cell population after paf-acether induced bronchoconstriction in baboons (abstract). Am Rev Respir Dis 1984; 129: A3.

Dent G, Ukena D, Barnes PJ. PAF receptors. In: Barnes PJ, Page CP, Henson PM. PAF and human disease. Blackwell, Oxford 1989, 58-116.

Dingsor G, Kramer J, Olsholt R, Soderstrom T. Flusonide nasal spray 0.025% in the prophylectic treatment of nasal polyposis after poltypectomy. Rhinology 1985; 23: 49-59.

Donovan R, Johansson SGO, Bennich H, Soothill JF Immunoglobulins in nasal polyp fluid. Int Arch Allergy appl Immunol 1970; 37: 154-66.

Drake-Lee AB, Barker THW, Thurley KW. Nasal polyps: 1 Scanning electron microscopy and artifact, 2 Fine structure of mast cells. J Laryngol Otol 1984; 98: 285-92.

Drettner B, Ebbesen A, Nilsson M. Prophylactive treatment with flusonide after polypectomy. Rhinology 1982; 20: 149-58.

Egesten A, Alumets J, von Mecklenburg C, Palmegren M, Olsson I. Localization of the eosinophil cationic protein, the major basic protein and the eosinophil peroxidase in human eosinophils by immunoelectron microscopic technique. 1986: J Histochem Cytochem 34 (11): 1399-403.

Enerback L, Pipkorn U, Granerus G. Intraepithelial migration of nasal mucosa mast cells in hay fever. 1986: Int Arch All Appl Immunol 80; 44-51.

Enokihara H, Hamaguchi H, Sakamaki H, Saito K, Furusawa S, Shishido H. Specific production of eosinophil colony stimulating factor from sensitized T cells from a patient with allergic eosinophilia. Brit J Haematol 1985: 59; 85-91.

Ernst PB, Underdown BJ, Bienenstock J. Immunity in mucosal tissues. In: Stites DP, Stobo JD, Wells JV, eds. Basic and clinical immunology. East Norwalk, Conn.: Appleton & Lange, 1987; 159-66.

Fauci AS, Dale DC. The effect of hydrocortisone on the kinetics of normal human lymphocytes. Blood 1975; 46: 235-43.

Fitzharris P, Moqbel R, Thorne KJ, Richardson BA, Hartnell A, Cromwell O, Butterworth EA, Kay AB. The effects of eosinophil activating factor on IgG-dependent sulphidopeptide leukotrine generation by human eosinophils. Clin Exp Immunol 1987; 66: 673-80.

Fokkens WJ, Vroom TM, Rijntjes E, Mulder PGH. CD1a(T6), HLA-DR-expressing cells, persumable Langerhans cells, in nasal mucosa. Allergy 1989; 44: 167-72.

Friedmann I, Osborn DA. (1982). Pathology of granulomas and neoplasms of the nose and paranasal sinuses, chapter 5, 28-35. Churchill, Davidson, Edinburgh.

Frigas E, Loegering DA, Solley GO, Farrow GM, Gleich GJ. Elevated levels of eosinophil granule major basic protein in the sputum of patients with bronchial asthma. Mayo clin Proc 1981: 56; 345-53.

Fukuda T, Numao T, Makino S. Specific binding of PAF by human eosinophils. J Allergy Clin Immunol (Abstract) 1987; 79: 172.

Fujisawa T, Abu-Ghazaleh R, Kita H, Sanderson CJ, Gleich GJ. Regulatory effect of cytokines on eosinophil degranulation. J Immunol 1989; 144: 642-46. Galli SJ, Lichtenstein LM (1988). In Allergy: Principles and Practice (3th Edn) (Middleton, Jr,E, Reed CE, Ellis EF, Adkinson NF, Yunginger JW, eds), pp 106-134, CV Mosby Co.

Ganzer U, Bachert C. Localisation of IgE synthesis in immediate-type allergy of the upper respiratiry tract. 1988: ORL 50; 257-64.

Gleich GJ, Loegering DA, Mann KG, Maldonado JE. Comparative properties of the Charcot-Leyden Crystal Protein and the Major Basic Protein from human eosinophils. J Clin Invest 1976: 57; 633-40.

Gleich GJ, Flavahan NA, Fujisawa T, Vanhoutte PM. The eosinophil as a mediator of damage to respiratory epithelium: A model for bronchial hyperreactivity. J Allergy Clin Immunol 1988; 81: 776-81.

Gonzales MC, Diaz P, Galleguillos FR, Ancic P, Cromwell O, Kay AB. Allergen-induced recruitment of bronchoalveolar (OKT4) and suppressor (OKT8) T cells in asthma: relative increases in OKT8 cells in single early responders compared with those in late-phase responders. Am Rev Respir Dis 1987; 136: 600-04.

Grantham JG, Meadows A, Gleich GJ. Chronic eosinophilic pneumonia. Evidence for eosinophil degranulation and release of major basic protein. Am J Med 1986: 80; 89-94.

Green DR, Gold J, St Martin S, Gershon R, Gershon RK. Microenvironmental immunoregulation: Possible role of contrasuppressor cells in maintaining immune respons in gut associated lymphoid tissues. Proc Natl Acad Sci USA 1982; 79: 889-92.

Griffen MP, McFadden ER, Ingram RH, Pardee S. Airway cooling in asthmatics and nonasthmatics during nasal and oral breathing. J Allergy Clin Immunol 1982; 69: 354-59.

Hajek M. Uber die pathologischen veranderungen der siebbein-knochen im gefolge der entzundlichen schleimhaut hypertrophie und der nasenpolypen. Arch Laryngol Rhinol 1896; 4: 277.

Hamaguchi Y, Ohi M, Sakakura Y, Mukojima T. Quantitation of nasal secretory IgA by enzyme-linked immunosorbent assay. Int Arch Allergy appl Immunol 1982; 69: 1-6.

Hamaguchi Y, Kanakura Y, Fujita J, et al. Interleukin 4 as an essential factor for in vitro clonal growth of murine connective tissue-type mast cells. J Exp Med 1987; 165: 268-73.

Hameleers DMH, Stoop AE, van der Ven I, Sminia T, van der Baan S, Biewenga J. Immunohistochemical characterisationof leukocytes in the nasal mucosa of ear, nose and throat patients and controls. EOS J Immunol Immunopharmacol 1990; 10: 58-63.

Harlin SL, Ansel DG, Lane SR, Myers J, Kephart GM, Gleich GJ. A clinical and pathologic study of chronic sinusitis: the role of the eosinophil. J Allergy Clin Immunol 1988; 81: 867-75.

Hartwig S, Linden M, Laurent C, Varko AK, Lindqvist N. Budesonide nasal spray as prophylactic treatment after polypectomy. J Laryngol Otolog 1988; 102: 148-51.

Hastie AT, Loegering DA, Gleich GJ, Kueppers F. The effect of purified human eosinophil major basic protein on mammalian ciliary activity. Am Rev Respir Dis 1987; 135: 848-53.

Henderson WR, Jong EC, Kelbanoff SJ. Binding of eosinophil peroxidase to mast cell granules with retention of peroxidase activity. J Immunol 1980a; 124; 1383-88.

Henderson WR, Chi EY, Klebanoff SJ. Eosinophil peroxidase-induced mast cell secretion. J Exp Med 1980b; 152: 265-79.

Henderson WR, Chi EY. Ultrastructural characterisation and morphometric analysis of human eosinophil degranulation. J Cell Sci 1985: 73; 33-48.

Henocq E. PAF-acether and eosinophils. Prog Biochem Pharmacol 1988; 22: 141-48.

Hirschberg H, Kaakinen A, Thorsby E. Presence of HLA-DR determinants on human macrophages. Nature 1976; 263: 63-64.

Hisamatsu K, Ganbo T, Nakazawa T, Murakami Y, Gleich GJ, Makiyama K, Koyama H. Cytotoxicity of human eosinophil granule major basic protein to human nasal mucosa in vitro. J Allergy Clin Immunol 1990; 86: 52-63.

Holgate ST, Robinson C, Church MK. (1988). In Allergy: Principles and Practice (3th Edn) (Middleton, Jr,E, Reed CE, Ellis EF, Adkinson NF, Yunginger JW, eds), pp 135-163, CV Mosby Co.

Holopainen E, Malmberg H, Binder E. Long term follow-up of intranasal beclomethasone treatment; A clinical and histological study. Acta Otolaryngol 1982; Suppl 386: 270-73.

Hosemann W, Michelson A, Weindler J, Mang H, Wigand ME. Einfluss der endonasalen nasennebenhohlenchirurgie auf die lungenfunction des patienten mit asthma bronchiale. Laryngol-Rhino-Otolog 1990; 69: 521-26.

Ihle JN, Keller J, Oroszalan S, et al. Biologic properties of homogenous interleukine 3. Demonstration of WEHI-3 growth factor activity, mast cell growth factor activity, P cell stimulating factor activity and histamine-producing cell-stimulating factor activity. J Immunol 1983; 131: 282-87.

Johansson SGO, Deuschl H. Immunoglobulins in nasal secretions with special reference to IgE. Int Arch Allergy appl Immunol 1976; 52: 364-75.

John AC, Merrett TG. The radioallergosorbent test (RAST) in nasal polypsis. J Laryngol Otolog 1979; 93: 889-98.

Jones E, Frenkiel S, Small P, Rochon L. Immunopathological characteristics of nasal polyps. 1987: J Otolaryngology 16,1; 19-22.

Jouvin-Marche E, Grych JM, Boullet C, Capron M, Benveniste J. Formation of PAF-acether by human eosinophils. Fed Proc 1984; 43: 1924.

Jung TTK, Juhn SK, Hwang D, Stewart R. Prostaglandins, leukotrienes, and other arachidonic acid metabolites in nasal polyps and nasal mucosa. Laryngoscope 1987; 97: 184-89.

Kajita T, Yui Y, Mita H, Taniguchi N, Saito H, Mishima T, Shida T. Release of leukotriene C4 from human eosinophils and its relation to the cell density. Int Arch Allergy appl Immunol 1985; 78: 406-10.

Kakoi H, Hiraide F. A histological study of formation and growth of nasal polyps. Acta Otolaryngol 1987; 103: 137-41.

Karlsson G, Rundcrantz H. A randomized trial of intranasal beclomethasone dipropionate after polypectomy. Rhinology 1982; 20: 144-48.

Kauffman HF, van der Belt B, de Monchy JGR, Boelens H, Koeter GH, de Vries K. Leukotriene C4 production by normal density and low density eosinophils of atopic individuals and other patients with eosinophilia. J Allergy Clin Immunol 1987; 79: 611-19.

Khalife J, Capron M, Cesbron JY, Tai PC, Taelman H, Prin L, Capron A. Role of specific IgE antibodies in peroxidase (EPO) release from human eosinophils. J Immunol 1986; 137: 1659-64.

Kloprogge ED, de Leeuw AJ, de Monchy JGR et al. Hypodence eosinophilic granulocytes in normal individuals and patients with asthma: generation of hypodence cell populations in vitro. J Allergy Clin Immunol 1989; 83(2): 393-400.

Korsrud FR, Brandtzaeg P. Immunology of human nasal mucosa. 1983: Rhinology 21; 203-12.

Krajina Z. A contribution to the aetiopathogenesis of nasal polyps. Pract Oto-Rhino-Laryngol 1963; 25: 241-46.

Krajina Z, Zirdum A. Histochemical analyses of nasal polyps. Acta Otolaryngol (Stockh) 1987; 103: 435-40.

Kroegel C, König W, Mollay C, Kreil G. Generation of eosinophil chemotactic factor (ECF) from various cell types by melettin. Mol Immunol 1981; 18: 227-36.

Lamas AM, Leon OG, Klunk DA, Schleimer RP. Glucocorticoids specially decrease of eosinophil survival. J Allergy Clin Immunol 1990; Suppl 85-1 part 2: 282.

Lanoff G, Daddono A, Johnson E. Nasal polyps in children: a ten-year study. Ann Allergy 1973; 31: 551-54.

Larssen PL, Tos M. Origin of nasal polyps. Laryngoscope 1991; 101: 305-12.

Lee TC, Malone B, Wasserman SI, Fitzgerald V, Snyder F. Activities that metabolize platelet activating factor in neutrophils and eosinophils from humans and the effect of a calciumionophore. Biophys Biochem Res Commun 1988; 105: 1303-10.

Lehner T, Avery J, Jones T. Separation and characterisation of a subset of human T8+ cells which function as antigen-presenting and contrasuppressor cells. Immunol 1985; 54: 713-22.

Lewis RA, Goetzl EJ, Drazen JM, Soter NA, Austen KF, Corey EJ. Functional characterization of synthetic leukotriene B4 and its stereochemicalm isomers. J Exp Med 1981; 154: 1243-48.

Lindahl G, Hedfors E, Klareskog L, Forsum U. Epithelial HLA-DR expression and T lymphocyte subsets in salivary glands in Sjogren's syndrome. Clin Exp Immunol 1985; 61: 475-82.

Lildholdt T, Fogstrup J, Gammelgaard N, Kortholm B, Ulsor C. Management of nasal polyps by steroid nose drops. Am J Rhinology 1991; 5-1: 25-27

Lindqvist N, Balle VH, Karma P, Karja J, Lindstrom D, Makinen J, Pukander J, Ruoppi P, Suonpaa J, Ostlund W, Pipkorn U. Long-term safety and efficacy of budesonide nasal aerosol in periennial rhinitis. Allergy 1986; 41: 179-86.

Little MM, Casale TB. PAF induced chemotaxis of eosinophils across and endothelial barrier. J Allergy Clin Immunol (Abstract) 1990; 85-1; 281.

Lundgren R, Soderberg M, Horstedt P, Stenling R. Morphological studies of bronchial mucosal biopsies from asthmatics before and after ten years of treatment with inhaled steroids. Eur Respir J 1988; 1: 883-89.

Mabry RL. Visual loss after intranasal corticosteroid injection. Arch Otolaryngol 1981; 107: 484-86.

Masuyama K, Samejima Y, Ishikawa T. Eosinophils in nasal secretion. Acta Otolaryngol 1988: Suppl 458: 181-89.

Metcalf D, Begley CG, Johnson GR, et al. Biologic properties in vitro of a recombinant human granulocyte-macrophage colony-stimulating factor. Blood 1986; 67: 37-45.

Metzgar RS, Bertoglio J, Anderson JK, Bonnard GD, Ruscetti FW. Detection of HLA-DRw (Ia-like) antigens on human Tlymphocytes grown in tissue culture. J Immunol 1979; 122: 949-53.

Miller AM, McGarry MP. A diffuse stimulator of eosinophilopoiesis produced by lymphoid cells as demonstrated with diffusion chambres. Blood 1976; 48: 293-300.

Mings R, Friedman WH, Lindford PA, Slavin RG. Five-year follow-up of the effects of bilateral intranasal sphenoidectomy in patients with sinusitis and asthma. Am J Rhinol 1988; 2: 13-16.

Miyajima A, Kiyatake S, Schreurs J, de Vries J, Arai N, Yokota T, Arai K. Coordinate regulation of immune and inflammatory responses by T cell derived lymphokines. FASEB J 1988; 2: 2462-73.

Motojima S, Dunnette SL, Frigas E, Gleich GJ. Creola bodies and eosinophil granule proteins in sputum. J Allergy Clin Immunol 1987; 79: 128.

Motojima S, Frigas E, Loegering DA, Gleich GJ. Toxicity of eosinophil cationic for guinea pig tracheal epithelium in vitro. Am Rev Respir Dis 1989; 139: 801-05.

Mullarky MF. Eosinophilic nonallergic rhinitis. J All Clin Immunology 1988; 82: 941-49.

Mygind M, Weeke B, Ullman S. Quantitive determination of immunoglobulins in nasal secretion. Int Arch Allergy appl Immunol 1975; 49: 99-107.

Nagy L, Lee TH, Goetzl EJ, Pickett WC, Kay AB. Complement receptor enhancement and chemotaxis of human neutrophils and eosinophils by leukotriens and other lipoxygenase products. Clin Exp Immunol 1982; 47: 541-47.

Nakashima T, Hamashima Y. Local immune system os nasal mucosa in inflammation. IgA distribution and secretory activity. 1980: Ann Otol 89; 140-46.

Nishimoto K, Ukai K, Harada T, Chun Shun J, Sakakura Y. Lymphocyte subsets of maxillary mucosa in chronic inflammation. Acta Otolaryngol (Stockh) 1988; 106: 291-98.

Ogasawara H, Yoshimura S, Kumoi T. Hydrgen peroxyde generation by eosinophils in allergic rhinitis. J Allergy Clin Immunol 1988; 81(1): 206.

Ogawa H, Kunkel SL, Fantone JC, Ward PA. Comparative study of eosinophil and neutrophil chemotaxis and enzyme release. Am J Pathol 1981a; 105: 149-55.

Ogawa H, Kunkel SL, Fantone JC, Ward PA. Digestion of the fifth component of complement by eosinophil lysosomal enzymes: production of eosinophil specific activity. Virchows Arch (Cell Pathol) 1981b; 38: 149-57.

Ogawa H. Atopic aspect of eosinophilic nasal polyposis and a possible mechanism of eosinophil accumulation. Acta Otolaryngol 1986: Suppl 430; 12-17.

Ogino S, Harada T. Aspirin induced asthma and nasal polyps. Acta Otolaryngol (Stockh) 1986; suppl 430: 21-27.

Olsson I, Venge P, Spitznagel JK, et al. Arginine-rich cationic proteins of human eosinophil granules: comparison of the constituents of eosinophilic and neutrophilic leukocytes. Lab Invest 1977: 36(5); 493-500.

Owen WF, Rothenberg ME, Silberstein DS, Gasson JC, Stevens L, Austen KF, Soberman RJ. Regulation of human eosinophil viability, density and function by granulocyte/ macrophage colony stimulating factor in the presensence of 3T3 fibroblasts. J Exp Med 1987; 166: 129-41.

Paludetti G, Maurizi M, Tassoni A, Tosti M, Altissimi G. Nasal polyps: a comparative study of morphologic and etiopathogenic aspects. Rhinology 1983; 21: 347-60.

Parrillo JE, Fauci AS. Mechanisms of glucocorticoid action on immune processes. Ann Rev Pharmacol Toxicol 1979; 19: 179-201.

Perkins JA, Blakeslee DB, Andrade P. Nasal polyps: A manifestation of allergy? Otolaryngol Head and Neck Surg 1989; 101: 641-45.

Peters MS, Gleich GJ, Dunette SL, Fukuda T. Ultrastructural study of eosinophils from patients with the hypereosinophilic syndrome: a morphological basis of hypodense eosinophils. Blood 1988; 71-3: 780-85.

Peterson AP, Altman LC, Hill JS et al. Glucocorticoid receptors in normal human eosinophils: comparison with neutrophils. J Allergy Clin Immunol 1981; 68(3): 212-17.

Peterson CGB, Skoog V, Venge P. Human eosinophil cationic proteins (ECP and EPX) and their suppressive effects on lymphocyte proliferation. Immunobiol 1986: 171; 1-13.

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 Eng J Medicine 1987; 316-24: 1506-10.

Prin L, Capron M, Tonnel AB, Bletry D, Capron AB. Heterogeneity of human peripheral blood eosinophils: variability in cell density and cytotoxic ability in relation to the level and the origin of hypereosinophilia. Int Arch Allergy appl Immunol 1983: 72; 336-346.

Prin L. Polynucleaire eosinophile et recepteur glucocorticoid. In: Journees Nationales de la societe francaise d'allergologie. Rapports et communications Lille CHU, 1989, 37-40.

Proctor DF, Adams GK. Physiology and pharmacology of nasal function and mucus secretion. Pharmacol Ther 1976; 2B: 492.

Pulido V, Garcia-Calderon PA. Some immunological parameters in serum and nasal secretion in subjects with vasomotor and allergic rhinitis and nasal polyps. A comparative study. 1983: Rhinology 21; 29-37.

Rachelefsky GS, Katz RM, Siegel SC. Chronic sinus disease with associated airway disease in children. Pediatrics 1984; 73: 526-29.

Romagnani S, Del Prete G, Maggi E, et al. Role of interleukins in induction and regulation of human IgE synthesis. Clin Immunopathol 1989; 50: 13-23. Rothenberg ME, Owen WF, Silberstein DS, et al. Cytokine regulation of human eosinophil viabilty, density and function. J Allergy Clin Immunol (Abstract) 1988; 81: 209.

Rotteveel FTM, Kokkelink I, van Lier RAW, Kuenen B, Meager A, Miedema F, Lucas CJ. Clonal analyses of functionally distinct human CD4+ T cell subsets. J Exp Med 1988; 168: 1659-73.

Ruhno J, Howie K, Anderson M, Anderson B, Vanzieleghem M, Hitch D, Lapp P, Denburg J, Dolowich J. The increased number of epithelial mast cells in nasal polyps and the adjacent turbinates is notallergy-dependent. Allergy 1990; 45: 370-74.

Sakaguchi I, Okuda M, Ushijima K, Sakaguchi Y, Tanigaito Y. Study of nasal surface basophilic cells in patients with nasal polyps. Acta Otolaryngol (Stockh) 1986; suppl 430: 28-32.

Samter M. Nasal polyps. An inquiry into the mechanism of formation. Arch Otolaryngol 1961; 73: 334-41.

Samuelsson B. Leukotrienes: Mediators of immediate hypersensitivity reactions and inflammation. Science 1983; 220: 568-75.

Sanderson CJ, Warren DJ, Strath M. Identification of a lymphokine that stimulates eosinophil differentiation in vitro. J Exp Med 1985; 162: 60-70.

Sasaki Y. Distribution of the degranulated and non-degranulated mast cell in nasal polyps. Acta Otolaryngol (Stockh) 1986; 430: 34-38.

Schauer U, Eckhart A, Muller R, Gemsa D, Rieger CHL. Enhanced leukotriene C4 production by peripheral eosinophilic granulocytes from children with asthma. Int Arch Allergy appl Immunol 1989; 90: 201-6.

Schleimer RP, Derse CP, Friedman B, Gilles S, Plaut M, Lichtenstein LM, McGlashan DW. Regulation of human basophil mediator release by cytokines. Interaction with antiinflammatory steroids. J Immunol 1989; 143: 1310-17.

Schram VL, Effron MZ. Nasal polyps in children. The Laryngoscope 1980; 90: 1488-95.

Schrezenmeier H, Fleischer B. A regulatory role for the CD4 and CD8 molecules in T cell activation. J Immunol 1988; 141: 398-403.

Selby WS, Janossy G, Mason DY, Jewell DP. Expression of HLA-DR antigens by colonic epithelium in inflammatory bowel disease. Clin Exp Immunol 1983; 53: 614-18.

Settipane GA Chafee FH. Nasal polyps in asthma and rhinitis: a review of 6037 patients. J Allergy Clin Immunol 1977; 59: 17-21.

Settipane GA. Nasal polyps: Epidemiology, pathology, immunology, and treatment. Am J Rhinology 1987; 1-3: 119-26.

Shaw RJ, Walsh GM, Cromwell O, Moqbel R, Spry CJF, Kay AB. Activated eosinophils generate SRS-A leukotrienes following IgG-dependent stimulation. Nature 1985; 316: 150-52.

Shult PA, Lega M, Jadidi S. The presence of hypodence eosinophils and diminished chemoluminiscence response in asthma. J Allergy Clin Immunol 1988; 81(2): 429-37.

Siegel SC. Topical intranasal corticosteroid therapy in rhinitis. J Allergy Clin Immunol 1988; 81: 984-91.

Sigal CE, Valona FH, Holtzman MJ, Goetzl EJ. Preferential human eosinophil chemotactic activity of platelet activating factor. J Clin Immunol 1987; 7: 179-84.

Slavin RG. Relationship of nasal disease and sinusitis to bronchial asthma. Ann Allergy 1982; 49: 76-79.

Slavin RG, Lindford PA, Friedman WH. Bilateral intranasal sphenoidectomy in the treatment of nasal polyps, sinusitis and bronchial asthma. J Allergy Clin Immunol 1983; 71: 156.

Small P, Barrett D, Frenkiel S, Rochon L, Cohen C, Black M. Local specific IgE production in nasal polyps associated with negativ skin tests and serum RAST. 1985: Ann Allergy 55; 736-39.

Sorensen H, Mygind N, Pedersen CB, Prytz S. Long-term treatment of nasal polyps with beclomethasone dipropionate aerosol. Acta Otolaryngol 1976; 82: 260-62.

Soter NA, Lewis RA, Corey EJ Austen KF. Local effects of synthetic leukotrienes (LTC4, LTD4, LTE4 and LTB4) in human skin. J Invest Dermatol 1983: 80(2); 115-19.

Spector SL, Wangaard CH, Farr RS. Aspirin and concomitant idiosyncrasies in adult asthmatic patients. J Allergy Clin Immunol 1979; 64-6 (1): 500-6.

Tai PC, Spry CJF. The mechanisms which produce vacuolated and degranulated eosinophils. BR J Haematol 1981: 49; 219-26.

Tai PC, Bakes DM, Barkens JR. Plasma membrane antigens on light density and activated human blood eosinophils. Clin Exp Immunol 1985; 60: 427-36.

Takada S, Engleman EG. Evidence for an association between CD8 molecules and the T cell receptor complex on cytotoxic T cells. J Immunol 1987; 139: 3231-35.

Takasaka T, Kaku Y Hozowa K. Mast cell degranulation in nasal polyps. Acya Otolaryng (Stockh) 1986; 430: 39-48.

Tamura N, Agrawal DK, Suliaman FA, Townley RG. Effects of platelet-activating factor on the chemotaxis of normodense eosinophils from normal subjects. Biochem Biophys Res Commun 1987; 142: 638-44.

Taniguchi N, Mita H, Saito H, Yui Y, Kaika T, Shida T. Increased generation of leukotriene C4 from eosinophils in asthmatic patients. Allergy 1985: 40; 571-73.

Tonnel AB, Gossett Ph, Joseph M, Lassalle PH, Dessant JP, Capron A. Alveolair macrophage and its participation in the inflammatory process of allergic asthma. Clin Respir Physiol 1986; 22 (suppl 22): 70-77.

Tos M, Mogensen C. Pathogenesis of nasal polyps. Rhinology 1977; 15: 87-95.

Ukena D, Kroegel C, Dent G, Yukawa T, Sybrecht G, Barnes PJ. PAF-receptors on eosinophils: identification with novel ligand, [3H]WEB 2086. Biochem Pharmacol 1989; 38: 1702-5.

Vadas MA, Nicola NA, Metcalf D. Activation of antibody-dependent cytotoxicity of human neutrophils and eosinophils by separate colony-stimulating factors. J Immunol 1983; 130: 795-99.

Vanderhock JY, Ekborg SL, Baily JM. Nonsteroidal anti-inflammatory drugs stimulate 15-lipoxygenase/ leukotriene pathway in human polymorphonuclear leukocytes. J Clin Immunol 1984; 74: 412-17.

