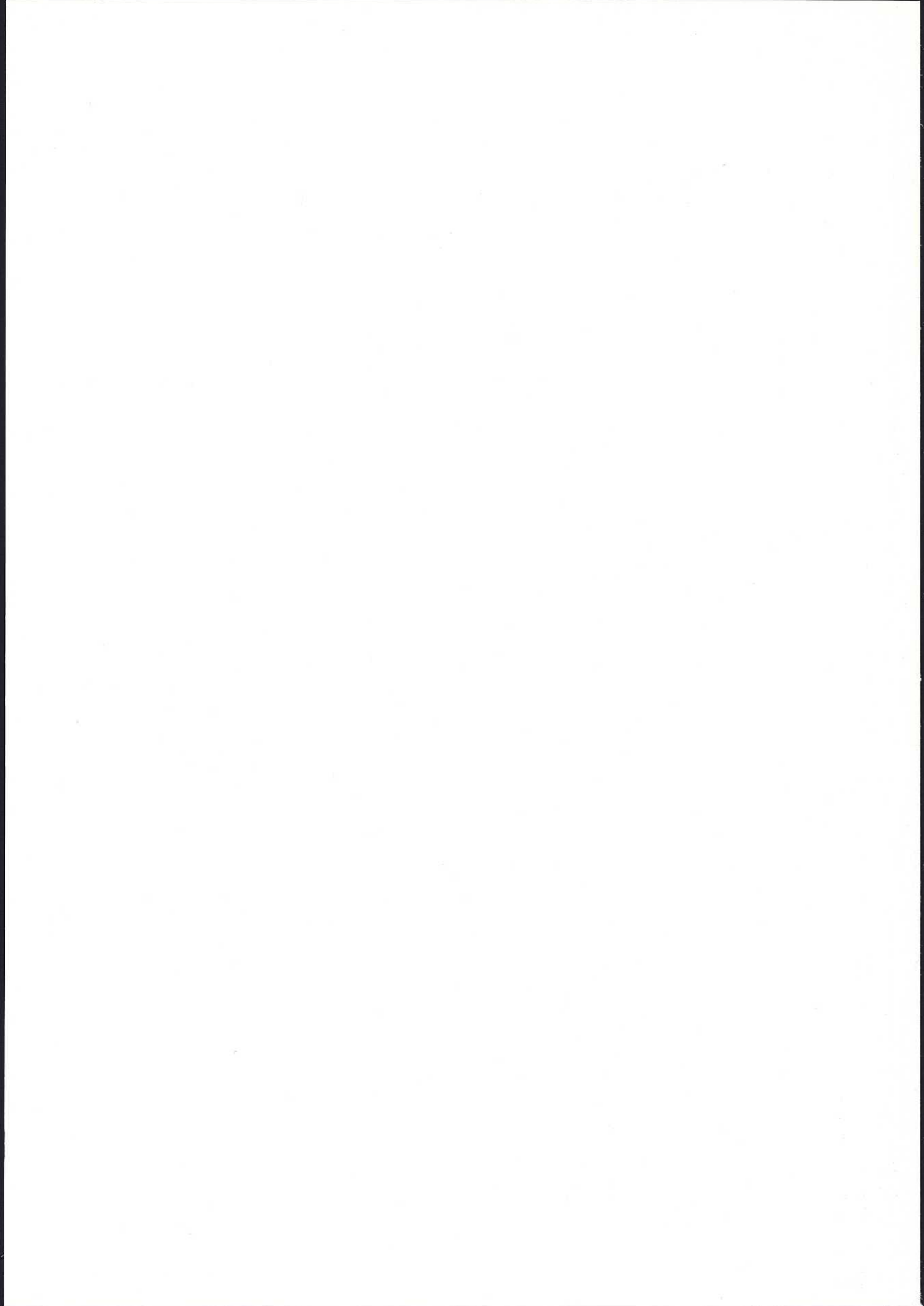# ALLERGIC INFLAMMATION IN THE NASAL MUCOSA

A Clinical, Morphological and
Biochemical Study in Allergic Rhinitis
with Special Reference to Mast Cells

Sigurdur Juliusson

Göteborg 1993

ABSTRACT

ALLERGIC INFLAMMATION IN THE NASAL MUCOSA
A Clinical, Morphological and Biochemical Study in Allergic Rhinitis with Special Reference to Mast Cells

SIGURDUR JULIUSSON, M.D., Departments of Otolaryngology Head & Neck Surgery and Pathology, University of Göteborg, Sahlgrenska Hospital, S-413 45 Göteborg, Sweden.

Mast cells are the primary effector cells in acute allergic reactions in mucosal membranes and in the skin. The acute symptoms after allergen exposure of the sensitised individual are mainly caused by mast-cell-derived mediators. The aim of this study was to investigate the function of the mast cell in allergic inflammation by morphological and biochemical analysis of the nasal mucosa and by clinical study of patients with seasonal allergic rhinitis before and after allergen provocation as well as before and during the pollen season. Nasal biopsies were performed and brush and lavage samples were collected from the nasal mucosa. Mast cells were morphologically identified based on their metachromatic properties on staining with toluidine blue as well as with immunohistological methods with monoclonal antibodies against IgE, and two mast cell proteases, tryptase and chymase. Sensitivity to aldehyde fixation on metachromacy was studied. The histamine content of tissue samples was determined by high performance liquid chromatography. The levels of histamine and tryptase in the returned fluid of nasal lavages were analysed by radioimmunoassay and the TAME-esterase activity in the lavage fluid was determined with a radiochemical method. The effects of topical treatment with corticosteroids were studied.

Mast cells were found in increased numbers in the nasal epithelium in patients with allergic rhinitis even when free of symptoms. This increase in intra-epithelial cells consisted mostly of cells containing tryptase and lacking chymase but also of cells containing both tryptase and chymase. There was also an increase in the stroma of mast cells containing tryptase only. The metachromatic staining properties of mast cells in the specimens from the allergic patients were found to be decreased and highly aldehyde sensitive. The numbers of mast cells in the epithelium before allergen provocation correlated with nasal symptoms after the provocation. After an initial decrease after the allergen challenge, the numbers of metachromatic, tryptase-containing and IgE-bearing cells increased as well as the histamine content of cellular material from the nasal epithelium. The levels of histamine and tryptase and the TAME-esterase activity in the lavage fluid increased after allergen provocation. After topical treatment with a corticosteroid a decrease was found in the post allergen challenge symptoms and tryptase levels in the lavage fluid as well as the density of those mast cells in the epithelium that contained tryptase but lacked chymase.

It was concluded that the mast cell plays an unequivocal role in the inflammation of allergic diseases in mucosal membranes. The functional properties of mast cells found at the site of the inflammation are altered. The lack of chymase and the decrease in metachromatic staining capacity combined with an increase in aldehyde sensitivity reflects a functional activation of the mast cells, rather than phenotypic differentiation related to anatomical site. The beneficial effects on allergic symptoms after topical corticosteroid treatment may to some extent be explained through a decrease in the density of mast cells in the shock organ.

Key words: Allergic rhinitis, nasal mucosa, allergen challenge, mast cells, eosinophil granulocytes, basophils, histamine, tryptase, chymase, immunohistochemsitry.

ALLERGIC INFLAMMATION IN THE NASAL MUCOSA
A Clinical, Morphological and Biochemical Study in Allergic Rhinitis with Special Reference to Mast Cells

Avhandlingen baseras på följande delarbeten:

I   Juliusson S, Pipkorn U, Karlsson G and Enerbäck L.
Mast cells and Eosinophils in the Allergic Mucosal Response to Allergen Challenge;
Changes in Distribution and Signs of Activation in Relation to Symptoms.
Journal of Allergy and Clinical Immunology 90: 898-909, 1992.

II   Juliusson S, Karlsson G, Bachert C and Enerbäck L.
Metachromatic, IgE-bearing and Tryptase-containing Cells on the Nasal Mucosal
Surface in Provoked Allergic Rhinitis.
Submitted for publication 1993.

III   Juliusson S, Holmberg K, Baumgarten CR, Olsson M, Enander I and Pipkorn U.
Tryptase in Nasal Lavage Fluid after Local Allergen Challenge; Relationship to
Histamine Levels and TAME-Esterase Activity.
Allergy 46: 459-465, 1991.

IV   Juliusson S, Holmberg K, Karlsson G, Enerbäck L and Pipkorn U.
Mast Cells and Mediators in the Nasal Mucosa after Allergen Challenge; Effects of
Four Weeks' Treatment with Topical Glucocorticoid.
Clinical and Experimental Allergy 23: 591-599, 1993.

V   Juliusson S, Aldenborg F and Enerbäck L.
Proteinase Content of Mast Cells of the Nasal Mucosa; Effects of Natural Allergen
Exposure and of Local Corticosteroid Treatment.
Submitted for publication 1993.

ALLERGIC INFLAMMATION
IN THE NASAL MUCOSA

A Clinical, Morphological and Biochemical Study
in Allergic Rhinitis with Special Reference to Mast Cells

Sigurdur Juliusson

Departments of
Otolaryngology Head & Neck Surgery
and Pathology
University of Göteborg, Sahlgrenska Hospital,
S-413 45 Göteborg, Sweden

Göteborg 1993

ABSTRACT

ALLERGIC INFLAMMATION IN THE NASAL MUCOSA. A Clinical, Morphological and Biochemical Study in Allergic Rhinitis with Special Reference to Mast Cells
SIGURDUR JULIUSSON, M.D., Departments of Otolaryngology Head & Neck Surgery, and Pathology, University of Göteborg, Sahlgrenska Hospital, S-413 45 Göteborg, Sweden.

Mast cells are the primary effector cells in acute allergic reactions in mucosal membranes and in the skin. The acute symptoms after allergen exposure of the sensitised individual are mainly caused by mast-cell-derived mediators. The aim of this study was to investigate the function of the mast cell in allergic inflammation by morphological and biochemical analysis of the nasal mucosa and by clinical study of patients with seasonal allergic rhinitis before and after allergen provocation as well as before and during the pollen season. Nasal biopsies were performed and brush and lavage samples were collected from the nasal mucosa. Mast cells were morphologically identified based on their metachromatic properties on staining with toluidine blue as well as with immunohistological methods with monoclonal antibodies against IgE, and two mast cell proteases, tryptase and chymase. Sensitivity to aldehyde fixation on metachromacy was studied. The histamine content of tissue samples was determined by high performance liquid chromatography. The levels of histamine and tryptase in the returned fluid of nasal lavages were analysed by radioimmunoassay and the TAME-esterase activity in the lavage fluid was determined with a radiochemical method. The effects of topical treatment with corticosteroids were studied.

Mast cells were found in increased numbers in the nasal epithelium in patients with allergic rhinitis even when free of symptoms. This increase in intra-epithelial cells consisted mostly of cells containing tryptase and lacking chymase but also of cells containing both tryptase and chymase. There was also an increase in the stroma of mast cells containing tryptase only. The metachromatic staining properties of mast cells in the specimens from the allergic patients were found to be decreased and highly aldehyde sensitive. The numbers of mast cells in the epithelium before allergen provocation correlated with nasal symptoms after the provocation. After an initial decrease after the allergen challenge, the numbers of metachromatic, tryptase-containing and IgE-bearing cells increased as well as the histamine content of cellular material from the nasal epithelium. The levels of histamine and tryptase and the TAME-esterase activity in the lavage fluid increased after allergen provocation. After topical treatment with a corticosteroid a decrease was found in the post allergen challenge symptoms and tryptase levels in the lavage fluid as well as the density of those mast cells in the epithelium that contained tryptase but lacked chymase.

It was concluded that the mast cell plays an unequivocal role in the inflammation of allergic diseases in mucosal membranes. The functional properties of mast cells found at the site of the inflammation are altered. The lack of chymase and the decrease in metachromatic staining capacity combined with an increase in aldehyde sensitivity reflects a functional activation of the mast cells, rather than phenotypic differentiation related to anatomical site. The beneficial effects on allergic symptoms after topical corticosteroid treatment may to some extent be explained through a decrease in the density of mast cells in the shock organ.

Key words: Allergic rhinitis, nasal mucosa, allergen challenge, mast cells, eosinophil granulocytes, basophils, histamine, tryptase, chymase, immunohistochemsitry.

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I   Juliusson S, Pipkorn U, Karlsson G and Enerbäck L.
Mast cells and Eosinophils in the Allergic Mucosal Response to Allergen Challenge; Changes in Distribution and Signs of Activation in Relation to Symptoms.
Journal of Allergy and Clinical Immunology 90: 898-909, 1992.

II   Juliusson S, Karlsson G, Bachert C and Enerbäck L.
Metachromatic, IgE-bearing and Tryptase-containing Cells on the Nasal Mucosal Surface in Provoked Allergic Rhinitis.
Submitted for publication 1993.

III   Juliusson S, Holmberg K, Baumgarten CR, Olsson M, Enander I and Pipkorn U.
Tryptase in Nasal Lavage Fluid after Local Allergen Challenge; Relationship to Histamine Levels and TAME-Esterase Activity.
Allergy 46: 459-465, 1991.

IV   Juliusson S, Holmberg K, Karlsson G, Enerbäck L and Pipkorn U.
Mast Cells and Mediators in the Nasal Mucosa after Allergen Challenge; Effects of Four Weeks' Treatment with Topical Glucocorticoid.
Clinical and Experimental Allergy 23: 591-599, 1993.

V   Juliusson S, Aldenborg F and Enerbäck L.
Proteinase Content of Mast Cells of the Nasal Mucosa; Effects of Natural Allergen Exposure and of Local Corticosteroid Treatment.
Submitted for publication 1993.

CONTENTS

ABBREVIATIONS

ACE	angiotensin converting enzyme
APAAP	alkaline phosphatase-anti-alkaline phosphatase
BU	biological units
CD	clusters of differentiation
cpm	counts per minute
CTMC	connective tissue mast cells
Da	Dalton
ECF-A	eosinophil chemotactic factor of anaphylaxis
GM-CSF	granulocyte/macrophage colony stimulating factor
h	hour(s)
HPLC	highly purified liquid chromatography
IFAA	iso-osmotic formaldehyde acetic acid
Ig	immunoglobulin
IL	interleukin
IFN	interferon
LPR	late-phase reactions
LTB_4	leukotriene B_4 (also LTC_4, LTD_4, LTE_4)
MBP	major basic protein
MC_T	tryptase only positive mast cells
MC_{TC}	tryptase and chymase positive mast cells
MMC	mucosal mast cells
min	minute(s)
mol wt	molecular weight
NCF-A	neutrophil chemotactic factor of anaphylaxis
PAF	platelet-activating factor
PAP	peroxidase-anti-peroxidase
PGD_2	prostaglandin D_2 (also PGE_2, PGF_2)
PGI_2	prostacyclin
PPD	purified protein derivative
RIA	radioimmunoassay
RIACT	radioimmunoassay on coated tubes
RNA	ribonucleic acid
SCF	stem cell factor
SEM	standard error of the mean
SRS-A	slow reacting substances of anaphylaxis
TAME	N-α-tosyl-L-arginine-methyl ester
TNF_α	tumor necrosis factor-α
TXA_2	thromboxane A_2

INTRODUCTION

The first known indication of allergic hyperactivity occurred about 5000 years ago. It was the first ruler of the first dynasty in ancient Egypt, Pharaoh Menes of Memphis, who died after having been stung by a hornet. As a sufferer of hay fever, Charles Blackley established in 1873 for the first time beyond any doubt that pollen caused hay fever (Blackley 1873). At the beginning of the 20th century von Pirquet and Shick proposed the term allergy (from the Greek words allos "changed" and ergos "action") for a changed reactivity to staphylococcal antitoxin serum in children (von Pirquet and Schick 1906).

Cooke and Coca coined the term atopy for a cluster of diseases, namely, asthma, hay fever, urticaria and anaphylactic reactions, all with a familial tendency of occurrence (Coca and Cooke 1923). Atopic dermatitis was later added to this list. Today the term atopy refers to a hereditary predisposition to overproduction of antibodies of the IgE type. The risk of developing an allergic disease is increased in parallel to an increased incidence of IgE-mediated disease in one or both parents (Kjellman 1977, Åberg et al. 1989). The atopic individual may suffer from one or more of the atopic diseases which characteristically start at different ages. The more atopic the individual, the earlier the first appearance of symptoms. Gastrointestinal allergy starts in infancy and is followed by atopic dermatitis. During childhood the individual develops atopic asthma while allergic rhinitis often starts in early adolescence. The sensitisation to the allergen persists, but the disease often decreases in intensity with age.

It seems certain that there has been an increase in the occurrence of both allergic rhinitis and asthma in many if not all the industrialised nations (Würthrich 1989, Åberg 1989). The differences in incidence from one community to another present a picture of a group of conditions where potentially alterable environmental factors certainly play a role (Peat et al. 1987, Åberg 1989). Today, allergy and other forms of hyperreactivity are so common in the Nordic countries that they constitute a major public health problem. Annually, nearly one-third of the population experiences some sort of hypersensitivity or allergy, and over a lifetime nearly half of the population experience a hypersensitive reaction. Some differences do exist between the scientific/medical concepts of allergy and the popular definition, however (Consensus Statement 1992).

Inhalation of allergens can cause allergic reactions in the nose and/or the bronchi of the sensitised individual. The nasal reaction causes sneezing, itching, congestion and rhinorrhea while the bronchial reaction is due to narrowing of the airways by constriction, mucosal swelling and increased secretion.

The central feature of allergic rhinitis is an inflammation of the nasal mucous membrane. The mast cell, with IgE on its surface, is the primary affector cell in this process. Activation of the cell initiates a cascade of reactions starting with the release of inflammatory mediators, such as histamine, leading to recruitment of new inflammatory cells resulting in an ongoing inflammatory process.

The mast cell's role in the pathophysiology of the allergic inflammation has received increased interest during the last decade. This is due to new knowledge on the heterogeneity of the mast cells, their origin in the bone marrow and their differentiation. The fact that mast cells produce and secrete cytokines has given them a potential role in cell regulation, orchestrating an autoregulation of the allergic inflammation. Furthermore, there has been an intense research on mast-cell derived mediators such as tryptase and chymase, two neutral esterases in the mast cell granule.

The over-all aim of this study was to elucidate the role of the mast cell in allergic inflammation. To achieve this, clinical, morphological and biochemical studies have been performed, both as allergen challenges in the laboratory and under natural allergen exposure during the pollen season.