Vancil ME. A historical survey of nasal polyposis. Laryngoscope 1969; 79: 435-45.

Venge P, Hakansson L, Peterson CG. Eosinophil activation in allergic disease. Int Arch All app Immunol 1987: 82; 333-37.

Virolainen E, Puhakka H. The effect of intranasal beclometasone dipropionate on the recurence of nasal polyps after ethmoidectomy. Rhinology 1980; 18: 9-18.

Vleming M, Stoop AE, Middelweerd MJ, de Vries N. Results of endoscopic sinus surgery for nasal polyps. AM J Rhinilogy (in press).

Wallen ND, Weiler DA, Gleich GJ, Kita H. Glucocorticoids inhibit rIL-5-enhenced in vitro survival of eosinophils. J Allergy Clin Immunol 1990; suppl 85-1 p2: 282.

Waller G, Weidenbecher M, Pesch HJ, Beankler H. Vergleichende klinische, histologische und immunologische untersuchungen zur Atiologie der polyposis nasi et sinuum. Laryngol Rhinol 1976; 55: 174-78.

Walsh GM, Nagakura T, Iikura Y. Flow-cytometric analyses of increased IgE uptake by normal eosinophils following activation with PAF-acether and other inflammatory mediators. Int Arch Allergy Appl Immunol 1989; 88: 194-96.

Wardlaw AJ, Kay AB. The role of the eosinophil in the pathogenesis of asthma. Allergy 1987; 42: 321-35.

Wardlaw AJ, Moqbel R, Kurihara K, et al. Role of PAF-acether in leucocyte activation and chemotaxis. In: Braquet P ed., The role of platelet activating factor in immune disorders. Basel, Karger, 1988: 1-9.

Watanabe K, Hasegawa M, Saito Y, Takayama S. Eosinophilic leukocytes in nasal allergy-movement of enzymes. Clin Allergy 1977; 7: 263-71.

Wayoff M, Moneret-Vautrin DA. Le syndrome d'hyperreactivite nasale (rhinites allergiques et vasomotrices). E.M.C. ORL 3, 20350 A-10.

Weiss JW, Drazen JM, Coles N, McFadden ER, Weller PF, Corey EJ, Lewis RA, Austen KF. Bronchoconstrictor effects of leukotriene (in humans). Science 1982: 9-216; 196-98.

Weller PF. Eosinophilia. J All Clin Immunolog 1984: 73; 1-14.

Whiteside TL, Rabin BS, Zettenberg J, Criep L. The presence of IgE on the surface of lymphocytes in nasal polyps.J Allergy Clin Immunol 1975; 55-3: 186-94.

Winqvist I, Olofsson T, Olsson I, Persson A, Hallberg T. Altered density, metabolism, and surface receptors of eosinophils in eosinphilia. Immunology 1982; 47: 531-35.

Winqvist I, Oloffsson I, Olsson I. Mechanisms for eosinophil degranulation; release of eosinophil cationic protein. Immunology 1984: 51; 1-8.

Winther B, Innes DJ, Mills SE, Mygand N, Zito D, Hayden FG. Lymphocyte subsets in normal airway mucosa of the nose. Arch Otolaryngol Head & Neck Surg 1987; 113: 59-62.

Wladislavosky-Wasserman P, Kern EB, Holley KE, Eisenbrey AB, Gleich GJ. Epithelial damage in nasal polyps. Clin Allergy 1984; 14: 241-47.

Yonge ES. Observations on the determining cause of formation of nasal polypi. Br Med J 1907; 12: 964.

Zuckerlandl E. Normale und pathologische anatomie der nasenhohle und ihrer pneumatischen anhange. Wien: Braumuller W, 1882, Vol 2.

Chapter 2

LYMPHOCYTES AND NONLYMPHOID CELLS IN THE NASAL MUCOSA OF PATIENTS WITH NASAL POLYPS AND OF HEALTHY SUBJECTS

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SUMMARY

Immunohistochemical stainings were performed on biopsy specimens of the middle and inferior turbinates of the nasal mucosa of 14 patients with nasal polyps and 16 healthy subjects. Significantly more CD8+ (T suppressor/cytotoxic) cells than CD4+ (T helper/inducer) cells were found in the lamina propria of the middle and inferior turbinates of patients with nasal polyps and in the inferior turbinates of healthy persons. The middle and inferior turbinates of healthy persons contained significantly more CD4+ cells than the middle and inferior turbinates of patients with nasal polyps. CD19+ B cells were hardly detected in the patients and healthy subjects. More HLA-DR+ cells were found in the middle than in the inferior turbinates, especially in the patients. Varying but small numbers of eosinophils, neutrophils, mastcells and plasma cells were found in patients and healthy subjects. The possible role of CD4+, CD8+ and HLA-DR+ cells in the nasal mucosa is discussed with regard to the pathogenesis of nasal polyps.

INTRODUCTION

The etiology and pathogenesis of nasal polyps are poorly understood. Inflammation probably plays an important role. In the inflamed nasal mucosa infiltration of lymphocytes is one of the characteristic findings (Nishimoto et al. 1988). Lymphocytes and their subsets are regulatory and effector cells in the complex process of the inflammatory response (Ernst et al. 1987). The study of these cells in the nasal mucosa may, therefore, provide information as to the development of nasal polyps.

Most studies on the occurrence of lymphocytes in the upper respiratory tract concern the tonsils (Brandtzaeg, 1984). Recently, Winther et al. (1987) described the lymphocyte distribution in the inferior turbinate of the normal human nasal mucosa, as studied by immunohistochemistry. Likewise, Nishimoto et al. (1988) reported on the distribution of lymphocytes and their subsets in the maxillary mucosa of patients with chronic sinusitis.

To our knowledge there is no study on the distribution of lymphocytes and their subsets in both the middle and inferior turbinates of patients with nasal polyps and of healthy subjects. With the availability of monoclonal antibodies against a variety of leukocyte determinants, it is possible to recognize lymphoid and non-lymphoid cell types (Shaw 1987, Zola 1987).

The aim of this study was to compare lymphoid and nonlymphoid cell populations in the middle and inferior turbinates of patients with nasal polyps and of healthy subjects by immunohistochemistry.

MATERIALS and METHODS

Patients and healthy persons

In this study 14 patients (aged 15-77 years, mean age: 39 years) who were operated upon for nasal polyps and 16 healthy subjects (aged 17-50 years, mean age: 28 years) who underwent an osteotomy for cosmetic reasons or malocclusion (Department of Maxillo-Facial Surgery) were studied. Four of the patients had an IgE-mediated allergy, i.e. positive skintests (› 2 mm, Phazet, Farmacia) and serum IgE ›100 IU/L (Table 1). None of the patients had an aspirin intolerance or had used locally applied corticosteroids during at least 6 weeks before entering the study. The healthy subjects had no IgE-mediated allergic rhinitis or other nasal complaints. They were nonsmokers, had a normal preoperative ear, nose and throat examination and none of them had suffered from a common cold at least 6 weeks previous to the moment of biopsy taking.

Table 1. Clinical data of the patients included in this study.

Patient	Sex	Age in years	IgE mediated allergy	Massive polyposis
1	M	25	- *	+
2	M	44	-	-
3	M	48	+	+
4	M	59	+	-
5	M	77	- *	+
6	F	46	-	-
7	F	40	+	+
8	M	38	+	+
9	F	15	-	+
10	F	24	-	-
11	M	40	-	-
12	F	18	-	-
13	F	48	-	-
14	M	25	-	+
	M=8 F=6		4/14=+	7/14=+

(* infiltration of eosinophils in the middle turbinate)

Tissues

The biopsy specimens were taken from the lateral part of the middle turbinate, not from polypous tissue, and from the inferior turbinate, about 1.5 cm behind the anterior part. They were immediately embedded in ornithine carbamyltransferase tissue compound (Miles, Elkhart, Ind) and frozen in liquid nitrogen. Frozen samples were stored at -70°C until used.

Immune reagents

The mouse monoclonal antibodies against human leukocytes used in this study are listed in table 2. The antibodies were appropriately diluted in 0.01 mol/L phosphate buffered saline (PBS, pH 7.4), containing 0.5% bovine serum albumin (BSA), and used in an indirect immunoperoxidase staining. The conjugate used was horseradish peroxidase-labelled rabbit antimouse-Ig (Dakopatts, Glostrup, Denmark). This was diluted 1:300 in PBS, containing 0.5% BSA and 1% normal human serum.

Table 2. Mouse monoclonal antibodies against human leukocytes used in this study.

antibody	manufacturer[1]	subtype	specificity
anti-CD2	CLB	IgG1	all peripheral T cells, 90% of the thymocytes
anti-CD4	Sanbio	IgG2	helper/inducer T cells and subpopulations of macrophages
anti-CD8	Sanbio	IgG2	suppressor/cytotoxic T cells
anti-CD19	Dakopatts	IgG1	precursor and mature B cells (no plasma cells)
anti-HLA-DR	CLB	IgG1	cells of the monocyte lineage, myeloblasts, promyelocytes and cells of the B-lymphocyte lineage

[1] CLB: Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands.
Sanbio BV-biological products, Uden, The Netherlands.
Dakopatts, Glostrup, Denmark.

Staining procedures

Six to 8 µm thick cryostat sections were picked up on gelatin coated microscope slides and allowed to dry overnight above silica gel. Sections were fixed in pure acetone for 10 minutes and, if necessary treated with 0.228% HIO_4 for 45 seconds to inactivate endogenous peroxidase, and incubated with the appropriate antibodies for 1h at room temperature. After washing in PBS, the sections were covered with the conjugate for 1 hpur at room temperature, washed again in PBS, and stained for peroxidase activity with 3,3'-di-aminobenzidine-

tetrahydrochloride (Sigma, St.Louis, Mo.) at a concentration of 0.5 mg/ml in Tris-HCl buffer, pH 7.6, containing 0.03% H_2O_2. To enhance the staining of the brown reaction product, slides were rinsed with, subsequently, distilled water and 0.9% NaCl, and incubated in 0.5% $CuSO_4$ in 0.9% NaCl for 10-15 min. at room temperture. After rinsing in distilled water the sections were counterstained with haematoxilin (3-10 seconds), dehydrated, and mounted in Entellan (Merck, Darmstadt, Germany). Tonsil sections were used as positive controls. Controls for nonspecific staining were incubated with 0.5% BSA in PBS or with the second stage conjugate only. For routine histological examination standard methylgreen-pyronin and haematoxilin-eosin stainings were performed.

Evaluation

All sections were examined with conventional light microscopy. The number of positively stained cells detected in the sections was expressed as 0, no positive cells; 1, few positive cells; 2, moderate number of positive cells; 3, moderate number of single and some clusters of positive cells; 4, many positive cells. To avoid overestimation, each section was evaluated in lower and higher magnifications (x100, x200, x400 oil immersion). Tissue sections damaged by the procedure of biopsy taking or preparation and specimens of one patient with too much endogenous peroxidase, other than from granulocytes, were excluded from the study. The sections were coded and evaluated independently by two investigators.

The data were statistically analysed by means of the two sided exact signed-rank test or by the two sided exact Wilcoxon rank-sum test.

RESULTS

In the lamina propria most CD2+ T lymphocytes (pan-T) were located in the subepithelium and around the glands. Evaluation of serial sections revealed that the number of CD2+ cells was less than the combined numbers of CD4+ and CD8+ cells. More CD8+ (T suppressor/cytotoxic) cells than CD4+ (T helper/inducer) cells were found in the middle and inferior turbinates of patients and of healthy subjects (Figs. 1 through 3). This predominance was significant ($p < 0.05$) in the middle and inferior turbinates of the patients and in the inferior turbinate of healthy subjects. More CD8+ ($p < 0.05$) and CD4+ cells (not significant) were found in the middle turbinates than in the inferior turbinates of the patients. In the middle turbinates of the healthy subjects significantly more CD4+ cells ($p < 0.05$) were found than in the middle turbinates of patients with nasal polyps. The same was true for the inferior turbinates (Fig. 3). In healthy subjects more CD4+ cells were found in the middle than in the inferior

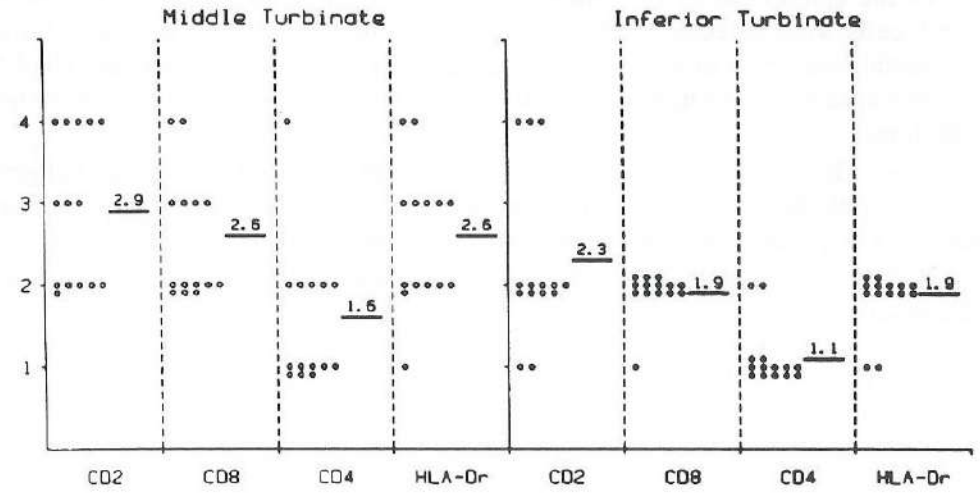

Fig. 1.

Quantification of CD2+, CD4+, CD8+ lymphocytes and HLA-Dr+ cells in the lamina propria of the middle and inferior turbinates in 14 patients with nasal polyps. The bars represent the mean values which are given above the bars.

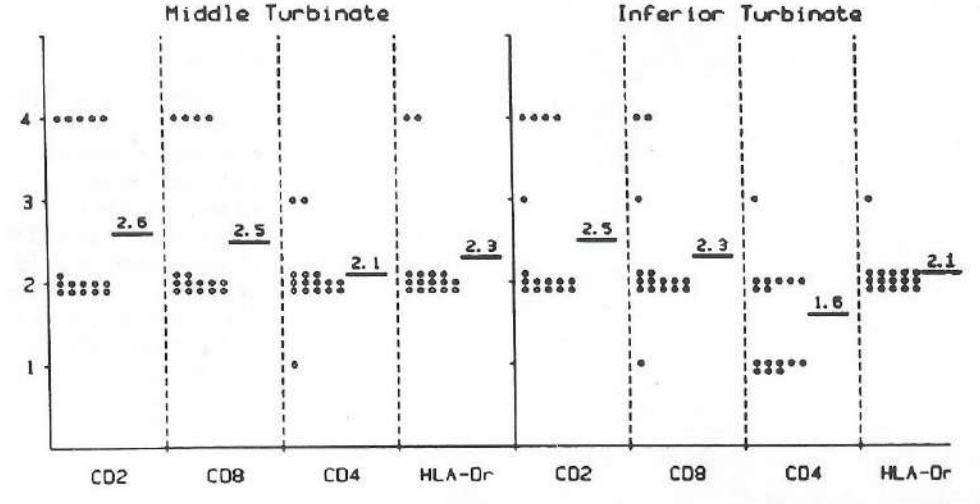

Fig. 2.

Quantification of CD2+, CD4+, CD8+ lymphocytes and HLA-Dr+ cells in the lamina propria of the middle and inferior turbinates in 16 healthy persons. The bars represent the mean values which are given above the bars.

turbinates ($p = 0.056$).

In the epithelium of the middle and inferior turbinates more CD8+ than CD4+ cells were detected in both groups, but their density in the epithelium was much lower than in the lamina propria. If the number of CD8+ and CD4+ cells in a specimen was high in the lamina propria, this was also observed in the epithelium.

More HLA-DR+ cells were found in the lamina propria and epithelium (also on epithelial cells) of the middle turbinates (Fig. 4) than in the inferior turbinates in patients ($p < 0.05$) and in healthy subjects (not significant).

CD19+ B cells were hardly found in the middle and inferior turbinates in both groups.

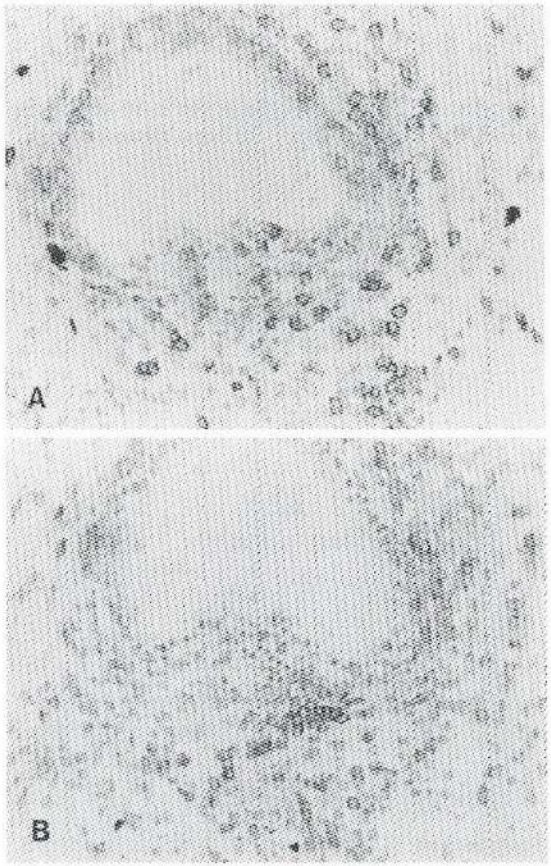

Fig. 3.

Cryostat sections of the nasal mucosa (middle turbinate) of patients with nasal polyps, stained for CD4 (T helper/inducer) and CD8 (T suppressor/cytotoxic) expression (x 200). More CD8+ cells (a), scored as 2 than CD4+ cells (b), scored as 1, were found.

In healthy persons varying but small numbers of eosinophils were detected in the lamina propria of the middle and inferior turbinates (scored as 0-1). Two of the lamina propria specimens of the middle turbinate of the patients were infiltrated with eosinophils (scored as 4), which were especially localized in the subepithelium and around the glands. These two patients had no IgE-mediated allergy. In the other patients eosinophils were scored as 0-1. Small numbers of neutrophils, mastcells and plasma cells (scored as 0-1) were found in the middle and inferior turbinates of patients and healthy subjects.

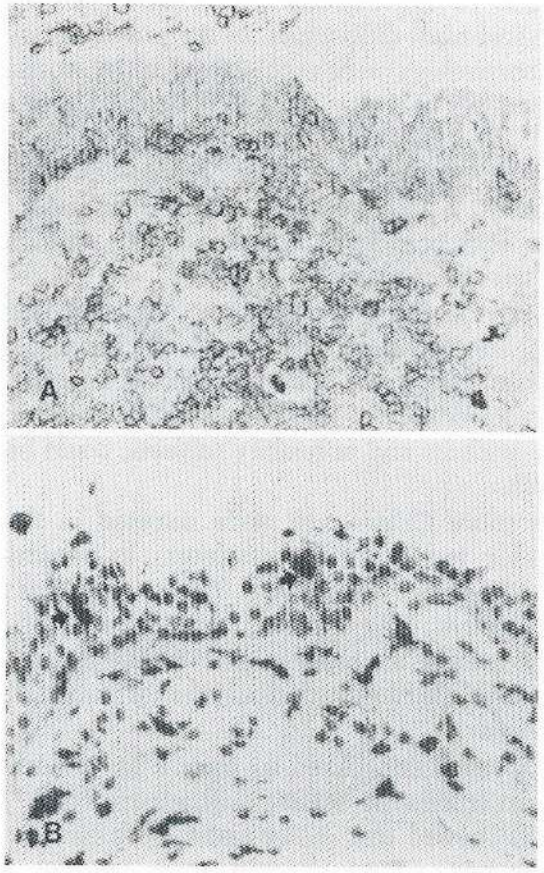

Fig. 4.

Cryostat sections of the nasal mucosa of patients with nasal polyps stained for HLA-Dr (x200). More HLA-Dr+ cells, scored as 4, were found in the middle turbinate (a) than in the inferior turbinate (b), scored as 2.

DISCUSSION

T lymphocytes and T cell factors play an important role in the regulation of the immune response and may be involved in the development of nasal polyps. Therefore, the nasal mucosa of patients with nasal polyps and of healthy subjects was investigated for its cellular composition. In the biopsies specimens, CD2+ (pan-T) cells occured in lower numbers than CD8+ and CD4+ T cells together. This could be in accordance with the findings of Ernst et al. (1987) who showed that intra-epithelial lymphocytes may lack the pan T cell marker, whereas these cells carry the CD8 marker (Hameleers et al. 1989). In the lamina propria of the middle and inferior turbinates of the patients and of the healthy subjects, CD8+ (T suppressor/cytotoxic) cells predominated over CD4+ (T helper/inducer) cells. However, Winther et al. (1987) found Leu-3+ (T helper/ inducer) cells to predominate over Leu-2+ (T suppressor/ cytotoxic) cells in the lamina propria of the inferior turbinate in healthy subjects. Their relatively high number of Leu-3+ cells may be due to the inclusion of CD4+ (Leu-3a) macrophages (Hume et al. 1987) or to technical differences. In the present study CD4+ cells with a macrophage-like morphology were excluded from the scores. Our results are in accordance with those of Nishimoto et al. (1988) who found a predominance of CD8+ cells over CD4+ cells in inflamed maxillary sinus mucosa. Also in the previous study by Hameleers et al. (1989) on intra-epithelial lymphocytes in the human nasal mucosa, a predominance of CD8+ cells over CD4+ cells was found in patients with nasal complaints, including some with an IgE-mediated hypersensitivity, and in healthy subjects.

The nasal mucosa is continuously exposed to antigenic and irritating agents. It is possible that CD8+ (T suppressor/cytotoxic) cells in the nasal mucosa are beneficial because of their suppressive effect on the induction of local inflammatory responses. The predominance of CD8+ over CD4+ cells in the middle and inferior turbinates, in patients and in healthy subjects, could be an expression of this suppressive benefit.

In patients and healthy subjects more CD4+ cells were detected in the middle turbinates than in the inferior turbinates. Furthermore, in healthy subjects, more CD4+ cells were detected in the middle and inferior turbinates compared with the numbers found in patients (Fig. 1 and 2). As a consequence of the relatively low number of CD4+ cells in the nasal mucosa of the patients an insufficiant humoral, resulting in chronic inflammation and the formation of nasal polyps immune response could occur.

Although CD4+ and CD8+ cells were considered to have a helper and suppressor function, respectively, recent studies by Takada & Engleman (1987) and Schrezenmeier & Fleischer (1988) showed that CD4 and CD8 molecules have more complex regulatory functions. CD4+ T cells may exercise a cytotoxic function (Rotteveel et al. 1988) and CD8+ T cells with cytotoxic or

contrasuppressor activity have been described. The latter cells appear to play an important role in the mucosal immune response (Green et al. 1982, Lehner et al. 1985, Lee et al. 1988). Further investigation of the functions of T cell subsets in normal and inflamed nasal mucosa is needed.

CD19+ B cells were hardly found in the middle and inferior turbinates in patients and in healthy subjects. Few to moderate numbers of plasma cells were detected in almost all specimens. These results are in accordance with the suggestion by Korsrud and Brandtzaeg (1981) that B cells are initially stimulated in the lymphoid tissue of the tonsils and migrate through lymph and blood into the respiratory mucosa where they differentiate into plasma cells. Nishimoto et al. (1988) found CD20+ B cells in the lamina propria of chronically inflamed maxillary sinus mucosa. The difference between their and our findings on B cells may be explained by the fact that CD20 is a stronger marker than CD19; moreover, CD20 is also found on dendritic cells (Zola 1987).

HLA-DR molecules can be found on macrophages (Hirschberg et al. 1976), on activated T cells (Metzger et al. 1979) and on epithelial cells (Selby et al. 1983, Lindahl et al. 1985). They play an important role in the immune regulation, since antigen is presented to T lymphocytes in combination with the HLA-DR antigen. In this study, HLA-DR+ cells were found in the lamina propria and epithelium (also on epithelial cells) in all specimens; most in the middle turbinates of the patients, which suggests locally increased immune reactivity. It should be remembered that nasal polyps originate from the ethmoid mucosa in the vicinity of the middle turbinate. HLA-DR+ gut epithelial cells were described to activate human CD8+ T cells (Mayer & Shlien 1987). The appearance of HLA-DR+ and CD8+ cells, in combination with a lower density of CD4+ cells, especially in the middle turbinate, could be of importance in the pathogenesis of nasal polyps. These parameters will be further studied in specimens of nasal polyps.

It is remarkable that, except in two patients, the number of eosinophils in the middle and inferior turbinates are similar in the patients and in the healthy subjects. This could be an indication that eosinophils are not a causal factor in the pathogenesis of nasal polyps. An IgE-mediated allergy was found in four of 14 patients only, whereas the two patients with an eosinophilic infiltration in the middle turbinate had no IgE-mediated allergy. These data indicate that an IgE-mediated allergy does not play a major role in the development of nasal polyps either.

References

Brandtzaeg P. (1984). Immune functions of human nasal mucosa and tonsils in health and disease. In: Bienenstock J, ed. Immunology of the lung and upper respiratory tract. New York: McGraw-Hill International Book Co, 1984: 28-95.

Ernst PB, Underdown BJ, Bienenstock J. (1987). Immunity in mucosal tissues. In: Stites DP, Stobo JD, Wells JV, eds. Basic and clinical immunology. Norwalk: Appleton and Lange, 1987: 159-66.

Green DR, Gold J, St. Martin S, Gershon R, Gershon RK. Microenvironmental immunoregulation: Possible role of contrasuppressor cells in maintaining immune responses in gut-associated lymphoid tissues. Proc Natl Acad Sci USA 1982; 79: 889-92.

Hameleers DMH, Stoop AE, van der Ven I, Biewenga J, van der Baan S, Sminia T. Intra-epithelial lymphocytes and non-lymphoid cells in the human nasal mucosa. Int Archs All appl Immunol 1989; 88: 317-22.

Hirschberg H, Kaakinen A, Thorsby E. Presence of HLA-DR determinants on human macrophages. Nature 1976; 263: 63-64.

Hume DA, Allan W, Hogan PG, Doe WF. Immunohistochemical characterization of macrophages in human liver and gastrointestinal tract: Expression of CD4, HLA-DR, OKM1, and mature macrophage marker 15F9 in normal and diseased tissue. J Leuk Biol 1987; 42: 474-84.

Korsrud FR, Brandtzaeg P. Immunohistochemical evaluation of J-chain expression by intra- and extra-follicular immunoglobulin producing human tonsillar cells. Scand J Immunol 1981; 13: 271-80.

Lee A, Sugerman H, Elson CO. Regulatory activity of the human CD8+ cell subset: a comparison of CD8+ cells from intestinal lamina propria and blood. Eur J Immunol 1988; 18: 21-27.

Lehner T, Avery J, Jones T. Separation and characterization of a subset of human T8+ cells which function as antigen-presenting and contrasuppressor cells. Immunology 1985; 54: 713-22.

Lindahl G, Hedfors E, Klareskog L, Forsum U. Epithelial HLA-DR expression and T lymphocyte subsets in salivary glands in Sjögren's syndrome. Clin Exp Immunol 1985; 61: 475-82.

Mayer L, Shlien R, Evidence for function of Ia molecules on gut epithelial cells in man. J Exp Med 1987; 166: 1471-83.

Metzgar RS, Bertoglio J, Anderson JK, Bonnard GD, Ruscetti FW. Detection of HLA-Drw (Ia-like) antigens on human T lymphocytes grown in tissue culture. J Immunol 1979; 122: 949-53.

Nishimoto K, Ukai K, Harada T, Chun Shun J, Sakakura Y. Lymphocyte subsets of maxillary mucosa in chronic inflammation. Acta Otolaryngol (Stockh) 1988; 106: 291-98.

Rotteveel FTM, Kokkelink I, van Lier RAW, Kuenen B, Meager A, Miedema F, Lucas CJ. Clonal analyses of functionally distinct human CD4+ T cell subsets. J Exp Med 1988; 168: 1659-73.

Schrezenmeier H, Fleischer B. A regulatory role for the CD4 and CD8 molecules in T cell activation. J Immunol 1988; 141: 398-403.

Selby WS, Janossy G, Mason DY, Jewell DP. Expression of HLA-DR antigens by colonic epithelium in inflammatory bowel disease. Clin Exp Immunol 1983; 53: 614-18.

Shaw S. Characterization of human leukocyte differentiation antigens. Imm Today 1987; 8: 1-3.

Takada S, Engleman EG. Evidence for an association between CD8 molecules and the T cell receptor complex on cytotoxic T cells. J Immunol 1987; 139: 3231-35.

Winther B, Innes DJ, Mills SE, Mygind N, Zito D, Hayden FG. Lymphocyte subsets in normal airway mucosa of the human nose. Arch Otolaryngol Head and Neck Surg 1987; 113: 59-62.

Zola H. The surface antigens of human B lymphocytes. Imm Today 1987; 8: 308-15.

Chapter 3

LYMPHOCYTES AND NONLYMPHOID CELLS IN HUMAN NASAL POLYPS

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SUMMARY

Immunohistochemical stainings were performed on polyp specimens of 48 patients and on mucosal biopsy specimens of the middle and inferior turbinates of 23 and 28 patients, respectively. Significantly more CD8+ (suppressor/ cytotoxic) than CD4+ (helper/ inducer) cells were found in the polyps. The number of CD2+, CD4+ and CD8+ lymphocytes in nasal polyps were very similar to the number in the macroscopically unaffected mucosa of the middle turbinates, whereas scores in the inferior turbinates were lower. In healthy subjects the differences of these lymphocytes were smaller. CD22+ B cells were detected in varying numbers in the polyps in more or less organized clusters. Significantly more HLA-DR+ cells were found in polyps and middle turbinates than in the inferior turbinates. Eosinophils were found in moderate to large numbers in polyps of 77% of the patients. Mast cells and plasma cells were detected in moderate numbers, whereas neutrophils were found in 35% of the patients. In the middle and inferior turbinates varying but small numbers of eosinophils, mast cells, plasma cells and neutrophils were found. In considering these findings, the role of chronic inflammation with T cell dependent disturbances is discussed with regard to the pathogenesis of nasal polyps.

INTRODUCTION

Inflammatory processes within the mucosa of the upper respiratory tract probably play an important role in the etiology and pathogenesis of nasal polyps (Slavin 1988).

Recent studies have indicated that some inflammatory diseases of the human respiratory mucosa may be associated with T lymphocyte dependent disturbances in peripheral blood (Drexhage et al. 1983, Knutsen et al. 1988) and also locally in the maxillary sinus mucosa (Nishimoto et al. 1988). T and B lymphocytes, which are regulatory and effector cells in the complex process of inflammatory responses (Ernst et al. 1987), are found in the nasal mucosa (Winther et al. 1987, Hameleers et al 1989, Stoop et al 1989). Also non-lymphoid cells, such as HLA-DR+ macrophages, could possibly play a role in immunoregulation (Brandtzaeg 1984) by antigen uptake and its presentation to T lymphocytes (Sertl et al. 1986). Moreover, HLA-DR+ epithelial cells, also present in the nasal mucosa (Hameleers et al. 1989, Stoop et al. 1989), may activate CD8+ T lymphocytes (Mayer & Shlien 1987). Granulocytes are involved in inflammatory reactions as phagocytic cells. In addition, they release inflammatory mediators from their granules and membrane-derived mediators (Leukotriens and platelet activating factor). The cytoplasmic granules of eosinophils contain strongly basic proteins, like major basic protein and eosinophil cationic protein. There is evidence that these proteins can damage the respiratory mucosa of the maxillary sinus (Harlin et al. 1988) and may cause the loss of bronchial epithelial cells in patients with astma (Gleich et al. 1988).

In a previous study, we described the distribution of lymphoid and nonlymphoid cells in the macroscopically unaffected mucosa of the middle and inferior turbinates of patients with nasal polyps and of healthy subjects (Stoop et al. 1989). The purpose of the present study was to compare the distribution of lymphocyte subpopulations and non-lymphoid cells in nasal polyps with the distribution in the macroscopically unaffected mucosa of the patients and healthy subjects.

MATERIAL and METHODS

Patients

In this study 48 patients were included (aged 15-92; mean age: 45 years). From these 48 patients polyp tissue was evaluated. From 23 of these patients biopsies of macroscopically unaffected mucosa of the middle and inferior turbinates were evaluated. From another five patients (in which the middle turbinate was no longer present because of previous surgery) the inferior turbinate was

evaluated. An IgE- mediated allergy for common inhalation antigens, that is, positive skin tests (diameter › 2 mm, Phazet, Pharmacia Diagnostics, Uppsala, Sweden) and/ or RAST-class ≥ 2 (0.35 PRU/ ml), was present in 25% of the patients. A chronic obstructive pulmonary disease (COPD, i.e. asthma/bronchitis and/or emphysema) was present in 29%, whereas 10% of the patients had an IgE mediated allergy and a COPD (Table 1). The clinical data of the patients, from which biopsies of the middle and inferior turbinates were taken (Table 1), demonstrate that the biopsy donors were representive of the whole group. The healthy subjects (n= 16, aged 17-50; mean age: 28 years) have been described previously (Stoop et al. 1989).

Table 1. Clinical data of the patients (n=48).

	Polyp tissue (n=48)	Eosinophil score ≥2 in the polyps	Middle turbinate tissue (n=23)	Inferior turbinate tissue (n=28)
COPD	14/48 (29%)	93%	7/23 (30%)	8/28 (29%)
IgE-mediated Allergy	12/48 (25%)	92%	6/23 (26%)	6/28 (21%)
COPD + IgE-mediated Allergy	5/48 (10%)	100%	4/23 (17%)	4/28 (14%)
No COPD or IgE-mediated Allergy	27/48 (56%)	67%	14/23 (61%)	18/28 (64%)

The percentages of patients concerned is given in parenthesis.

Tissue preparation

The polyps and tissue biopsies were frozen in liquid nitrogen immediately after resection. Frozen samples were stored at -70o until used. Cryostat sections of 6-8 µm were prepared (Stoop et al. 1989).

Immune reagents

The mouse monoclonal antibodies against human leukocytes used in this study are listed in table 2. The antibodies were appropriately diluted in 0.01 mol/L PBS (pH 7.4) containing 0.5% BSA, and used in an indirect immunoperoxidase staining. The conjugate used was horseradish peroxidase-labelled rabbit antimouse IgG (Dakopatts, Glostrup, Denmark). This was diluted 1:300 in PBS containing 0.5% BSA and 1% normal human serum.

Table 2. Mouse monoclonal antibodies against human leukocytes used in this study.

Antibody	manufacturer	subtype	specificity
anti-CD2	CLB	IgG1	peripheral T cells, 90% of the thymocytes
anti-CD4	Sanbio	IgG2	helper/inducer T cells and subpopulations of macrophages
anti-CD8	Sanbio	IgG2	suppressor/cytotoxic T cells
anti-CD22	CLB	IgG1	B cells
anti-HLA-DR	CLB	IgG1	cells of the monocyte lineage, myeloblasts, promyelocytes and cells of the B-lymphocyte lineage

[1] CLB: Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands.
Sanbio BV-biological products, Uden, The Netherlands.
Dakopatts, Glostrup, Denmark.

Staining procedures

The staining procedures were described previously (Stoop et al. 1989). In short: Sections were fixed in pure aceton for 10 minutes, if necessary treated with 0.23% HIO4 for 45 seconds to inactivate endogenous peroxidase (Kelly et al. 1987) and incubated with the appropriate antibodies. The slides were subsequently incubated with the conjugate and stained for peroxidase activity with 3,3,-di-aminobenzidine-tetrahydrochloride (Sigma, St.Louis, Mo, USA). The sections were washed between the incubations. Control sections were incubated with 0.5% BSA in PBS and with the conjugate. Tonsil sections were used as positive controls. For routine histological examinations standard Methylgreen-Pyronin, May-Grünwald/Giemsa and Haematoxilin-Eosin stainings were performed.

Evaluation

The sections were coded and evaluated independently by two persons with conventional light microscopy at lower and higher magnifications (x100, x200, x400 oil immersion). The number of stained cells in the sections was expressed as 0, no positive cells; 1, few positive cells; 2, moderate number of positive cells; 3, moderate number of single cells and some clusters of positive cells; 4, many positive cells, including cell clusters. The scores on the cryostat sections were related to cell density.

The data were statistically analysed by means of the two sided exact signed-rank test or by the two sided exact Wilcoxon rank-sum test.

RESULTS

General features

The cryostat sections showed that 35% of the polyps was mainly covered with a pseudostratified (ciliated) epithelium. Pseudostratified epithelium and stratified squamous epithelium was found in 59%, whereas in 6% of the polyps only stratified squamous epithelium was observed. Most polyps revealed an interstitium composed of highly edematous connective tissue with hardly any glands. In some polyps, cystic formations were observed in the stroma.

Lymphocytes and their subsets

CD2+ (pan T) lymphocytes were scattered throughout the stroma of the polyps in moderate to large numbers, although most lymphocytes were detected in the

subepithelium and around the glands. At both locations, small, more or less organized, clusters of lymphocytes were observed (Fig. 1). A significant predominance of CD8+ (suppressor/ cytotoxic) cells over CD4+ (helper/ inducer) cells was found ($p < 0.05$; Fig. 2 and 3). Few CD8+ and CD4+ cells were found in the epithelium, in which again CD8+ cells outnumbered the CD4+ cells.

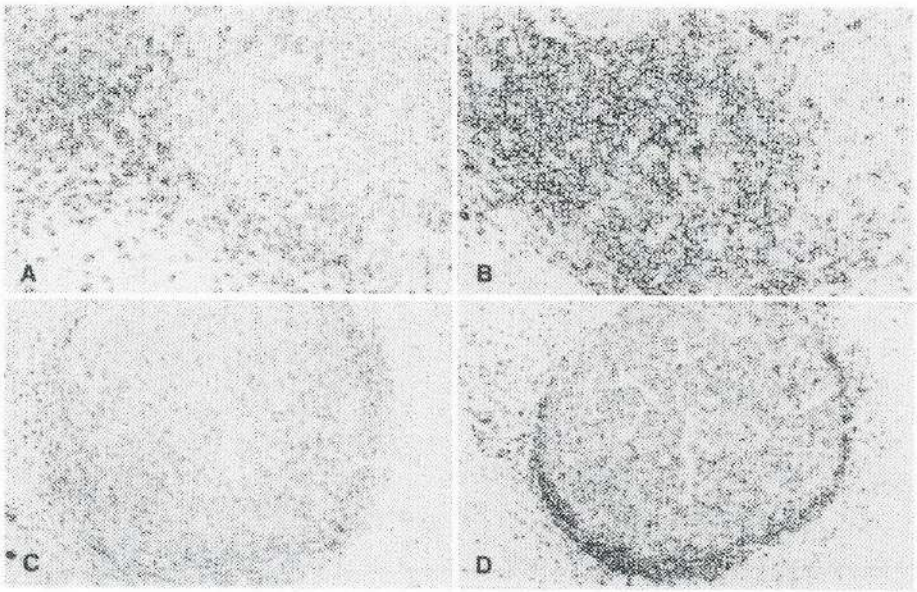

Fig. 1.

Cryostat sections of nasal polyps with a cluster (A,B) and a solitairy follicle (C,D) stained for CD2+ (A, C) and CD22+ (B,D) cells. Original magnification x100.

The numbers of lymphocytes in nasal polyps were very similar to numbers in the macroscopically unaffected mucosa of the middle turbinates, whereas scores in the mucosa of the inferior turbinates of the patients were significantly lower for CD8+ cells ($p < 0.05$) and lower for CD2+ and CD4+ cells (not significant; Fig. 2 and 3). The middle and inferior turbinates of healthy subjects contained significantly more CD4+ cells than the middle and inferior turbinates of the patients ($p < 0.05$).

CD19+ (early B cell marker) cells were hardly found in the stroma of the polyps, whereas CD22+ cells (wich is a stronger marker on mature B cells)

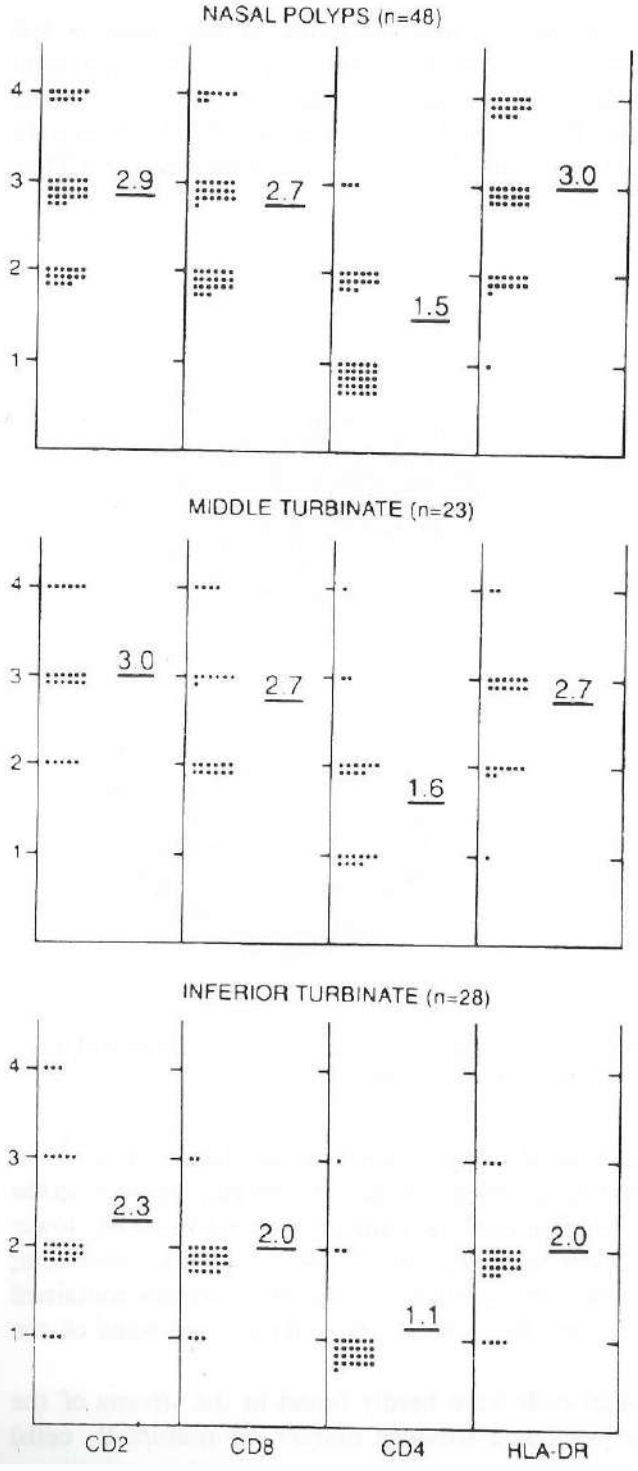

Fig. 2.

Quantification of CD2+, CD4+, CD8+ and HLA-DR+ cells in the stroma of the nasal polyps and the middle and inferior turbinates of the patients. The bars represent the mean values that are listed above the bars.

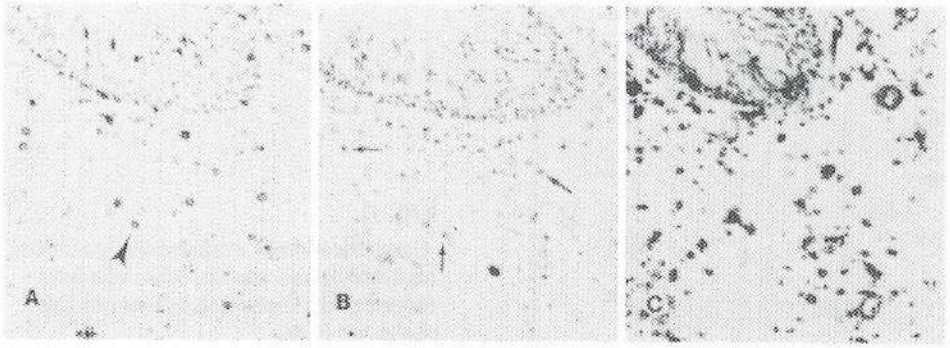

Fig. 3.

Cryostat sections of a nasal polyp stained for CD8+, CD4+ and HLA-DR+ cells. Original magnification 200x. More CD8+ cells (A, scored as 2, e.g. arrow head) than CD4+ cells (B, scored as 1, arrows) are found. HLA-DR+ cells (C) were scored as 3.

were seen in small to moderate numbers (mean score 1.7). Thirteen polyps contained large B cell clusters that resembled B cell follicles. One polyp contained a solitary B cell follicle surrounded by T cells (Fig.1). Small and varying numbers of CD22+ cells were detected in the middle and inferior turbinates of the patients. Plasma cells were found in varying numbers in the polyps and tissue biopsy specimens.

Nonlymphoid cells

Moderate to large numbers of HLA-DR+ cells were found in the epithelium and submucosa of the nasal polyps and in the mucosa of the middle turbinates. HLA-DR+ cells scored significantly lower in the mucosa of the inferior turbinates of the patients ($p < 0.05$; Fig. 2). The HLA-DR antigen was present on most epithelial cells and on part of the lymphocytes (B-lymphocytes and activated T cells) and nonlymphoid cells (e.g., activated macrophages).

Eosinophils were found in moderate to large numbers (score ≥2) in 77% of the polyps (Fig. 4). They were generally localized in the subepithelium and around the vessels and glands. Small clusters of eosinophils were seen at these locations. Eosinophils were scored ≥2 in 92% of the sections of patients with an IgE-mediated allergy and in 93% of the patients with COPD. In patients without an IgE mediated allergy or COPD eosinophils were scored ≥2 in 67% (Table 1).

Neutrophils were scarce in polyp tissue; they were found in 35% of the patients only. Mast cells were found in small to moderate numbers in all polyps (Fig. 4).

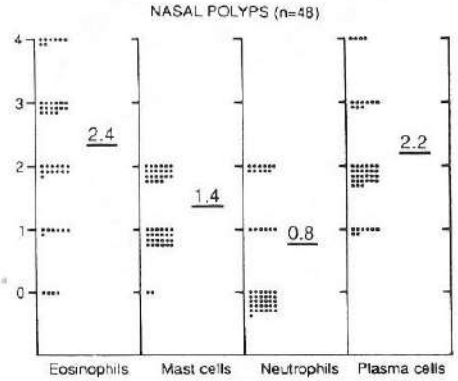

Fig. 4.

Qauntification of eosinophils, mast cells, neutrophils and plasma cells. The bars represent the mean values that are listed above the bars.

DISCUSSION

Infection and IgE-mediated allergy are thought to play a role in the pathogenesis of nasal polyps (Wilson 1976). However, in patients with a proved IgE-mediated allergy, nasal polyps are not more frequent than in patients without an IgE-mediated allergy (Caplin et al. 1971, Sakaguchi 1986). In this study, 25% of the patients had an IgE-mediated allergy. Recently Drake-Lee et al. (1988) reported that mast cells are more frequent in the submucosa than in the epithelium of nasal polyps, which also indicates that an IgE-mediated degranulation of mast cells is at least doubtfull as a causal factor. Presently, the role of infection and the subsequent inflammatory response, including release of cell mediators, is seen as the most likely cause (Slavin 1988). Therefore, the distribution of cells which participate in the inflammatory response was studied.

The similarity between the scores on the middle and inferior turbinates in our previous study (Stoop et al. 1989) and in the present study shows that the scoring method is reliable.

Significantly more CD8+ (suppressor/ cytotoxic) than CD4+ (helper/ inducer) cells were found especially in the polyps and the unaffected mucosa of the middle turbinates of the patients. It is possible that the high number of CD8+ cells could be beneficial because of their suppressive and downregulating effect on the (chronic) local inflammatory response, although the precise role of these cells is still a matter of controversy (Takada & Engleman 1987, Schrezenmeier & Fleischer 1988).

The relatively low number of CD4+ cells in polyps and in the unaffected middle turbinates of the patients, in combination with the high number of CD8+ cells in these tissues, could perhaps indirectly result in a less sufficient humoral immune response but is certainly evidence of a altered (T) cell mediated immune defence. The fact that significantly more CD4+ cells are found in healthy subjects than in patients with nasal polyps (Stoop et al. 1989)emphasizes this hypothesis.

Nasal polyps originate from the mucosa of the ethmoid and the middle turbinate only. In this respect it is interesting to note that the numbers of T lymphocytes in the polyps and in the macroscopically unaffected mucosa of the middle turbinates of the patients are very similar (Fig. 2). In addition, the difference between the polyps/ middle turbinates and the inferior turbinates of the patients and the difference between the polyps/ middle turbinates of the patients and the middle turbinates of the healthy subjects (Stoop et al. 1989), are an indication that the formation of nasal polyps is associated with local T cell dependent disturbances.

Polyp specimens of 13 patients contained clusters of B cells, and in one polyp, even a solitary follicle was observed. Such B cell structures have not been described in polyps previously and are indicative of a chronic inflammatory process.

This finding is in agreement with the abundant presence of HLA-DR+ cells in the stroma of nasal polyps and in the mucosa of the macroscopically unaffected middle turbinates, as well as in the epithelium of these tissues. HLA-DR+ cells possibly play a role in uptake and presentation of antigens. HLA-DR+ epithelial cells may also activate CD8+ cells, as shown by Mayer & Shlien (1987), although this study was performed on gut epithelial cells. This could suppress the chronic inflammatory reaction in the polyps and middle turbinates of the patients.

Mast cells, which were seen in almost all cryostat sections, release factors like eosinophil chemotactic factor of anaphylaxis and arachidonic acid metabolites. Indeed, Smith (1987) found that the 15-lipoxygenase activity of the arachidonic acid pathway is, at average, 30 times higher in nasal polyps than in normal nasal mucosa and in chronically inflamed sinus mucosa. In addition, eosinophil cemotactic factor is not only chemotactic but also induces activation of eosinophils (Tamura et al. 1988). Neutrophils were detected in relatively small numbers in the polyps of only 35% of the patients. Because neutrophils are especially associated with acute inflammatory reactions, this finding also indicates that nasal polyps are associated with chronic inflammatory processes.

The moderate to high infiltration of eosinophils (scores ≥2) in 77% of the nasal polyps is indicative of a chronic inflammation and is in accordance with the high prevalence of nasal polyps in patients with eosinophilic non-allergic rhinitis (nares, Mullarky 1988). Eosinophils and their mediators can damage the respiratory epithelium of the paranasal sinuses as shown by Harlin et al. (1988).

The same authors describe a significant association of sinus mucosa eosinophilia and asthma. De Monchy et al. (1985) found that eosinophils and their mediators are involved in the late asthmatic reaction. Moreover, eosinophils and their products may cause the loss of bronchial epithelial cells in asthmatic patients (Gleich et al. 1988). In the present study 13 of 14 patients with COPD had an eosinophil score ≥ 2 in their polyps. In the pathogenesis of COPD and nasal polyps, the local infiltration of eosinophils is probably caused by a common factor. Eosinophils were often found at the same sites as the lymphocytes. Data from in vivo (Keidan et al. 1985) and in vitro studies suggest that eosinopoetic factors are produced by the CD8+ cells (Chikkappa & Philips 1984) and CD4+ cells (Hirashima et al. 1986).

In conclusion, the data presented support the theory that the pathogenesis of nasal polyps is associated with chronic inflammation and T cell-dependent disturbances in specific sites of the (para)nasal mucosa.

References

Brandtzaeg P. Immune functions of human nasal mucosa and tonsils in health and disease. In: Bienenstock J, Ed. Immunology of the lung and upper respiratory tract. New York: McGraw-Hill International, 1984: 28-95.

Caplin I, Haynes JT, Spahn J. Are polyps an allergic phenomenon? Ann Allergy 1971; 29: 631-34.

Chikkappa G, Philips PG. Regulation of normal human blood neutrophilic, macrophagic and eosinophilic committed stem cells proliferation by autologous blood T lymphocytes subsets. Blood 1984; 63: 356-61.

Drake Lee AB, Chevreton E, Lowe D. The effect of different fixations on the distribution and numbers of mast cells in patients with nasal polyps. J Laryngol Otology 1988; 102: 1099-1101.

Drexhage HA, v d Plassche EM, Kokje M, Leezenberg HA. Abnormalities in cell mediated immune functions to haemophilus influenzae in chronic purulent infections of the upper respiratory tract. Clin Immunol Immunopath 1983; 28: 218-28.

Ernst PB, Underdown BJ, Bienenstock J. Immunity in mucosal tissues. In: Stites DP, Stobo JD, Wells JV, eds. Basic and clinical immunology. Norwalk: Appleton and Lange, 1987: 159-66.

Gleich GJ, Flavahan NA, Fujisawa T, Vanhoutte PM. The eosinophil as a mediator of damage to respiratory epithelium: A model for bronchial hyperreactivity. J Allergy Clin Immunlog 1988; 81/5 1: 776-81.

Hameleers DMH, Stoop AE, van der Ven , Biewenga J, van der Baan S, Sminia T. Intra-epithelial lymphocytes and non-lymphoid cells in the human nasal mucosa. Int Archs Allergy Appl Immunol 1989; 88: 317-22.

Harlin SL, Ansel DG, Lane SR, Myers J, Kephart GM, Gleich GJ. A clinical and pathologic study of chronic sinusites: The role of the eosinophil. J Allergy Clin Immunol 1988; 81: 867-75.