NASAL AIRWAYS

The specialised anatomy of the nose is important for its function: heating, humidification and filtration of inhaled air; the sense of smell; and conservation of exhaled water and heat in the relatively cold anterior part of the nose. The nasal mucosa constitutes the first line of defence for the body interior, having a high capacity to remove inhaled microorganisms, allergens and irritating substances. The efficacy of the nasal filter depends upon the size of the particles. Most particles larger than 10 µm (pollen grains) will be trapped in the nose, while most particles smaller than 2 µm (mould spores) pass through the nose. Deposited particles are cleared from the nose within 10-30 min by mucociliary transport (Mygind and Bisgaard 1990).

The vestibulum nasi is lined by skin. The nasal cavity is lined by a ciliated pseudostratified columnar epithelium consisting of nonciliated epithelial cells, ciliated epithelial cells (with 4-6 µm long cilia), goblet cells and basal cells, all resting on a basement membrane. Beneath the basement membrane is the lamina propria, consisting of connective tissue, glands, blood vessels and nerves. Fibroblasts and macrophages form the most common cell types but varying numbers of leukocytes, lymphocytes, plasma cells, and mast cells are seen in the stroma (Petruson et al. 1984). The main constituent of nasal fluid is water (95-97%), but it also contains mucin (2.5-3%) and electrolytes (1-2%) as well as actively secreted IgA. Small molecules like histamine (mol wt 111 Da) readily penetrate the epithelial lining and small proteins (e.g allergens with a mol wt of 10,000 to 40,000 Da) from pollen grains may be soluble in the nasal surface liquid and may penetrate into the lamina propria to some extent (Okuda 1977). The wind-borne pollen grains (15-50 µm) probably do not penetrate the epithelial lining (Okuda 1977, Wihl and Mygind 1977).

The nasal cavity is richly supplied with blood that comes from branches of both the internal and the external carotid arteries. The mucosa has a well-developed capillary network. There is a subepithelial layer of capillaries, which have been shown to be partly of a fenestrated type (Cauna and Hinderer 1969). The cavernous sinusoids, which are especially abundant in the middle and inferior turbinates (Cauna 1982), are situated in the deepest parts of the lamina propria. A third functional and structural entity in the lower parts of the lamina propria is the arteriovenous anastomoses, which allow the blood to pass the capillary bed and are probably involved in thermoregulation (Cole 1982). The blood volume is regulated by the tone of the capacitance vessels (venous sinusoids and large veins), while the blood flow is regulated by the resistance vessels (small arteries and arterioli). The post-capillary venules are responsible for the inflammatory, mediator-induced increase in permeability of plasma proteins (Persson 1991).

The sensory nerve supply of the nasal cavities is derived from the ophthalmic and maxillary divisions of the trigeminal nerve. The non-myelinated nerve-endings are situated in the intra and subepithelial area (Cauna 1982). The sympathetic nerve fibres emerge from the stellate ganglion and mainly innervate the nasal mucosal vessels (Cauna 1982). The preganglionic transmitter substance is acetylcholine, which acts on nicotinic receptors, whereas the postganglionic transmitter is noradrenaline. Stimulation of α-adrenoceptors causes contraction of the vascular smooth muscles. The parasympathetic nerves originate in the superior salivatory nucleus. The transmitter is acetylcholine, which acts on nicotinic receptors preganglionically and muscarinic receptors postganglionically. Parasympathetic fibres are particularly numerous in the glands (Cauna et al. 1972) but the vessels are also innervated (Cauna 1982). Substance P, neurokinin A and K and calcitonin gene-related peptide are among a number of neuropeptides that are found in the autonomic and sensory nerve fibres of the airways in man. They are believed to participate in local axon reflexes upon stimulation of the nerve endings.
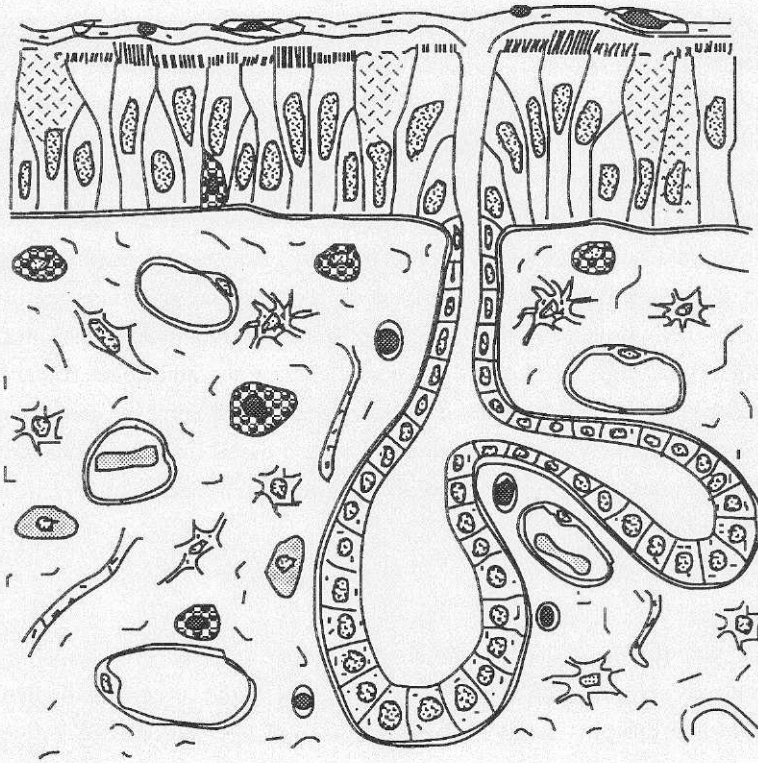

Figure 1. Illustration of the nasal mucosa. Mucus covers the surface of the pseudostratified epithelium. Ciliated cells, non-ciliated cells, goblet cells, and basal cells rest on the basement membrene. Fibroblasts, macrophages, leukocytes, lymphocytes, plasma cells, and mast cells are seen in the stroma between seromucous glands and capillaries. Adapted from Petruson et al. (1984).

ALLERGIC INFLAMMATION

Inflammatory reaction

Inflammation is the response of vascularised tissue to injury and is usually beneficial since it serves to resolve and repair the effect of damage. The factors that initiate inflammation are diverse and include infectious agents (bacteria, viruses and parasites), chemical agents (drugs, toxins and industrial agents), mechanical and ischemic injury, and immunological reactions, such as allergy and autoimmunity. The histopathological features of inflammation consist of changes in blood flow and caliber of small blood vessels and an increase in vascular permeability, the formation of an inflammatory exudate, and the escape of leukocytes from the blood into the extravascular spaces. This acute phase is followed by infiltration of lymphocytes and monocytes/macrophages together with proliferation of blood vessels and connective tissue. The memory capabilities of the immune system are conveyed by lymphocytes, but a specialised biological amplification system involving cellular and humoral components is necessary for host defence. The clearest example of the cells and mediators of the allergic response participating in host defence is the protective role of IgE, mast cells, basophils and eosinophils in helminth infection (Lee et al. 1986, Taverne 1989). When an adaptive immune response occurs in an exaggerated or inappropriate form causing tissue damage, the term hypersensitivity is applied. Coombs and Gell have classified hypersensitivity reactions into four types according to symptom pattern (Coombs and Gell 1968). Type I, or immediate hypersensitivity, is dependent on the specific triggering of IgE-sensitised mast cells by antigen resulting in the release of pharmacological mediators of inflammation. The allergic reaction has also been classified into immediate, late and delayed reaction, according to the time of onset of symptoms. Considering the complexity of the immune response, such classifications can at most be a useful over-simplification and it is now evident that allergy is an inflammatory response involving all cellular elements of the immune system.

IgE

The characteristic feature of allergic inflammation is the exaggerated IgE response, directed against innocuous antigens such as pollen. Following the initial contact of allergen with the mucosa there is a complex series of events before IgE is produced and before allergic symptoms result after a second contact with the same allergen. IgE production by B cells involves antigen presentation via antigen-presenting cells, T cell help and IL-4 stimulation of B cells to become IgE-producing. Different types of receptors for IgE have been described, a high-affinity IgE-receptor (FcɛI-R), classically described on mast cells and basophils, and a

low-affinity IgE-receptor (FcℰII-R), found on a variety of cells including lymphocytes, eosinophils, macrophages and thrombocytes, reviewed by Siraganian (1993).

Mast cells

Mast cells are found in loose connective tissue of most organs, except the brain, and are predominant in skin, airways and the gastro-intestinal tract. Mast cells exhibit a diversity of histological, biochemical and functional properties (Enerbäck et al. 1989). The role of mast cells in humans under non-pathological conditions is unknown but recent evidence of their cytokine production indicates that they may have a function in the growth and development of other cells (cf. Schwartz and Huff 1993). Mast cells are found in increased numbers in many pathological disorders such as allergic asthma (Gibson et al. 1993), allergic rhino-conjunctivitis, atopic dermatitis, parasitic infection, mastocytosis, arthritis, scleroderma and fibrosis (Schwartz and Huff 1993). With IgE on its surface the mast cell is the primay effector cell in allergic diseases in mucous membranes. The contact between the allergen and the mast cells is enhanced by an allergen induced redistribution of the cells from the stroma to the epithelium (Enerbäck et al. 1986a, Enerbäck et al. 1986b). Situated superficially in the respiratory epithelium, the mast cell is ideally located to respond to inhaled allergen. By cross-linking of adjacent IgE-molecules on the mast cell surface, the allergen triggers the cell to degranulate. Upon stimulation, mast cells release a spectrum of preformed and newly formed membrane-derived mediators (See Figure 2).

Mast cells are derived from pluripotential haematopoietic stem cells (Schwartz and Huff 1993). They undergo only part of their differentiation in bone marrow and complete there differentiation in peripheral tissue microenvironments rich in fibroblasts or stromal cells. For humans it is not clear which cytokines stimulate mast cell maturation and development. The first mast-cell-specific marker detected on the mast cell precursors is Kit, a receptor tyrosine kinase highly expressed on mast cells (Galli et al. 1992). Kit[+], IL-3^{-} progenitors are thought to be released from the bone marrow and their further development is dependent on stem cell factor (SCF), the ligand for the Kit, found on fibroblasts (Irani et al. 1992, Agis et al. 1993). Recent studies show that IL-3 does not enhance maturation of SCF-dependent fetal liver mast cells and when cultured with SCF and IL-4, a marked inhibition of mast cell differentiation was seen (Nilsson et al. 1993).

Morphology

Mature human tissue mast cells are usually 9 to 12 µm in diameter and can be round, spindle-shaped, or spider-like in shape and up to 20 µm long. Mast cell nuclei are rather long unsegmented ovals, and eccentrically placed, and rarely show mitotic figures in tissue (Galli 1984). There are thin elongated folds of their plasma membrane and cytoplasmic filaments and lipid bodies are found. As in basophils, free and membrane-bound ribosomes, and Golgi

structures are diminished in mast cells compare with other cells. Mast cells have numerous cytoplasmic granules (about 200), which vary remarkably in their shape and ultrastructural patterns. The granules can be filled with scrolls, crystals, particles, in reticular patterns or combinations of these patterns (Dvorak et al. 1983a, Enerbäck et al. 1986b). When stimulated *in vitro*, human mast cells undergo complex intracytoplasmic changes. Numerous degranulation channels are formed and granule matrix materials are solubilised prior to the development of multiple openings to the exterior of the degranulation channels (Dvorak et al. 1983b).

Different types of mast cells

Mast cells of two major types have emerged as a consistent observation in human and rodent tissues. Recognition of this complexity is crucial for an understanding of mast cell biology and, potentially, for treating mast-cell-associated disease. It has been recognised for many years that mast cell populations in both humans and rodents differ in their ability to be identified using standard metachromatic dyes such as toluidine blue following formaldehyde fixation (Enerbäck 1966b, Pipkorn et al. 1988c). While in the rat intestinal mucosa the entire mast cell population is "formalin sensitive", there are a number of other differences between mast cells in specific tissue sites. Several nomenclatures are used to describe particular mast cell types. However, none of these is optimal, because the nomenclature does not relate to known functional differences between the different cell types.

In rodents, mucosal mast cells (MMC) and connective tissue mast cells (CTMC) are the terms currently in most common use (Enerbäck 1966b). These mast cell types have been discriminated from one another based on proteoglycan content, neutral protease expression, arachidonic acid metabolites, and surface antigenic determinants (Schwartz and Huff 1993). The MMC lose their metachromatic staining property after treatment with strong aldehyde fixatives (Enerbäck 1966b). The proteoglycan in CTMC is heparin while MMC have chondroitin sulphates (Yurt et al. 1977, Kusche et al. 1988). The content of neutral protease in the granules qualitatively appears to distinguish rat CTMC, which contain chymase I and carboxypeptidase A, from rat MMC, which contain chymase II (Gibson and Miller 1986).

In humans, subsets of mast cells have been identified based on similar staining characteristics which depend upon mast cell proteoglycan content (Pipkorn et al. 1988c). Schwartz and coworkers have defined mast cells in man in terms of their content of specific protease enzymes. The term tryptase positive mast cells (MC_T) is used for cells with tryptase alone, and the term tryptase and chymase positive mast cells (MC_{TC}) for those cells which contain both tryptase and chymase (Irani et al. 1986b). MC_T have been described as the predominant population in the intestinal and respiratory mucosa while MC_{TC} predominate the skin and some other sites (Irani et al. 1986a, Irani et al. 1986b). The consequences of the lack of chymase in the MC_T cells are not known.

Histamine

Histamine, β-imidazolethylamine (mol wt 111 Da) is an intracellular mediator exerting potent effects on various target tissues. It is produced by decarboxylation of histidine. Ubiquitously distributed in mammalian tissues, histamine acting on histamine H_1 and H_2-receptors has a wide range of functions and is one of the major mediators of immediate hypersensitivity (Siraganian 1982, Wasserman 1983, Schwartz 1987). Histamine is also an important mediator in the central nervous system and the gastric mucosa (Sandvik et al. 1987). Stored in large amounts in metachromatic granules of mast cells and basophils, it is spontaneously released at low levels and actively released from these cells after challenge with allergens to which the allergic patient is sensitised, or with "non-specific" histamine releasers such as substance P, polyamines, opiates and a range of lymphokines and cytokines. As an extracellular mediator, histamine functions as a local hormone that is rapidly metabolised by either of two enzymatic pathways, methylation (70%) by histamine N-methyltransferase, or oxidation (30%) (Holgate et al. 1993).

Neutral proteases

Mast cell neutral proteases are a unique series of proteolytic enzymes that comprise most of the protein of the mast cell granule. The biological importance of these enzymes is not yet determined but studies demonstrate that they are a heterogeneous population giving a basis for a mast cell classification according to the neutral protease content (Irani et al. 1986a, Irani et al. 1986b).

Tryptase was the first neutral protease to be identified in human mast cells (Glenner and Cohen 1960). Human tryptase is a tetramer with a molecular weight of 134 kDa, composed of subunits of 31-35 kDa (Schwartz et al. 1981). Tryptase is contained in all mast cells, it constitutes approximately 20% of the total cellular protein content and is stored within the secretory granules in association with heparin proteoglycan. In MC_T cells levels of tryptase are around 10 pg per cell and in MC_{TC} cells they are up to 35 pg per cell (Schwartz et al. 1987). Very small quantities of tryptase, around 0.04 pg per cell, are found in human basophils (Castells et al. 1987). When the mast cell is stimulated for exocytosis, tryptase is released into the extracellular enviroment. After anaphylactic reactions, human tryptase diffuses into the systemic circulation and can be quantitated by serum immunoassay (Schwartz et al. 1987, Schwartz et al. 1989). Tryptase, in the absence of heparin, has a very short half-life of activity (Schwartz and Bradford 1986). Since the proteoglycans are large molecules and would be predicted to diffuse poorly, substantial tryptase activity is thought to be limited to the local area of mast cell activation. There is no known major biological function of this enzyme. Potential biological properties are fibrinogenolysis, high molecular weight kininogen destruction, prostromelysin activation, cleavage of C3 to C3a and neuropeptide degradation (cf. Holgate et al. 1993).

Chymase (mol wt 30 kDa) is a family of enzymes with chymotrypsin-like activity (Schechter et al. 1986). While being demonstrable only in certain subpopulations of higher mammalian mast cells (MC$_{TC}$), chymases are the dominant protease in rodent mast cells (Gibson and Miller 1986). Chymase is a monomer, stored active, presumably bound to heparin and secreted with histamine. It converts angiotensin I to angiotensin II approximately four times more efficiently than does angiotensin converting enzyme (ACE) (Reiley et al. 1982, Wintroub et al. 1984). Chymase also inactivates bradykinin and attacks the lamina lucida of the basement membrane at the dermal-epidermal junction of human skin. Its activity is inhibited by several biological inhibitors (Holgate et al. 1993).

Carboxypeptidase enzymes are the most recent enzymes to be localised to and purified from human mast cells. In man it has been found in MC$_{TC}$-cells and has similar activity as chymase but closely associated with the heparin proteoglycan, which in turn largely remains bound to the extracellular surface of the mast cell after degranulation (Goldstein et al. 1989).

Other enzymes and chemotactic factors

Mast cells also contain different acid hydrolases and oxidative enzymes as well as chemotactic factors like eosinophil chemotactic factor of anaphylaxis (ECF-A) and neutrophil chemotactic factor of anaphylaxis (NCF-A) (Holgate et al. 1993).

Proteoglycans

Proteoglycans comprise a central protein core from which long carbohydrate side chains issue radially, giving the proteoglycan its characteristic physiochemical properties. Intracellular proteoglycans form the structural basis of lysosomal granules into which the Golgi apparatus secretes various other chemical substances pertinent to the cell's function. The proteoglycan content of cells varies between different cell types and species. The proteoglycan in human mast cells is heparin (60 kDa). Histamine is bound to the carboxyl groups of glucuronic and iduronic acid, whereas the neutral proteases are bound to the anionic carboxyl and sulphate groups of the glycosaminoglycans. Heparin acts as an anticoagulant by enhancing the ability of antithrombin 3 to inhibit the proteases involved in the coagulation cascade. (Holgate et al. 1993).

Cytokines

Numerous studies have demonstrated that immunological activation of murine bone-marrow-cultured mast cells can lead to the expression of a number of cytokines (Gordon et al. 1990, Galli et al. 1991). These include IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, granulocyte/macrophage colony stimulating factor (GM-CSF) and tumour necrosis factor (TNF)-α. The production of cytokines by human mast cells has not been as extensively studied as the murine counterpart, but several studies have appeared which suggest a similar pattern in humans. For example,

human lung and skin mast cells have been shown to produce TNF-α (Walsh et al. 1991, Ohkawara et al. 1992). Human mast cells have also been shown to produce IL-4, IL-5 and IL-6 (Bradding et al. 1991). Although the significance of IL production by human mast cells is not known, the fact stands that thay produce and secrete cytokines. This gives the mast cell a potential role in cell regulation, the possibility of orchestrating an autoregulation of the allergic inflammation (See Figure 2).