Hirashima M, Sakata K, Tashiro K, Ohmori J, Iyama K, Tsuda H, Nagai T, Hiraoka T, Kimura T. Spontaneous production of eosinophil chemotactic factors by T lymphocytes from patients with subcutaneous angeoblastic lymphoid hyperplasia with eosinophilia. Clin Imm Immunopathol 1986; 39(2): 231-41.

Keidan AJ, Catovsky D, Tavares De Castro J. Hypereosinophilic syndrome preceding T cell lymphoblastic lymphoma. Clin Lab Hematol 1984; 7: 83-88.

Kelly J, Whelan CA, Weir DG, Feighery C. Removal of endogenous peroxidase from cryostat sections for immunoperoxidase visualisation of monoclonal antibodies. J Immunol Methods 1987; 96: 127-32.

Knutsen AP, Slavin RG, Roodman ST, Mueller KR, Marino NL. Decreased T helper cell function in patients with cystic fibrosis. Int Arch Allergy Appl Immunol 1988; 85: 208-12.

Mayer L, Shlien R. Evidence for function of Ia molecules on gut epithelial cells in man. J Exp Med 1987; 166: 1471-83.

Monchy de JGR, Kauffman HF, Venge P, Koëter GH, Jansen HM, Sluiter HJ, Vries de K. Bronchoalveolair eosinophilia during allergen-induced late astmatic reactions. Am Rev Respir Dis 1985; 131: 373-76.

Mullarkey MF. Eosinophilic nonallergic rhinitis. J Allergy Clin Immunol 1988; 82: 941-49.

Nishimoto K, Ukai K, Harada T, Chun Shun J, Sakakura Y. Lymphocyte subsets of maxillary mucosa in chronic inflammation. Acta Otolaryngol (Stockh) 1988; 106: 291-98.

Sakaguchi K. Study of nasal surface basophilic cells in patients with nasal polyps. Acta Otolaryng (Stockh) 1986; Suppl 430: 28-33.

Schrezenmeier H, Fleischer B. A regulatory role for the CD4 and CD8 molecules in T cell activation. J Immunol 1988; 141: 398-403.

Sertl K, Takemura T, Tschachler E, Ferrans V, Kaliner M, Shevach E. Dendritic cells with antigen-presenting capability reside in airway epithelium, lung parenchyma and visceral pleura. J Exp Med 1986; 163: 436-51.

Slavin RG. Sinusitis in adults and its relation to allergic rhinitis, asthma and nasal polyps. J Allergy Clin Immunol 1988; 82: 950-56.

Smith DM. Comparison of arachidonic acid metabolism in nasal polyps and eosinophils. Int Arch Allergy Appl Immunol 1987; 82: 83-88.

Stoop AE, Hameleers DMH, van Run PEM, Biewenga J, van der Baan S. Lymphocytes and non-lymphoid cells in the nasal mucosa of patients with nasal polyps and of healthy persons. J Allergy Clin Immunol 1989; 84: 734-41. Takada S, Engleman EG. Evidence for an association between CD8 molecules and the T cell receptor complex on cytotoxic T cells. J Immunol 1987; 139: 3231-35.

Tamura N, Agrawal DK, Townley RG. Leukotriene C4 production from human eosinophils in vitro. Role of eosinophil chemotactic factors on eosinphil activation. J Immunol 1988; 141(12): 4291-97.

Wilson JA. Nasal polyps. Clin Otolaryngol 1976; 1: 4-6.

Winther B, Innes DJ, Mills SE, Mygind N, Zito D, Hayden FG. Lymphocyte subsets in normal airway mucosa of the human nose. Arch Otolaryngol Head and Neck Surg 1987; 113: 59-62.

Chapter 4

CLINICAL ASPECTS AND DISTRIBUTION OF IMMUNOLOGICALLY ACTIVE CELLS IN THE NASAL MUCOSA OF PATIENTS WITH NASAL POLYPS AFTER ENDOSCOPIC SINUS SURGERY AND TREATMENT WITH TOPICAL STEROIDS

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SUMMARY

Clinical parameters of 72 patients who were operated upon for nasal polyps were evaluated as well as biopsy specimens of the mucosa of the middle and inferior turbinates of 41 of these patients. Biopsies were taken at the time of endoscopic sinus surgery (ESS), after 6 months and in 23 of these patients after 1 year. During the follow-up period the patients were treated with topical corticosteroids (budesonide). At time of ESS significantly more CD8+ (suppressor/cytotoxic) cells than CD4+ (helper/inducer) cells were found in the middle and inferior turbinates. At 6 months significantly more CD4+ cells were found than at time of ESS, whereas at 1 year the number of CD4+ cells had decreased and was lower than at 6 months. These data support the theory that the occurrence of nasal polyps is associated with T cell dependent disturbances. Clinical evaluation revealed that most of the patients with chronic airway obstruction (CAO) had a better lungfuntion or used less lungmedication postoperatively. It is concluded that ESS combined with topical corticosteroids has a positive effect on the pathology in the upper and lower respiratory tract.

INTRODUCTION

The development of nasal polyps is related to inflammatory reactions in the nasal mucosa, although the exact etiology and pathogenesis are still elusive. Nasal polyps reccur frequently after surgery. Topical corticosteriods are considered to postpone c.q. prevent recurrences (Virolainen & Puhakka 1980, Drettner et al. 1982, Karlsson & Rundcrantz 1982, Dingsor et al. 1985, Hartwig et al. 1988) although the precise mechanisms involved remain incompletely understood (Siegel 1988).

T and B lymphocytes are regulatory and effector cells in the complex process of inflammatory responses (Ernst et al. 1987). These cells are found in the normal and inflamed nasal mucosa of patients with nasal polyps and healthy controls (Nishimoto et al. 1988, Stoop et al. 1989, 1991). Local changes in T cell numbers possibly result in an altered (T) cell mediated immune defence and may be related to the formation of nasal polyps. Also nonlymphoid cells, such as HLA-DR+ cells, possibly play a role in immunoregulation by antigen uptake and its presentation to lymphoid cells (Sertl et al. 1986).

The purpose of this study was to compare the distribution of lymphocytes and non-lymphoid cells in the macroscopically unaffected mucosa of the middle and inferior turbinates of patients with nasal polyps at time of endoscopic sinus surgery (ESS), after 6 months and 1 year. During the follow-up period the patients were treated with topical corticosteroids. Moreover, clinical parameters of the patients were evaluated.

MATERIAL and METHODS

Patients

In this study 72 patients (age 16-72 years; mean age, 44 years) who were operated upon for nasal polyps were clinically evaluated. From 41 of these patients biopsies were taken of the macroscopically unaffected mucosa of the middle and inferior turbinates at the time of endoscopic sinus surgery (ESS) and after 6 months. From 23 patients biopsies were also taken after 1 year. The clinical data (i.e. presence of chronic airway obstruction (CAO) and/ or an IgE-mediated allergy, extensive polyposis and recurrence rate) of the biopsy donors were representive for the whole group (Table 1). During the follow-up period all patients were treated with topical corticosteroids (budesonide 400 µg daily). Because the beneficial effect of corticosteroids in the treatment for nasal polyps is generally acknowledged, it was considered not ethical to study a control group not using topical corticosteroids. Extensive polyposis was seen in 44% of the patients. An IgE-mediated allergy for common inhalation allergens, i.e. positive

Table 1. Clinical data of the patients included in this study.

	Clinical data at the time of ESS	Recurrence rate after 6 months	Recurrence rate after 1 year
Patients:	n= 72.	36/72 (50%)	40/72 (56%)
Subgroups: Patients with:			
CAO	32 (44%)	16/32 (50%)	20/32 (63%)
IgE-mediated Allergy	20 (28%)	8/20 (40%)	13/20 (65%)
CAO + IgE-mediated Allergy	13 (18%)	6/13 (46%)	8/13 (62%)
Extensive Polyposis	32 (44%)	19/32 (59%)	22/32 (69%)
Extensive Polyposis + IgE-mediated Allergy	11 (15%)	7/11 (64%)	9/11 (82%)
Extensive Polyposis + CAO	17 (24%)	10/17 (59%)	11/17 (65%)
Extensive Polyposis + CAO + IgE-mediated Allergy	9 (13%)	5/9 (56%)	7/9 (78%)
No CAO, No IgE-mediated Allergy No extensive polyposis	19 (26%)	9/19 (47%)	13/19 (68%)

skintests (diameter › 2 mm, Phazet, Pharmacia Diagnostics Uppsala, Sweden) and/ or RAST class ›2 (0.35 PRU/ ml) was present in 28% of the patients. A chronic airway obstruction (CAO, i.e. asthma/ bronchitis and/or emphysema) was present in 44%, whereas 18% of the patients had an IgE mediated allergy *and* a CAO (Table 1). A lungfunction test was performed preoperatively and at 6 months on all the patients with CAO. On 20 of the CAO patients another lungfunction test was performed after 1 year. An aselective group of 19 patients *without* CAO underwent a lungfunction test at the time of ESS and at 6 months.

Ten of these patients underwent a lungfunction test at 1 year.

Tissue preparation and Immune reagents

Cryostat sections of 6-8 µm were prepared, incubated with mouse monoclonal antibodies (MAB) and stained as described previously (Stoop et al. 1991). The MAB against human leukocyte antigens used in this study are listed in table 2. Tonsil sections were used as positive controls. For routine histologic examination hematoxilin-eosin and toluidine blue stainings were performed.

Evaluation

The sections were evaluated with conventional light microscopy (100x, 200x, 400x oil immersion). The number of stained cells in the sections was expressed as 0: no positive cells, 1: few positive cells, 2: moderate number of positive cells, 3: moderate number of single cells and some clusters of positive cells, 4: many positive cells, including cell clusters. The scores on the cryostat sections were related to cell density and statistically analysed (exact Wilcoxon's signed rank test and Fisher's exact test).

Table 2. Mouse monoclonal antibodies against human leukocytes used in this study.

antibody	manufacturer[1]	subtype	specificity
anti-CD2	CLB	IgG1	all peripheral T cells, 90% of the thymocytes
anti-CD4	Sanbio	IgG2	helper/inducer T cells and subpopulations of macrophages
anti-CD8	Sanbio	IgG2	suppressor/cytotoxic T cells
anti-CD19	Dakopatts	IgG1	precursor and mature B cells (no plasma cells)
anti-CD22	CLB	IgG1	B cells
anti-HLA-DR	CLB	IgG1	cells of the monocyte lineage, myeloblasts, promyelocytes and cells of the B-lymphocyte lineage

[1] CLB: Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands.
Sanbio BV-biological products, Uden, The Netherlands.
Dakopatts, Glostrup, Denmark.

RESULTS

Lymphocytes and nonlymphoid cells

CD2+ (pan T) lymphocytes were found in moderate to large numbers in the lamina propria of the middle and inferior turbinates. At time of ESS a significant predominance of CD8+ (suppressor/cytotoxic) cells over CD4+ (helper/inducer) cells was found ($p < 0.01$) in the mucosa of the middle and inferior turbinates of the patients (Fig. 1). Moreover, higher scores of CD4+ and CD8+ cells were found in the middle than in the inferior turbinates. At 6 months significantly more CD4+ cells ($p < 0.01$) were found in the macroscopically unaffected mucosa of the middle and inferior turbinates than at time of ESS (Fig. 1,2). At 1 year more CD4+ cells were found in the middle and inferior turbinates than at the time of ESS (n.s.), but less than at 6 months (n.s.). No correlations were found between scores of CD4+ cells and a recurrence of nasal polyps, or between ratios of CD4+:CD8+ cells and a recurrence at 6 months and 1 year.

In the epithelium of the middle and inferior turbinates the ratios of CD4+ and CD8+ lymphocytes were the same as in the subepithelial stroma, but the densities of CD4+ and CD8+ cells were much lower in the epithelium than in the lamina propria.

CD22+ cells (B cells) and plasma cells were seen in small to moderate numbers in the lamina propria of the middle and inferior turbinates at time of ESS. No differences in number of CD22+ cells could be detected between biopsies taken at the time of ESS, at 6 months or 1 year.

Moderate to large numbers of HLA-DR+ cells were found in the epithelium and lamina propria of the middle turbinates whereas numbers in the inferior turbinates were significantly lower ($p < 0.01$). The HLA-DR antigen was present on part of the epithelial cells, lymphocytes and non-lymphoid cells. After 6 months and 1 year of treatment no significant changes in scores of HLA-DR+ cells were found.

Varying but small numbers of eosinophils and neutrophils were found in the middle and inferior turbinates at time of ESS, at 6 months and 1 year. No relationship was found between the numbers of eosinophils and recurrence rate of nasal polyps in the group of 41 patients.

Mast cells were found in varying numbers in the middle and inferior turbinates. No differences were detected between the time of ESS, 6 months and 1 year thereafter.

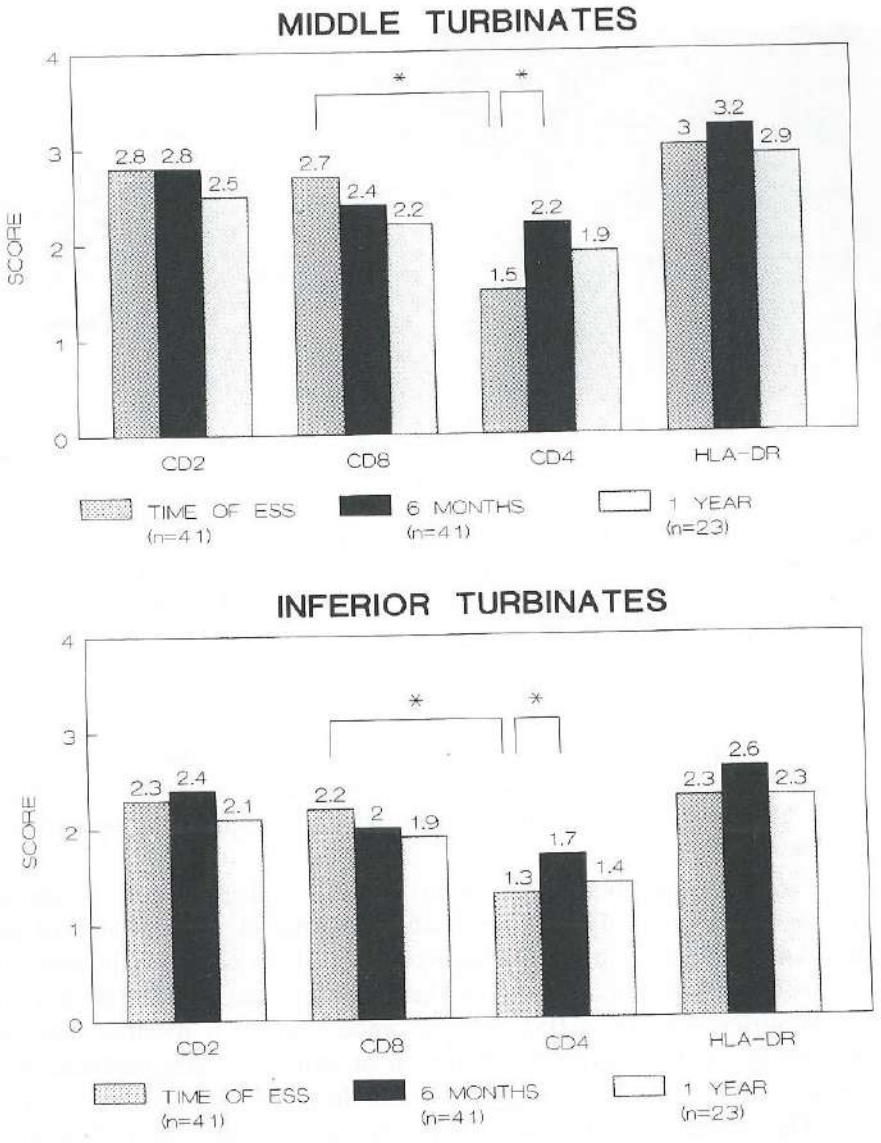

Fig 1.

Quantification of CD2+, CD4+, CD8+ and HLA-DR+ cells in the lamina propria of the middle and inferior turbinates at the time of ESS and at 6 and 12 months. The histograms represent the mean values of the scores. Significant differences ($p < 0.01$) between scores are indicated by *. No standard deviations are given because biopsies of single patients were compared at different moments.

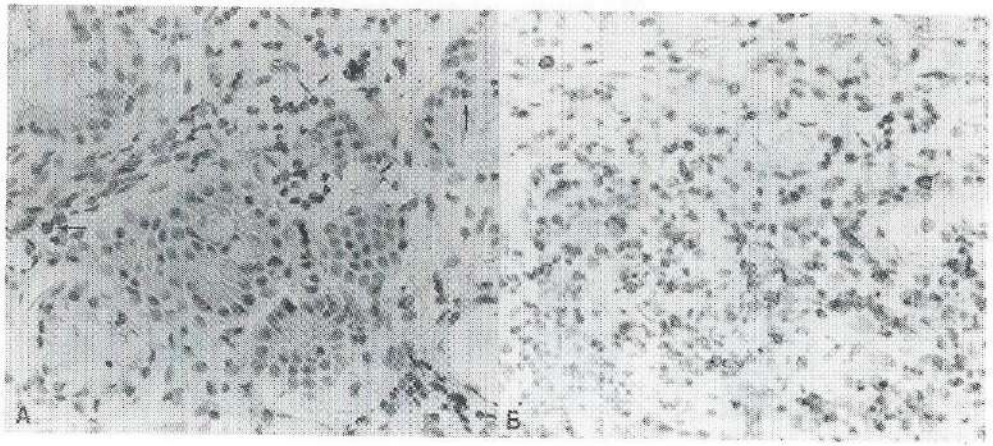

Fig 2.
Cryostat sections of CD4+ from a patient taken at the time of ESS and at 6 months (Original magnification x 200) showing less CD4+ (T helper/inducer) cells in the lamina propria of the middle turbinate at the time of ESS (A, scored as 1, arrows) than at 6 months (B, scored as 2).

Clinical features

The clinical data of the patients are given in table 1. Only in patients with extensive polyposis in combination with an IgE-mediated allergy a significantly ($p = 0.05$) higher recurrence rate was found at 1 year as compared to the restgroup.

Most of the patients (63%) with CAO had a better postoperative lungfuntion (increase of Forced Expiratory Volume (1 second) › 10%) and/or used less lungmedication after 6 months (Table 3). In contrast, in 84% of the patients without CAO no change of lungfunction was found at 6 months. Significant differences ($p < 0.05$) in recurrence rates at 6 months were found between patients with a better lungfunction or using less lungmedication (25% recurrence) and patients with a deteriorated lungfunction (100% recurrence, Table 3). The aforementioned two groups showed no significant differences in recurrence rate at 1 year.

Table 3. Follow-up of the patients on which lungfunction tests were performed pre-peratively and after 6 months and 1 year.

	Patients with CAO		Patiets without CAO	
Lungfunction:	6 Months (n=32)	1 Year (n=20)	6 Months (n=19)	1 Year (n=10)
Improved: Better lungfunction (and/ or less lung-medication)	20 (63%)	5 (25%)	2 (11%)	0
{recurrence rate}	5/20 (25%)*	2/5 (40%)	1/2 (50%)	0
No change of lung-function (and medication)	7 (22%)	11 (55%)	16 (84%)	9 (90%)
{recurrence rate}	3/7 (43%)	7/11 (64%)	9/16 (56%)	6/9 (67%)
Deteriorated: Decrease of lungfunction	5 (16%)	4 (20%)	1 (5%)	1 (10%)
{recurrence rate}	5/5 (100%)*	4/4 (100%)	1/1 (100%)	1/1 (100%)

* A significant difference ($p < 0.05$) was found between these subgroups.

DISCUSSION

Although corticosteroids may cause a redistribution of lymphocytes (Fauci & Dale 1975), so far little attention has been payed to the influence of these drugs on the distribution of lymphocytes and their subsets in the upper respiratory tract. Therefore, we investigated the cellular distribution in the macroscopically unaffected mucosa of the middle and inferior turbinates of patients with nasal polyps at time of ESS and after follow-up periods of 6 months and 1 year, during which time the patients were treated with topical corticosteroids.

The relatively low numbers of CD4+ (helper/inducer) cells in the middle turbinates and nasal polyps (Stoop et al. 1991) at the time of ESS suggest a less sufficient immune response against infectious agents, resulting in a chronic inflammatory reaction. The increase in numbers of CD4+ cells during the 6 months after ESS, especially pronounced in the middle turbinates, could result in a more effective immune response in the nasal mucosa. At 6 months the

same ratios of CD4+: CD8+ cells were found in healthy subjects (Stoop et al. 1989). Inflammatory responses may become more adequate and consequently a recurrence of nasal polyps may be prevented or postponed. However, less CD4+ cells were found at 1 year than at 6 months. No correlation could be found between scores of CD4+ cells and recurrence rate at 6 months and 1 year. These data suggest that, although there seems to be an association between the numbers of CD4+ lymphocytes and the presence of nasal polyps, the pathogenesis of nasal polyps is more complex. Also, in the literature the functional role of CD4+ and CD8+ cells remains controversy (Lehner et al. 1985, Lee et al. 1988, Rotteveel et al. 1988, Schrezenmeier & Fleischer 1988).

HLA-DR+ cells probably play a role in the uptake and presentation of antigens. The abundant presence of HLA-DR+ cells, especially in the middle turbinates, is an indication of an active inflammatory reaction in the vicinity of the ethmoid complex where nasal polyps originate from. After ESS and treatment with topical steroids, however, no significant changes in scores of HLA-DR+ cells were detected. On the other hand, HLA-DR+ cells may activate CD8+ cells, as shown in gut (Mayer & Shlien 1987), and thereby suppress the chronic inflammatory reaction, especially in the middle turbinates.

Clinical evaluation at 6 months and 1 year revealed a recurrence of nasal polyps in 50% and 56% of the patients respectively (Table 1). These percentages are slightly higher than those mentioned by Virolainen et al. (1980). Patients with CAO had a recurrence rate of 50% and 56% at 6 months and 1 year, respectively. This is in accordance with data from Vleming et al. (in press), whereas Drake-Lee et al. (1984) diagnosed a recurrence rate for nasal polyps of only 24.5% in patients with asthma. Only patients with extensive polyposis, in combination with an IgE-mediated allergy, have a higher risk of a recurrence of nasal polyps at 1 year (Table 1).

An association of nasal and paranasal sinus diseases with bronchial asthma has been described (Slavin 1982, Racnelefsky et al. 1984, Mings et al. 1988) Slavin et al. (1983) described a subjective improvement of the asthmatic state in 66% of patients from whom nasal polyps were surgically removed. Hosemann et al. (1990) found an improvement in the lungfunction and/ or in the use of less lungmedication in 77% of the patients with asthma who underwent ESS for chronic sinusitis. In our study a better postoperative lungfunction, or the use of less medication was seen in 63% and 25% of the patients with CAO at 6 months and 1 year, respectively (Table 3). Only 25% and 40% of these patients had a recurrence of nasal polyps at 6 months and 1 year, respectively, whereas *all* the patients with a deteriorated lungfunction had a recurrence at 6 months and 1 year. These differences, which are significant at 6 months, are indicative of the relationship between the upper and lower respiratory tracts and show that ESS and the use of topical steroids in the upper respiratory tract have a beneficial effect on the lower respiratory tract.

In conclusion, the data presented show that the occurrence of nasal polyps is probably associated with T cell dependent disturbances, as shown by the low numbers of CD4+ cells in sites of the (para)nasal mucosa where nasal polyps originate from. After ESS and treatment with topical corticosteroids the numbers of CD4+ cells increase, at least initially. Furthermore, a positive effect on the upper and lower respiratory tract is found after ESS and treatment with topical corticosteroids.

References

Dingsor G,Kramer J, Olsholt R, Soderstrom T. Flusonide nasal spray 0.025% in the prophylactic treatment of nasal polypsis after polypectomy. Rhinology 1985; 23: 49-59.

Drake-Lee AB, Lowe D, Swanston A, Grace A. Clinical profile and recurrence of nasal polyps. J Laryngol Otolog 1984; 98: 783-93.

Drettner B, Ebbesen A, Nilsson M. Prophylactive treatment with flusonide after polypectomy. Rhinology 1982; 20: 149-58.

Ernst PB, Underdown BJ, Bienenstock J. Immunity in mucosal tissues. In: Stites DP, Stobo JD, Wells JV, eds. Basic and clinical immunology. East norwalk, Conn.: Applrton / Lange, 1987: 159-66.

Fauci AS, Dale DC. The effect of hydrocortisone on the kinetics of normal human lymphocytes. Blood 1975; 46: 235-43.

Hartwig S, Linden M, Laurent C, Varko AK, Lindqvist N. Budesonide nasal spray as prophylactic treatment after polypectomy. J Laryngol Otolog 1988; 102: 148-51.

Hosemann W, Michelson A, Weindler J, Mang H, Wigand ME. Einfluss der endonasalen Nasennebenhohlenchirurgie auf die Lungenfunction des Patienten mit Asthma bronchiale. Laryngo-Rhino-Otol. 1990; 69: 521-26.

Jantti-Alanko S, Holopainen E, Malmberg H. Recurrence of nasal polyps after surgical treatment. Rhinology 1989; Suppl 8: 59-64.

Karlsson G, Rundcrantz H. A randomized trial of intranasal beclomethasone dipropionate after polypectomy. Rhinology 1982; 20: 144-48.

Lee A, Sugerman H, Elson CO. Regulatory activity of the human CD8+ cell subset: a comparison of CD8+ cells from intestinal lamina propria and blood. Eur J Immunol 1988; 18: 21-27.

Lehner T, Avery J, Jones T. Separation and characterization of a subset of human T8+ cells which function as antigen-presenting and contrasuppressor cells. Immunology 1985; 54: 713-22.

Mayer L, Shlien R. Evidence for function of Ia molecules on gut epithelial gut cells in man. J Exp Med 1987; 166: 1471-83.

Mings R, FRiedman WH, Linford PA, Slavin RG. Five-year follow-up of the effects of bilateral intranasal sphenoethmoidectomy in patients with sinusitis and asthma. Am J Rhinology 1988; 2: 13-16.

Nishimoto K, Ukai, Chun Shun J, Sakakura Y. Lymphocyte subsets of maxillary mucosa in chronic inflammation. Acta Otolaryngol (Stockh) 1988; 106: 291-98.

Rachelefsky GS, Katz RM, Siegel SC. Chronic sinus diseasewith associated airway disease in children. Pediatrics 1984; 73: 526-29.

Rotteveel FTM, Kokkelink I, van Lier RAW, Kuenen B, Meager A, Miedema F, Lucas CJ. Clonal analyses of functionally distinct human CD4+ T cell subsets. J Exp Med 1988; 168: 1659-73.

Schrezenmeier H, Fleischer B. A regulatory role for the CD4 and CD8 molecules in T cell activation. J Immunol 1988; 141: 398-403.

Sertl K, Takemura T, Tschachler E, Ferrans V, Kaliner M, Shevach E. Dendritic cells with antigen presenting capability reside in airway epithelium, lung parenchyma, and viscveral pleura. J Exp Med 1986; 163: 436-51.