Newly generated mediators

Diverse products of the complex pathways of oxidative metabolism of arachidonic acid are potent mediators of a wide range of physiological and pathological processes in humans, see Table 1.

Mediator	Pharmacological actions
PGD_2	Bronchoconstrictor; peripheral vasodilator; coronary and pulmonary vasoconstrictor; inhibition of platelet aggregation; neutrophil chemo-attractant; augmentation of basophil histamine release.
PGF_2	Bronchoconstrictor; peripheral vasodilatator; coronary vasoconstrictor; inhibitor of platelet aggregation.
TXA_2	Vasoconstrictor; aggregates platelets; bronchoconstrictor.
LTB_4	Neutrophil chemotaxis; adherence and degranulation; augmentation of vascular permeability.
LTC_4	Bronchoconstrictor; increases vascular permeability; arteriolar constrictor.
LTD_4	Bronchoconstrictor; increases vascular permeability.
LTE_4	Weak bronchoconstrictor; enhances bronchial responsiveness; increases vascular permeability.
PAF	Aggregates platelets; chemotaxis and degranulation of eosinophils and neutrophils; increases vascular permeability; bronchoconstrictor; hypotensive.

Table 1. Pharmacologic activities of newly generated mediators (From Holgate et al. 1993).

Cyclooxygenase is associated with the endoplasmic reticulum of most cells. It catalyses the incorporation of molecular oxygen into the arachidonic acid molecule and promotes ring closure to form unstable intermediates that are converted to the different prostaglandins (PGD_2, PGE_2 and PGF_2), prostacyclin (PGI_2) and thromboxane A2 (TXA_2). Arachidonic acid may also be metabolised by a variety of lipoxygenase enzymes. The 5-lipoxygenase pathway gives rise to the leukotrienes (LTB_4, LTC_4, LTD_4 and LTE_4). Together LTC_4, LTD_4 and LTE_4 comprise slow reacting substances of anaphylaxis (SRS-A). Platelet-activating factor (PAF), a phospholipid, is stucturally different from the arachidonic acid-derived products but is also synthesised, from an inactive precursor, upon activation of phospholipase A_2 (Holgate et al. 1993).
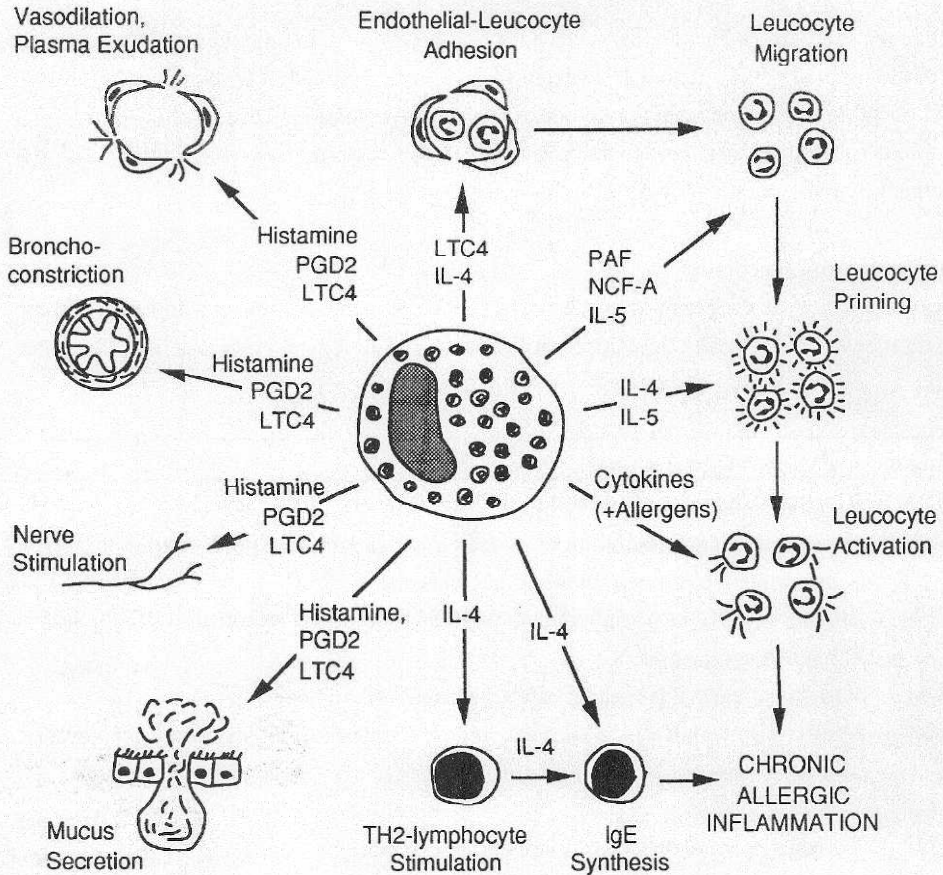
Figure 2. Schematic presentation of the role of the mast cell in acute and chronic allergic inflammation (Adapted from Holgate et al. 1993).

Basophil granulocytes

Basophil granulocytes are normally found in very small numbers in the circulation. They have been implicated in the allergic inflammation of mucosal membranes, especially the nose (Schleimer et al. 1985). The mast cell and the basophil granulocyte share many properties but the relationship between these two cell types is unclear. Basophils differentiate from pluripotent stem cells and IL-3 has been consistently found to stimulate the growth of basophils (Saito et al. 1988). Besides IL-3, GM-CSF also induces basophilic differentiation along with other cells of granulocytic lineages such as eosinophils. Mature human basophils are usually small round cells 5 to 7 µm in diameter. They have short surface processes and many, large cytoplasmic granules that contain electron-dense particles, and, rarely, large crystals on electron microscopy. The granules are basophilic on routine laboratory staining

(Giemsa, Wright´s), as well as with specific stains such as toluidine blue and alcian blue. The nucleus is segmented, usually bilobular, and the chromatin is heavily condensed. Endoplasmic reticulum and Golgi apparatus are inconspicuous. Upon activation individual granules of basophils fuse with the cell membrane and release their content extracellularly (Dvorak et al. 1983a, Dvorak et al. 1983b). The granules contain histamine and chondroitin sulpfate A and a small amount of tryptase (Castells et al. 1987). Furthermore, basophils produce LTC_4 but not PGD_2 (Schwartz and Huff 1993).

Metachromatic cells in the nasal mucosa

Cells with basophil, metachromatic granules have been repeatedly observed in the nasal epithelium and on the mucosal surface in patients with allergic rhinitis. This was first described by Bryan and Bryan (1959). Okuda interpreted the intraepithelial cells as mast cells but described another cell in the mucous blanket as basophils (Okuda et al. 1983, Okuda et al. 1985). Since then, metachromatic cells in the nasal mucosa have variously been addressed as basophils or mast cells (Davies et al. 1987, Bascom et al. 1988, Liu 1988, Pipkorn et al. 1988b, Pipkorn et al. 1989). The presence of highly sulphated proteoglycans in secretory granules of mast cells and basophils results in metachromasia when these cells are stained with basic dyes. These staining techniques reflect the structral properties of the proteoglycan core of the granules, especially the glycosaminoglycan composition. Staining with toluidine blue results in a shift in the absorbtion spectra of the dye to a shorter wavelength giving the metachromacy, a change in the color from blue toward violet (Enerbäck 1986). The morphological criteria for the identification of mature and immature basophils and mast cells are related to the function of the cells. Expressions of activation and release reaction may alter the morphology of basophils and mast cells under basal conditions, and as a result may create confusion in the identification of these cells. Until a specific marker for mast cells as well as basophils is found, the different metachromatic cells cannot be classified without reservations. The mast cell is traditionally identified by the metachromasia of its secretory granules (Ehrlich 1879, Enerbäck and Norrby 1989). The mast cell can also be identified using anti-IgE-antibodies (Callerame and Condemi 1974, Feltkamp-Vroom et al. 1975, Stallman et al. 1977, Rognum and Brandtzaeg 1989, Bachert et al. 1990) and anti-tryptase-antibodies (Schwartz 1985). Basophils contain metachromatic granules, histamine and high-affinity IgE receptors (Ishizaka et al. 1970) but only a minimal amount of tryptase (Castells et al. 1987), suggesting that this enzyme can be used as a selective marker for the tissue mast cell (Irani et al. 1986b). In a recent report, metachromatic cells recovered from nasal lavage fluid after allergen challenge have been found to carry CD18, a typical leukocyte marker, indicating a basophil lineage (Iliopoulos et al. 1992).

Eosinophil granulocytes

The eosinophil granulocyte is a bone-marrow-derived, cytotoxic cell which usually presents in low number in human peripheral blood with the exception of allergic, parasitic and some uncommon diseases such as the idiopathic hypereosinophilic syndrome, in which it is commonly found in increased numbers both in peripheral blood and in affected tissues. IL-3 promotes eosinophil differentiation of pluripotent stem cells. IL-5 induces selective differentiation and proliferation of eosinophils, and activation synergistically enhanced by GM-CSF. PAF is the strongest among a group of known eosinophil chemotactic factors. Since its discovery in Paul Ehrlich (1879), a role for the eosinophil granulocyte in the defence against parasites has been proposed by several investigators. The role played by the eosinophil in this defence has remained enigmatic, however, although direct killing of the parasite by the eosinophil has been suggested. The proposed capacity to kill parasites would imply that the eosinophil is capable of tissue destruction and this putative destructive ability might in some situations also be turned against the host, for instance in asthma. The protein content of the human eosinophil granules is dominated by the presence of four major proteins as depicted in Table 2. These proteins have high isoelectric points, for some of them above pH 11, and have been shown *in vitro* to kill parasites such as *Schistosoma mansoni*, *Trichinella spiralis* and *Trypanosoma cruzi*.

Protein	Molecular weight (kDa)
Eosinophil cationic protein (ECP)	18.5-22
Eosinophil peroxidase (EPO)	67
Eosinophil protein-X (EPX) or eosinophil derived neurotoxin (EDN)	23
Major basic protein (MBP)	9.2

Table 2. The major proteins of human eosinophil granules (From Venge and Peterson 1989).

Two populations of granules can be distinguished morphologically in human eosinophils. One is the large, crystalloid-containing population which is also peroxidase positive and the other is made up of small peroxidase-negative granules. The crystalloid structure is made up of major basic protein (MBP). Depending on the type of stimuli and activation stage of the cell, the granule proteins may be released selectively, although they are contained in the same granule population. Eosinophils produce LTC4 and LTD4 and studies indicate that they also produce cytokines such as IL-3, GM-CSF and transforming growth factor (TGF)-α and β, a multifactoral regulator of cell growth and associated with diseases characterised by fibrosis as reviewed by Venge and Peterson (1989) and Sur et al. (1993).

T-helper cells

Two sub-populations of T-helper lymphocytes (CD4+ cells) have been described, with distinct cytokine profiles (Mosmann et al. 1986). The characteristic cytokines of the first group (TH1 cells) are IL-2 and IFN-γ and the second group (TH2 cells) produce IL-4, IL-5, IL-6 and IL-10. Both cell types secrete IL-3 and GM-CSF (Mossman and Moore 1991). Isolated T-cell clones specific for allergens produce TH2-cell cytokine profile whereas T-cell clones specific for purified protein derivative (PPD) of *Mycobacterium tuberculosis* produce TH1-cell cytokines. T lymphocytes from asthmatic patients bear the receptor for IL-2, a phenotypic sign of activation (Azzawi et al. 1990). In broncheoalveolar lavage fluid from patients with atopic asthma, there is a predominance of TH2 cells (Robinson et al. 1992). There is an increase in the expression of messenger RNA for the TH2 cytokines after nasal allergen provocation (Durham et al. 1992). As illustrated in Figure 3, the interleukines of TH2 cells promote allergic inflammation: IL-3 promotes eosinophil and basophil growth and differention; IL-4 stimulate B cell proliferation and IgE production, and favour mast cell activation; and IL-5 enhance eosinophil differentiation, vascular adhesion and survival. The two T helper cell subgroups have opposite effects on IgE production, as IL-4 and IL-6 stimulate and INF-γ suppress IgE production of B cells. Furthermore, IL-10 suppress the development of TH1 cells (Mosmann 1991). Because of these functional characteristics, TH2 cells are strongly implicated in the pathogenesis of allergic inflammation but TH1 cells in delayed-type hypersensitivity reactions (Del-Prete 1992, Corrigan and Kay 1993).
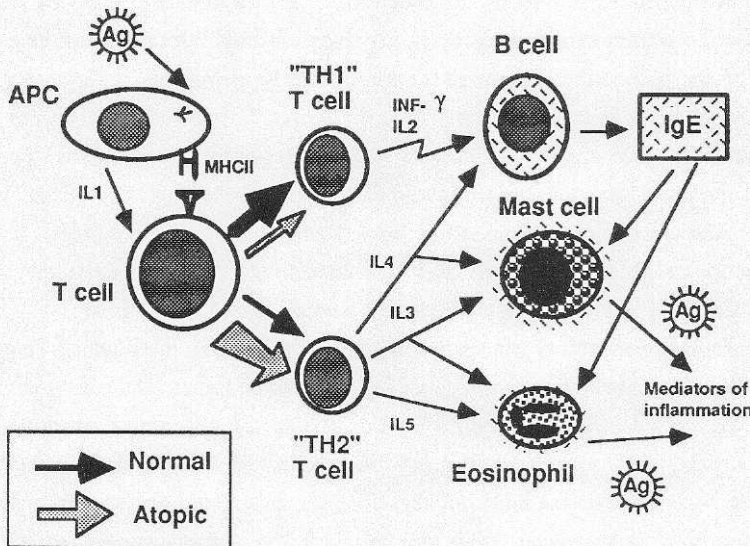

Figure 3 Schematic presentation of the "TH1 - TH2" theory. Ag = allergen; APC = antigen presenting cell; MHC II = major histocompatibility complex II; see text.

Adhesion molecules

Cell trafic is controlled by adhesion molecules, expressed on all surfaces that allow cell-to-cell contact. These glycoproteins have a variety of functions, which include promoting adhesion of one cell to another or to a tissue matrix, activating cells and promoting cellular migration and infiltration. Human sensitised pulmonary endothelial cells increase intercellular adhesion molecule (ICAM)-1 expression after allergen challenge (Gibson et al. 1991). ICAM-1 promotes recruitment of eosinophils and lymphocytes. Human basophils express ICAM-1, but mast cells express it to a much lesser degree (Valent and Bettelheim 1992). The vascular cell adhesion molecule (VCAM)-1 promotes adhesion of lymphocytes, monocytes, eosinophils, and basophils to endothelium. The expression of VCAM-1 is induced by IL-4 which thereby selectively recruits eosinophils, basophils and possibly mast cells but not neutrophils to the allergic inflammation (Schleimer et al. 1992). On the other hand, an endothelial cell adhesion molecule (ELAM)-1, expressed by TNF-α -stimulated endothelial cells, promotes neutrophil adhesion and migration. Endothelial cells express E-selectin after degranulation of human mast cells (Matis et al. 1990). Thus, a role for human mast cells as "gatekeepers" of the endothelium is established and indicates that mast cell mediators other than amines can influence endothelium in the inflammatory process. (Reviewed by Calderon and Lockey 1992)

Allergic rhinitis

Most patients develop allergic rhinitis during childhood and young adulthood. The pattern of the disease is determined in part by the spectrum of sensitivities exhibited by the patient. Thus, the most common symptom complex involves seasonal allergies were exacerbations correspond to the pollinating seasons of the trees in early spring, grasses in late spring and early summer and weeds in late summer to early autum. Conversely, year-round allergens such as dust mites and animal emanations can cause perennial symptoms. The allergic response in the nasal mucosa comprises: episodic sneezing, itching, rhinorrhea, and nasal congestion. Associated symptoms include palatal and eye itching, conjunctival swelling, post-nasal drip, coughing, and the irrepressible need to sneeze. The symptoms of allergic rhinitis are caused by the following processes: 1) Vasodilatation causes mucosal swelling and increased vascular permeability causes oedema fluid to collect in the mucosa, causing nasal congestion and also contributing to the secretions in the nasal lumen. Decreased vascular tone and increased permeability are caused by the actions of vasoactive amines including histamine, prostaglandins and leukotrienes, bradykinin and PAF, and secondarily by the release of neuropeptides on the endothelial cells in the superficial post-capillary venules. 2) Mucous secretion contributes to host-defence functions of the secretions. Glands are stimulated directly by prostaglandins, and reflexly by histamine. 3) Neural reflexes stimulated by histamine and possibly by other mast cell mediators cause the pruritus and sneezing

reflexes, as well as glandular secretion. 4) Late-phase reactions contribute to the oedema and irritability of the nose. Late-phase reactions are caused by inflammatory factors released from mast cells and include chemotactic factors, PAF, eicosanoids, and a number of inflammatory cytokines which are also synthesised and released by lymphocytes. These factors lead to the expression of adhesion molecules, the attraction of inflammatory cells and the infiltration of the mucosa with neutrophils, eosinophils, basophils, thrombocytes, lymphocytes, macrophages, and mast cells. This inflammation plays a major role in the increased irritability of the nose characteristically seen during the allergy season. The spectrum of symptoms of allergic rhinitis is therefore caused by both acute and chronic events as reviewed by Kaliner (1993).

Treating allergic rhinitis

After a diagnosis is established, the most important thing is to inform the patient about the nature of the disease. Elimination of the offending agent is of course the most adequate measure, but when environmental control cannot be accomplished symptomatic pharmacological treatment tends to be the treatment of first choice. Immunotherapy is most effective in young monoallergic patients but has for several reasons mostly been used in severe cases where pharmacological treatment has not given the expected results. The immunomodulation shifts the T-helper cell reaction towards the TH1-like spectrum of cytokines where IFNγ has its negative effect on the IgE-production of the B-cell (Durham 1993). The possibility of potential benefits of surgical intervention should be considered for every patient. Pharmaceutical treatment is aimed at two targets, preventing or reversing the acute events and preventing the late responses which cause the chronic inflammatory changes. Sodium cromoglycate is a widely used antiallergic drug that is thought to "stabilise" mast cells and prevent exocytosis (Leung et al. 1988). Antihistamines are competitive inhibitors acting on the H_1-receptor with high affinity. Their direct antiallergic action has recently been reviewed by Simons and Simons (1993). Only glucocorticoids will be discussed in more detail here.

Glucocorticoid effects

Glucocorticoids are widely used in the treatment of rhinitis. They have been found to decrease the symptoms that arise immediately after allergen provocation. The nasal symptoms occurring 2 - 11 h after allergen provocation can be completely eliminated by topical pretreatment with corticosteroids (Reviewed by Mygind 1993). Corticosteroids have a multitude of actions that may be potentially beneficial in allergic airway diseases. They decrease inflammatory cell recruitment and activation (Schleimer 1990), upregulate β_2 receptors (Svedmyr 1990) and decrease microvascular permeability (Williams and Yardwood 1990) and they may decrease mucus production (Lundgren et al. 1990). In terms of molecular

mechanism, steroids are thought to act by binding to a glucocorticoid intracellular receptor, which is then activated and binds to the cell nucleus, on to the regulatory glucocorticoid receptor elements associated with several genes (Munck et al. 1990). These glucocorticoid response elements then up or down regulate production of messenger RNA (mRNA), which eventually leads to increases or decreases in protein production. The proteins affected include enzymes and cell surface receptors. Such a mechanism of action, with gene regulation and then protein synthesis, will probably take some hours or even days to produce a clinical effect. Since glucocorticoids dramatically reduce allergic inflammation (Pipkorn et al. 1987a, Pipkorn et al. 1987b, Andersson et al. 1988, Gomez et al. 1988, Walden et al. 1988, Bisgaard et al. 1990, Burke et al. 1992), their impact on mast cells has been of considerable interest. Their ability to alter transcription of a family of genes is well known, but their role in IgE-receptor-mediated activation of mast cells and basophils remains controversial (cf. Schleimer 1993). Although *in vivo* experimental models demonstrate a reduced anaphylactic activity as the result of prior therapy with glucocorticoids, *in vitro* studies on mast cells have been less convincing (Cohan et al. 1989).

Monitoring the inflammatory reaction in the human nasal mucosa

As yet, there is no single parameter which can be used to measure the severity of the inflammatory reaction in the airway mucosa. The symptoms experienced in allergic inflammatory nasal disease can all be results of disorders not regarded as inflammatory reactions. The mere presence of inflammatory cells (neutrophils, eosinophils, mast cells, platelets, lymphocytes and basophils) and their products in and/or on the nasal mucosa may not be equal to inflammation. The cells may be present without affecting the tissue, or rather in order to fulfil tissue repair. It is only when it can be ascertained that the cells are activated and actively contribute to the inflammatory reaction that they may define airway inflammation and symptoms. Even so, the relationship between cell numbers and the degree of inflammation is uncertain. A series of diagnostic modalities can help to study nasal disorders. These include history, physical examination, rhinoscopy, rhinomanometry, acoustic rhinometry, rhinostereometry, nasal challenges, biochemical determinations, immunology studies, *in vivo* and *in vitro* testing for specific IgE, imaging, blood flow and ciliary function analysis, and examination of nasal morphology. The selection of sampling method for morphological and biochemical studies depends on the aims of the study. Some of the considerations are: the age of the patient, the need for repeated sampling, the site and thickness of the nasal mucosa, and the requirement for simultaneous biochemical and morphological studies. Thus, a number of methods have evolved, such as blown secretions, smears taken with cotton wool swabs, imprints, brushing, nasal scrapings, nasal lavages and biopsy, each method having its pros and cons (Pipkorn and Karlsson 1988).

AIMS OF THE INVESTIGATION

The purpose of this study was to evaluate:

- the correlation between the mast cell, its mediators and allergic symptoms (I - IV)

- the cellular kinetics after allergen challenge and during natural pollen exposure (I, II, V)

- the occurrence of the basophil granulocyte in allergic inflammation (II, V)

- the effect of corticosteroid treatment on the mast cell and its mediators (IV, V)

- the mast cell sub-populations in allergic inflammation (V).

MATERIAL AND METHODS

Design of the Studies

Study I.   An allergen challenge was performed in ten asymptomatic patients with strictly seasonal allergic rhinitis. For comparison, seven nonallergics were challenged with allergen and seven allergics challenged with diluent. Cell samples, using the brush technique and nasal lavage were taken before challenge and at 2-hourly intervals during 12 h. The subjects rated their nasal symptoms before each sampling and 15 min after the challenge. The cell suspensions were cytocentrifuged onto microscope slides for enumeration of metachromatic cells in toluidine-blue-stained samples and differential counting of eosinophils, neutrophils and epithelial cells by light microscopy. Histamine was determined in the cell pellets.

Study II.   Nasal brush and lavage samples were obtained before a single nasal allergen challenge and every 2 h for 12 h after the challenge in 10 allergics and 3 controls. The subjects rated their nasal symptoms before each sampling and 15 min after the challenge. The total numbers of IgE-bearing and tryptase-containing cells and the histamine content of the cell pellets were studied in brush samples. Toluidine-blue-stained brush and lavage samples were used to differentiate the metachromatic cells into mast cells or basophils according to their morphology.

Study III   A nasal allergen challenge with 3 allergen doses was performed in 25 patients with strictly seasonal allergic rhinitis and 6 normal controls. A nasal lavage technique was used to monitor changes in local levels of tryptase, histamine and TAME-esterase activity.

Study IV.   Twenty-six patients with strictly seasonal allergic rhinitis entered a double-blind, placebo-controlled, randomised, parallel group study of the effect of 4 weeks´ treatment with topically-applied fluticasone propionate followed by a 4-week follow-up period. Allergen challenges with nasal lavages were performed before the start of the study and every second week for 8 weeks. The lavage fluid was analysed for histamine and tryptase levels and TAME-esterase activity. The patients were subjected to pretreatment nasal biopsy at a preparatory visit two weeks before the start of the study and post-treatment nasal biopsy directly after the allergen challenge after the treatment period. The biopsies were analysed for mast cell density and histamine content.

Study V.   The distribution and density of metachromatic cells and mast cells containing chymase plus tryptase (MC_{TC}) or tryptase only (MC_T) were studied in nasal mucosa using dye-binding techniques and immunohistochemistry. Biopsies were obtained from 17 subjects

with birch pollen allergy before the pollen season and during the season, and from 9 healthy controls. Six patients were treated with intranasal glucocorticosteroid before and during the season in an open study.

Subjects

Normal subjects

A total of 22 healthy individuals participated in these studies. The subjects in Studies I and II were partly identical. They were all non-atopic subjects with no history of allergic disease, or of chronic or recurrent nasal diseases. Furthermore, they had no symptoms of acute airway disease at the time of the challenge procedure. No medication was allowed during the studies. (I, II, III, V)

Allergic patients

A total of 60 patients were recruited for these studies. The subjects in Studies I and II were partly identical as were the subjects in Studies III and IV. All patients had a history of seasonal allergic rhinitis due to birch or grass pollen, a positive skin prick test and a positive nasal provocation test to birch or timothy pollen. The subjects in Studies I and II had strictly grass pollen allergy. The subjects in Studies III and IV had birch and/or grass pollen allergy. The subjects in Study V had birch pollen or birch-and-grass pollen allergy. The exclusion criteria were nasal polyps, asthma, dermal manifestation of their atopic disease or pregnancy. No medication was allowed during the studies except for the study drugs in Studies IV and V. (I - V)

Nasal allergen provocations

Studies I - IV took place during pollen-free winter months and were designed as an open investigation regarding the challenge agents (Pipkorn et al. 1987). Both nasal cavities were challenged in Studies I and II, with either one spray of timothy pollen allergen extract, 10,000 Biological Units (BU)/ml, or the diluent (Pharmalgen®, Pharmacia Diagnostics Norden AB, Uppsala, Sweden) from a metered dose pump delivering 0.13 ml in each actuation. A unilateral challenge procedure involving nasal lavages for the harvesting of mediators was used in Studies III and IV. The procedure started with 3 quick lavages with 10 ml of saline, 5 ml into each nasal cavity, with the head tilted backwards during closure of the soft palate. Lavages were then performed at constant 10 min interval. Each of the lavages, starting with the fourth, was immediately followed by the administration of a challenge agent, the sequence being oxymetazoline, diluent, diluent, 100 BU/ml of allergen (birch or timothy), 1,000 BU/ml and, finally, 10,000 BU/ml. Each lavage was designed to harvest the result of the preceding challenge (Naclerio et al. 1983). At each of 5 visits (study weeks 0 to 8) in

Study IV, unilateral nasal allergen challenges were performed on alternate sides, starting with the right side. The challenge procedure was the same as in Study III. The mechanical pump used for the challenges delivered 0.13 ml in each spray.

Natural allergen exposure

In Study V the subjects were observed for a period of 2-3 weeks into the pollen season until the patients had developed symptoms of their allergic disease and the daily pollen news reported moderate levels of birch pollen (Associate Professor Sven-Olof Strandhede, Department of Taxonomy, University of Göteborg, Sweden).

Assessment of symptoms

During the challenge procedures in Studies I to IV, the subjects continuously registered their nasal symptoms on a symptom chart using a 5-point scale: 0 = no, 1 = mild, 2 = moderate, 3 = severe and 4 = intolerable symptoms. The symptoms recorded were nasal congestion, rhinorrhea and sneezing and in Studies III and IV nasal itching was also recorded. Furthermore, the number of sneezes was registered. A composite symptom score was calculated as the sum of the scores for each symptom. The sneeze score was calculated as follows: 0 sneezes = 0, 1 to 4 sneezes = 1, 5 to 9 sneezes = 2, 10 to 19 sneezes = 3 and 20 or more sneezes = 4. No systematic symptom assessment was made in Study V.

Material sampling methods

The nasal lavage method

In Studies I and II the nasal lavage was performed using a compressible plastic container containing 12 ml of sterile saline with 0.1% of human serum albumin (Greiff et al. 1990). Both nasal cavities were lavaged twice. In Studies III and IV a nasal lavage provocation procedure was used as described above (Naclerio et al. 1983).

The brush method

In Studies I and II a 5.5 mm diameter nylon brush (Doft AB, Östhammar, Sweden), was used for cell sampling (Pipkorn et al. 1988a). The brush was placed between the nasal septum and the inferior turbinate and removed gently. No anaesthesia was used. The brush was then shaken vigorously in 2 ml of buffered salt solution and the cell suspension used for analysis.

Nasal biopsy

In Studies IV and Study V nasal mucosal specimens were obtained after topical anaesthesia. A biopsy was performed 1 cm behind the anterior edge of the inferior turbinate using a pair of cup forceps (Pipkorn and Karlsson 1988).

Analysis of biochemical markers

TAME-esterase activity

In Studies III and IV N-α-tosyl-L-arginine-methyl-ester-(TAME)-esterase activity, i.e. enzymes with arginine esterase activity, was measured in returned lavage fluid using a radiochemical method essentially as described by Imanari et al. (1976) and adapted for the nasal lavage procedure (Naclerio et al. 1983). The method is based on the enzymatic liberation of ^{3}H-methanol from the synthetic substrate [^{3}H]-TAME. A 40 μl aliquot of lavage sample and 10 μl of a 0.2 M Tris buffer, pH 8.0, were put into a 1.5 ml propylene microtube. The enzymatic reaction was then started by addition of 10 μl of [^{3}H]-TAME; i.e. 0.38 x 10^{-4} mg of [^{3}H]-TAME. The microtube was placed in a counting vial containing 10 ml of Econofluor (a lipophilic scintillation solution) and 50 μl of the stop solution (1 vol. glacial acetic acid and 9 vol. 0.02 M TAME; i.e. 0.38 mg of TAME). The counting vial was capped, and after 1 hour at room temperature the reaction was terminated by shaking. The liberated ^{3}H-methanol was then partitioned into the lipophilic Econofluor solution, while TAME was dissolved in the hydrophilic suspension (mainly lavage sample and stop solution). The radioactivity in the lipophilic phase influenced the Econofluor solution to produce flashes, which were counted in a liquid scintillation spectrometer for 2 minutes. The activity was expressed as cpm (counts per minute). The enzymes which are active in this assay are designated TAME-esterases. All the samples from each person were assayed the same day.

Tryptase

In Studies III and IV tryptase in returned nasal lavage fluid was analysed using a radioimmunoassay on coated tubes (RIACT) with two monoclonal antibodies for tryptase (Wenzel et al. 1986, Enander et al. 1991). In the RIACT (Pharmacia Diagnostics AB, Uppsala, Sweden), 50 μl of nasal lavage fluid, 50 μl of sample diluent and 50 μl of ^{125}I-labelled anti-tryptase antibody were added simultaneously to plastic tubes coated with anti-tryptase antibody. After incubation overnight, the tubes were washed three times and then counted in a gamma counter. The lower limit of detection for the assay is less than 0.5 ng/ml. The inter-assay coefficient of variation is 2.3% and the intra-assay coefficient of variation is 2.8%. Recovery from spiked samples is 101.8% ± 7.4% (Enander et al. 1991).

Histamine

HPLC

In Studies I and II cell pellets and in Study IV biopsies were analysed using highly purified liquid chromatography (HPLC). After thawing, the specimens were homogenised in 0.4 molar perchloric acid followed by neutralisation of the extract with potassium carbonate with the precipitation of potassium perchlorate. Histamine was purified by reverse-phase, high-performance liquid chromatography using a C18 column with 15 mM citrate buffer at pH 3 containing 5% methanol as a mobile phase and pentane sulphonic acid (5 mM) as the counter ion. Histamine was detected and assayed using a two-stage, post-column, derivative-forming procedure using o-phthalaldehyde as the fluorescent reagent (Allenmark et al. 1985). The limit of detection for the assay is 1 ng/ml. The intra-assay and interassay coefficient of variation for this method is less than 5%. The mean range of recovery in added samples is 98% to 102%.

RIA

In Studies III and IV histamine in returned lavage fluid was determined by a solid-phase radioimmunoassay (RIA) (Morel and Delaage 1988, McBride and Kaliner 1989). In the RIA (Immunotech, Marseilles, France), succinyl glycin is used as a reagent to acylate histamine in the sample. The acylated histamine competes with ^{125}I-labelled acylated histamine for the binding site of the monoclonal antibody. The limit of detection for the assay is 0.2 nM. The intra-assay and the interassay coefficient of variation for this method is less than 8% and 11% respectively.

Morphological methods

Fixation techniques

The biopsy specimens in Study IV were fixed in Carnoy´s fluid (60% absolute ethanol, 30% chlorophorm and 10% glacial acetic acid) for two hours followed by absolute ethanol for 12 to 24 h. In Study V the biopsy specimens were fixed in iso-osmotic formaldehyde acetic acid (IFAA) (0.6% formaldehyde and 0.5% acetic acid in distilled water) for 12h followed by 12h in 70% ethanol. In Studies I and II cytological samples were air dried after cytocentrifugation.

Staining techniques

Dye binding procedures

For staining of metachromatic cells in cytospin samples in Studies I and III and tissue sections in Studies IV and V, toluidine blue was used in a 0.5% aqueous solution at pH 0.5. The staining time was 30 min. The staining resulted in a violet-blue colour of the positively stained cells. In Study V the degree of aldehyde-induced blocking of cationic dye-binding

(Enerbäck et al. 1989) was assessed by counting the mast cells in serial sections fixed in IFAA and stained with toluidine blue for 30 min directly or after post fixation in 4% formaldehyde solution overnight and then stained for 30 min (Aldenborg and Enerbäck 1988). Giemsa staining of cytospin preparations in Study I gave dark-blue nuclei of all cells, and highly red eosinophils.

Immunohistochemistry

In Study II the monoclonal anti-human-tryptase-antibody was used with the peroxidase-anti-peroxidase (PAP) immunochemical technique to visualise tryptase-positive cells in cytospin samples. Positively stained cells are brown. Specimens were counterstained with toluidine blue giving a faintly stained background. The monoclonal anti-human IgE and the alkaline phosphatase-anti-alkaline phosphatase (APAAP) immunochemical technique was used to visualise IgE-bearing cells in Study II. The method using Fast Red as the peroxidase substrate gives positively labelled red staining cells against the background, counterstained blue with hemalaun (Bachert et al. 1990). The immunohistochemical double-labelling technique used on tissue sections in Study V results in a blue staining of MC_T and brown coloured MC_{TC} (Irani et al. 1986b). Mast cell chymase was visualised with the biotin-conjugated monoclonal anti-human-chymase antibody and the method gave a brown peroxidase reaction as the end-stage product. Tryptase was visualised by incubating the slides with the alkaline phosphatase-conjugated mouse monoclonal anti-human mast cell tryptase antibody. A blue staining product was produced by incubating the slides with fast blue RR. Measures for negative and positive controls were included in the immunohistochemical staining procedures.

Counting methods

In Study I the total number of cells was estimated by light microscopy and differential counting of eosinophils, neutrophils and epithelial cells was done on Giemsa-stained cytospin specimens. Furthermore, eosinophils were divided into non-vacuolated and vacuolated eosinophils, on the basis of the number of cytoplasmic vacuoles. In the toluidine-blue-stained cytospin specimens in Studies I and II, and the anti-tryptase and anti-IgE-immunolabelled cytospin specimens in Study II, the total number of positively stained cells was counted on each specimen. Furthermore, in Study II, the metachromatic cells on toluidine-blue-stained cytospin specimens were assigned to four categories, "basophils", "mast cells", "unidentifiable cells" and "questionable metachromatic cells", based on morphological criteria (Bascom et al. 1988). In Studies IV and V, sections of biopsy samples were surveyed for immunopositive or metachromatic cells by light microscopy. In Study IV the cells and area of whole biopsy sections were surveyed. The epithelium and the subepithelial stroma was studied in Study V. Furthermore, the area surveyed for cells was estimated. Biopsy cell counts were expressed as cells/mm^2. Light microscopy was undertaken on coded specimens.

Investigational drugs

Fluticasone propionate

In Study IV 26 patients were randomly allocated to receive, under double-blind, parallell group conditions, fluticasone propionate aqueous nasal spray, 50 µg per actuation (13 patients) or identical placebo (13 patients), two sprays into each nostril once daily for 4 weeks. The total daily dose of fluticasone propionate was 200 µg. The fluorinated, topically-active glucocorticoid fluticasone propionate (Glaxo Laboratories Ltd., Greenford, Middlesex, England) has been shown to be safe and effective in the treatment of both seasonal and perennial rhinitis (Harding 1990, Banov et al. 1991, Nathan et al. 1991).

Budesonide

In Study V 6 patients were given nasal sprays containing budesonide. The dosage was 2 actuations into each nostril morning and afternoon. Each actuation contained 50 µg of budesonide, giving a total daily dose of 400 µg. The patients were instructed to start their treatment about 2 weeks before the expected start of the pollen season and then to continue until the second visit. Budesonide is a non-halogenated glucocorticoid with high local antiinflammatory activity and low systemic activity (Gruvstad and Bengtsson 1980, Gordon et al. 1987). Topically administered, the drug is effective in seasonal and perennial rhinitis (Day et al. 1990, Pedersen and Dahl 1990).