Siegel SC. topical intranasal corticosteroid therapy in rhinitis. J All Clin Immunol 1988; 81: 984-91.

Slavin RG. Relationship of nasal disease and sinusitis to bronchial asthma. Ann Allergy 1982; 49: 76-79.

Slavin RG, Linford PA, Friedman WH. Bilateral intranasal sphenoethmoidectomy in the treatment of nasal polyps, sinusitis and bronchial asthma. J Allergy Clin Immunol 1983; 71: 156.

Stoop AE, Hameleers DMH, van Run P, Biewenga J, van der Baan S. Lymphocytes and nonlymphoid cells in the nasal mucosa of patients with nasal polyps and of healthy subjects. J Allergy Clin Immunol 1989; 84: 734-41.

Stoop AE, van der Heyden HAMD, Biewenga J, van der Baan S. Lymphocytes and nonlymphoid cells in nasal polyps. J Allergy Clin Immunol 1991; 87: 470-75.

Virolainen E, Puhakka H. The effect of intranasal beclomethasone dipropionate on the recurrence of nasal polyps after ethmoidectomy. Rhinology 1980; 18: 9-18.

Vleming M, Stoop AE, Middelweerd MJ, de Vries N. Results of Endoscopic Sinus Surgery for Nasal Polyps. Am J Rhinol 1992; (in press).

Chapter 5

EOSINOPHILS IN NASAL POLYPS AND NASAL MUCOSA: AN IMMUNOHISTOCHEMICAL STUDY

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(submitted for publication)

SUMMARY

Immunohistochemical stainings were performed on nasal polyps and biopsy specimens of the macroscopically unaffected mucosa of the middle and inferior turbinates of patients with nasal polyps at the time of endoscopic sinus surgery (ESS), after 6 months, and after 1 year. During the follow-up period the 46 patients were treated with topical corticosteroids. At time of ESS significantly more BMK13+, EG1+ and EG2+ eosinophils were found in the polyps than in the macroscopically unaffected mucosa of the middle and inferior turbinates of the patients. Moreover, significantly more BMK13+, EG1+ and EG2+ eosinophils were found in the mucosa of the middle turbinates than in the inferior turbinates of the patients. The inferior turbinates of the patients contained similar numbers of BMK13+ and EG1+ eosinophils as the middle and inferior turbinates of 10 healthy subjects, but no EG2+ eosinophils were detected in the biopsies of the healthy subjects. Six months after ESS and treatment with topical corticosteroids, recurrences of polyps and the macroscopically unaffected mucosa of the middle and inferior turbinates of the patients contained lower numbers of BMK13+, EG1+ and especially of EG2+ eosinophils than at time of ESS. After 1 year, slightly lower numbers of BMK13+, EG1+ and EG2+ eosinophils were found in recurrences of nasal polyps than in recurrences after 6 months. No more significant differences in scores were found between the macroscopically unaffected mucosa of the middle and inferior turbinates and recurrences of nasal polyps after 1 year. The reduction of eosinophil infiltration and activity in nasal polyps and nasal mucosa after ESS and treatment with topical corticosteroids is probably of importance to postpone or prevent recurrences of nasal polyps.

INTRODUCTION

Nasal polyps originate from the respiratory mucosa of the ethmoid and middle turbinate only. Chronic inflammation at these specific sites of the nasal mucosa probably plays an important role in the development of nasal polyps (Stoop et al. 1989, 1991). Several inflammatory diseases of the respiratory mucosa, like eosinophilic nonallergic rhinitis (ENR) and asthma, are associated with increased numbers of eosinophilic leukocytes in tissues with or without blood eosinophilia (Weller 1984). In nasal polyps a moderate to high infiltration of eosinophils is found (Ogawa 1986, Stoop et al. 1989). In patients with an aspirin intolerance and nasal polyps, a marked infiltration of the polyps with eosinophils is always found (Ogino & Harada 1986).

On activation, eosinophils release inflammatory products from their granules, e.g. major basic protein (MBP), eosinophilic cationic protein (ECP) and eosinophil peroxidase (EPO), as well as leucotrienes and platelet activating factor (PAF). When released, these products damage the respiratory epithelium of the upper and lower respiratory tract (Gleich et al. 1988, Harlin et al. 1988).

Eosinophils exist in different densities: from normodense to hypodense. Hypodense eosinophils are probably activated and more toxic. They contain significantly less MBP and have smaller granula than normodense eosinophils which may explain the hypodensity (Peters et al. 1988). Moreover, hypodense eosinophils have a significantly greater chemotactic response to PAF (Fukuda & Gleich 1989) and have a higher oxygen consumption than normodense eosinophils (Winqvist et al. 1982).

The treatment of nasal polyps, surgically and/or with medicins, forms a dilemma because of the high recurrence rate. Several authors have demonstrated that the use of topical corticosteroids may prevent or postpone a recurrence of nasal polyps (Virolainen & Puhakka 1980, Drettner et al. 1982, Karlsson & Rundcrantz 1982, Dingsor et al. 1985, Hartwig et al. 1988). Corticosteroids have anti-inflammatory properties, they reduce the edema and the influx of inflammatory cells in the respiratory mucosa (Lundgren et al. 1988, Siegel 1988). Human eosinophils have glucocorticoid receptors (Peterson et al. 1981). Glucocorticosteroids may exert an inhibitory effect on eosinophil survival (Lamas et al. 1990, Wallen et al. 1991), which results in a shorter exposition of the mucosa to eosinophils and probably also in a reduction of numbers of eosinophils in the tissues. The beneficial effect of corticosteroids may not be due to a direct effect on eosinophil degranulation because no difference in inhibition of degranulation by corticosteroids was found between normodense and hypodense eosinophils (Kita et al. 1991).

The aim of this study was to investigate the effect of endoscopic sinus surgery (ESS) and treatment with topical corticosteroids on the distribution of activated and non-activated eosinophils in nasal polyps and the macroscopically

unaffected mucosa of the middle and inferior turbinates of patients with nasal polyps at the time of surgery, and after a follow-up period of 6 months and 1 year. For control the mucosa of the middle and inferior turbinates of 10 healthy subjects was investigated.

MATERIAL and METHODS

Patients

Forty six patients (age 15-74 years; mean age 44 years), who were operated upon for nasal polyps, were evaluated. None of the patients used topical corticosteroids preoperatively. A chronic airway obstruction (CAO, i.e. asthma/ bronchitis and/ or emphysema) was present in 46% of the patients, whereas an IgE-mediated allergy for inhalation allergens, i.e. positive skin tests (diameter › 2 mm, Phazet, Pharmacia Diagnostics Uppsala, Sweden) and/ or RAST class › 2 (0.35 PRU/ ml) was found in 35% of the patients. In 63% of the patients an elevated blood eosinophil count was found (table 1).

Polyp tissue, taken at the time of ESS and after 6 months and 1 year in case of recurrences, was evaluated by light microscopy. From 16 patients initial polyp tissue was evaluated by electron microscopy. From 37 and 44 patients respectively, biopsies of the macroscopically unaffected mucosa of the middle and inferior turbinates were taken under endoscopic view at the time of ESS. During the follow-up period all patients were treated with topical corticosteroids (budesonide 400 µg daily). Because the beneficial effect of corticosteroids in the treatment for nasal polyps is generally acknowledged, we found it not ethical to study a control group without topical corticosteroid treatment. For clinical follow-up the patients underwent a nose endoscopy 6 months and 1 year after ESS. Twenty three patients (50%) developed a recurrence after 6 months. After a follow-up period of 6 months biopsies of the macroscopically unaffected middle and inferior turbinates were taken from 37 and 42 patients respectively. Two patients did not accept another biopsy from the inferior turbinate.

After 1 year follow-up 28 patients could be evaluated. The rest of the initial 46 patients did not accept another biopsy or stopped the treatment with topical corticosteroids because they did well. Fifteen of the 28 patients (54%) developed a recurrence after 1 year. Biopsies of the macroscopically unaffected middle and inferior turbinates from 19 and 21 patients respectively could be evaluated. Unless the limited number of biopsy donors after 1 year, which could imply a certain selection, the percentages of the patients with an IgE-mediated allergy and/ or CAO were similar as in the original group.

From 10 healthy subjects (age 17-45 years, mean age 27 years) biopsies were taken from the middle and inferior turbinates. These subjects had no IgE-

Table 1. Clinical data of the patients included in this study.

	Clinical data at the time of surgery. (46 patients)	Recurrence rate after 6 months (Follow-up: 46 patients)	Recurrence rate after 1 year (Follow-up: 28 patients)
Patients	n= 46	50% (23/46)	54% (15/28*)
Clinical subgroups:			
Patients with:			
IgE-mediated allergy	35% (16/46)	63% (10/16)	44% (4/9)
CAO	46% (21/46)	57% (12/21)	58% (7/12)
Elevated blood eosinophils	63% (29/46)	59% (17/29)	60% (9/15)
CAO + Elevated blood eosinophils	81% (17/21)	59% (10/17)	50% (4/8)

* After 1 year of follow-up only 28 patients could be evalated.

Table 2. Monoclonal antibodies used in this study.

Antibody	specificity
anti-BMK13	pan-eosinophil marker, binds to Major Basic Protein (MBP) in resting and activated eosinophils
anti-EG1	stains storage and secreted forms of Eosinophil Cationic Protein (ECP)
anti-EG2	stains the secreted form of ECP, hence it stains activated eosinophils

Source: Sanbio, BV-biological products, Uden, The Netherlands.

mediated allergy nor nasal complaints. They were nonsmokers and had a normal ear-, nose- and throat examination. None of the subjects had suffered from a common cold at least 6 weeks previous to the moment the biopsy was obtained.

Tissue preparation and Immune reagents

The tissue biopsies were frozen in liquid nitrogen immediately after resection. Frozen samples were stored at -70°C until used. Cryostat sections of 6-8 µm were prepared, incubated with mouse monoclonal antibodies (MAB) and stained as described previously (Stoop et al. 1989). The MAB against human leukocyte antigens used in this study are listed in Table 2. The antibodies were appropriately diluted in 0.01M phosphate buffered saline (PBS), pH 7.4, containing 0.5% BSA, and used in an indirect immunoperoxidase staining. The conjugate used was horseradish peroxidase labeled rabbit anti-mouse IgG (Dakopatts, Glostrup, Denmark). This was diluted 1:300 in PBS containing 0.5% BSA and 1% normal human serum. Tonsil sections were used as positive controls. Controls for nonspecific staining were incubated with 0.5% BSA in PBS or with the second stage conjugate only. For routine histologic examination standard hematoxilin-eosin stainings were performed.

For electron microscopy the biopsies were immediately immersed in 1.5% glutaraldehyde in 0.1 M Sorensen buffer adjusted to pH 7.6. After 24 hours the tissue fragments were washed and postfixated in 1% O_3O_4, dehydrated in ethanol and embedded in Epon. Semi-thin sections were stained with toluidine blue. These sections were used for selection of areas with eosinophils. The selected areas were than trimmed for ultramicrotomy. Ultrathin sections were contrasted with 5% uranyl acetate and lead citrate before examination in a Philips EM.

Evaluation

The sections were coded and evaluated by two persons independently with conventional light microscopy (x100, x200, x400 oil immersion). The number of stained cells in the sections was expressed as 0, no positive cells; 1, few positive cells; 2, moderate number of positive cells; 3, moderate number of single cells with clusters of positive cells; 4, many positive cells, including cell clusters. The scores on the cryostat sections were related to the total density of cells.

The scores were compared intra-individually at different moments and statistically analysed by means of the exact Wilcoxon-s signed rank test or the McNemar-s test. To compare percentages between different clinical subgroups the Fisher-s exact test was used.

RESULTS

At time of ESS, BMK13+ and EG1+ eosinophils were found in moderate to large numbers in the polyps (mean scores 2.7 and 2.5 respectively, Fig. 1). The eosinophils were generally localized in the subepithelium and around the vessels and glands. Most of the MBK13+ and EG1+ eosinophils were also EG2+ (mean score 2.1, Fig. 1 and 2). Electron microscopic examination demonstrated that at time of ESS almost all eosinophils in the polyps were hypodense with small granules and a smaller granule area than in normodense eosinophils (Fig. 3). Moreover, partial lucency of the granule matrix, which is an indication for degranulation of the granules, was a consistent finding in most of the eosinophils.

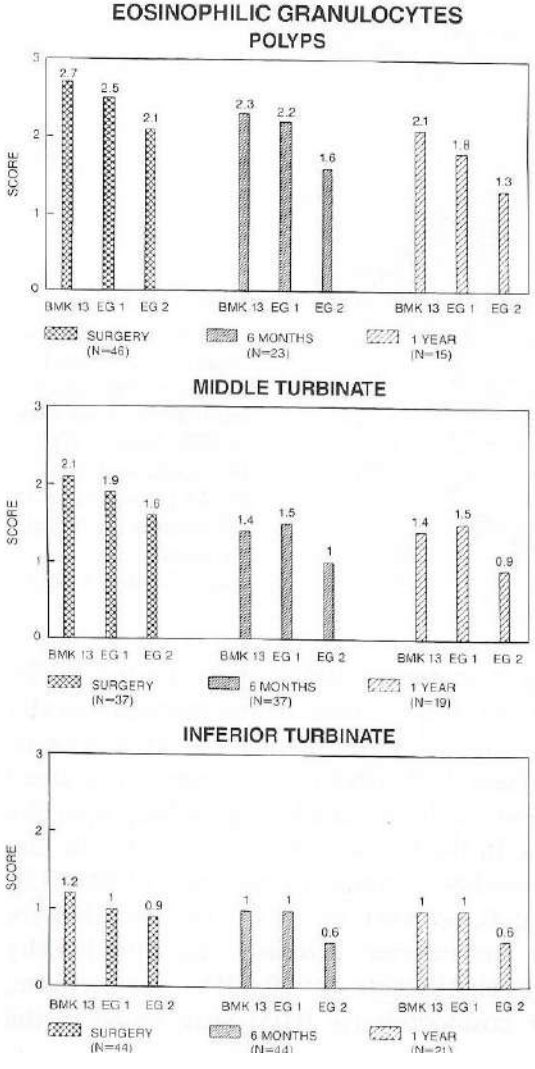

Fig. 1.

Quantification of BMK13+, EG1+ and EG2+ eosinophils in nasal polyps and the lamina propria of the middle and inferior turbinates at the time of surgery and after 6 and 12 months. The histograms represent the the mean values of the scores. No standard deviatons are given because biopsies of single patients are compared at different moments.

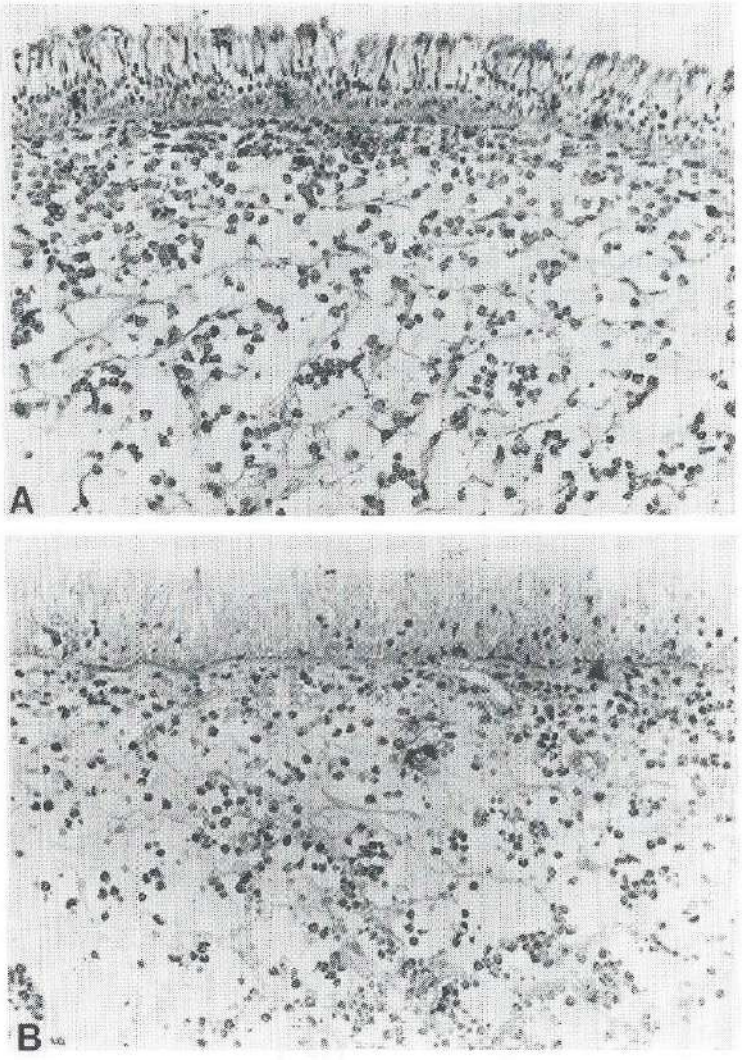

Fig. 2.

Cryostat sections of a nasal polyp stained for EG1+ and EG2+ eosinophils at the time of ESS. Many EG1+ (A, scored as 4) and EG2+ (B, scored as 3) eosinophils are found. (Original magnification x200.)

At time of ESS, significantly higher scores of BMK13+, EG1+ and EG2+ eosinophils ($p < 0.001$) were found in the polyps than in the macroscopically unaffected mucosa of the middle and inferior turbinates of the same patients (Fig. 1). The lamina propria of the middle turbinates of the patients contained significantly more BMK13+, EG1+ and EG2+ eosinophils ($p < 0.001$) than the inferior turbinates of the same patients. In the lamina propria of the middle and inferior turbinates of the healthy subjects low to moderate numbers of BMK13+ and EG1+ eosinophils were found (Fig. 4). however, no EG2+ eosinophils were detected in the mucosa of the middle and inferior turbinates from the healthy subjects. This difference is highly significant ($p < 0.001$). Furthermore, significantly less BMK13+ and EG1+ eosinophils ($p < 0.05$) were found in the

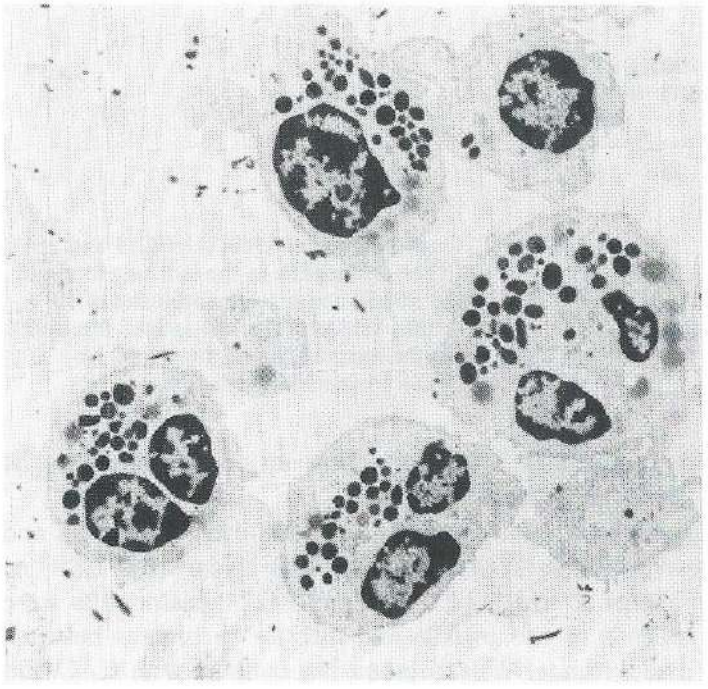

Fig. 3.

Morphology of nasal polyp eosinophils by electron microscopic examination (x7200). Nearly all the eosinophils are hypodense with small granules and a smaller granule area than in normodense eosinophils. Note the partial lucency of the granule matrix, which is a consistent finding in most of the eosinophils and is an indication for degranulation of the granules.

middle turbinates of the healthy subjects than in the middle turbinates of the patients at time of ESS. The scores for BMK13+ and EG1+ eosinophils in the inferior turbinates of patients and healthy subjects were not different, nor were the eosinophil scores different between the middle and inferior turbinates of the healthy subjects.

If polyps recurred after 6 months, lower scores of BMK13+ ($p < 0.05$), EG1+ (n.s.) and EG2+ ($p < 0.001$) eosinophils were found than at time of ESS (Fig. 1). The scores of BMK13+, EG1+ and EG2+ eosinophils in recurrences after 6 months were still significantly higher ($p < 0.05$) than in the macroscopically unaffected mucosa of the middle turbinates of the same patients. Significantly lower scores of BMK13+, EG1+ and EG2+ eosinophils ($p < 0.05$) were found in the macroscopically unaffected mucosa of the middle turbinates after 6 months than at time of ESS, whereas in the mucosa of the inferior turbinates of the patients only significantly lower scores of EG2+ eosinophils ($p < 0.05$) were found (Fig.1).

Slightly lower scores (n.s.) of BMK13+, EG1+ and EG2+ eosinophils were found in the recurrences of polyps after 1 year as compared to polyp tissue taken after 6 months of follow-up (Fig. 1). The scores of BMK13+, EG1+ and EG2+ eosinophils in recurrences after 1 year were no more significantly different from the macroscopically unaffected mucosa of the middle turbinates of the same patients. In the mucosa of the middle and inferior turbinates, no

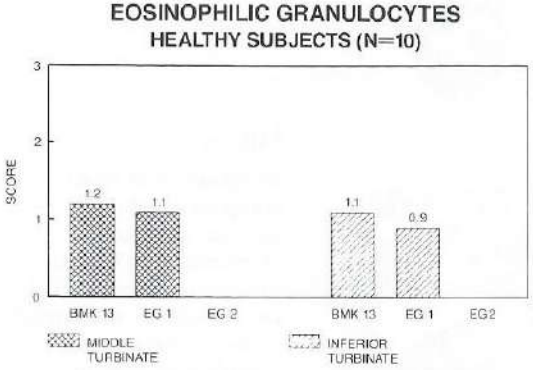

Fig 4.

Quantification of BMK13+, EG1+ and EG2+ eosinophils in the lamina propria of the middle and inferior turbinates of 10 healthy subjects. The histograms represent the the mean values of the scores. No EG2+ eosinophils are found.

differences in scores were found after 1 year of follow-up as compared to the scores after 6 months in the same patients (Fig. 1).

Clinical evaluation revealed a recurrence rate of 50% after 6 months and 54% after 1 year of follow-up (Table 1). At 6 months and 1 year after ESS no significant differences in scores of BMK13+, EG1+ and EG2+ eosinophils were found in the polyps or mucosa of the middle and inferior turbinates between patients with or without a recurrence. When comparing patients with CAO and patients without CAO, the former had significantly higher scores of EG2+ eosinophils ($p < 0.05$) in the macroscopically unaffected mucosa of the middle turbinates and in recurrences of nasal polyps after 1 year. No differences in recurrence rates were found in patients with or without CAO and/or an IgE-mediated allergy.

An elevated blood eosinophil count was found in 81% of the patients with CAO and in 48% of the patients without CAO. The former had significantly more EG2+ eosinophils in recurrences of polyps after 1 year ($p < 0.05$) than the patients with a normal blood eosinophil count. The recurrence rate after 6 months and 1 year was not different between these patients groups.

DISCUSSION

Eosinophils play an important role in chronic inflammatory processes. The cytoplasmic granules of eosinophils contain strongly basic proteins, like major basic protein (MBP) and eosinophilic cationic protein (ECP). These toxic proteins can damage the respiratory mucosa of the upper- and lower respiratory tract (Gleich et al. 1988, Harlin et al. 1988). Moreover, eosinophils may release mediators like platelet activating factor (PAF) which increases vascular permeability, is a chemoattractant for eosinophils in the human upper respiratory tract (Miadonna et al. 1991) and stimulates the release of leucotriene C_4 (LTC_4) and MBP by human eosinophils (Shaw et al. 1985, Henocq 1988).

Because chronic inflammation probably plays an important role in the development of nasal polyps, we studied the effect of ESS and treatment with topical corticosteroids on the distribution and activation of eosinophils in nasal polyps and in the macroscopically unaffected mucosa of the middle and inferior turbinates of patients with nasal polyps and healthy subjects.

The moderate to high infiltration of eosinophils in 77% of the nasal polyps, and the fact that almost all the eosinophils were activated (EG2+) and hypodense (on EM examination) is indicative of an active inflammatory process in the polyps. In addition, significantly more (activated) eosinophils were found in the polyps than in the macroscopically unaffected mucosa of the midlle and inferior turbinates of the patients. This indicates that the inflammatory process is most severe in polyp tissue. Moreover, in the mucosa of the middle turbinates of the patients significantly more activated eosinophils were found than in the mucosa of the inferior turbinates of the same patients at the time of ESS. This suggests that the inflammatory process is more severe in the nasal mucosa of the middle than the inferior turbinate and could explain why nasal polyps originate in the viscinity of the ethmoid complex only.

Whereas in the respiratory mucosa of healthy subjects low to moderate numbers of MBK13+ and EG1+ eosinophils were found, no EG2+ eosinophils were detected, suggesting that only after activation eosinophils cause inflammation. Their activation, rather than the high number of eosinophils, probably plays a role in the inflammatory process which may lead to nasal pathology (Fukuda & Gleich 1989). The data are in accordance with studies in which a considerable degree of eosinophilia was found in normal controls without any nasal symptoms (Pech et al. 1983, Fokkens et al. 1990).

After 6 months of follow-up, the scores of BMK13+, EG1+ and of EG2+ eosinophils especially had decreased in the macroscopically unaffected mucosa of the middle turbinates and in polyp tissue when a recurrence was present. After 1 year of follow-up, the scores of the activated and nonactivated eosinophils in recurrences of nasal polyps were even lower than after 6 months. In addition, no more significantly different scores of eosinophils were found between recurrences of nasal polyps and the macroscopically unaffected mucosa of the middle and inferior turbinates of the same patients after 1 year. This reduction in numbers of eosinophils in the polyps and nasal mucosa is in accordance with studies in which an inhibitory effect of corticosteroids on eosinophil survival was found (Lamas et al. 1990, Wallen et al. 1991), possibly resulting in a shorter exposure of tissues to eosinophils. Moreover, the reduction of eosinophils (especially of activated eosinophils) suggests that the severity of the inflammatory reaction is reduced probably due to the effect of topical steroids. This may be important to postpone recurrences of nasal polyps.

Eosinophils and their mediators are involved in asthmatic reactions (de Monchy et al 1985, Bruijnzeel 1989). A significant association between sinus

mucosa eosinophilia and asthma has been described by Harlin et al. (1988). There is evidence that eosinophils in asthmatic patients have an increased ability to release LTC_4 and granule proteins (ECP) (Taniguchi et al 1985, Schauer et al. 1989, Carlson et al 1991). Moreover, the number of hypodense blood eosinophils is increased in patients with atopic asthma as compared to healthy volunteers (Kloprogge et al. 1989).

Significantly higher scores of EG2+ eosinophils were found after 1 year in recurrences of nasal polyps and in the macroscopically unaffected mucosa of the middle turbinates from patients with CAO as compared to patients without CAO. Moreover, in patients with an elevated blood eosinophil count, significantly more EG2+ eosinophils were detected in recurrences of polyps after 1 year than in patients with a normal eosinophilic blood count. This suggests a more severe inflammatory process in polyps and the nasal mucosa of patients with CAO and patients with blood eosinophilia. Despite these findings, no difference in recurrence rate was found between these patients groups. An explanation could be the relatively short follow-up period of 1 year.

In conclusion, the data presented show that in healthy subjects eosinophils in the nasal mucosa are not activated, whereas in nasal polyps and in nasal mucosa of patients with nasal polyps a considerable part of the eosinophils is activated (EG2+; hypodense). After ESS and treatment with topical corticosteroids, lower numbers of activated eosinophils are found in nasal polyps and the mucosa of the middle and inferior turbinates. This reduction is probably important to postpone or prevent recurrences of nasal polyps after surgery.

References

Bruijnzeel PLB.Contribution of eosinophil-derived mediators in asthma. Int Arch Allergy appl Immunol 1989; 90: 57-63.

Carlson M, Hakansson L, Peterson C, Stalenheim G, Venge P. Secretion of granule proteins from eosinophils and neutrophils is increased in asthma. J Allergy Clin Immunol 1991; 87: 27-33.

Dingsor G, Kramer J, Olsholt R, Soderstrom T. Flusonide nasal spray 0.025% in the prophylactic treatment of nasal polypsis after polypectomy. Rhinology 1985; 23: 49-59.

Drettner B, Ebbesen A, Nilsson M. Prophylactive treatment with flusonide after polypectomy. Rhinology 1982; 20: 149-58.

Fokkens WJ, Holm AF, Rijntjes E, Mulder PGH, Vroom TM. Characterisation 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 1990; 93: 66-72.

Fukuda T, Gleich GJ. Heterogeneity of human eosinophils. J Allergy Clin Immunol 1989; 83: 369-73.

Gleich GJ, Flavahan NA, Fujisawa T, Vanhoutte PM. The eosinophil as a mediator of damage to respiratory epithelium: a model for bronchial hyperreactivity (Aspen Allergy Conference). J Allergy Clin Immunol 1988; 81: 776-81.

Harlin SL, Ansel DG, Lane SL, Myers J, Kephart GM, Gleich GJ. A clinical and pathologic study of chronic sinusitis: the role of the eosinophil. J Allergy Clin Immunol 1988; 81: 867-75.

Hartwig S, Linden M, Laurent C, Varko AK, Lindqvist N. Budesonide nasal spray as prophylactic treatment after polypectomy. J Laryngol Otolog 1988; 102: 148-51.

Henocq E. PAF-acether and eosinophils. Prog Biochem Pharmacol 1988; 22: 141-48

Karlsson G, Rundcrantz H. A randomized trial of intranasal beclomethasone dipropionate after polypectomy. Rhinology 1982; 20: 144-48.

Kita H, Abu-Ghazaleh R, Sanderson CJ, Gleich GJ. Effect of steroids on immunoglobulin-induced eosinophil degranulation. J Allergy Clin Immunol 1991; 87: 70-7.

Kloprogge E, de Leeuw AJ, de Monchy JCR, Kauffman HF. Hypodense eosinophilic granulocytes in normal individuals and patients with asthma: Generation of hypodense cell populations in vitro. J Allergy Clin Immunol 1989; 83: 393-400.

Lamas AM, Leon OG, Klunk DA, Schleimer RP. Glucocorticoids specially decrease of eosinophil survival. J Allergy Clin Immunol 1990; suppl 85-1 part 2; 282.

Lundgren R, Soderberg M, Horstedt P, Stenling R. Morphological studies of bronchial mucosa biopsies from asthmatics before and after ten years of treatment with inhaled steroids. Eur Respir J 1988; 1: 883-89.

Miadonna A, Palumbo G, Lorini M, Tech M, Arquati M, Tedeschi A. Nasal neutrophilia after local insufflation of PAF-acether. J Allergy Clin Immunol 1991; 87-1: 146.

de Monchy JCR, Kauffman HF, Venge P, et al. Bronchoalveolair eosinophilia during allergen-induced late asthmatic reactions. Am Rev Respir Dis 1985; 131: 373-76.

Ogawa H. Atopic aspects of eosinophilic nasal polyposis and a possible mechanism of eosinophilic accumulation. Acta Otolaryngol 1986; Suppl 430: 12-17. Ogino S, Harada T. Aspirin induced asthma and nasal polyps. Acta Otolaryngol (Stockh) 1986; suppl 430: 21-27.

Pech A, Besson J, Banis C, et al. Etude histologique morphometrique de 117 muqueuses nasales au cours de rhinites chroniques allergiques et non allergiques. Interpretation statistique. J Fr Otorhinolaryngol 1983; 32: 371-77.

Peters MS, Gleich GJ, Dunette SL, Fukuda T. Ultrastructural study of eosinophils from patients with the hypereosinophilic syndrome: A morphologic basis of hypodense eosinophils. Blood 1988; 71-3: 780-85.

Peterson AP, Altman LC, Hills JS, et al. Glucocorticoid receptors in human eosinophils: comparison with neutrophils. J Allergy Clin Immunol 1981; 68-3: 212-17.

Schauer U, Eckhart A, Muller R, et al. Enhanced leukotriene C4 production by peripheral eosinophilic granulocytes from children with asthma. Int Arch Allergy appl Immunol 1989; 90:201-6.

Shaw RJ, Walsh GM, Cromwell O, Moqbel R, Spry CJF, Kay AB. Activated eosinophils generate SRS-A leukotrienes following IgG-dependent stimulation. Nature 1985; 316: 150-52.

Siegel SC. topical intranasal corticosteroid therapy in rhinitis. J All Clin Immunol 1988; 81: 984-91.

Stoop AE, Hameleers DMH, v. Run PEM, Biewenga J, v.d. Baan S. Lymphocytes and nonlymphoid cells in the nasal mucosa of patients with nasal polyps and of healthy subjects. J Allergy Clin Immunol 1989; 84: 734-41.

Stoop AE, v.d. Heijden HAMD, Biewenga J, v.d. Baan S. Lymphocytes and nonlymphoid cells in human nasal polyps. J Allergy Clin Immunol 1991; 87 2: 470-75. Taniguchi N, Mita H, Saito H, Yui Y, Kaika T, Shida T. Allergy 1985; 40: 571.

Virolainen E, Puhakka H. The effect of intranasal beclomethasone dipropionate on the recurrence of nasal polyps after ethmoidectomy. Rhinology 1980; 18: 9-18.

Wallen ND, Weiler DA, Gleich GJ, Kita H. Glucocoticoids inhibit rIL-5-enhanced in vitro survival of eosinophils. J Allergy Clin Immunol 1991; 87-1 part 2; 173.

Weller PF. Eosinophilia. J Allergy Clin Immunol 1984; 73:1.

Winqvist I, Oloffsson I, Olsson I, Persson A, Hallberg T. Altered density, metabolism and surface receptors of eosinophils in eosinophilia. Immunology 1982; 47: 531-39.

Chapter 6A

NASAL SECRETIONS FROM PATIENTS WITH POLYPS AND HEALTHY INDIVIDUALS, COLLECTED WITH A NEW ASPIRATION SYSTEM: EVALUATION OF TOTAL PROTEIN AND IMMUNOGLOBULIN CONCENTRATIONS.

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SUMMARY

This study was designed, first, to test a new system for aspiration of human nasal secretions and, secondly, to evaluate protein and immunoglobulin concentrations in these secretions at different levels of secretory activity. The direct aspiration system combines the advantages of minimal irritation of the mucosa with the facility to determine concentrations per gram of secretion. The total protein and immunoglobulin concentrations were inversely related to the amount of secretion obtained. Variations in fluid secretion throughout the day may be responsible for this relationship. The inverse relationship was much more significant in patients with nasal polyps, in which higher concentrations were found, than in healthy subjects. Ratios of immunoglobulin to total protein were independent of the amount of secretion obtained. Compared to the controls, the ratios of IgM and IgG to protein in the secretions of the patients were significantly increased. The secretory immunoglobulin A to total protein ratios were only slightly higher in the patient's secretions.

INTRODUCTION

The secretory layer lining the mucosal surfaces in the respiratory tract contains proteins such as lactoferrin and secretory immunoglobulin A (sIgA). These, respectively, inhibit bacterial growth and protect the respiratory mucosa from invasion by micro-organisms. In pathological conditions such as infections or allergic diseases the composition of nasal secretions can vary considerably (Bachert et al. 1989). Investigations on nasal secretions have been hampered by the difficulty of obtaining large enough and representative samples (Holt & Kern 1986). Using the nasal lavage method an unknown dilution factor is introduced and, consequently, the immunoglobulin levels can only be expressed relative to the protein concentrations (Salvaggio et al. 1973, Hobday et al. 1971). Nasal secretions have also been collected by absorption onto filter paper (Mygind & Wihl 1976) or by aspiration after stimulation with cellulose sponges (Holt & Kern 1986). In both these methods the mucosa is irritated considerably and the samples obtained may not be representative. We have developed a direct aspiration method which induces minimal stimulation of the nasal mucosa. The samples collected by this method are diluted but the dilution factor can be determined, thus enabling the measurement of absolute concentrations. The system was evaluated by the assay of protein and immunoglobulin concentrations in nasal secretions of healthy individuals and patients with nasal polyps.

MATERIALS and METHODS

Patients and samples

Seventeen patients with nasal polyps (7 men and 10 women; aged 17-68 years) and 18 healthy individuals (9 men and 9 women; aged 20-52 years) were included in this study. The latter had no history of IgE mediated allergy or other nasal complaints, nor were abnormalities detected by routine ear- nose- and throat examination. Four of the patients had an IgE-mediated allergy as confirmed by specific IgE radio-immuno sorbent assay (RAST) and/or positive skin test. Chronic airway obstruction (CAO; asthma, bronchitis) was diagnosed in three other patients. The nasal secretions were obtained from the patients prior to surgical removal of polyps. The samples were collected by repeated aspiration from the middle meatus and from the floor of both nasal cavities, into a preweighed sampling tube. Secretions were sampled between 0930h and 1500h.

The aspiration system

Nasal secretions were collected with an aspiration system which was developed in-house (Fig. 1), consisting of a metal cap (a) with a metal aspiration tube (b). The cap contains O-rings (c) to permit the leakage-free fitting on a standard disposable collection tube (d). This tube (diameter 9.1 mm, height 70 mm) can be replaced easily. The cap is to be connected to a vacuum pump by two adaptors (e) and standard plastic tubing (f; inner diameter 4 mm) and the replaceble metal aspiration tube (inner diameter 0,5 mm) fits closely into a hole in the cap. Its internal diameter is small enough to prevent loss of secretion but large enough to allow aspiration of the more viscous secretions. The total length of the aspiration tube is 132 mm. The 42 mm length within the collection tube prevents suction of the sample directly into the pump. For easy manipulation, the aspiration tube has a length of 80 mm outside the collection tube and has been constructed with an angle of 45°. This part of the aspiration tube is siliconized on the outside to diminish irritation or damage of the nasal mucosa. Both cap and aspiration tube can be cleaned and sterilized.

The secretions remaining in the aspiration tube after sampling are washed into the collection tube by aspiration of a known aliquot (0.5 mL) of phosphate buffered saline (PBS). The amount of secretion sampled was determined by weighing the collection tube and the tube with the aliquot of PBS before and after sampling. Collection of secretion with this method was well tolerated by the patients. The samples were cooled on ice immediately after sampling, then mixed on a Vortex mixer, centrifuged at 120 x g and 1500 x g and 4°C to remove any cells and cell debris. The samples were stored at -20°C untill analysed.

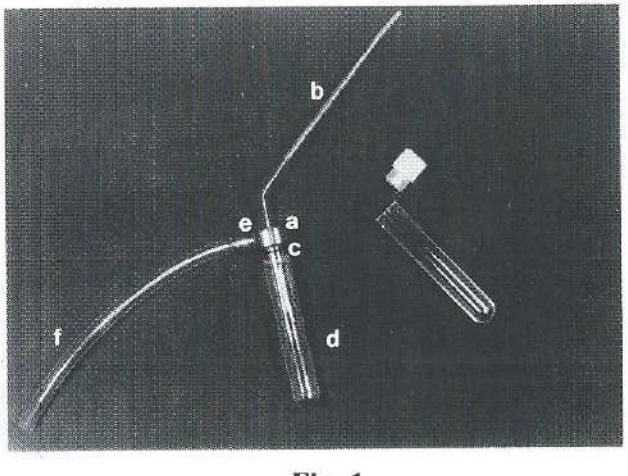

Fig. 1.
The aspiration system.

Immunoglobulin and protein analyses

Total protein was determined by the bicinchoninic acid (BCA) method (Pierce, Chemical Company, Rockford, USA). The sIgA and IgM concentrations were measured by enzyme linked immunosorbent assays (ELISA). For secretory IgA (sIgA) measurements microtiter plates (Greiner, Frickenhausen, Germany) were coated with 200 µl of purified monoclonal anti-secretory component antibodies (mab H 194.4.1; Haaijman et al. 1986) at 2 µg/ml and subsequently incubated with serial dilutions of the samples or with sIgA standard solutions. Bound sIgA was detected by incubation with appropriately diluted monoclonal anti-IgA (mab Hisa 43; Biewenga et al. 1986), then with horse-radish peroxidase (HRP)-conjugated goat anti-mouse IgG (Cappel, Cochranville, USA) and by staining with O-phenylene-diamine-dihydrochloride (Abbott Laboratories, Diagnostics Div., North Chicago, USA). The plates were washed between the incubation steps.

In the IgM-ELISA (van Kamp & Wolters 1989) 220 µl 1:1000 diluted swine anti-human IgM antibodies (Orion Diagnostica, Esporo, Finland) were used to coat microtiter plates. IgM bound from standards and samples was detected using HRP-conjugated rabbit anti-human IgM antibodies (Dakopatts, Copenhagen, Denmark). The staining method was as described above. IgG was measured using low-concentration radial immunodiffusion plates with appropriate standard solutions (Behringwerke, Marburg, Germany) according to the instructions. Protein and immunoglobulin concentrations were expressed as the mean of duplicate analyses.

Statistical methods

The dependency of protein, IgM, IgG and sIgA concentrations on the weight of secretion for both the control and patient's group was investigated by the multiple linear regression method. For each variable the null hypothesis of parallel regression lines between the two groups was tested first. If this hypothesis was not rejected, it was assumed that the regression slopes were equal. However, if the null hypothesis of parallel regression lines was rejected, a separate regression slope was estimated for each group.

RESULTS

The aspiration procedure was tested in a pilot study on nine healthy individuals and the reliability of the weighing procedure was evaluated. Sampling could be done in about 1 min with minimal stimulation of the mucosa as judged from the reaction of the subjects and the fact that only few samples were bloodstained. Such samples were excluded from the study. The system was used in adults and in children above 7 years. Because of the fourfold weighing procedure weights around and below 10 mg became unreliable. Therefore, for statistical purpose in the present study only samples weighing more than 15 mg were used.

The amount of secretion obtained from the healthy individuals varied between 11 and 176 mg (median 57.5 mg; n = 18) and from the patients between 8 and 150 mg (median 43.6; n = 17). Fourteen patient and 17 control samples weighed more than 15 mg and were thus included in the statistical analysis (Table 1). It was considered that weight errors introduced by the weighing procedure would cause higher variation in concentrations of the smaller samples rather than the larger ones. The concentrations the samples were plotted against their weights. The resulting figures (Fig. 2) showed an inverse relationship, which was much weaker in for the healthy subjects than for the polyp patients.

Table 1. Protein, IgM, IgG and sIgA concentrations in nasal secretions of patients with nasal polyps and healthy individuals.

	weigth of secretion (mg)	concentrations protein mg/g	IgM µg/g	IgG µg/g	sIgA µg/g
patients (n=14)	range 18.5-150	6.4-129.4	58.2-1,785	143-27,840	607-9,989
mean	66	40.6	572.6	5,489	3,610
SD	43	35.6	517.1	7,218	3,178
median	55	29.9	329	3,207	2,157
controls (n=17)					
range	19.0-176	3.4- 12.9	14.2-157.7	50.7-1,148	179-1,589
mean	73	8.9	57.8	442.6	652.1
SD	45	3.7	38.5	316.1	377.8
median	63	9.5	51.6	369.2	507.3

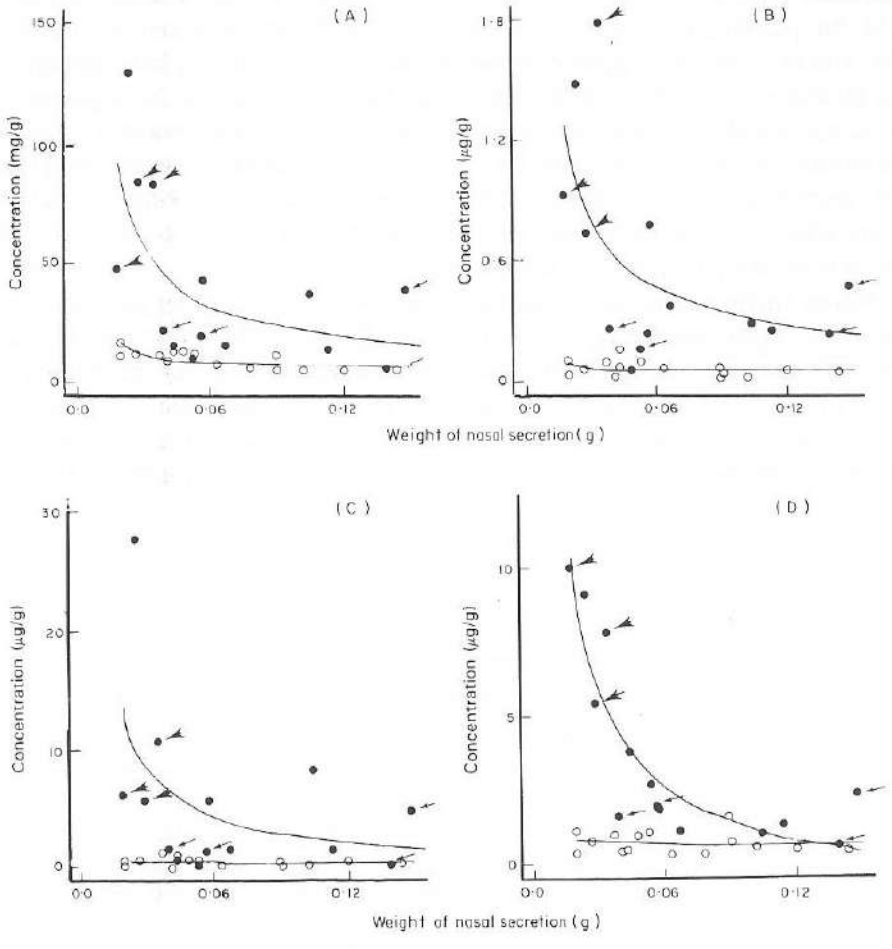

Fig. 2.
Protein and immunoglobulin concentrations for healthy individuals (open circles) and patients with nasal polyps (closed circles). (* protein expressed as mg/g; immunoglobulins as µg/g of secretion). A: total protein, B: IgM, C: IgG, D: sIgA. CAO patients (arrows) and allergic patients (arrow heads) are indicated. Inserts show the regression lines for the healthy individuals (lower lines) and patients (upper lines).

Graphical inspection of the data showed that protein, IgM, IgG and sIgA depended on weight of secretion, irrespective of weight errors. When comparing control and patient's groups after reciprocal transformation, the null hypothesis of parallel regression had to be rejected for each of the concentrations ($p = 0.0114$ for protein, $p = 0.0114$ for IgM, $p = 0.0406$ for IgG and $p < 0.0001$ for sIgA). Hence, separate regression slopes were estimated for both groups. The concentrations of protein, IgM, IgG and sIgA increased in both groups with decreasing weight of the secretions, however, this phenomenon was more pronounced for the patients than for the controls. Relative to sample weights, the nasal secretions of the three CAO patients had very high protein and immunoglobulin concentrations, whereas these were much lower in the nasal secretions of three of the four allergic patients.

When immunoglobulin concentrations were expressed as µg per mg total protein and again plotted against sample weight the null hypothesis of parallel regression lines was not rejected. Hence, the regression lines for the patients' and control groups were compared on the assumption that the regression slopes were homogeneous. The ratios for sIgA to protein were within the range of the controls except for four patients, one of which was a CAO patient (Table 2). The

Table 2. Immunoglobulin levels in nasal secretions of patients with nasal polyps and healthy individuals expressed as µg/mg of protein.

	weight of secretion (mg)	immunoglobulin to protein ratios for IgM	IgG	sIgA
patients (n=17)				
range	8-150	3.3-36.0	12-222	27-262
mean	56	15.8	98	108
SD	45	8.5	62	70
median	44	12.8	89	85
controls (n=18)				
range	11-176	1.7-12.2	5.4-100	22-144
mean	70	6.4	47	77
SD	46	3.3	26	36
median	57	6.6	42	73
significance p	0.0351[1]	0.0002[2]	0.0039[2]	0.15[2]

Significance was determined by ([1]) the two-sided tail-probability from Wilcoxon's rank-sum test or ([2]) by the curve fitting method.

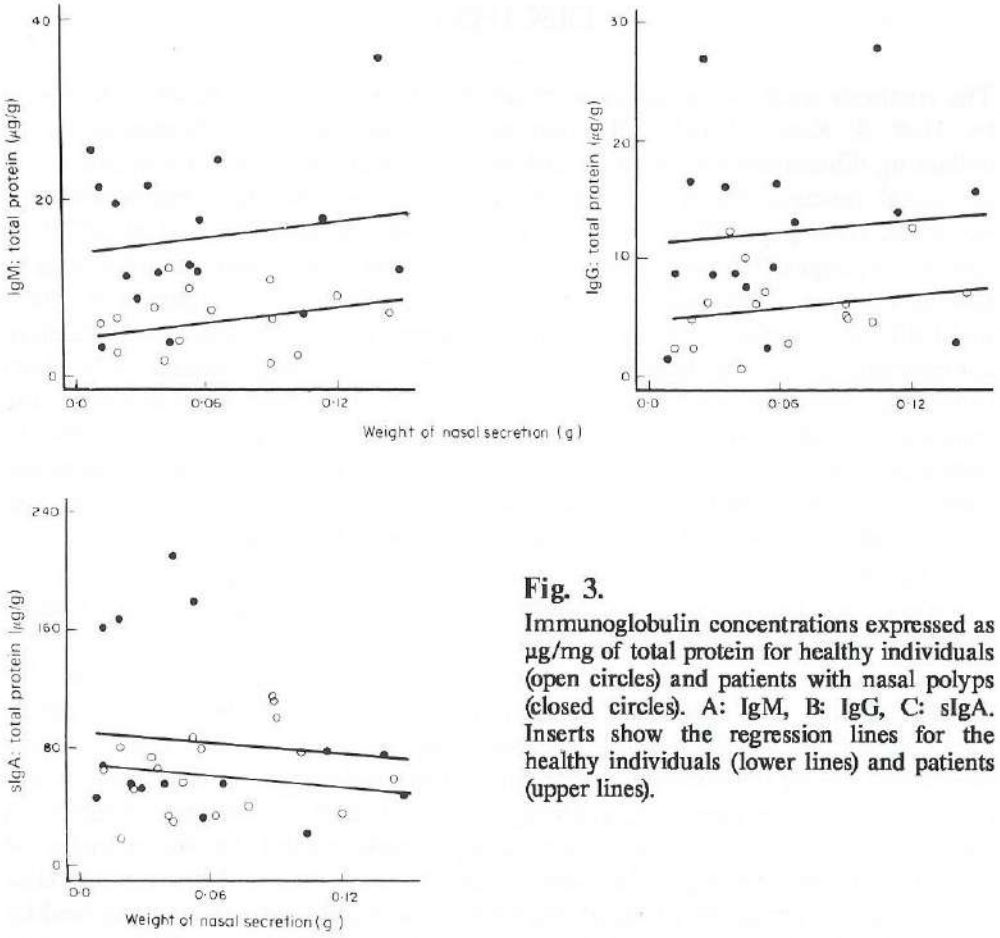

Fig. 3.
Immunoglobulin concentrations expressed as µg/mg of total protein for healthy individuals (open circles) and patients with nasal polyps (closed circles). A: IgM, B: IgG, C: sIgA. Inserts show the regression lines for the healthy individuals (lower lines) and patients (upper lines).

distance between the fitted lines (Fig. 3) did not deviate significantly from zero for the sIgA measurements ($p = 0.15$), indicating that the sIgA to protein ratios in nasal secretion of patients with nasal polyps and healthy individuals do not differ. However, for the IgM and IgG measurements the distance between the two fitted lines was significant ($p = 0.0002$ and $p = 0.0039$, respectively) and showed that on an average the IgM measurements were 9.6 µg/mg, and the IgG measurements 51.8 µg/mg protein higher for the patients than for the controls. Two of the three CAO patients showed IgM and IgG to protein values greater than the highest ratio within the control group. One of the four allergic patients had an IgG to protein ratio higher than the highest control value and for another allergic patient this was true for the IgM to protein ratio. The three immunoglobulins formed 5-20% of the proteins in the nasal secretions of the controls and 10-36% in the nasal secretions of the patients.

DISCUSSION

The methods used for sampling of nasal secretions have been recently reviewed by Holt & Kern (1986). All previous methods have the disadvantages of collecting diluted samples with an unknown dilution factor and/or stimulation of the nasal mucosa. Stimulation of the nasal mucosa during samplin with for examples filter paper (Mygind et al. 1976), cotton swabs (Sasaki et al. 1977) or cellulose sponges (Holt & Kern 1986) may provide a non-representative sample. Lavage methods (Salvaggio et al. 1973, Hobday et al. 1971, Holmes et al. 1987) yield diluted samples with an unknown dilution factor, on which only relative concentrations can be determined. Our method has the advantage of a short sampling time with little irritation, resulting in minimal stimulation of the mucosa. In addition, although obtaining diluted samples, we are able to determine the dilution factors. Our method is only limited in cases in which the nasal cavity contains very little secretion. The method has already been used without major problems for the collecting of over 100 samples.

The present study showed an inverse relationship between the amount of secretion present in the nasal cavity and the protein and immunoglobulin concentrations in these secretions (Fig. 2). "Simple" mean or median values (as shown in Table 1) cannot be used to compare patients' with controls because of the dependency on the amount of secretion. Therefore, the method of multiple linear regression was used and this showed that the dependency of concentration on sample weight differed for protein and immunoglobulins between patient and control groups. Furthermore, a strong increase in protein and immunoglobulin concentrations was found with decreasing sample weight for the samples of patients with nasal polyps. The exact difference in protein and immunoglobulin concentrations per gram of nasal secretion between the patients and the healthy subjects has to be determined in larger groups because of the unequal regression slopes.