Oxymetazoline chloride

In Studies III and IV, oxymetazoline chloride, an α_2-adrenoceptor agonist, was used topically. It was administered with a nasal mechanical spray pump delivering 100 µl (two actuations of 50 µl) into each nasal cavity. The concentration used was 0.5 mg/ml, which is that recommended and clinically used for adults.

Statistics

The statistical evaluation was performed on a microcomputer using a statistical software package. In Study I, an analysis of variance (ANOVA) was used to evaluate whether observations made at the different times differed from one another. In Studies I, II, IV and Study V, Wilcoxon's signed rank sum test and Mann-Whitney´s U test were used for nonparametric and in Study III, Student's t-test for parametric evaluation of changes within and between groups. In Studies I, II, IV and V, Spearman´s correlation coefficient test and in Study III, regression analysis was used to evaluate correlation between the different observations. p-values of < 0.05 were considered significant.

RESULTS

Study I. Mast cells and Eosinophils in the Allergic Mucosal Response to Allergen Challenge: Changes in Distribution and Signs of Activation in Relation to Symptoms

The patients reacted promptly to the allergen challenge with an increase in nasal symptoms. In brush samples from the allergics challenged with allergen, the numbers of metachromatic cells increased to a maximum of 8-fold at 10 h. In the lavage specimens, no metachromatic cells were observed before provocation, but they progressively increased in number 2 to 12 h after provocation. Cell pellet histamine content decreased temporarily 2-4 h after challenge ($p<0.05$) in brush samples from allergen-challenged allergics. The local metachromatic cell density before challenge, as reflected in the brush specimens, correlated with nasal congestion, sneezing and the degree of eosinophilia. Three of the patients challenged with allergen differed from the remaining seven in that their brush samples obtained before allergen challenge contained a comparatively large number of metachromatic cells and eosinophils and they developed intense symptoms after challenge plus an intense eosinophil response after 4-10 h and a pronounced mast cell response 10-12 h after challenge. There was a temporary decrease in the histamine-per-cell ratio from those three patients with significant numbers of mast cells in the pre-challenge samples, as shown in Figure 4.
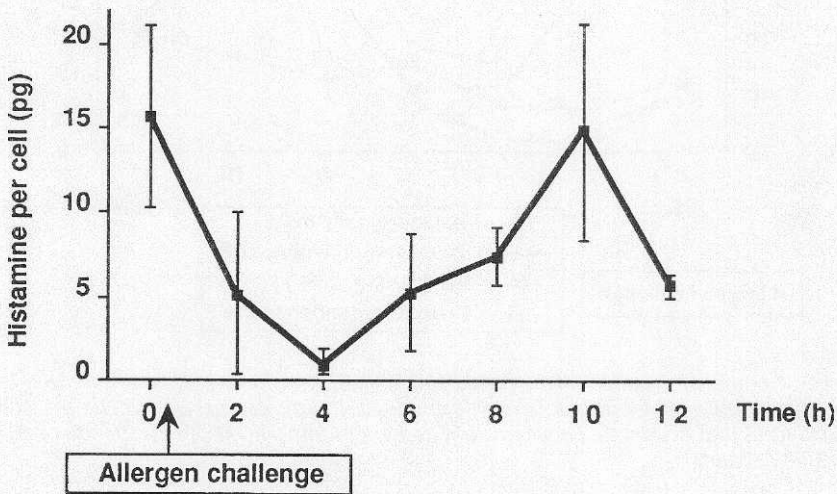

Figure 4. The histamine content of metachromatic cells in the brush samples from the three patients that had high cell numbers in the pre-challenge brush samples. The histamine-per-cell content is calculated from the number of metachromatic cells per slide and the histamine content of the cell pellets and corrected for volume of the sample.

Study II. Metachromatic, IgE-bearing and Tryptase-containing Cells on the Nasal Mucosal Surface in Provoked Allergic Rhinitis

After an initial decrease at 2 h, the numbers of metachromatic, IgE-bearing and tryptase-containing cells as well as the histamine content of the cells in the brush samples increased during the subsequent observation hours (Figure 5). The morphology of the metachromatic cells in the prechallenge brush samples agreed with that of mast cells but corresponded with mast cells and basophils in brush and lavage samples at 8 to 12 hours. There was a statistically significant correlation between all four parameters. There was a significant correlation between the cell numbers from all three staining methods as well as histamine values in pre-challenge samples and symptoms, expressed as numbers of sneezes 15 min after allergen challenge (n=10).
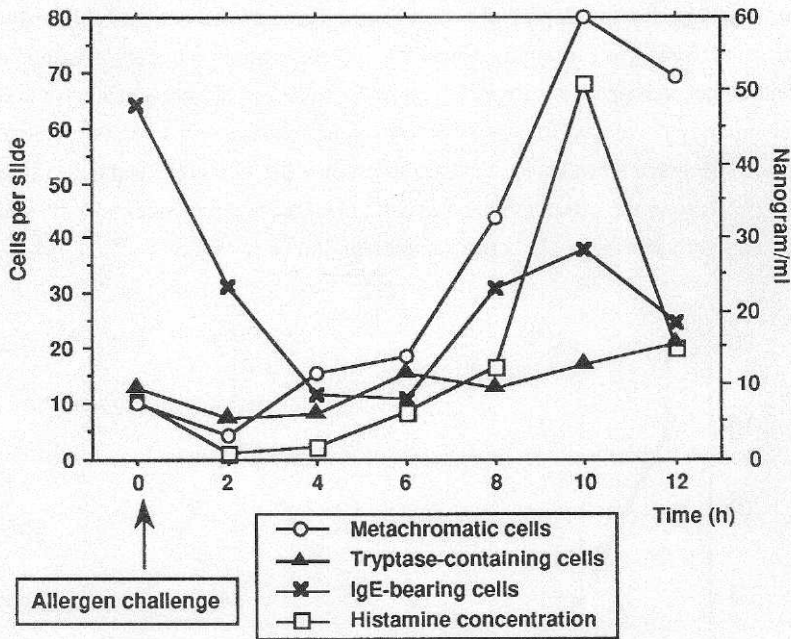

Figure 5. Combined graph showing means for four mast cell markers in brush samples from 10 allergics challenged with allergen. Morphological data expressed as cells per slide (left side ordinate) and histamine concentration as nanograms per ml of resuspended cell pellets (right side ordinate).

Study III. Tryptase in Nasal Lavage Fluid after Local Allergen Challenge; Relationship to Histamine Levels and TAME-Esterase Activity

The allergen challenge resulted in significant increases in the levels of all three markers, tryptase, histamine and TAME-esterase, following the allergen challenge. The increase in TAME-esterase activity as well as in the levels of tryptase was dose-dependent. A 3-fold increase in histamine was noted after the first allergen dose but no further rise was seen. In the individual measurements after the challenges there was a highly significant correlation between the TAME-esterase levels and the tryptase levels, while the generation of histamine and tryptase was not significantly correlated. On the other hand, when comparing the cumulative generation of the three markers, significant correlations were found between all three markers (Figure 6). Allergen challenges in 6 non-allergic controls did not result in any increase in tryptase levels.
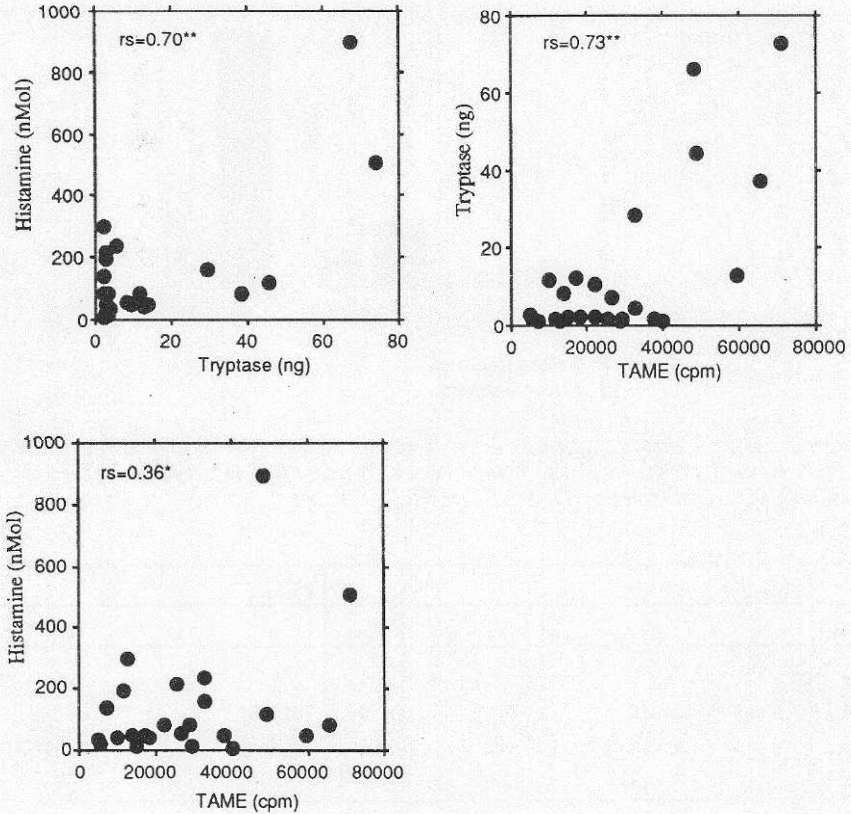

Figure 6. Combined figure showing the relationships between the cumulative generation of histamine, tryptase and TAME in nasal lavage fluid after allergen challenge. Correlation coefficients (r_S) and degree of statistical significance are shown (* = $p<0.05$, ** = $p<0.01$).

Study IV. Mast Cells and Mediators in the Nasal Mucosa after Allergen Challenge: Effects of Four Weeks' Treatment with Topical Glucocorticoid

Treatment with fluticasone propionate did not influence mast-cell density, the tissue histamine concentration, the lavage histamine levels or the TAME-esterase activity, while a reduction in nasal symptoms and tryptase in nasal lavage fluid was revealed (Figure 7).

The reaction to the allergen challenge in the untreated group was rather constant from one week to the other. There was a strong of positive correlation in the TAME-esterase activity and symptom score between the different weeks, as shown in Tables 2.
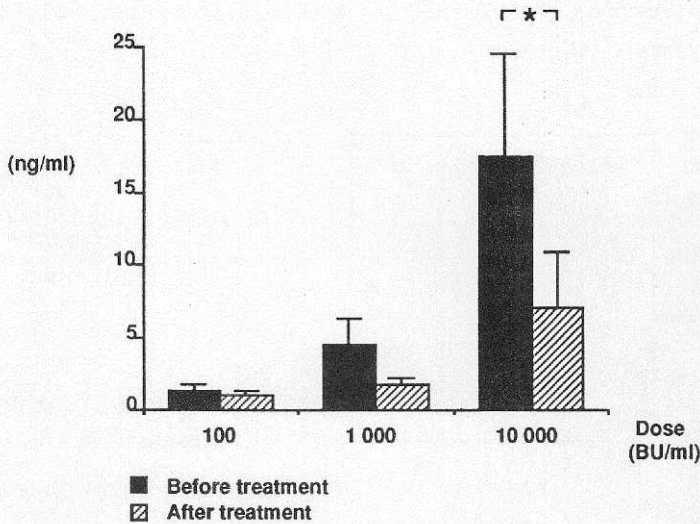

*Figure 7. Graph showing tryptase levels (mean ± SEM) in returned lavage fluid from the actively treated group (n = 12) before (week 0) and after the treatment period (week 4) (*p<0.05, before vs after).*

	Sneezes					TAME			
Weeks	0	2	4	6	Weeks	0	2	4	6
2	0.798 **	1			2	0.623*	1		
4	0.901 **	0.915 **	1		4	0.586*	0.873***	1	
6	0.912 **	0.853 **	0.890 **	1	6	0.703**	0.677*	0.405	1
8	0.760 **	0.912 **	0.806 **	0.809 **	8	0.517	0.611*	0.756**	0.293

*Table 2. Correlation of total number of sneezes and total TAME-esterase activity in the lavage fluid at each visit (weeks 0 to 8) for the placebo-treated group (n = 13). Results are shown as r_S (*p = <0.05, ** = p<0.01, *** = p<0.001).*

Study V. Proteinase Content of Mast Cells in the Nasal Mucosa; Effects of Natural Allergen Exposure and Local Corticosteroid Treatment

Occasional MC_T and MC_{TC} but no metachromatic cells were detected in the epithelium in 3 of 9 controls. In the stroma in the controls, MC_T constituted 2.4 % of the immunopositive cells. The density of epithelial MC_T, MC_{TC} and metachromatic cells as well as stromal MC_T was higher in the allergic patients than in the controls pre season (Figure 8). In the allergics, MC_T comprised 92.4% of the epithelial and 25.9% of the stromal immunolabelled cells pre season. Allergen exposure did not result in statistically significant numerical changes in epithelial or stromal immunopositive or metachromatic cells. Epithelial MC_T were reduced after glucocorticoid treatment. Serial sections displayed higher numbers of immunopositive cells than of metachromatic cells in the controls and in allergic patients. The metachromatic cells comprised from 33% (epithelium) to 90% (stroma) of the immunopositive cells in atopic mucosa before and during the season. About 87% (epithelium) to 73% (stroma) of the metachromatic cells in the allergic patients and 47% (stroma) in the controls were sensitive to aldehyde fixation. There was a correlation between the proportions of MC_T and of aldehyde-sensitive metachromatic cells.
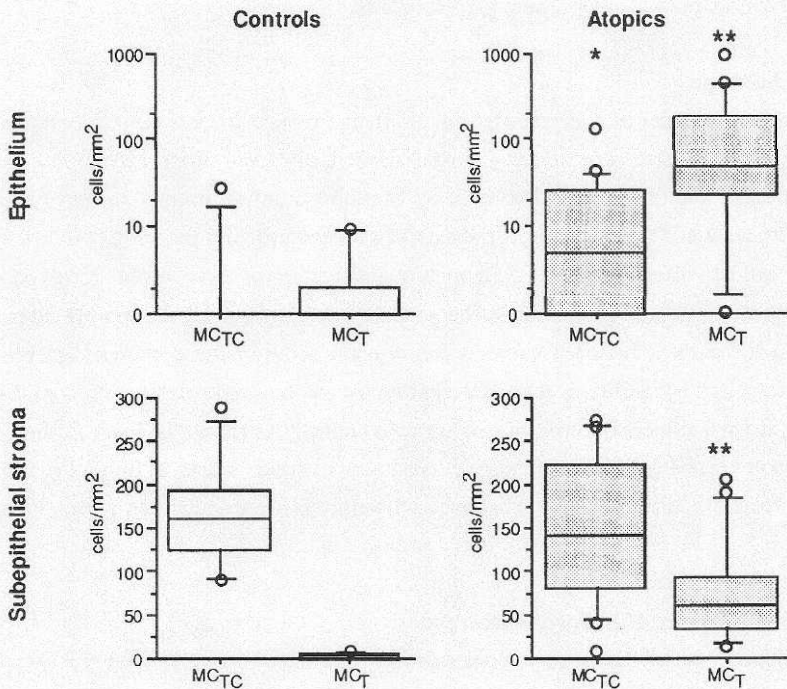

Figure 8. Box graphs showing medians and interquartile range for the density of MC_{TC} and MC_T in pre-seasonal nasal biopsies in 9 controls and 17 allergics. (= p<0.05, ** = p<0.01, atopics vs controls.)*

METHODOLOGICAL CONSIDERATIONS

Subject selection

The allergic individuals were recruited among medical and engineering students, and among patients at the ENT clinic. No attempt was made to take into account the severity of the disease as an inclusion criterion. All patients reacted to the allergen challenge with an increase in nasal symptoms as well as in the objective parameters. As can be expected in an unselected patient material, the individual reactions varied considerably. The healthy controls were recruited from medical students and the hospital staff.

Assessment of symptoms

The first parameter utilised for monitoring of the results after nasal challenge, the subjective assessment of symptoms, remains valid (Blackley 1873). The symptoms quantitated are nasal congestion, rhinorrhea and nasal irritation. Counting sneezes provides a relatively more objective symptom index. The subjects assessed their symptoms on a five-point scale. This method may be too rough when it comes to the correlation between the symptoms and the different biological parameters measured and a visual analogue scale may be a better method to use in this situation as it gives more graded results.·

Allergen challenge

Quantitating the amount of allergen used for challenge represents an essential component of any experimental system. The delivery system used (I - IV) is a liquid extract in a metered dose nasal spray pump that avoids the tactile stimulus from an extract-soaked paper disc placed in the nose and excludes the variability of systems utilising the subject its own effort for delivery of the offending agent. Although quantification of the stimulus received by the subject is preferable, quantification of the stimulus delivered is an acceptable alternative. Anatomical differences between subjects can explain some of the apparent differences in subject reactivity. By utilising repeated challenges in the study design, this problem is minimised as each subject becomes his or her own control. As shown in Table 2, there was a high degree of correlation between the reactions in the different weeks in Study IV. It may be concluded from this that the lavage challenge procedure is a stable method giving validity to the results.

Methods for sampling of biological material

Biopsy is the method of choice to demonstrate the presence of a specific cell type in a tissue, but has its drawbacks as the number of time-points that can be obtained is limited. When studying the kinetics of putative cellular changes in the nasal mucosa, non-traumatic cell-sampling techniques are preferable since repeated samples are required. The lavage technique

collects cells floating on the nasal mucosal surface and the brush technique harvests a proportion of cells within the epithelial lining (Pipkorn et al. 1988a, Greiff et al. 1990). Both techniques are non-traumatic, do not require anaesthetics, are easily tolerated and can be used for quantitative evaluation. As the interindividual variation in cell yield is greater than the intra-individual variation between different observations (Studies I and II), these methods can be used with preference for kinetic studies with repeated observations using each individual as his or her own control. Nasal lavage has been used for measuring changes in histamine and other mast-cell-associated mediators in nasal secretions before and after allergen challenge (Naclerio et al. 1983, Naclerio et al. 1985). This method results in a variable dilution factor that precludes the determination of mediator concentrations in nasal secretions. The levels of mediators in nasal lavage obtained reflect the total amount of the mediator collected, which reflects the amount of mediator released. By the use of paper discs (Flowers et al. 1990) or by adding a tracer to the lavage fluid and calculating the dilution factor (Linder et al. 1983), the exact concentration of the mediator in the secretion can be determined. If resulting nasal secretions contain no significant levels of a mediator, as is the case for tryptase, unlike histamine, it will be easier to demonstrate an antigen-induced increase in lavage than in a paper disc as a wider mucosal area is surveyed.

Drugs used in the study

Fluticasone propionate and budesonide are both corticosteroids developed for topical use. They both have high activity in skin vasoconstriction tests and have been shown to be safe and give good clinical results (Phillipps 1990). To avoid total occlusion of the nasal cavity after allergen challenge, making lavage impossible, oxymetazoline was used in the lavage challenge model. The nasal decongestion does of course influence the degree of blockage the patients can experience (Naclerio et al. 1983). Despite this influence, the actively treated group reported less nasal congestion than the placebo group on the challenge after 4 weeks of treatment (IV). In the biopsy procedure, anaesthetics in combination with decongestants are used to eliminate pain and bleeding after the biopsy. Theoretically, this may affect the cells in the specimen as well as the volume of the tissue and thereby the results. Biopsy without these manoeuvres would probably not be approved by the ethics committee and make subject recruitment difficult. For all drugs used in this study, the doses are the recommended for clinical use.

Staining and cell counting methods

The methods used in this study identify distinctive properties of the mast cells: the granular content of glycosaminoglycan, chymase and tryptase, and IgE antibodies bound to high-affinity IgE receptors. These properties, with the exception of the proteases, are shared with the closely related blood basophil. Tryptase appears to be more or less specific for the mast cell (Schwartz et al. 1987). Under normal or undisturbed conditions, close correlation

between cell numbers obtained using these methods is to be expected. Selective changes in any of these properties, resulting in changes or absence of such correlation, are the likely effects of functional activation of the cells, e.g. release of mediators or redistribution of IgE. The double-labelling technique for the identification of the MC_T and MC_{TC} is based on the principle that the chymase labelling and the resulting staining product effectively prevent the tryptase epitopes, present in all mast cells, from binding to the anti-tryptase antibody in the second labelling cycle (Irani et al. 1989) (V). In the cytological samples the total number of positively stained cells was counted and no consideration given to the total number of cells on each slide (I, II). The prerequisite for this is that the samples have a relatively stable number of cells. By doing a differential count, this interobservation variability is eliminated. In the brush and lavage cytospin samples, the positively stained cells were too few to make a differential count practical. Furthermore, the metachromatic, IgE- and tryptase-staining methods used in Study II, all have different dyes for the background staining, thereby staining different proportions of the cells, making it impossible to compare results of differential counts obtained with different staining methods. In Study V, the point-counting method and the use of a square eyepiece graticule to determine the area surveyed for cells have the advantage that the area and the cells are simultaneously measured by the observer.

Histamine, TAME and tryptase analysis

Histamine has been shown to be unstable in biological solutions. It is deactivated and the analyses use for detecting histamine also react to other amines (McBride et al. 1988). The RIA used in Studies III and IV is 50 times as sensitive as the HPLC used in Studies I, II and IV. The Immunotech-RIA$^{®}$ is specific to histamine and does not determine the first degradation product of histamine, methylhistamine, in contrast to the traditional OPT-reaction-based methods and the Pharmacia-RIA$^{®}$ (Hammar et al. 1990). The significance of this remains to be evaluated. (III, IV)

DISCUSSION

The upper and lower airways possess structural similarities and also appear to possess similar characteristics of allergic inflammation. Furthermore, the upper airways are far more accessible for investigations *in vivo* than the lower. Thus, the nasal mucosa is suitable for investigations of human airways in health and disease. Seasonal allergic rhinitis lends itself well to study for several reasons. Firstly, it affects a large percentage of the population, thus making it relatively easy to acquire a sufficient number of patients for study. Secondly, most patients are young and free of other underlying diseases that may confound the interpretation of results. Thirdly, the subjects can be studied during a period of the year when they are free from symptoms. Nasal challenge was chosen as the experimental method because it can answer questions about the pathophysiology of the disease and the action of drugs. In the laboratory full control can be achieved regarding the dose of challenge agent and timing of the challenge. One should be aware of the fact that challenge experiments are artificial situations which cannot mimic the continuous exposure occurring during natural exposure. Thus, certain important events may only be apparent during natural allergen exposure. It is therefore important to confirm results from the laboratory in the natural disease. Pollen is one of the major allergen sources in Scandinavia. At least one in every ten Scandinavians has a nasal allergy and it is estimated that pollen allergy accounts for half of these cases (Åberg 1989, Varjonen et al. 1992). Thus patients with seasonal allergic rhinitis provide a relevant population for the study of allergic inflammation.

Correlation between mast cells, mediators and symptoms

The differences between the allergic patients' reactions to allergen versus diluent and the difference between the allergic patients and healthy controls regarding mast-cell density and the liberation of mast-cell markers are apparent. The allergics had increased numbers of mast cells in their mucosa even in the resting stage and the patients reacted to the allergen challenges with an increase in symptoms and biological markers but the healthy subjects did not. Control challenges with diluent prove that these changes were specific for the allergen. Challenging the nose with histamine generates a symptom pattern relevant to that seen after allergen challenge (Pipkorn 1988). An increase in the nasal lavage level of histamine was observed (III, IV) and the histamine/metachromatic cell quotient decreased after the allergen challenge, suggesting a secretion of the cellular histamine. The tissue histamine correlated with the tissue mast-cell density and the total number of mast cells per cytospin slide from brush samples correlated with the cellular histamine as well as the symptoms. It is therefore reasonable to conclude that the post-allergen challenge increase in symptoms is caused by mast-cell degranulation and secretion of mediators such as histamine.

The cellular kinetics after allergen provocation and during the pollen season

There are many reports of an increase in the numbers of mast cells and basophils in nasal mucosa of allergic patients as a reaction to allergen exposure (Okuda et al. 1983, Otsuka et al. 1985, Enerbäck et al. 1986a, Davies et al. 1987, Viegas et al. 1987, Bascom et al. 1988, Gomez et al. 1988, Liu 1988, Pipkorn et al. 1988b, Enerbäck et al. 1989, Pipkorn et al. 1989, Bachert et al. 1990, Borres et al. 1990, Lozewicz et al. 1990, Bentley et al. 1992, Iliopoulos et al. 1992). In this Study, several methods of identifying these cells have been employed in a parallel manner and all have shown an increase in the mast cell markers 8 to 12 hours after allergen challenge. This supports the hypothesis that a redistribution of mast cells towards the mucosal surface is part of the allergic inflammation in mucous membranes. On the other hand, the study on the effect of the pollen season did not show any statistically significant changes in mast cell density in either epithelium or stroma in non-steroid-treated patients. Results in line with this are found in the literature (Wihl and Mygind 1977, Pipkorn et al. 1988d, Lozewicz et al. 1992, Varney et al. 1992). Differences in study design, patient selection, cell counting method or fixation and staining techniques (Trotter et al. 1990, Nakamura et al. 1991) as well as differences in timing of the studies, with different degrees of mast-cell secretion and mediator depletion, interfering with the demonstrability of the mast cells, all influence the results. In the pre-challenge samples in Study II a larger number of IgE-positive cells than metachromatic or tryptase-containing cells was found. The correlation between IgE-positive cells and the histamine content of the cell pellets was also poor. These findings can be interpreted as a sign of degranulation of mast cells in the epithelium. Such "ghost cells" could represent cells that have lost their granules but still bear IgE molecules linked to the Fc_ε-receptors on their surface (Fokkens et al. 1992). The possibility that these IgE-positive cells could represent non-mast cells, with low-affinity Fc_ε-receptors, appears less likely on the grounds of morphology and the fact that the immunohistochemical staining method does not detect cells with a low IgE content (Rognum and Brandtzaeg 1989, Bachert et al. 1990). The fact that the IgE-positive cells did not increase in number as much as the metachromatic cells during the late observation hours could indicate that the latter must reach a certain stage of maturation before they acquire IgE in amounts detectable by immunohistochemical staining methods.

Release of mediators

An increasing number of papers are reporting that the levels of tryptase in nasal lavage fluid are increased in patients with allergic rhinitis, both during native disease and after local allergen challenge (Castells and Schwartz 1988, Proud et al. 1992, Rasp et al. 1993). In Studies III and IV tryptase was compared with other markers of the allergic inflammatory response, TAME-esterase activity and histamine. The relationship between the total generation of tryptase and histamine indicates that these two markers are released together. Furthermore, there was a high degree of correlation between the individual levels of TAME-

esterase and tryptase. This was not surprising since 25-30 % of the TAME-esterase activity measured in the challenge situation represents tryptase activity, while the remainder largely represents plasma kallikrein activity (Baumgarten et al. 1986). In the resting stage, levels of tryptase are not above the limit of detection. This appears to contrast with the findings relating to histamine. High and variable baseline histamine levels in nasal lavage fluid are constantly reported (Naclerio et al. 1983, Pipkorn et al. 1987a, Pipkorn et al. 1987b, Bisgaard et al. 1988, Linder et al. 1988). In Study I the allergen challenge resulted in significantly lower histamine cell pellet contents and histamine/metachromatic cell quotient. This was taken as evidence of mast-cell activation and the loss of intracellular histamine. These findings lend further support to the concept that mast-cell activation is an essential ingredient in the immediate allergic response.

The effects of topical corticosteroid treatment

Reduction of the numbers of mast cells (Otsuka et al. 1986, Gomez et al. 1988) and mast-cell markers in nasal lavage fluid (Pipkorn et al. 1987a) have previously been recorded after topical glucocorticosteroid treatment of allergic nasal mucosa. In other publications on the effect on mast cells after corticosteroid pretreatment, varying results have been reported (Pipkorn 1983, Lozewicz et al. 1992). The results of Study IV show that the actively treated patients reported fewer symptoms than the placebo group. There was also a reduction in the release of tryptase after 4 weeks´ treatment with topical corticosteroid. The specificity of tryptase in mast-cell-mediated reactions is demonstrated in this study as it was the only marker able to manifest the effect of the corticosteroid treatment. In Study V the epithelial numbers of MC_T were reduced after steroid treatment. The reduction of MC_{TC} numbers did not reach statistical significance, however, probably due to the small number of subjects treated. In Study IV no change in mast-cell density after the treatment period was detected. Perhaps a more specific means of mast-cell detection, such as using immunolabelling with tryptase and chymase antibodies, or separate analysis of cellular findings in epithelium and stroma, would reveal other results. The changes in immunoregulation after glucocorticoid treatment are complex and may include all phases of the allergic response. Corticosteroid treatment does not seem to inhibit the release of histamine and other mediators from human mast cells isolated from skin, intestine and airways after challenge with anti-IgE (Cohan et al. 1989). The reduction in numbers of epithelial mast cells induced by the glucocorticosteroid treatment may thus be due to the inhibition of the production of mast-cell growth factors by mucosal T lymphocytes either by a direct action on the T-helper cells (Culpepper and Lee 1985, Burke et al. 1992) or by acting on the antigen-presenting cells (Martinet et al. 1992).

The heterogeneity among the patients

The heterogeneity of the patient population is worth special consideration. Although in Studies I - V the patients showed a wide spectrum of reactions and the results varied between

patients, this heterogeneity was best demonstrated in Studies I and II. Three of the patients had elevated pre-challenge numbers of eosinophils and mast cells and high numbers of IgE-bearing and tryptase-containing cells and reacted to the challenge with the most pronounced response. These findings suggest that a group of asymptomatic allergic individuals have an activated inflammatory system readily capable of vivid reactions to an offending antigen. Possible explanations for such a forceful inflammatory reaction may be sought in a different duration of the allergic disease, different degree of atopic heredity or a subclinical sensitisation to other allergens such as perennial allergens.

The identity of the metachromatic cells

It is of considerable biological interest to be able to decide the identity of the metachromatic cells in the nasal mucosa and the role of mast cells and basophils in mucosal allergy. Based on light-microscopic morphology and ultrastructural observations as well as on the finding of a naphthol-AS-D chloroacetate esterase activity, studies have indicated that the majority of metachromatic cells in the nasal epithelium are mast cells rather than blood basophils (Pipkorn et al. 1988c). Supported on histological and biochemical studies, basophils have been implicated in the late-phase reaction (Naclerio et al. 1985, Bascom et al. 1988, Iliopoulos et al. 1992). In Study II, most of the metachromatic cells of the pre-challenge brush samples had a mast-cell-like morphology and their numbers correlated with the number of tryptase-positive cells. In samples taken 8 h to 12 h after the allergen challenge, metachromatic cells were far more numerous than tryptase-containing cells. In these samples an increase was noted in cells with lobulated nuclei, suggesting a basophil derivation. There was also an influx of rather small but strongly metachromatic cells which had no counterpart in the anti-IgE and anti-tryptase-stained specimens. The finding of an increase in cells resembling basophils in the late-phase samples is in agreement with reports that basophils may be a major source of metachromatic cells in the late-phase reaction (Naclerio et al. 1987). An increase in the cells that had the morphology of mast cells and an increase in tryptase-containing cells was also noted. Cells containing tryptase in such quantity that it enables visualisation with immunochemical staining methods using anti-tryptase antibodies are probably not basophils (Castells et al. 1987). These findings taken together can only be interpreted as an indication of an influx of mast cells as well as of basophils into the allergic nasal mucosa during the late-phase reaction. In Study V the biopsy specimens contained more tryptase-containing cells than metachromatic cells. This can be an indication that the basophils are not a major source of metachromatic cells in these samples. The exact nature of the metachromatic cells can only be determined by using specific morphological markers for basophilic granulocytes as well as for mast cells. It has been reported that metachromatic cells recovered from nasal lavage fluid after allergen challenge carried CD18, a typical leukocyte marker, indicating a basophil lineage (Iliopoulos et al. 1992).

The mast cell heterogeneity - sign of functional differentiation

In the rat, a strict compartmental segregation exists between the mucosal mast cell (MMC) and the connective tissue mast cell (CTMC). In Study V nasal mucosa in non-atopic controls was found to contain predominantly MC_{TC} while high fractions of MC_T were detected in allergic nasal mucosa, both in the stroma and, predominantly, in the epithelium. Furthermore, intra-epithelial MC_{TC} were found in allergic patients as well as non-atopic individuals. These findings conflict with the hypothesis that mast cells of the MC_T category may be the human equivalent of the MMC found in rodents and MC_{TC} the counterpart of the rodent CTMC (Miller and Schwartz 1989, Irani and Schwartz 1990). It would be more reasonable to interpret these findings as that the lack of chymase reflects functional activation of the mast cells rather than phenotypic differentiation. The correlation between numbers of protease-containing mast cells and metachromatic cells was strong. Allergic nasal epithelium exhibited the largest difference in metachromatic cells and immunopositive mast cell density. The glycosaminoglycan content of some mast cells therefore appears to be too low to be demonstrable with toluidine blue staining whereas the mast cells may contain high enough quantities of protease to permit immune labelling, indicating that the mast cell content of proteases and glycosaminoglycan can vary independently. Stimulated or newly recruited mast cells may thus display asynchrony in the acquisition or secretion of glycosaminoglycan and protease. A correlation between the fractions of aldehyde-sensitive mast cells and the MC_T was found. Absence of chymase thus appears to decrease the dye-binding ability of the glycosaminoglycan and increase the dye-binding blocking effect of aldehyde fixation. This is an indication of altered functional characteristics of the mast cells in allergic inflammation rather than a sign of phenotypic differentiation related to anatomical site.

Mast cell function in the allergic inflammation

The atopic patients displayed high numbers of epithelial mast cells and a recruitment of mast cells into the epithelium after allergen challenge (I, II, V). This phenomenon is of fundamental pathophysiological importance in the establishment of contact between aero-allergens and the mast cells in allergic conditions. Interestingly, both MC_T and MC_{TC} were seen in the epithelium of normal subjects and in the allergic patients (V). MC_T was, however, clearly the dominating type of intra-epithelial mast cell in allergic patients. The metachromatic properties of these cells were also found to be reduced and highly aldehyde sensitive. These findings as well as the detection of high levels of the mast cell mediators histamine and tryptase in nasal lavage fluid from allergic patients after allergen challenge (III, IV), make it evident that the mast cells are actively involved in the allergic inflammation, are functionally altered and serve as secretory cells giving rise to the symptoms of an acute allergic reaction. The theories about how mast cells contribute to allergic disease are undergoing dramatic changes. This study focuses on the participation of mast cells in the acute allergic reaction. A concept is put forward of the mast cell as a functionally altered,

primary effector cell with secretory activities responsible for the early allergic symptoms. The recognition of mast cells as a source of chemotactic factors and multifunctional cytokines, acting on other inflammatory cells and the endothelial cells to regulate cell traffic, as well as the capacity of the mast cell for autoregulation of the IgE-mast cell-eosinophil response has led to the development of a concept of a cell that is capable of orchestrating an ongoing allergic inflammation.

Future tasks

Clinically asymptomatic subjects should be studied regarding fluctuation in morphological and biological markers. This might help to understand the so called "baseline", that all changes in the different parameters are compared with. The heterogeneity among the allergic patients needs to be analysed to find the distinguishing criteria for the group of patients that are high responders to allergen challenge. It is not known whether these patients are liable to become more allergic, multiallergic or develop allergy in other locations of the airways. This group might have special clinical requirements regarding medication or controls. There are no studies comparing the reaction to allergen exposure in multiallergic patients with the reaction in monoallergic patients. The exact nature of metachromatic cells in the nasal mucosa should be clarified using specific markers for mast cells as well as basophils. The kinetics of the tryptase release in relation to the histamine release after nasal allergen challenge is not clear. It should be analysed how these markers appear in and on the mucosal surface, both during the acute phase of the allergic reaction and over a longer time span, thereby increasing our knowledge of the function of the mast cells in the subsequent phases in the allergic inflammation.

Although the disease studied here is seasonal allergic rhinitis, the methods applied can be utilised for the study of other clinical entities, e.g. perennial allergic rhinitis, occupational rhinitis due to exposure to chemical or physical irritants, vasomotoric rhinitis and infectious rhinitis. Mast cells have an active function in the acute allergic response and in chronic allergic inflammation. With an increased understanding of the different types of mast cells, the mediators they produce and how they are regulated, we will be in a better position to control the diseases in which these cells are implicated.

SUMMARY

- Mast cells are increased in number in the nasal mucosal epithelium in patients with allergic rhinitis.

- The intra-epithelial mast-cell density correlates with the degree of symptoms after allergen provocation as a sign that these cells are of pivotal importance for the symptoms.

- The histamine content of the mast cells decreases and mast-cell markers in nasal lavage fluid increase after allergen provocation as a sign of degranulation of the cell as a reaction to the provocation.