Mygind & Thomsen (1976) found diurnal variations in nasal protein and immunoglobulin concentrations. Since our samples were collected within a few hours around midday eventual diurnal variations would be small and, therefore, are not expected to produce the inverse relationship as shown. Besides the above diurnal variations in protein concentrations, there may be variations in the amount of secretion produced (Harada et al. 1984). This might well explain the variations found by Mygind & Thomsen (1976). In this study the sIgA concentrations in nasal secretions of healthy individuals are somewhat lower than found previously by Harada et al. (1984). This could be due to the use of different reference preparations. The extremely high IgG and IgM concentrations in the nasal secretions of the patients are indicative for considerable leakage of plasma proteins from the tissues in nasal polyposis (Donovan et al. 1970, Mygind et al. 1975). sIgA, which is produced locally, was also increased although less than IgG and IgM. Nasal polyps contain much interstitial fluid and few secretory glands. This may explain the considerable leakage of IgM and IgG into the nasal secretions, while the sIgA concentrations remain relatively low. Nevertheless, the data suggest increased sIgA production within the nasal mucosa of the patients.

On account of the simultaneously increase in protein and immunoglobulin concentrations in the patients' nasal secretions, the difference between patients' and control groups is less pronounced when comparing immunoglobulin to protein ratios. Moreover, the immunoglobulin to protein ratios are independent of sample weights, providing a means of determining the differences between patients' and control groups (Table 2). In spite of the high protein content, control and patients' groups differ significantly for IgM to protein and IgG to protein ratios. This again demonstrates the extreme increase in the IgM and IgG concentrations. The sIgA to protein ratio is not significantly different between patient and control groups. The high sIgA concentrations are not apparant in this ratio because of the simultanious high protein concentrations. In polyp interstitial fluid we found IgG and IgM to protein ratios in the same range as in the nasal secretions (median values of 15 and 153, respectively; n = 20) and sIgA to protein ratios much lower than in the nasal secretions (median value 18, n = 19; data not shown). These findings are in accordance with the above data and the suggested leakage of proteins from the tissues.

We are presently studying a larger group of patients with nasal polyps to correlate the protein and immunoglobulin concentrations in nasal secretions with clinical data. Relative to their weight nasal secretions of the allergic patients had lower protein and immunoglobulin concentrations than those of the patients with CAO. Whether this is characteristic for these patient groups is under investigation.

The results of this study lead to the conclusion that analytical data on nasal secretions should be related to the amount of secretion present in the nasal cavity to enable detectionof the increased secretion of locally produced proteins such as sIgA. In adddition, the proposed diurnal variation in nasal protein and immunoglobulin secretion has to be reconsidered, because it merely may be a variation in secretion of fluid.

References

Bachert C, Becker W, Ganzer U. The role of nasal secretions in allergic disease of the nose. Arch. Otorhinolaryngol. 1989; 246:173-82.

Biewenga J, Faber A, Pronk JC, Haaijman JJ. Production and characterization of pepsin fragments of human IgA1 to determine domain-specificity of monoclonal antibodies. Immunol. 1986; 59:153-58.

Donovan R, Johansson SGO, Bennich H, Soothill JF. Immunoglobulins in nasal polyp fluid. Int. Arch. Allergy 1970; 37:154-66.

Haaijman JJ, Deen C, Radl J. Determination of different molecular forms of human IgA1 and IgA2 with monoclonal antibodies In: Immunoregulation in aging. 1986; pg 285-94. Eds. A. Facchini, J.J. Haaijman and G. Labo.

Harada T, Hamaguchi Y, Sakakura Y, Miyoshi Y. Circadian variation of secretory IgA in nasal secretions from normal subjects. Acta Otolaryngol. 1984; 97:359-62.

Hobday JD, Cake M, Turner KJ. A comparison of the immunoglobulins IgA, IgG and IgE in nasal secretions from normal and asthmatic children. Clin. exp. Immunol. 1971; 9:577-83.

Holmes MJ, Callow KA, Parry HE An improved method for recovery of secretory immunoglobulins and lymphocytes fronm the nasal mucosa. J. Immunol. Meth. 1987; 98:183-87.

Holt JJ, Kern EB. A new method of collecting nasal secre tions. Otolaryngol. Head & Neck Surg. 1986; 94:403-04.

Mygind M, Weeke B, Ullman S. Quantitative determination of immunoglobulins in nasal secretion. Int. Archs Allergy appl. Immunol. 1975; 49:99-107.

Mygind M, Thomsen J. Diurnal variation of nasal protein concentration. Acta Otolaryngol. 1976; 82:219-21.

Mygind M, Wihl JA. Concentration of immunoglobulins in nasal secretion from children with recurrent infections in the upper airways. Acta Otolaryngol. 1976; 82:216-18.

Salvaggio J, Lopez M, Arquembourg P, Waldman R, Sly M. Salivary, nasal wash, and sputum IgA concentrations in atopic and nonatopic individuals. J. Allergy Clin. Immunol. 1973; 51:335-43.

Sasaki Y, Araki A, Koga K. The mast cell and eosinophil in nasal secretion. Ann. Allergy 1977; 39:106-09.

Van Kamp GJ, Wolters ECh. CSF-IgM measurement in neurovenereological disease. Clin. Chim. Acta 1989; 183:295-300.

Chapter 6B

IMMUNOGLOBULIN CONCENTRATIONS IN NASAL SECRETIONS OF PATIENTS WITH NASAL POLYPS.

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(submitted for publication)

SUMMARY

Nasal secretions of 26 healthy subjects and 41 patients who were operated upon for nasal polyps were evaluated. Compared to the healthy subjects, patients had highly increased concentrations of total protein, albumin, IgM, sIgA (all p< 0.001) and IgG (P< 0.05). Treatment with endoscopic sinus surgery and topical steroids (budesonide) decreased sIgA within 6 months (P< 0.001) and IgM (P< 0.001) and IgG (P< 0.05) within 1 year. Also IgE levels decreased, but not significantly. Since protein concentrations may be dependent on amount of secretion obtained, concentrations were also expressed as relative to total protein concentration. For patients, albumin, IgM, IgG and sIgA to total protein ratios were higher than in healthy subjects (P< 0.05). Ratios for albumin (P< 0.005), sIgA (P< 0.001), IgG (ns) and IgM (ns) decreased within 6 months of treatment and thereafter increased slightly, probably due to a relatively strong decrease of total protein concentrations. Clinical evaluation revealed that patients with chronic airway obstruction (CAO) had higher initial sIgA levels than those without CAO (P< 0.001), whereas the recurrence rates of the polyps were the same in both groups. Patients with an IgE-mediated allergy had slightly higher sIgA than those without such condition; the recurrence rate was highest in the former group (75% vs 48%). The data presented suggest that nasal polyposis is associated with locally increased sIgA production and increased leakage of IgM and IgG from the tissues and support the hypothesis that local inflammatory processes play a role in the pathogenesis of nasal polyps.

INTRODUCTION

Immune and inflammatory reactions in the upper respiratory tract are reflected by the presence of inflammatory cells in the nasal mucosa (Hameleers et al. 1989, 1990, Stoop et al. 1989, 1991) as well as in protein and immunoglobulin composition of nasal secretions (Bachert et al. 1989). For example, concentrations of immunoglobulins in nasal secretions are higher in patients with an IgE-mediated rhinopathy as compared to those with a non-IgE-mediated rhinopathy (Illum & Balle 1978, Pulido & Garcia 1983, Swart et al. 1991). In nasal secretions of patients with nasal polyps considerably increased levels of IgA, IgG, IgM and IgE are found (Donovan & Johansson 1970, Mygind et al. 1975, Pulido & Garcia 1983, Biewenga et al. 1991). In these patients the high immunoglobulin levels in nasal secretions are probably due to leakage of serum proteins from the polyp tissues, because high concentrations of immunoglobulins are also present in the interstitial fluid of nasal polyps (Waller et al. 1976, Yarumchuk et al. 1991).

Corticosteroids have anti-inflammatory properties. They reduce the vasopermeability and the influx of inflammatory cells in the respiratory mucosa (Lundgren et al. 1988, Siegel 1988). Corticosteroids also influence immunoglobulin levels in human secretions. Wira et al. (1990) found decreased IgA levels in saliva and vaginal secretions after systemic treatment with dexamethason, whereas Sörensen et al. (1976) demonstrated that during treatment with topical corticosteroids albumin, IgG and IgE concentrations decreased in nasal secretions of patients with nasal polyps. Unfortunately, investigations on nasal secretions were hampered by the difficulty of obtaining large enough and representative samples. With the nasal lavage method (Rossen et al. 1966, Miadonna et al. 1983, Raphael et al. 1988) an unknown dilution factor is introduced and immunoglobulin levels can only be expressed relative to the protein concentrations. In the direct aspiration method used in this study absolute concentrations can be measured.

The aim of this study was to investigate the effect of treatment with endoscopic sinus surgery (ESS) and topical corticosteroids on protein and immunoglobulin levels in nasal secretions of patients with nasal polyps with or without associated respiratory pathology.

MATERIAL and METHODS

Patients

Forty one patients (aged 16-72 years, mean age 44 year) operated upon for nasal polyps by ESS are included in this study. An IgE-mediated allergy for common

Table 1. Clinical data of the patients.

	% of patients per clinical subgroup	% of patients with a recurrence at 6 months
Patients (n=41)		56% (23/41)
IgE-mediated allergy	29% (12/41)	75% (9/12)
CAO	46% (19/41)	63% (12/19)
No IgE-mediated allergy or CAO	34% (14/41)	43% (6/14)

inhalation allergens, i.e. positive skin tests (diameter › 2 mm, Phazet, Pharmacia Diagnostics Uppsala, Sweden) and/ or RAST class › 2 (›0.35 PRU/ ml) was present in 29% of the patients (Table 1). A chronic airway obstruction (CAO, i.e. asthma/ bronchitis and/ or emphysema) was present in 46% of the patients. Serum levels of IgA, IgG and IgM were within the normal ranges: 0.6-4.5 g/l, 6.4-19.2 g/l and 0.4-3.6 g/l, respectively, in all patients. Nasal secretions were collected from the patients at time of surgery and after a follow-up period of 6 months and 1 year, during which time the patients were treated with topical corticosteroids (budesonide 400 µgram daily). None of the patients was treated with corticosteroids preoperatively for at least 2 months. For clinical follow-up patients underwent a nose endoscopy at 6 months and 1 year. At 6 months 56% of the patients had developed a recurrence. Because 2 of these patients had no recurrence after 1 year of follow-up, and 2 patients without recurrence after 6 months had a recurrence after 1 year, the recurrence rate after 1 year was exactly the same as at 6 months.

Control nasal secretions were taken from 26 healthy subjects (12 male, 14 female, aged 19-38 years; mean age 25 years). The healthy subjects were nonsmokers, had no IgE-mediated allergy or CAO, a normal ENT examination and had had no common cold for at least 1 month prior to the collection of nasal secretion.

Nasal secretions

Nasal secretions were collected with the direct aspiration system described previously (Biewenga et al. 1991). Sample weights were determined from the weights of the collection tubes before and after sampling and the weights of added phosphate buffered saline (0.1 M phosphate, 0.15 M NaCl, pH 7.4). The samples were cooled on ice immediately after sampling, then mixed on a Vortex mixer, centrifuged at 120 x g and 1500 x g at 4° C to remove any cells and cell debris subsequently. Samples with more than an occasional erythrocyte in the cytospin preparations of the first centrifugation step were excluded from the study, because this could indicate that the mucosa had been injured during sampling. The samples were then stored at -20° C untill analysed.

Protein and immunoglobulin analyses

Immunoglobulin and protein measurements were described previously (Swart et al. 1991). In short: Albumin was measured nephelometrically with the Beckman Array System (Beckman Instruments Inc., Brea, CA, USA). Total protein was determined by the bicinchoninic acid (BCA) method (Pierce, Chemical Company, Oud Beierland, NL). IgG concentrations were determined by radial immunodiffusion on low concentration plates (LC-Partigen IgG, Behringwerke AG, Marburg, Germany). IgM was measured by enzyme-linked immunosorbent assays (ELISA) as described before (van Kamp & Wolters 1989). Secretory-IgA (sIgA) was measured (by ELISA) on microtiter plates coated with goat-anti-mouse immunoglobulins (CLB, Amsterdam, The Netherlands) and then incubated with mouse monoclonal anti-secretory component antibodies (purified H 194-4.1, TNO, Rijswijk, The Netherlands, kindly provided by Dr. J.J. Haaijman). Bound sIgA was detected by HRP-conjugated rabbit-anti-human IgA (DAKO, P 216). IgE concentrations were determined with the IgE Fast Test (3M Diagnostic Systems, Santa Clara, CA, USA), according to the manufacturers instructions.

Evaluation and Statistics

Concentrations were expressed in mg (for total protein) or in µg (for albumin and immunoglobulins) per gram of secretion and relative to total protein as µg/ mg protein. Values were compared using either Wilcoxon's Rank sum test (patients v.s. controls) or Wilcoxon's Sign-Rank test (time of surgery v.s. follow-up). P-values ‹ 0.05 were considered significant.

RESULTS

The total protein, albumin and immunoglobulin contents of the nasal secretions are summarized in Table 2 for all patients and healthy subjects. As compared to healthy subjects, all patients had highly increased concentrations of albumin (P‹ 0.001), sIgA (P‹ 0.001), IgG (P‹ 0.05) and IgM (P‹ 0.001) at time of surgery. At 6 months after surgery and treatment with topical corticosteroids lower protein and immunoglobulin concentrations were found in nasal secretions of the patients, although this decrease was significant only for sIgA (P‹ 0.001). At 6

months, protein and immunoglobulin concentrations in the patients were still significantly higher than in healthy subjects (at least p< 0.05).

After 1 year significantly lower concentrations of albumin, sIgA, IgG and IgM were found than at time of surgery (Table 2). Nevertheless, concentrations

Table 2. Total protein, albumin, sIgA, IgG, IgM and IgE concentrations in nasal secretions of patients with nasal polyps and healthy controls.

	Time of surgery (n=41)	After 6 months (n=41)	After 1 year (n=34)	Healthy controls (n=25)
Weight of secretion Mean value (mg)	67	89 (ns)	132 (ns)	46
(range)	11-250	10-296	23-277	11-176
Total protein Mean value (mg/g)	40.6	32.7 (ns)	18.9 (ns)	10.7
(range)	2.2-190.2	3.0-336	3.5-60.1	3.4-35.7
Albumin Mean value (µg/g)	10298	6698 (ns)	5383 (P<0.05)	1757
(range)	58-79905	58-39720	88-33836	309-4804
sIgA Mean value (µg/g)	7590	2933 (P<0.001)	2043 (P<0.001)	1182
(range)	444-102266	440-8602	166-7340	310-1617
IgG Mean value (µg/g)	4346	3186 (ns)	** 1884 (P<0.005)	669
(range)	2-28086	2-21199	7-9516	51-3358
IgM Mean value (µg/g)	541	400 (ns)	248 (P<0.001)	100
(range)	3.6-3237.5	5.1-2426.3	7.3-1362.8	14-447
IgE Mean value (IU/g)	285.2	161.0	133.6	n.d.
(range)	<2-2464.7	<2-1554.8	<2-1395.5	

P values concern differences in concentration during follow-up as compared to the time of surgery (ns = not significant).

** only value, not significantly different between patients and healthy subjects.

of albumin (P< 0.001), sIgA (P< 0.001) and IgM (P< 0.05) in patients were still significantly higher than in healthy subjects. The IgE concentrations were not statistically evaluated because of undetectable low levels and an extremely wide range in the patients. No correlation was found between high IgE levels in nasal secretions and the occurrence of an IgE-mediated allergy. From 6 months to 1 year after surgery the concentrations further decreased, however, these decreases were not significant.

Because protein concentrations may be dependent on the amount of secretion produced, albumin and immunoglobulin levels were also determined relative to total protein concentration (Table 3). At time of surgery albumin, sIgA, IgG and IgM to total protein ratios were increased as compared to ratios in healthy subjects (for all parameters at least P< 0.05). In patients the ratios decreased for albumin (P<0.05) and sIgA (P< 0.001) but not significantly for IgG and IgM during the first 6 months of treatment. They increased slightly between 6 months and 1 year so that no significant differences remained with the initial

Table 3. Immunoglobulin levels in nasal secretions of patients with nasal polyps and healthy controls expressed as µg/mg of protein.

	Time of surgery (n=41)	6 months (n=41)	1 year (n=34)	healthy controls (n=25)
Albumin	212.5	153.4 (P<0.05)	182.7 (ns)	111.7
(range)	18-604	3-336	26-709	26-290
sIgA	194.6	** 107.1 (P<0.001)	148.0 (ns)	78.9
(range)	18-735	17-301	4-362	27-170
IgG	77.0	** 69.6 (ns)	77.8 (ns)	52.2
(range)	2-215	2-302	0.2-200	2-107
IgM	12.2	10.5 (ns)	10.8 (ns)	7.2
(range)	0.8-36.8	1.0-25.3	2.1-27.5	1.7-15.2

P values concern differences in immunoglobulin to total protein ratios during follow-up as compared to time of surgery (ns = not significant).

** only values not significantly different between patients and healthy subjects.

values. At 6 months, only IgM to total protein ratio was significantly higher (P< 0.05) when comparing patients with healthy subjects, but at 1 year albumin, sIgA, IgG and IgM to total protein ratios were again significantly higher (P< 0.05) in patients than in healthy subjects. During this time the mean total protein concentrations had dropped by 40%, whereas sIgA and albumin had dropped by 30% and 20%, respectively.

Clinical evaluation further showed that patients with and without an IgE-mediated allergy did not differ with regard to albumin and immunoglobulin concentrations at any time, except for sIgA. In the patients with an IgE-mediated allergy (n=12) the values for sIgA/g of secretion were higher (P< 0.05) than in patients without an IgE-mediated allergy (n=29) after 6 months of follow-up. In addition, their recurrence rate of 75% (9/12) at 6 months was higher (not significant) than for patients without an IgE-mediated allergy, which was 48% (14/29). sIgA concentrations per gram of secretion also differed (P< 0.001) between patients with (n=19) and without CAO (n=22), both at the time of surgery and after 6 months. At 1 year, also higher concentrations of IgG (P< 0.05) and IgM (P< 0.05) were found in patients with CAO than in patients without CAO. Patients with CAO had a recurrence rate at 6 months of 63%, those without CAO of 50% (not significant). At 1 year the recurrence rates of these patients were the same. No differences in immunoglobulin to total protein ratios were found between patients with- or without CAO.

With respect to recurrence, higher albumin, IgG and IgM concentrations (p< 0.05) were found in patients with a recurrence at 6 months (n=23) than in patients without a recurrence (n=18), but after 1 year of therapy no significant differences were found between these groups. As compared to healthy subjects, patients with a recurrence at 6 months (23/41= 56%) had significantly higher sIgA, IgG and IgM concentrations (all parameters at least P< 0.05). At 1 year, IgG concentrations were no longer significantly higher than in healthy subjects. However, patients without a recurrence differed from healthy subjects only by significantly higher sIgA concentrations at 6 months and 1 year.

Finally, the question remained wether the initial concentration or ratio to protein of any parameter might be of any predictive value for the development of recurrences. Therefore, patients who had developed a recurrence at 6 months were compared to those without a recurrence. Surprisingly, only the initial albumin concentrations per gram of secretion at time of surgery were higher (P< 0.05) in the former (n=23) than in the latter group (n=18).

DISCUSSION

In earlier studies we have demonstrated an inverse relationship between the amount of secretion present in the nasal cavity and the protein and immunoglobulin concentrations in these secretions (Biewenga et al. 1991, Swart et al. 1991). This relationship was especially found for amounts of secretion below 50 mg in patients and 30 mg in healthy controls. Because in this study the amounts of the secretions were higher, especially in the patients, concentrations in nasal secretions of the patients were compared irrespective of the amount of secretion sampled.

For healthy controls the amount of secretion obtained is generally lower which is consistent with relatively high concentrations. Nonetheless, albumin, sIgA, IgG and IgM concentrations in nasal secretions of patients were significantly higher than in healthy subjects. This suggests leakage of albumin, IgG and IgM as well as an increased local production of sIgA in nasal polyps and probably also the surrounding mucosa. This conclusion is supported by the findings that patients with a recurrence had higher IgG and IgM concentrations than those without a recurrence, whereas both groups had high sIgA levels as compared to healthy subjects. These findings are in accordance with previous studies in which higher levels of albumin (Keith et al. 1991) IgA, IgG and IgM were found in nasal secretions of patients with nasal polyps than in healthy controls (Pulida & Garcia 1983). The decrease in total protein, albumin, IgG and IgM concentrations at 6 months after surgical and medical treatment, is probably due to a reduction of local inflammatory processes. Concentrations of sIgA had also decreased significantly at 6 months. This suggests that treatment (surgery and/or topical corticosteroids) reduce local production of sIgA through a direct or indirect effect on B lymphocytes. After 1 year concentrations of sIgA, IgG and IgM were significantly lower than at time of surgery, suggesting a remarkable decrease of local production and of leakage of serum proteins from the tissues. These findings are in accordance with those of Sörensen et al.(1976), who found a decrease of albumin and IgG in nasal secretions of patients with nasal polyps after treatment with topical corticosteroids.

Differences between patients and healthy subjects were less pronounced when comparing immunoglobulin to protein ratios. In nasal secretions of the patients albumin, sIgA, IgG and IgM to protein ratios were significantly higher (all P< 0.05) at time of surgery than in healthy subjects. This demonstrates the increased local production of sIgA in the patients and relatively large leakage of IgG and IgM from the polyps. Although it is generally accepted that higher concentrations of IgG are due to an increased permeability of the respiratory membranes, recent studies indicate that additional local production of IgG may occur, at least in the lungs (Out et al. 1991).

In patients immunoglobulin to protein ratios were lower at 6 months than

at time of surgery which was significant for albumin (P< 0.001) and sIgA (P< 0.001) but not significant for IgG and IgM. Furthermore, sIgA and IgG to protein ratios in patients at 6 months were no longer different from ratio's in healthy subjects. This "normalisation" indicates that local production of sIgA and leakage of IgG and IgM from the tissues had decreased and that the local inflammatory response is less severe after surgery and treatment with topical steroids.

Albumin, sIgA and IgG to protein ratios increased from 6 months to 1 year and became again significantly higher than in the healthy subjects. This could be due to the relatively strong decrease in total protein concentration. However, these shifts can also reflect changes in local immune reactivity. The effect of topical corticosteroids may be partially extinguished. Because, in the present study recurrence rates at 1 year were the same as at 6 months, this increase can not be correlated with recurrences of nasal polyps.

Inflammatory processes like IgE-mediated allergy and CAO probably induce the local production of sIgA as seen in patients within these clinical subgroups. Higher concentrations of sIgA at the time of surgery and at 6 months were found as compared to patients without CAO or an IgE-mediated allergy. In patients with a recurrence at 6 months, higher concentrations of albumin, IgG and IgM were found than in patients without a recurrence. These data suggest more leakage of these proteins from polyps than from the mucosa of the patients without recurrence.

Because in patients who developed a recurrence after 6 months had only significantly higher initial albumin concentrations at time of surgery, it is concluded that concentrations of immunoglobulins before treatment do not correlate with the rate of recurrence of nasal polyps.

In conclusion, nasal secretions of patients with nasal polyps contain significantly more albumin, IgG, IgM and sIgA than nasal secretions of healthy subjects. After treatment with surgery and topical corticosteroids for a period of 1 year the concentrations of IgG, IgM and sIgA decrease, at least initially. The data support the hypothesis that local inflammatory processes play a role in the pathogenesis of nasal polyps. Initial concentrations of immunoglobulins before treatment do not correlate with the rate of recurrence of nasal polyps.

References

Bachert C, Becker W, Ganzer U. The role of nasal secretions in allergic disease in the nose. Arch Otorhinolaryngol 1989; 246: 173-82.

Biewenga J, Stoop AE, Baker HE, Swart SJ, Nauta JJP, van Kamp GJ, van der Baan S. Nasal secretions from patients with polyps and from healthy individuals, collected with a new aspiration system, and analysed for total protein and immunoglobulin concentrations. Annals of Clinical Biochemistry 1991; 28: 260-66.

Donovan R, Johansson SGO, Bennich H, Soothill JF. Immunoglobulins in nasal polyp fluid. Int Arch Allergy Appl Immunol 1970; 37: 154-66.

Hameleers DMH, Stoop AE, v.d. Ven I, Biewenga J, v.d. Baan S, Sminia T. Intraepithelial lymphocytes and nonlymphoid cells in the human nasal mucosa. Int Arch Allergy Appl Immunol 1989; 88: 317-22.

Hameleers DHM, Stoop AE, v.d. Ven I, Sminia T, v.d. Baan S, Biewenga J. An immuno- and enzyme-histochemical study of the human nasal mucosa of ear, nose and throat patients and controls. EOS J Immunol Immunopharmacol. 1990; Vol 10, no 2: 58-63.

Illum P, Balle V. Immunoglobulins in nasal secretions and nasal mucosa in perinnial rhinitis. Acta Otolaryngol 1978; 86: 135-41.

van Kamp GJ, Wolters Ch. CSf-IgM measurement in neurovenereological disease. Clin Chem Acta 1989; 183: 295-300.

Keith P, Wong D, Liehl E, Ceska M, Denburg J, Dolovich J. In vivo assessment of airway inflammation in nasal polyposis, presence of Il-8 and albumin in nasal lavage fluid. J Allergy Clin Immunol 1991; 87 (2); 139.

Lundgren R, Soderberg M, Horstedt P, Stenling R. Morphological studies of bronchial mucosa biopsies from asthmatics before and after ten years of treatment with inhaled steroids. Eur Respir J 1988; 1: 883-89.

Miadonna A, Leggieri A, Tedeschi A, Zanussi C. Clinical significance of specific IgE determination on nasal secretions. Clin Allergy 1983; 13: 155-64.

Mygind M, Weeke B, Ullman S. Quantitative determination of immuno globulins in nasal secretions. Int Arch Allergy Appl Immunol 1975; 49: 99-107.

Out TA, van de Graaf EA, van den Berg NJ, Jansen HM. IgG subclasses in bronchoalveolar lavage fluid from patients with asthma. Scand J Immunol 1991; 33: 719-27.

Pulido V, Garcia-Calderon PA. Some immunological parameters in serum and nasal secretions in subjects with vasomotor and allergic rhinitis and nasal polyps: a comparative study. Rhinology 1983; 21: 29-37.

Raphael GD, Druce HM, Baraniuk JN, Kaliner MA. Pathophysiology of rhinitis: I. Assessment of the sources of protein in metacholine-induced nasal secretions. AM Rev Resp Dis 1988; 138: 413-20.