- The increase in metachromatic cells with the morphology of mast cells and tryptase-positive cells in the nasal epithelium 8-10 h and 12 h respectively after the provocation is a sign of recruitment of mast cells into the shock organ.

- Metachromatic cells with morphology resembling the basophilic granulocyte appear in the epithelium in increased numbers 8 - 12 h after allergen challenge.

- The higher number of immunopositive cells compared with metachromatic cells in biopsy specimens from allergic patients indicate that the mast cell content of proteases and glycosaminoglycans can vary independently.

- The decrease in demonastrable glycosaminoglycans and the increased aldehyde sensitivity of mast cells from allergic patients as well as the fact that this increase consists mostly of cells containing tryptase, but lacking chymase, is a sign of altered functional characteristica of the mast cells in allergic inflammation.

- The decrease in the post-allergen-challenge nasal lavage fluid tryptase levels and the decrease in the density of mast cells containing tryptase but lacking chymase in the nasal epithelium after topical corticosteroid treatment may explain the clinically beneficial effects of this treatment.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to all patients who have participated in this study and to:

Associate Professor Göran Karlsson, my tutor, for scientific guidance and never-failing encouragement;

Professor Lennart Enerbäck, my supervisor, for his skilful scientific guidance in mast cell research;

The late Associate Professor Ulf Pipkorn, my first tutor, for introducing me to the exciting field of rhino-allergology. His work in the science of rhinology will always be remembered;

Professor Christer Lundberg, my chief, for his interest and concern throughout the work of this study;

Associate Professors Hans Rundcrantz, Mats Bende and Björn Petruson for introducing me to the fascinating and diverse fields of rhinology;

Kenneth Holmberg and Frank Aldenborg, my co-workers during this study;

Gun Augustsson, Anita Olofsson, Elisabet Warnhammar and Sture Bergström for their excellent laboratory assistance;

Jeanette Kliger and John Gulliver for revising the English text;

Professor Stefán Skaftason for introducing me to the field of oto-rhino-laryngology;

and last but not least my family Anna, Eyjólfur, Kristján and Júlíus Már for their loving patience and support.

REFERENCES

Agis H, Willheim M, Sperr WR, Wilfing A, Geissler K, Spittler A, Boltz-Nitulescu G, Lechner K, Valent P. The circulating mast cell precursor is a CD34+, CD14-; CD17-, colony-forming cell. Allergy 48: Suppl 16: 111, 1993.

Aldenborg F, Enerbäck L. Histochemical heterogeneity of dermal mast cells in athymic and normal rats. Histochem J 20: 19-28, 1988.

Allenmark S, Bergström S, Enerbäck L. A selective post-column O-phthalaldehyde derivatization system for the determination of histamine in biological material by high-performance liquid chromatography. Anal Biochem 144: 98-103, 1985.

Andersson M, Andersson P, Pipkorn U. Topical glucocorticosteroids and allergen-induced increase in nasal reactivity: relationship between treatment time and inhibitory effect. J Allergy Clin Immunol 82: 1019-26, 1988.

Azzawi M, Bradley B, Jeffery PK, Frew AJ, Wardlaw AJ, Knowles G, Assoufi B, Collins JV, Durham S, Kay AB. Identification of activated T-lymphocytes and eosinophils in bronchial biopsies in stable asthma. Am Rev Respir Dis 142: 1407-13, 1990.

Bachert C, Prohaska P, Pipkorn U. IgE-positive mast cells on the human nasal mucosal surface in response to allergen exposure. Rhinology 28: 149-158, 1990.

Banov C, Blumenthal M, Frazer H, Gold B, LaForce C, Miller M, Morgan W, Pearman D, Southern D, Woehler T, Beam J, Rogers P. Treatment of perennial allergic rhinitis with fluticasone propionate. Ann Allergy 66: 97, 1991.

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

Baumgarten CR, Nichols RC, Naclerio RM, Lichtenstein LM, Norman PS, Proud D. Plasma kallikrein during experimentally induced allergic rhinitis: Role in kinin formation and contribution to TAME-esterase activity in nasal secretions. J Immunol 137: 977-982, 1986.

Bentley AM, Jacobson MR, Cumberworth V, Barkans JR, Moqbel R, Schwartz LB, Irani A-MA, Kay AB, Durham SR. Immunohistology of the nasal mucosa in seasonal allergic rhinitis: Increases in activated eosinophils and epithelial mast cells. J Allergy Clin Immunol 89: 877-83, 1992.

Bisgaard H, Grønborg H, Mygind N, Dahl R, Venge P. Allergen-induced increase of eosinophil cationic protein in nasal lavage fluid: Effect of the glucocortcoid budesonide. J Allergy Clin Immunol 85: 891-895, 1990.

Bisgaard H, Robinson C, Romeling F, Mygind N, Church M, Holgate ST. Leukotriene C4 and histamine in the early allergic reaction in the nose. Allergy 43: 219-227, 1988.

Blackley CH. Experimental research on the cause and nature of catarrhus activus. In: Blackley CH. Hay fever or hay asthma. Baillière, Tindall and Fox, London. 1873.

Borres MP, Irander K, Björksten B. Metachromatic cells in nasal mucosa after allergen challenge. Allergy 45: 98-103, 1990.

Bradding P, Feather IH, Howarth PH, Mueller R, Roberts JA, Britten K, Bews JPA, Hunt TC, Okayama Y, Heusser CH, Bullock GR, Church MK, Holgate ST. Interleukin 4 is localized to and released by human mast cells. J Exp Med 176: 1381-1386, 1991.

Bryan WTK, Bryan MP. Significance of mast cells in nasal secretions. Trans Am Acad Ophtalmol Otolaryngol 63: 613-627, 1959.

Burke C, Power CK, Norris A, Condez A, Schmekel B, Poulter LW. Lung function and immunopathological changes after inhaled corticosteroid therapy in asthma. Eur Respir J 5: 73-79, 1992.

Calderon E, Lockey RF. A possible role for adhesion molecules in asthma. J Allergy Clin Immunol 90: 852-65, 1992.

Callerame ML, Condemi JJ. Demonstration of IgE on human-skin mast cells using anti-IgE[125I] and refined light microscopy. Am J Clin Pathol 62: 823-829, 1974.

Castells M, Schwartz LB. Tryptase levels in nasal-lavage fluid as an indicator of the immediate allergic response. J Allergy Clin Immunol 82: 348-355, 1988.

Castells MC, Irani AM, Schwartz LB. Evaluation of human peripheral blood leukocytes for mast cell tryptase. J Immunol 138: 2184-2189, 1987.

Cauna N. Blood and nerve supply of the nasal lining. In: Proctor DF, Andersen IB, eds. The nose: upper airway physiology and the atmospheric environment. Elsevier Biomedical Press, Amsterdam. 45-69, 1982.

Cauna N, Cauna D, Hinderer KH. Innervation of human nasal glands. J Neurocytol 1: 49-60, 1972.

Cauna N, Hinderer KH. Fine structure of blood vessels of the human nasal repiratory mucosa. Ann Otol Rhinol Laryngol 78: 865-879, 1969.

Coca AF, Cooke RA. On the classification of the phenomena of hypersensitiveness. J Immunol 8: 163-82, 1923.

Cohan VL, Undem BJ, Fox CC, Adkinson NF, Lichtenstein LM, Schleimer RP. Dexamethasone does not inhibit the release of mediators from human mast cells residing in airway, intestine, or skin. Am Rev Respir Dis 140: 951-954, 1989.

Cole P. Modification of inspired air. In: Proctor DF, Andersen IB, eds. The nose: upper airway physiology and the atmospheric environment. Elsevier Biomedical Press, Amsterdam. 351-375, 1982.

Consensus Statement. Investigation of allergy and other hypersensitivity. The Board for Nordic Collaboration in Medical Research. Spri, Stockholm, Sweden. 1992.

Coombs RRA, Gell PGH. Classification of allergic reactions responsible for clinical hypersensitivity. In: Gell PGH, Coombs RRA, ed. Clinical Aspects of Immunology. Blackwell Scientific, Oxford, England. 1968.

Corrigan CJ, Kay AB. Lymphocytes. In: Middleton E Jr., Reed CE, Ellis EF, Adkinson NF Jr., Yunginger JQ, Busse WW, eds. Allergy. Principles and Practice. I. Mosby-Year Book, Inc., St. Louis, Missouri. 206-211, 1993.

Culpepper JA, Lee F. Regulation of IL 3 expression by glucocorticoids in cloned murine T lymphocytes. J Immunol 135: 3191-7, 1985.

Davies RJ, Gaitland D, Brooks J, Gomez E, Viegas M. Effect of pollen season on nasal mast cell. Br Med J 294: 414, 1987.

Day JH, Andersson CB, Briscoe MP. Efficacy and safety of intranasal budesonide in the treatment of perennial rhinitis in adults and children. Ann Allergy 64: 445-450, 1990.

Del-Prete G. Human Th1 and Th2 lymphocytes: their role in the pathophysiology of atopy. Allergy 47: 450-5, 1992.

Durham SR. Basic mechanisms of immunotherapy. Allergy 48: Suppl 16: 15, 1993.

Durham SR, Ying S, Varney VA, Jacobson MR, Sudderick RM, MacKay IS, Kay AB, Hamid QA. Cytokine messenger RNA expression for IL-2, IL-4, IL-5 and granulocyte/macrophage-colony-stimulating factor in the nasal mucosa after local allergen provocation: relationship to tissue eosinophilia. J Immunol 148: 2390-2394, 1992.

Dvorak AM, Dvorak HF, Galli SJ. Ultrastructural criteria for identification of mast cells and basophils in humans, guinea pigs and mice. Am Rev Respir Dis 128: 49-52, 1983a.

Dvorak AM, Galli SJ, Schulman ES, Lichtenstein LM, Dvorak HF. Basophil and mast cell degranulation: Ultrastructural analysis of mechanisms of mediator release. Federation Proceedings 42: 2510-2515, 1983b.

Ehrlich P. Beträge zur Kenntniss der granulirten Bindegewebszellen und der eosinophilen Leukocythen. Arch Anat Physiol, Physiol Abt. 166-169, 1879.

Enander I, Matsson P, Nystrand J, Andersson AS, Eklund E, Bradford TR, Schwartz LB. A new radioimmunoassay for human mast cell tryptase using monoclonal antibodies. J Immunol Methods 138: 39-46, 1991.

Enerbäck L. Mast cells in rat gastrointestinal mucosa. II. Dye-binding and metachromatic properties. Acta Path Microbiol Scand 66: 303-312, 1966a.

Enerbäck L. Mast cells in rat gastrointestinal mucosa. I. Effects of fixation. Acta Path Microbiol Scand 66: 289-302, 1966b.

Enerbäck L. Mast cells. In: Spicer SS, ed. Histochemistry in pathologic diagnosis. Marcel Dekker, New York. 695-727, 1986.

Enerbäck L, Karlsson G, Pipkorn U. Nasal mast cell response to natural allergen exposure. Int Arch Allergy Appl Immunol 88: 209-11, 1989.

Enerbäck L, Norrby K. The Mast Cell. In: Iversen OH, ed. Cell Kinetics of the Inflammatory Reaction. 79. Springer-Verlag, Berlin. 169-204, 1989.

Enerbäck L, Pipkorn U, Aldenborg F, Wingren U. Mast cell heterogeneity in man: properties and function of human mucosal mast cells. In: Galli SJ, Austen KF, eds. Mast cell and basophil differentiation and function in health and disease. Raven Press, Ltd., New York. 27-37, 1989.

Enerbäck L, Pipkorn U, Granerus G. Intraepithelial migration of nasal mucosal mast cells in hay fever. Int Archs Allergy Appl Immun 80: 44-51, 1986a.

Enerbäck L, Pipkorn U, Olofsson A. Intraepithelial migration of mucosal mast cells in hay fever: ultrastructural observations. Int Arch Allergy Appl Immunol 81: 289-97, 1986b.

Feltkamp-Vroom TM, Stallman PJ, Aalberse RC, Reerink-Brongers EE. Immunofluorescence studies on renal tissue, tonsils, adenoids, nasal polyps, and skin of atopic and nonatopic patients, with special reference to IgE. Clin Immunol Immunopath 4: 392-404, 1975.

Flowers BK, Proud D, Kagey-Sobotka A, Lichtenstein LM, Naclerio R. Localized antigen challenge of the nasal mucosa. Arch Otolaryngol Head Neck Surg 116: 1407-1410, 1990.

Fokkens WJ, Godthelp T, Holm AF, Blom H, Mulder PGH, Vroom TM, Rijntjes E. Dynamics of mast cells in the nasal mucosa of patients with allergic rhinitis and non-allergic controls: a biopsy study. Clin Exp Allergy 22: 701-710, 1992.

Galli SG, Gordon JR, Wershil BK. Cytokine production by mast cells and basophils. Curr Opin Immunol 3: 865-72, 1991.

Galli SJ. Basophils and mast cells: morphologic insights into their biology, secretory patterns, and function. In: Ishizaka K, ed. Progress in Allergy. 34. Karger, Basel. 1-141, 1984.

Galli SJ, Tsai M, Gordon JR, Geissler EN, Wershil BK. Analyzing mast cell development and function using mice carrying mutations at W/c-kit or Sl/MGF (SCF) loci. Ann N Y Acad Sci 664: 69-88, 1992.

Gibson PG, Allen CJ, Yang JP, Wong BJO, Dolovich J, Denburg J, Hargreave FE. Intraepithelial mast cells in allergic and nonallergic asthma. Assessment using bronchial brushings. Am Rev Respir Dis 148: 80-86, 1993.

Gibson PG, Manning PJ, O'Byrne PM, Girgis-Gabardo A, Dolovich J, Denburg JA, Hargreave FE. Allergen-induced asthmatic responses: Relationship between increases in airway reponsiveness and increses in circulating eosinophils, basophils and their progenitors. Am Rev Respir Dis 143: 331-335, 1991.

Gibson S, Miller HR. Mast cell subsets in the rat distinguished immunohistochemically by their content of serine proteinases. Immunology 58: 101-104, 1986.

Glenner GC, Cohen LA. Histochemical demonstration of species-specific trypsin-like enzyme in mast cells. Nature (London) 185: 846-847, 1960.

Goldstein SM, Kaempfer CE, Kealey JT, Wintroub BU. Human mast cell carboxypeptidase. Purification and characterization. J Clin Invest 83: 1630-1636, 1989.

Gomez E, Clague JE, Gatland D, Davies RJ. Effect of topical corticosteroids on seasonally induced increases in nasal mast cells. Br Med J 296: 1572-1573, 1988.

Gordon ACH, McDonald CF, Thomson SA, Frame MH, Pottage A, Crompton GK. Dose of inhaled budesonide required to produce clinical suppression of plasma cortisol. Eur J Respir Dis 71: 10-14, 1987.

Gordon JR, Burd PR, Galli SJ. Mast cells as a source of multifunctional cytokines. Immunol Today 11: 485-64, 1990.

Greiff L, Pipkorn U, Alkner U, Persson CG. The 'nasal pool' device applies controlled concentrations of solutes on human nasal airway mucosa and samples its surface exudations/secretions. Clin Exp Allergy 20: 253-9, 1990.

Gruvstad E, Bengtsson B. A comparison of a new steroid, budesonide, with other topical corticosteroids in vasoconstriction assay. Drugs Exp Clin Res 6: 385-390, 1980.

Hammar E, Berglund A, Hedin A, Norrman A, Rustas K, Ytterström U, Åkerblom E. An immunoassy for histamine based on monoclonal antibodies. J Immunol Methods 128: 51-58, 1990.

Harding SM. The human pharmacology of fluticasone propionate. Respir Med 84: Suppl A: 25-29, 1990.

Holgate ST, Robinson C, Church MK. Mediators of immediate hypersensitivity. In: Middleton E Jr., Reed CE, Ellis EF, Adkinson NF Jr., Yunginger JQ, Busse WW, eds. Allergy. Principles and Practice. I. Mosby-Year Book, Inc., St. Louis, Missouri. 267-301, 1993.

Iliopoulos O, Baroody FM, Naclerio RM, Bochner BS, Kagey-Sobotka A, Lichtenstein LM. Histamine-containing cells obtained from the nose hours after antigen challenge have functional and phenotypic characteristics of basophils. J Immunol 148: 2223-2228, 1992.

Imanari T, Kaizu T, Yoshida H, Yates K, Pierce JV, Pisano JJ. Radiochemical assays for human urinary, salivary, and plasma kallekrein. In: Pisano JJ, Austen KF, eds. Chemistry and biology of the kallekrein system in health and disease. D.C.L. Government Printing Office, Washington. 205-13, 1976.

Irani A-MA, Bradford TR, Kepley CL, Schechter NM, Schwartz LB. Detection of MCT and MCTC types of human mast cells by immunohistochemistry using new monoclonal anti-tryptase and anti-chymase antibodies. J Histochem Cytochem 37: 1509-15, 1989.

Irani A-MA, Craig SS, DeBlois G, Hutchinson L, Sismanis A, Schechter NM, Schwartz L. Distribution of mast cell subsets in human tissues. Clin Res 34: 277A, 1986a.

Irani AA, Schechter NM, Craig SS, DeBlois G, Schwartz LB. Two types of human mast cells that have distinct neutral protease compositions. Proc Natl Acad Sci USA 83: 4464-8, 1986b.

Irani AM, Nilsson G, Miettinen U, Craig SS, Ashman LK, Ishizaka T, Zsebo KM, Schwartz LB. Recombinant human stem cell factor stimulates differentiation of mast cells from dispersed human fetal liver cells. Blood 80: 3009-21, 1992.

Irani AM, Schwartz LB. Neutral proteases as indicators of human mast cell heterogeneity. Monogr Allergy 27: 146-162, 1990.

Ishizaka K, Tomioka H, Ishizaka T. Mechanisms of passive sensitization. I. Presence of IgE and IgG molecules on human leukocytes. J Immunol 105: 1459-1467, 1970.

Kaliner MA. Allergic rhinitis. In: Mygind N, Naclerio RM, eds. Allergic and non-allergic rhinitis. Clinical aspects. Munksgaard, Copenhagen. 153-158, 1993.

Kjellman NIM. Atopic disease in seven-year-old children. Acta Paediatr Scand 66: 465-71, 1977.

Kusche M, Lindahl U, Enerbäck L, Rodén L. Identification of oversulphated galactosaminoglycans in intestinal-mucosal mast cells of rats infected with the nematode worm *Nippostrongylus brasiliensis*. Biochem J 253: 885-893, 1988.

Lee TDG, Swieter M, Befus AD. Mast cell responses to helminth infection. Parasitology Today 2: 186-191, 1986.

Leung KBP, Flint KC, Brostoff J, Hudspith BN, McI Johnson N, Lau HYA, Liu WL, Pearce FL. Effects of sodium cromoglycate and nedocromil sodium on histamine secretion from human lung mast cells. Thorax 43: 756-761, 1988.

Linder A, Ronquist G, Deuschl H. Random distribution of exogenous lithium in nasal secretion and its application in substance determination. Acta Otolaryngol Scand (Stockholm) 96: 287-293, 1983.

Linder A, Strandberg K, Deuschl H. Variations in histamine concentrations in nasal secretion in patients with allergic rhinitis. Allergy 43: 119-126, 1988.

Liu C-M. Seasonal variation of nasal surface basophilic cells and eosinophils in Japanese cedar pollinosis. Rhinology 26: 167-173, 1988.

Lozewicz S, Gomez E, Claugue J, Gatland D, Davies RJ. Allergen-iduced changes in the nasal mucous membrane in seasonal allergic rhinitis: Effect of nedocromil sodium. J Allergy Clin Immunol 85: 125-31, 1990.

Lozewicz S, Wang J, Duddle J, Thomas K, Chalstray S, Reilly G, Devalia JL, Davies RJ. Topical glucocorticoids inhibit activation by allergen in the upper respiratory tract. J Allergy Clin Immunol 89: 951-7, 1992.

Lundgren JD, Kaliner MA, Shelhamer JH. Mechanisms by which glucocorticosteroids inhibit secretion of mucus in asthmatic airways. Am Rev Respir Dis 141: 52-8, 1990.

Martinet N, Vaillant P, Charles T, Lambert J, Martinet Y. Dexamethasone modulation of tumor necrosis factor-α (cachectin) release by activated normal human alveolar macrophages. Eur Respir J 5: 67-72, 1992.

Matis WL, Lavker RM, Murphy GF. Substance P induces the expression of an endothelial-leukocyte adhesion molecule by microvascular endothelium. J Invest Dermatol 94: 492-495, 1990.

McBride P, Bradley D, Kaliner M. Evaluation of a radioimmunoassay for histamine measurement in biological fluids. J Allergy Clin Immunol 82: 638-646, 1988.

McBride PT, Kaliner MA. Histamine determination: Immunologic methods. ACI News 2: 50-54, 1989.

Miller JS, Schwartz LB. Human mast cell proteases and mast cell heterogeneity. Curr Opin Immunol 1: 637-642, 1989.

Morel AM, Delaage MA. Immunoanalysis of histamine through a novel chemical derivation. J Allergy Clin Immunol 82: 646-654, 1988.

Mosmann TR. Regulation of immune responses by T cells with different cytokine secretion phenotypes: Role of a new cytokine, cytokine synthesis inhibitory factor (IL10). Int Arch Allergy Appl Immunol 94: 110-5, 1991.

Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol 136: 2348-2357, 1986.

Mossman TR, Moore KW. The role of IL-10 in crossregulation of Th1 and Th2 reponses. Immunol Today 12: A49-A53, 1991.

Munck A, Mendel DB, Smith LI, Orti E. Glucocorticoid receptors and actions. Am Rev Respir Dis 141: 2-10, 1990.

Mygind N. Glucocorticosteroids and rhinitis. Allergy 48: 476-490, 1993.

Mygind N, Bisgaard H. Applied anatomy of the airways. In: Mygind N, Pipkorn U, Dahl R, eds. Rhinitis and asthma: Similarities and differences. Munksgaard, Copenhagen. 21-36, 1990.

Naclerio RM, Kagey-Sobotka A, Lichtenstein LM, Togias AG, Iliopoulos O, Pipkorn U, Bascom R, Norman PS, Proud D. Observations on nasal late phase reactions. Immunol Invest 16: 649-685, 1987.

Naclerio RM, Meier HL, Kagey-Sobotka A, Adkinson NF, Meyers DA, Norman PS, Lichtenstein LM. Mediator release after nasal airway challenge with allergen. Am Rev Respir Dis 128: 597-602, 1983.

Naclerio RM, Proud D, Togias AG, Adkinson N Jr., Meyers DA, Kagey-Sobotka A, Plaut M, Norman PS, Lichtenstein LM. Inflammatory mediators in late antigen-induced rhinitis. N Engl J Med 313: 65-70, 1985.

Nakamura A, Kawabori S, Unno T. Study on staining methods for mast cells in the nasal mucosa. Auris Nasus Larynx (Tokyo) 18: 359-366, 1991.

Nathan RA, Bronsky EA, Fireman P, Grossman J, LaForce CF, Lemanske RF, Pearlman DS, Ratner PH, Rogenes PR. Once daily fluticasone propionate aqueous nasal spray for seasonal allergic rhinitis. Ann Allergy 67: 332-8, 1991.

Nilsson G, Irani A-M, Schwartz LB. Regulation of growth and differentiation of human mast cells in vitro. Allergy 48: Suppl 16: 109, 1993.

Ohkawara Y, Yamauchi K, Tanno Y, Tamura G, Ohtami H, Nagura H, Ohkuda K, Takishima T. Human lung mast cells and pulmonary macrophages produce tumor necrosis factor-α in sensitized lung tissue after IgE receptor triggering. Am J Respir Cell Mol Biol 7: 385-92, 1992.

Okuda M. Mechanisms in nasal allergy. Part I. ORL Digest 39: 26-33, 1977.

Okuda M, Ohtsuka H, Kawabori S. Basophil leukocytes and mast cells in the nose. Eur J Respir Dis 64: Suppl 128: 7-14, 1983.

Okuda M, Sakaguchi Y, Suzuki F, Ohtsuka H, Kawabori S. Ultrastructural heterogeneity of the basophilic cells in the allergic nasal mucosa. Ann Allergy 54: 152-157, 1985.

Otsuka H, Denburg J, Dolovich J, Hitch D, Lapp P, Rajan RS, Bienenstock J, Befus D. Heterogeneity of metachromatic cells in human nose: significance of mucosal mast cells. J Allergy Clin Immunol 76: 695-702, 1985.

Otsuka H, Denburg JA, Befus AD, Hitch D, Lapp P, Rajan RS, Bienenstock J, Dolovich J. Effect of beclomethasone dipropionate on nasal metachromatic cell sub-populations. Clin Allergy 16: 589-595, 1986.

Peat JK, Britton WJ, Salome CM, J. WA. Bronchial hyperresponsiveness in two populations of Australian schoolchildren. III. Effect of exposure to environmental allergens. Clin Allergy 17: 291-300, 1987.

Persson CGA. Plasma exudation in the airways: Mechanisms and function. Eur Respir J 4: 1268-1274, 1991.

Pedersen B, Dahl R. Nasal inhalation of the glucocorticoid budesonide from a spacer for the treatment of patients with pollen rhinitis and asthma. Allergy 45: 451-456, 1990.

Petruson B, Hansson H-A, Karlsson G. Structural and functional aspects of cells in the nasal mucociliary system. Arch Otolaryngol 110: 576-581, 1984.

Phillipps GH. Structure-activity relationships of topically active steroids: The selection of fluticasone propionate. Respir Med 84: Suppl A: 19-23, 1990.

Pipkorn U. Effect of topical glucocorticoid treatment on nasal mucosal mast cells in allergic rhinitis. Allergy 38: 125-9, 1983.

Pipkorn U. Nasal provocation. Clin Rev Allergy 6: 285-302, 1988.

Pipkorn U, Bisgaard H, Wihl JÅ. Nasal provocation testing and lavage technique. In: Mygind N, Pipkorn U, eds. Allergic and vasomotor rhinitis: Pathophysiological aspects. Munksgaard, Copenhagen. 149-164, 1987.

Pipkorn U, Karlsson G. Methods for obtaining specimens from the nasal mucosa for morphological and biochemical analysis. Eur Respir J 1: 856-862, 1988.

Pipkorn U, Karlsson G, Enerbäck L. A brush method to harvest cells from the nasal mucosa for microscopic and biochemical analysis. J Immunol Methods 112: 37-42, 1988a.

Pipkorn U, Karlsson G, Enerbäck L. The cellular response of the human allergic mucosa to natural allergen exposure. J Allergy Clin Immunol 82: 1046-54, 1988b.

Pipkorn U, Karlsson G, Enerbäck L. Phenotypic expression of proteoglycan in mast cells of the human nasal mucosa. Histochem J 20: 519-25, 1988c.

Pipkorn U, Karlsson G, Enerbäck L. Secretory activity of nasal mucosal mast cells and histamine release in hay fever. Int Arch Allergy Appl Immunol 87: 349-60, 1988d.

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

Pipkorn U, Proud D, Lichtenstein LM, Kagey SA, Norman PS, Naclerio RM. Inhibition of mediator release in allergic rhinitis by pretreatment with topical glucocorticosteroids. N Engl J Med 316: 1506-10, 1987a.

Pipkorn U, Proud D, Lichtenstein LM, Schleimer RP, Peters SP, Adkinson NFJ, Kagey-Sobotka A, Norman PS, Naclerio RM. Effect of short-term systemic glucocorticoid treatment on human nasal mediator release after antigen challenge. J Clin Invest 80: 957-61, 1987b.

Proud D, Bailey GS, Naclerio RM, Reynolds CJ, Cruz AA, Eggleston PA, Lichtenstein LM, Togias AG. Tryptase and histamine as markers to evaluate mast cell activation during the responses to nasal challenge with allergen, cold, dry air, and hyperosmolar solutions. J Allergy Clin Immunol 89: 1098-1110, 1992.

Rasp G, Hochstrasser K. Tryptase in nasal fluid is a useful marker of allergic rhinitis. Allergy 48: 72-74, 1993.

Reiley CF, Tewksbury DA, Schechter NM, Travis J. Rapid conversion of angiotensin I to angiotensin II by neutrophil and mast cell proteinases. J Biol Chem 257: 8619-22, 1982.

Robinson DS, Hamid Q, Ying S, Tsicopoulos A, Barkans J, Bentley AM, Corrigan C, Durham SR, Kay AB. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. N Engl J Med 326: 298-304, 1992.

Rognum TO, Brandtzaeg P. IgE-positive cells in human intestinal mucosa are mainly mast cells. Int Arch Allergy Appl Immunol 89: 256-260, 1989.

Saito H, Hatake K, Dvorak AM, Leiferman KM, Donnenberg AD, Arai N, Ishizaka K, Ishizaka T. Selective differentiation and proliferation of hematopoietic cells induced by recombinant human interleukins. Proc Natl Acad Sci (USA) 85: 2288-2292, 1988.

Sandvik AK, Waldum HL, Kleveland PM, Schulze-Sognen B. Gastrin produces an immediate and dose dependent histamine release preceding acid secretion the totally isolated vasculary perfuced rat stomach. Scand J Gastroenterol 22: 803-808, 1987.

Schechter NM, Choi JK, Slavin DA, Deresienski DT, Sayama S, Dong G, Lavker RM, Proud D, Lazarus GS. Identification of chymotrypsin-like proteinase in human mast cells. J Immunol 137: 962-970, 1986.

Schleimer RP. Effects of glucocorticoids on inflammatory cells relevant to their therapeutic applications in asthma. Am Rev Respir Dis 141: 559-69, 1990.

Schleimer RP. Glucocorticosteroids: Their mechanisms of action and use in allergic diseases. In: Middleton E Jr., Reed CE, Ellis EF, Adkinson NF Jr., Yunginger JQ, Busse WW, eds. Allergy. Principles and Practice. I. Mosby-Year Book, Inc., St. Louis, Missouri. 893-925, 1993.

Schleimer RP, Fox CC, Naclerio RM, Plaut M, Creticos PS, Togias AG, Warner JA, Kagey-Sobotka A, Lichtenstein LM. Role of human basophils and mast cells in the pathogenesis of allergic diseases. J Allergy Clin Immunol 76: 369-374, 1985.

Schleimer RP, Sterbinsky SA, Kaiser J, Bickel CA, Klunk DA, Tomioka K, Newman W, Luscinskas FW, Gimbrone MA Jr., McIntyre BW, Bochner BS. IL-4 induces adherence of human eosinophils and basophils but not neutrophils to endothelium. Association with expression of VACM-1. J Immunol 148: 1086-1092, 1992.

Schwartz LB. Monoclonal antibodies against human mast cell tryptase demonstrate shared antigenic sites on subunits of tryptase and selective localization of the enzyme to mast cells. J Immunol 134: 526-31, 1985.

Schwartz LB. Mediators of human mast cells and human mast cell subsets. Ann Allergy 58: 226-35, 1987.

Schwartz LB, Bradford TR. Regulation of tryptase from human lung mast cells by heparin. Stabilization of the active tetramer. J Biol Chem 261: 7372-7379, 1986.

Schwartz LB, Huff T. Biology of mast cells and basophils. In: Middleton E Jr., Reed CE, Ellis EF, Adkinson NF Jr., Yunginger JQ, Busse WW, eds. Allergy. Principles and Practice. I. 4 ed. Mosby-Year Book, Inc., St. Louis, Missouri. 135-168, 1993.

Schwartz LB, Irani AM, Roller K, Castells MC, Schechter NM. Quantitation of histamine, tryptase, and chymase in dispersed human T and TC mast cells. J Immunol 138: 2611-2615, 1987.

Schwartz LB, Lewis RA, Austen KF. Tryptase from human pulmonary mast cells: Purification and characterization. J Biol Chem 256: 11939-43, 1981.

Schwartz LB, Metcalfe DD, Miller J, Earl H, Sullivan T. Tryptase levels as an indicator of mast cell activation in systemic anaphylaxis and mastocytosis. New Engl J Med 316: 1622-1626, 1987.

Schwartz LB, Yunginger JW, Miller J, Bokhari R, Dull D. Time course of appearance and disappearance of human mast cell tryptase in the circulation after anaphylaxis. J Clin Invest 83: 1551-5, 1989.

Simons FET, Simons KJ. Antihistamines. In: Middleton E Jr., Reed CE, Ellis EF, Adkinson NF Jr., Yunginger JQ, Busse WW, eds. Allergy. Principles and Practice. Mosby-Year Book, Inc., St. Louis, Missouri. 1993.

Siraganian RP. Histamine secretion from mast cells and basophils. TIPS 4: 432-437, 1982.

Siraganian RP. Mechanism of IgE-mediated hypersensitivity. In: Middleton E Jr., Reed CE, Ellis EF, Adkinson NF Jr., Yunginger JQ, Busse WW, eds. Allergy. Principles and Practice. I. 4 ed. Mosby-Year Book, Inc., St. Louis, Missouri. 105-134, 1993.

Stallman PJ, Wagenaar SS, Swierenga J, van der Wal RJ, Feltkamp-Vroom TM. Cell-bound IgE on human mast cells and basophilic granulocytes in atopic and nonatopic subjects. Int Archs Allergy Appl Immun 54: 443-450, 1977.

Sur S, Adolphson CR, Gleich GJ. Eosinophils. Biochemical and cellular aspects. In: Middleton E Jr., Reed CE, Ellis EF, Adkinson NF Jr., Yunginger JQ, Busse W W., eds. Allergy. Principles and Practice. I. Mosby-Year Book, Inc., St. Louis, Missouri. 169-200, 1993.

Svedmyr N. Action of corticosteroids on beta-adrenergic receptors. Am Rev Respir Dis 141: 31-8, 1990.

Taverne J. Immunity to protozoa and worms. In: Roitt IM, Brostoff J, Male DK, ed. Immunology. 2 ed. Gower Medical Publishing, London. 17.1-21, 1989.

Trotter CM, Salter DM, Wilson JA, Hall GH. A comparison of methods for nasal mast cell demonstration. Rhinology 28: 17-23, 1990.

Valent P, Bettelheim P. Cell surface structures on human basophils and mast cells: Biochemical and functional characterization. Adv Immunol 52: 333-423, 1992.

Varjonen E, Kalimo K, Lammintausta K, Terho P. Prevalence of atopic disorders among adolescents in Turku, Finland. Allergy 47: 243-248, 1992.

Varney VA, Jacobson MR, Sudderick RM, Robinson DS, Irani AA, Schwartz LB, MacKay IS, Kay AB, Durham SR. Immunohistology of the nasal mucosa following allergen-induced rhinitis. Am Rev Respir Dis 146: 170-6, 1992.

Venge P, Peterson CGB. Eosinophil biochemistry and killing mechanisms. In: Morley J, Colditz I, eds. Eosinophils in Asthma. Academic Press, London. 163-177, 1989.

Viegas M, Gomez E, Brooks J, Davies RJ. Changes in nasal mast cell numbers in and out of the pollen season. Int Arch Allergy Appl Immunol 82: 275-276, 1987.

von Pirquet C, Schick B. Die serum Krankheit. Deuticke, Wien. 1906.

Walden SM, Proud D, Bascom R, Lichtenstein LM, Kagey-Sobotka A, Adkinson N Jr., Naclerio RM. Experimentally induced nasal allergic responses. J Allergy Clin Immunol 81: 940-949, 1988.

Walsh LJ, Trinchieri G, Waldorf HA, Whitaker D, Murphy GF. Human dermal mast cells contain and release tumor necrosis factor-α which induces endothelial leukocyte adhesion molecule-1. Proc Natl Acad Sci USA 88: 4220-4224, 1991.

Wasserman SI. Mediators of immediate-hypersensitivity. J Allergy Clin Immun 72: 101-115, 1983.

Wenzel S, Irani AM, Sanders JM, Bradford TR, Schwartz LB. Immunoassay of tryptase from human mast cells. J Immunol Methods 86: 139-142, 1986.

Wihl J-Å, Mygind N. Studies on the allergen-challenged human nasal mucosa. Acta Otolaryngol (Stockh) 84: 281-286, 1977.

Williams TJ, Yardwood H. Effect of glucocorticoids on microvascular permeability. Am Rev Respir Dis 141: 39-43, 1990.

Wintroub BU, Schechter NB, Lazarus GS, Kaempfer CE, Schwartz LB. Angiotensin I conversion by human and rat chymotryptic proteases. J Invest Dermatol 83: 336-339, 1984.

Würthrich B. Epidemology of the allergic diseases: Are they really on the increase? Int Arch Allergy Appl Immunol 90: 3-10, 1989.

Yurt RW, Leid R Jr., Austen KF. Native heparin from rat peritoneal mast cells. J Biol Chem 252: 518-521, 1977.

Åberg N. Asthma and allergic rhinitis in Swedish conscripts. Clin Exp Allergy 19: 59-63, 1989.

Åberg N, Engström I, Lindberg U. Allergic diseases in Swedish school children. Acta Pediatr Scand 78: 246-52, 1989.

GÖTEBORGS UNIVERSITET
GÖTEBORGS UNIVERSITETSBIBLIOTEK