Rossen RD, Schade AL, Butler WY, Kasel JA. The proteins in nasal secretions: A longitudinal study of A-globulin, G-globulin, albumin, siderophilin and total protein concentrations in nasal washings from adult male volunteers. J Clin Invest 1966; 45(5): 768-76.

Siegel SC. Topical intranasal corticosteroid therapy in rhinitis. J Allergy Clin Immunol 1988; 81: 984-91.

Sorensen H, Mygind N, Pederson CB, Prytz S. Long-term treatment of nasal polyps with beclomethasone dipropionate aerosol. Acta Otolaryngol 1976; 82: 260-62.

Stoop AE, Hameleers DMH, v. Run P, Biewenga J, v.d. Baan S. Lymphocytes and nonlymphoid cells in the nasal mucosa of patients with nasal polyps and of healthy subjects. J Allergy Clin Immunol 1989; 84: 734-41.

Stoop AE, v.d. Heijden HAMD, Biewenga J, v.d. Baan S. Lymphocytes and nonlymphoid cells in human nasal polyps. J Allergy Clin Immunol 1991; 87: 470-75.

Swart SJ, van der Baan S, Steenbergen JJE, Nauta JJP, van Kamp GJ, Biewenga J. Immunoglobulin concentrations in nasal secretions differ between patients with an IgE-mediated rhinopathy and a non IgE-mediated rhinopathy. J Allergy Clin Immunol 1991; 88: 612-19.

Waller G, Weidenbecher M, Pesch HJ, Beankler H. Vergleichende klinische, histologische und immunologische Untersuchungen zur Aetiologie der Poliposis nasi et sinuum. Laryngol Rhinol 1976; 55: 174-78.

Wira CR, Sandoe CP, Steele MG. Glucocorticoid regulation of the humoral immune system. I. In vivo effects of dexamethasone on IgA and IgG in serum and at mucosal surfaces. J Immunol 1990; 144: 142-46.

Yarumchuk K, McCulluogh J, Ownby D. Immunologic evaluation of nasal polyps. Am J Rhinol 1991; 5-1: 19-23.

Chapter 7

GENERAL CONCLUSIONS

Nasal polyps are a local phenomenon: they originate nearly always from the mucosa of the ethmoid sinuses and middle turbinates. Although chronic inflammatory processes seem to play an important role in the development of nasal polyps, the exact mechanisms involved are poorly understood. Inflammatory reactions in the upper respiratory tract are reflected in the presence of inflammatory cells in the nasal mucosa and the immunoglobulin composition of nasal secretions.

The aim of this study was to investigate whether local immunological processes are associated with the development of nasal polyps. Because corticosteroids can prevent or postpone recurrences of nasal polyps after surgical treatment, we further studied the effects of treatment with topical steroids (budesonide 400 µg daily) after endoscopic sinus surgery during a follow-up period of 6 months and 1 year.

Immunologically active cells in nasal polyps and nasal mucosa

In this thesis we focussed on cells which play an important role in inflammatory reactions in the nasal mucosa and nasal polyps: *Lymphocytes,* which are regulator and effector cells in cellular and humoral immune responses and associated with inflammatory reactions; *eosinophils,* which enhance local inflammatory reactions; and *HLA-DR+ cells,* which play a role in antigen presentation. A study of these cells may provide information as to the development of nasal polyps. Although mast cells may also contribute to inflammatory processes in nasal polyps and nasal mucosa, evaluation of these cells was beyond the scope of this study.

Lymphocytes

In healthy subjects more CD8+ (T suppressor/ cytotoxic) than CD4+ (T helper/ inducer) cells were found in the mucosa of the middle and inferior turbinates, but in nasal polyps and in the macroscopically unaffected mucosa of the middle and inferior turbinates of patients with nasal polyps the predominance of CD8+ cells over CD4+ cells was stronger (**chapter 2 and 3**). Considering the fact that nasal polyps are a local phenomenon, it is interesting to note that the numbers of CD8+ and CD4+ cells in nasal polyps and the macroscopically unaffected mucosa of the middle turbinates of patients were very similar, whereas smaller numbers of these lymphocytes are found in the mucosa of the inferior

turbinates of the patients (**chapter 3**). Furthermore, in the mucosa of the middle turbinates of healthy subjects significantly more CD4+ cells were detected than in the macroscopically unaffected middle turbinates of the patients at time of surgery. CD4+ cells can stimulate B lymphocytes to develop into mature plasma cells. The relatively low number of CD4+ cells in the patients could, on the one hand, result in an ineffective (insufficient) humoral immune response. However, in nasal secretions of patients with nasal polyps high concentrations of immunoglobulins were found (**chapter 6A and 6B**), which is an indication of an active humoral immune response. On the other hand, supressor-inducer cells are also CD4+. It is also possible that, because of the low numbers of CD4+ cells, CD8+ (T suppressor/ cytotoxic) cells are not stimulated adequatly and have no suppressive effect on the (chronic) inflammatory response. In both cases the chronic inflammatory reaction could sustain and consequently induce the formation of nasal polyps. Although the precise role of CD4+ and CD8+ cells is still a matter of controversy, the aforementioned data emphasize that local (T) cell-mediated mechanisms are involved in the pathogenesis of nasal polyps. Moreover, it is suggested that a change in cellular composition, especially in the macroscopically unaffected middle turbinates, precedes the development of nasal polyps.

Six months after surgery and treatment with topical steroids the number of CD4+ (helper/ inducer) cells had increased, especially in the macroscopically unaffected mucosa of the middle turbinates of the patients (**chapter 4**). Furthermore, the CD8+ : CD4+ cell ratio's in the mucosa of the patients approached the ratio's found in the healthy subjects, mainly due to an increase in density of CD4+ cells in the mucosa of the patients. It is attractive to suggest that the increase of CD4+ cells plays a role to prevent or postpone recurrences of nasal polyps. However, no correlation was found between recurrence rates and numbers of CD4+ cells, or between recurrences and ratio's of CD8+:CD4+ cells. This suggests that besides T cell-mediated mechanisms other, as yet unknown, processes play a role in the development of nasal polyps. Further investigations are needed to determine the functional role of lymphocytes in nasal polyps and in the normal and inflamed nasal mucosa.

Eosinophils

Infiltration of eosinophils is a striking feature in many nasal polyps. Eosinophils play an important role in chronic inflammatory processes, their inflammatory products can damage the respiratory mucosa of the upper and lower respiratory tract. Eosinophils probably play an important role in the pathogenesis of nasal polyps.

The finding of significantly more eosinophils in nasal polyps than in the macroscopically unaffected mucosa of the middle and inferior turbinates of

patients with nasal polyps, and the fact that almost all the eosinophils were activated (**chapter 5**), indicate that eosinophils contribute to an active local inflammatory process in nasal polyps. Because low to moderate numbers of, only non-activated, eosinophils were detected in the nasal mucosa of healthy subjects, it is suggested that the activation stage rather than the number of eosinophils is important in the inflammatory reactions in the nasal mucosa.

The decrease in number of activated eosinophils in the nasal mucosa of patients and in polyps recurring after surgery and treatment with topical steroids (**chapter 5**), indicates a reduction in local inflammatory reactions. This, again, emphasizes the role of activated eosinophils in the pathogenesis of nasal polyps.

The fact that surgery and treatment with topical steroids had an effect on infiltration and activation stage of eosinophils opens new ways to develop medication directed to reduce eosinophil activation. Platelet activating factor (PAF), which is released by eosinophils, mast cells and macrophages, increases vascular permeability, causes further influx of eosinophils and plays a role in eosinophil activation. Reduction of eosinophil activation through competitive binding of PAF could be a step forwards in the treatment of nasal polyps.

HLA-DR+ cells

HLA-DR+ cells, which play a role in presentation of antigens, were abundantly present in the stroma and epithelium of nasal polyps and in the macroscopically unaffected middle turbinates, whereas significantly less HLA-DR+ cells were found in the inferior turbinates of the patients (**chapter 2 and 3**). This suggests an increased local inflammatory reaction in the viscinity of the ethmoid complex and the middle turbinate where nasal polyps originate from. After treatment with surgery and topical steroids, however, no significant changes in numbers of HLA-DR+ cells were detected (**chapter 4**). This is in contrast to the change in numbers of lymphocytes and eosinophils after treatment. The heterogenity of HLA-DR+ cells (activated T cells, macrophages, dendritic cells, Langerhans cells and epithelial cells) could be responsible for this striking feature. Further investigations as to the functional role of these cells in nasal polyposis are needed.

Immunoglobulins in nasal secretions

To get a better insight into the humoral immune response in patients with nasal polyps, albumin, total protein and immunoglobulin (sIgA, IgG, IgM and IgE) concentrations in nasal secretions from healthy subjects and patients with nasal polyps were evaluated. An inverse relationship was found between the amount of secretion present in the nasal cavity and the total protein and immunoglobulin concentrations in nasal secretions, especially in patients

(**chapter 6A**). Because in patients sample weights were considerably higher than in healthy subjects we also determined immunoglobulin to total protein ratio's, these ratio's being independent of sample weights.

The significantly increased amounts of albumin and immunoglobulins (expressed as µg/g of secretion, as well as µg/ mg of protein) in nasal secretions obtained from patients at the time of surgery as compared to healthy subjects (**chapter 6A and 6B**) suggest an increased leakage and/ or local production of albumin, sIgA, IgG and IgM in nasal polyposis. The decrease of albumin and immunoglobulins after 6 months of therapy supports the earlier conclusion (**chapter 4 and 5**) that local inflammatory reactions have been reduced by surgery and treatment with topical steroids.

After 6 months of treatment, sIgA and IgG to protein ratios were no longer significantly different from ratio's in healthy subjects. This indicates that the humoral immune response is reduced by the therapy to more or less normal levels.

Concentrations of albumin and immunoglobulins decreased further between 6 months and 1 year of treatment, whereas ratio's of albumin, sIgA and IgG to protein increased and became again significantly higher than in healthy subjects (**chapter 6B**). Although the increased ratio's of albumin, sIgA and IgG can reflect changes in local immune reactivity, they may also be due to the relatively strong decrease in total protein concentration between 6 months and 1 year of follow-up. Because recurrence rates of nasal polyps were the same at 1 year as at 6 months, the decrease of total protein concentration could not be correlated to leakage from polyps. The relatively strong decrease in total protein concentration is probably due to the treatment with topical steroids which reduce leakage of proteins by reduction of vasopermeability. In addition, the further decrease of immunoglobulin concentrations, and the fact that no higher recurrence rates were found after 1 year than at 6 months, indicate that 1 year after surgery treatment with topical steroids still has beneficial effects. Therefore, we advocate to use topical steroids for at least 1 year after surgery for nasal polyps. To determine long term effects of topical steroids patients will be evaluated after 2 years of follow-up.

Clinical Aspects

Clinical evaluation after 6 months and 1 year of follow-up revealed a recurrence of nasal polyps in 50% and 56% of the patients, respectively (**chapter 3**). Only patients with extensive polyposis, in combination with an IgE-mediated allergy, had a higher risk of a recurrence (82%) at 1 year than the whole group of 72 patients. The IgE-mediated allergy, which normally is associated with an inflammatory reaction, probably enhances inflammation in the nasal mucosa and thereby the development of recurrences of nasal polyps. Therefore, it is important that an IgE-mediated allergy is treated adequately.

In order to demonstrate the possible relationship between pathology in the upper- and lower respiratory tract in patients with nasal polyps, we studied lungfunctions pre- and postoperatively. A better postoperative lungfunction (Forced Expiratory Volume/ 1 second › 10%), or the use of less medication, was seen in 63% and 25% of the patients with CAO at 6 months and 1 year after surgery and treatment with topical steroids (**chapter 3**). Only 25% and 40% of these patients had a recurrence at 6 months and 1 year, respectively, whereas all the patients with a deteriorated lungfunction had a recurrence at 6 months and 1 year. These differences are indicative of a relationship between disease in the upper and lower respiratory tract and show that surgery and the use of topical steroids in the upper respiratory tract also have a beneficial effect on pathology in the lower respiratory tract. Nevertheless, the exact mechanisms involved in this relationship remain unclear.

The data presented give evidence that local immunological and inflammatory processes play an important role in the development of nasal polyps. Surgical treatment combined with topical steroids give a reduction of the inflammatory process in the nasal mucosa and of immunoglobulins levels in nasal secretions. This may be of importance to postpone or prevent recurrences of nasal polyps. Furthermore, reduction of inflammatory reactions in the upper respiratory tract improved the pathology in the lower respiratory tract. The notion that T lymphocytes and activated eosinophils play a role in the pathogenesis of nasal polyps, and the fact that surgical treatment combined with topical steroids has an effect on these cells, opens new ways to develop medication directed at keypoints in the interaction and activation of T lymphocytes and eosinophils.

SUMMARY

Although inflammatory processes within the mucosa of the upper respiratory tract seem to play a role in the etiology and pathogenesis of nasal polyps, the exact mechanisms involved are poorly understood. Nasal polyps originate nearly always from the mucosa of the ethmoid sinuses and middle turbinates. Chronic inflammation at these specific sites probably plays an important role in the development of nasal polyps.

The aim of the study was to investigate whether local immunological processes are associated with the development of nasal polyps.

In **Chapter 1** a summary of the current knowledge of cellular and humoral aspects of nasal polyps and the nasal mucosa is given. Moreover the treatment of nasal polyps is discussed.

Chapter 2 and **chapter 3** describe cell populations detected by immunohistochemical stainings performed on nasal polyps and biopsy specimens of the nasal mucosa of the middle and inferior turbinates of patients with nasal polyps and healthy subjects. Significantly more CD8+ (T suppressor/ cytotoxic) cells than CD4+ (helper/ inducer) cells were found in nasal polyps, the lamina propria of the middle and inferior turbinates of the patients and in the inferior turbinates of the healthy subjects. In the middle turbinates of healthy subjects no significant differences between scores of CD8+ and CD4+ cells were observed. The numbers of CD4+ and CD8+ cells in nasal polyps were very similar to the number in the macroscopically unaffected mucosa of the middle turbinates of the patients, whereas scores in the inferior turbinates were lower. The middle and inferior turbinates of healthy subjects contained significantly more CD4+ cells than the middle and inferior turbinates of patients with nasal polyps. Significantly more HLA-DR+ cells were found in polyps and the middle turbinates than in the inferior turbinates of the patients. The possible role of CD4+, CD8+ and HLA-DR+ cells in these tissues is dicussed with regard to the pathogenesis of nasal polyps.

Chapter 4 provides an evaluation of clinical parameters in relation to histology of biopsy specimens of the mucosa of the middle and inferior turbinates of patients who were operated upon for nasal polyps. Postoperatively the patients were treated with topical steroids. After 6 months of follow-up more CD4+ (helper/ inducer) cells were found than at time of surgery, especially in the middle turbinates, whereas at 1 year the number of CD4+ cells had decreased and was lower than at 6 months. This could suggest that the occurrence of nasal polyps is probably associated with T cell dependent disturbances. However, no correlation was found between scores of CD4+ cells and recurrence rates after 6 months and 1 year. Clinical evaluation revealed that most of the patients (63%) with chronic airway obstruction (CAO) had a better lungfunction or used less lungmedication postoperatively. It is concluded that

surgery combined with topical steroids has a positive effect on pathology in the upper- and lower respiratory tract.

Chapter 5 describes the distribution of activated and non-activated eosinophils in nasal polyps and the nasal mucosa of patients and healthy subjects. After surgery and treatment with topical steroids a reduction of eosinophil infiltration and activation in recurrences of nasal polyps and the nasal mucosa of the patients was found. Moreover, no activated eosinophils were detected in the nasal mucosa of healthy subjects. Activated eosinophils probably play a role in the pathogenesis of nasal polyps.

Chapter 6A and 6B provides data on nasal secretions of patients with nasal polyps and of healthy individuals. Compared to healthy subjects, patients had highly increased concentrations of total protein, IgM, sIgA and IgG. After surgery and treatment with topical steroids concentrations of IgM, sIgA and IgG decreased significantly. It is suggested that nasal polyposis is associated with a locally increased production and/ or leakage of IgM, sIgA, and IgG. Treatment with surgery and topical steroids reduces the production of immunoglobulins to more or less normal levels.

In **Conclusion,** the studies in this thesis show that local immunological and inflammatory processes play a role in the development of nasal polyps. Furthermore, the reduction of inflammatory reactions after surgery and treatment with topical steroids improves pathology in the upper- and lower respiratory tract.

SAMENVATTING

Algemeen wordt aangenomen dat ontstekingsprocessen in het respiratoire slijmvlies van de bovenste luchtwegen een belangrijke rol spelen bij de etiologie en pathogenese van neuspoliepen. De exacte werkingsmechanismen worden echter nog niet geheel begrepen. Omdat neuspoliepen vrijwel altijd ontstaan uit het slijmvlies van het ethmoid en de middelste neusschelp spelen locale factoren waarschijnlijk een rol.

Het doel van de studie was te onderzoeken of lokale immunologische processen een rol spelen bij de ontwikkeling van neuspoliepen.

In **Hoofdstuk 1** wordt een kort overzicht gegeven over de huidige kennis van cellulaire en humorale aspecten van neuspoliepn en neusmucosa. Tevens wordt de behandeling van neuspoliepen besproken.

Hoofdstuk 2 en 3 beschrijven celpopulaties die d.m.v. immunohistochemische kleuringstechnieken werden gevonden in neuspoliepen en biopten van het slijmvlies van de middelste en onderste neusschelpen van patienten met neuspoliepen en gezonde controle personen. Significant meer CD8+ (suppressor/ cytotoxische) cellen dan CD4+ (helper/ inducer) cellen werden gevonden in neuspoliepen, in de lamina propria van de middelste en onderste neusschelpen van patienten en in de onderste neusschelpen van gezonde personen. In de middelste neusschelpen van gezonde personen werden geen significante verschillen gevonden tussen CD4+ en CD8+ cellen. Het aantal CD4+ en CD8+ cellen in neuspoliepen waren vrijwel gelijk aan de aantallen die werden gevonden in de macroscopisch normaal uitziende middelste neusschelpen van patienten, terwijl lagere scores werden gevonden in de onderste neusschelpen. Het slijmvlies van de middelste en onderste neusschelpen van gezonde personen bevatte significant meer CD4+ cellen dan de middelste en onderste neusschelpen van patienten met poliepen. In poliepen en het slijmvlies van de middelste neusschelpen werden significant meer HLA-DR+ cellen gevonden dan in de onderste neusschelpen van patiente. De mogelijke rol die CD4+, CD8+ en HLA-DR+ cellen spelen met betrekking tot de pathogenese van neuspoliepen wordt besproken.

Hoofdstuk 4 geeft een evaluatie van klinische parameters in relatie tot de histologie van neusbiopten van het slijmvlies van de middelste en onderste neusschelpen van patienten met neuspoliepen tijdens en na operatie. Postoperatief werden alle patienten behandeld met locale corticosteroiden. Na 6 maanden follow-up werden meer CD4+ cellen gevonden dan op het tijdstip van de operatie, m.n. in de middelste neusschelpen, terwijl na 1 jaar het aantal CD4+ cellen daalde en lager was dan na 6 maanden. Dit zou een indicatie kunnen zijn dat het ontstaan van neuspoliepen mogelijk is geassocieerd met T cell afhankelijke veranderingen. Er werd echter geen correlatie gevonden tussen de lage aantallen CD4+ cellen en het ontstaan van een recidief na 6 maanden of 1

jaar. Klinische evaluatie liet zien dat de meeste patienten met CARA (63%) postoperatief een betere longfunctie hadden of duidelijk minder longmedicatie gebruikte. Chirurgie, gecombineerd met locale corticosteroiden hebben dus een positief effect op zowel de bovenste als de onderste luchtwegen.

Hoofdstuk 5 beschrijft de verdeling van geactiveerde en niet-geactiveerde eosinofiele granulocyten in neuspoliepen en het neusslijmvlies van patienten en gezonde personen. Na chirurgie en behandeling met locale corticosteroiden werden in het neusslijmvlies van patienten en in recidief poliepen m.n. minder geactiveerde eosinofielen gevonden. Verder werden geen geactiveerde eosinofielen gezien in het neusslijmvlies van gezonde personen. Geactiveerde eosinofielen spelen waarschijnlijk een belangrijke rol bij de pathogenese van neuspoliepen.

Hoofdstuk 6A en 6B verschaffen gegevens betreffende neussecreten van patienten met neuspoliepen en van gezonde personen. In vergelijking tot gezonde personen werden bij patienten sterk verhoogde concentraties totaal proteine, IgM, sIgA en IgG gevonden. Na chirurgie en behandeling met locale corticosteroiden werden significant lagere concentraties IgM, sIgA en IgG gevonden. Gesuggereerd wordt dat polyposis nasi is geassocieerd met een verhoogde lekkage, c.q. locale productie van IgM, sIgA en IgG. Behandeling met chirurgie en locale corticosteroiden verminderd de productie van immunoglobulines tot een min of meer normaal niveau.

Concluderend kan worden gesteld dat locale ontstekings- en immunologische processen een rol spelen bij de pathogenese van neuspoliepen. De vermindering van de inflammatoire reactie in poliepen en de neusmucosa, na chirurgie en behandeling met locale corticosteroiden, heeft een positief effect op zowel de bovenste- als ook op de onderste luchtwegen.

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CURRICULUM VITAE

De auteur van dit proefschrift werd op 28 februari 1959 geboren te Utrecht. Na het behalen van het Atheneum examen aan het College Blaucapel te Utrecht in 1977, werd de studie Geneeskunde aan de Rijksuniversiteit Groningen begonnen. In 1985 werd het arts-examen behaald, waarna hij 1 jaar werkte als arts-assitent in het Streekziekenhuis Midden Twente, Hengelo. Na 9 maanden als AGNIO te hebben gewerkt, werd hij van 1 juli 1987 tot 1 juli 1991 opgeleid tot Keel-, Neus-, en Oorarts in het Academisch Ziekenhuis van de Vrije Universiteit te Amsterdam (opleider prof.dr. G.B. Snow). Van 1 juli 1989 tot 1 januari 1990 werd een deel van deze opleiging in het Westeinde Ziekenhuis te Den Haag volbracht (opleider dr. I.B. Tan). Vanaf 1 juli 1991 is hij werkzaam als Chef de Clinique in het St. Clara Ziekenhuis te Rotterdam (dr. H. Jongert en A.O. Korff), alwaar hij zich op 1 januari 1993 zal vestigen.

PUBLICATIES

Stoop AE, Hameleers DMH, v. Run P, Biewenga J, v.d. Baan S. Lymphocytes and nonlymphoid cells in the nasal mucosa of patients with nasal polyps and of healthy subjects. J Allergy Clin Immunol 1989; 84: 734-41.

Hameleers DMH, Stoop AE, v.d. Ven I, Biewenga J, v.d. Baan S, Sminia T. Intraepithelial lymphocytes and nonlymphoid cells in the human nasal mucosa. Int Arch Allergy Appl Immunol 1989; 88: 317-22.

Hameleers DMH, Biewenga J, Van der Ven I, Stoop AE, Van der Baan S, Sminia T. Lymphocytes and non-lymphoid cells in the human nasal mucosa. NER Allergy Proc 1989; 9: 455.

Biewenga J, Stoop AE, Hameleers DMH, Van der Baan S. Lymphoid and nonlymphoid cells in the nasal mucosa of patients with nasal polyps, patients with other nasal complaints and healthy controls. Allergologie 1989; 12: 295.

Hameleers DHM, Stoop AE, v.d. Ven I, Sminia T, v.d. Baan S, Biewenga J. An immuno- and enzyme-histochemical study of the human nasal mucosa of ear, nose and throat patients and controls. EOS J Immunol Immunopharmacol. 1990; Vol 10, no 2: 58-63.

Stoop AE, v.d. Heijden HAMD, Biewenga J, v.d. Baan S. Lymphocytes and nonlymphoid cells in human nasal polyps. J Allergy Clin Immunol 1991; 87: 470-75.

Biewenga J, Stoop AE, Baker HE, Swart SJ, Nauta JJP, van Kamp GJ, van der Baan S. Nasal secretions from patients with polyps and from healthy individyals, collected with a new aspiration system, and analysed for total protein and immunoglobulin concentrations. Annals of Clinical Biochemistry 1991; 28: 260-66.

Vleming M, Stoop AE, Middelweerd MJ, de Vries N. Results of Endoscopic Sinus Surgery for Nasal Polyps. Am J Rhinol 1992; (in press).

Stoop AE, v.d. Heijden HAMD, Biewenga J, v.d. Baan S. Clinical aspects and distribution of immunologically active cells in the nasal mucosa of patients with nasal polyps after endoscopic sinus surgery and treatment with topical steroids. Eur Arch Oto-Rhino-Laryngol 1992; (in press).

Stoop AE, v.d. Heijden HAMD, Biewenga J, v.d. Baan S. Eosinophils in nasal polyps and nasal mucosa: an immunohistochemical study. (submitted for publication).

Stoop AE, v.d. Heijden HAMD, Biewenga J, v.d. Baan S. Immunoglobulin concentrations in nasal secretions of patients with nasal polyps. (submitted for publication).

STELLINGEN

behorende bij het proefschrift

Immunological aspects of nasal polyps

A.E. Stoop

1. Bij het ontstaan van neuspoliepen spelen locale T cel gemedieerde mechanismen een rol.

2. Vermindering van ontstekingsprocessen in de bovenste luchtwegen wordt weerspiegeld in klinische verbetering van pathologie in de onderste luchtwegen bij patienten met CARA.

3. Bij ontstekingsreacties in het neusslijmvlies speelt niet zozeer het aantal eosinofielen een rol alswel het activatiestadium van deze cellen.

4. Het verdient aanbeveling onderzoek te verrichten naar medicijnen die het "platelet activating factor" competatief kunnen binden.

5. Daling van het absolute aantal CD4+ cellen in HIV-seropositieven is van prognostisch belang en wordt algemeen gebruikt als parameter voor interventie met antiretrovirale middelen *(AIDS 1991; 5: 43-47)*.

6. Gezien de toenemende veroudering van de nederlandse bevolking is tijdige signalering van doof- en blindheid van groot belang voor het bepalen van het beleid ten aanzien van- en de hulpverlening aan ouderen ter voorkoming van onhoudbare situaties.

7. Dromen worden biochemisch bepaald *(Harvard Gazette, januari 1992)*.

8. Omdat opiaten niet alleen via receptoren in het centraal zenuwstelsel werken verdient het de voorkeur bij pijnbestrijding deze middelen, indien mogelijk, locaal toe te dienen.

9. De "dubbele" kwalificatie van kaakchirurgen zal in de toekomst wellicht voor "drukte aan het hoofd" zorgen.

10. Erfelijke eigenschappen, levenservaring, karakter en de wijze waarop irritaties worden verwerkt zijn factoren die het immuunsysteem kunnen beinvloeden.

11. Verschillende rassen komen alleen voor bij planten en dieren.

12. De snelle vooruitgang op het gebied van de genetische manipulatie zal er toch nooit toe leiden dat er een echte "Vlinderdas" ontstaat.