# Studying histamine, IL-5, IgE and TGF-beta1 in the pathogenesis of nasal polyposis

Ph.D. Dissertation

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# Contents

# ABBREVIATIONS

| | |
|---|---|
| 14C-SAM | *S*-Adenosyl methionine |
| AEC | amino-ehtylcarbasole |
| AP | atopic polyp |
| Bis-Tris-HCl | bisulphate-trisulphate-hydrogene-chloride |
| BLT1 | leukotriene B4 receptor type |
| BSA | bovine serum albumine |
| C3aR | complement 3a receptor |
| C5aR | complement 5a receptor |
| CCR1 | C-C chemokine receptor type |
| CCR3 | C-C chemokine receptor type 3 |
| CD4 | cluster of differentiation 4 |
| CD8 | cluster of differentiation 8 |
| CNM | control nasal mucosa |
| CNS | central nervous system |
| CT | computer tomography |
| CXCR2 | C-X-C chemokine receptor type 2 |
| CXCR4 | C-X-C chemokine receptor type 4 |
| CysLT1 | leukotriene D4 receptor type 1 |
| DAG | 1,2-diacyl glycerol |
| DAO | diamino-oxydase |
| Dpm | disintegrations per minute |
| ECL | electrochemoluminescense detection system |
| ECM | extracellular matrix |
| ECP | eosinophil cationic protein |
| EGTA | ethylene glycol tetraacetic acid |
| ELISA | enzyme linked immunoassay |
| EOS | eosinophil |
| FESS | functional endoscopic sinus surgery |
| f-MLFR | N-formyl-methionyl-leucyl-phenylalanine receptor |
| GI | gastrointestinal |

| | |
|---|---|
| GM-CSF | granulocyte-macrophage colony stimulating factor |
| GPCR | G-protein coupled receptor |
| H1R | H1 histamine receptor |
| H2R | H2 histamine receptor |
| H3R | H3 histamine receptor |
| H4R | H4 histamine receptor |
| HA | histamine |
| HDC | histidine-decarboxylase |
| HNMT | histamine-N-metyl-transferase |
| HPLC | high pressure liquid chromatography |
| HRP | horseradish peroxidase |
| ICAM | intercellular adhesion molecules |
| IDV | integrated density value |
| IgE | immunoglobulin E |
| IgG1 | immunoglobulin G1 |
| IL-4 | interleukin-4 |
| IL-5 | interleukin-5 |
| IL-6 | interleukin-6 |
| IL-8 | interleukin-8 |
| IP3 | inositol triphosphate |
| IP4 | inositol tetraphosphate |
| LAP | latency associated protein |
| LTBP | latent TGF-beta1 binding protein |
| M6P-R | mannose 6-phosphate/IGF-II receptor |
| MC | mast cell |
| mRNA | messenger ribonucleic acid |
| NaF | sodium fluoride |
| NAP | non-atopic-polyp |
| NK | natural killer |
| NO | nitric oxide |
| NP | nasal polyp |
| NSAID | non-steroidal-antiinflammatory-drugs |

| | |
|---|---|
| PAFR | platelet activating factor receptor |
| PBS | phosphate-buffer-saline |
| PDGF | platelet-derived-growth-factor |
| PIP2 | phosphatidyl inositol diphosphate |
| PKC | protein kinase C |
| Plasm | plasmin |
| Plasmgn | plasminogen |
| PLC | phospholipase C |
| PMFS | phenylmethylsulfonyl fluoride |
| PTX | pertussis toxin |
| PPO | poly-phenylene oxide |
| RANTES | regulated upon activation normal T cell expressed and secreted |
| RNA | ribonucleic acid |
| RP-HPLC | reverse phase high performance liquid chormatography |
| RT-PCR | reverse transcription polymerase chain reaction |
| SD | standard deviation |
| SDS-PAGE | sodium-dodecyl-sulphate-polyacrylamide gel |
| Tgase | transglutaminase |
| TGF-Rs | TGF-beta serine/threonine kinase receptors |
| TGF-beta1 | transforming growth factor beta1 |
| TH2 | T helper 2 |
| TM | transmembrane |
| TRIS | trishydroxymethylaminomethane |
| TSP-1 | thormbosondin-1 |
| uPA | urokinase type plasminogen activator |
| uPAR | urokinase tiype plasminogen activator receptor |
| VCAM-1 | vascular cell adhesion molecule-1 |
| VEGF | vascular-endothelial-growth-factor |

# Összefoglalás

Az orrpolip az orrnyálkahártya jóindulatú kitüremkedése az orrjáratokból az orrüregbe, jellemzője a krónikus eosinophil-sejtes gyulladás.

A feltételezett pathomechanizmusban résztvevő molekulák közöl célunk volt, az IgE, IL-5, TGF-beta1, és a hisztamin vizsgálata az orrpolip pathogenezisében, illetve meghatározni, hogy a hisztamin hatásainak közvetítésében mely hisztamin-receptorok vesznek részt.

Kutatásaink során a szöveti IgE szintet szignifikáns mértékben emelkedettnek találtuk polipszövetben kontroll orrnyálkahártyához képest. A szöveti IL-5 szint szintén szignifikáns mértékben magasabbnak bizonyult polipszövetben, de az allergiás és nem allergiás polipok között nem találtunk különbséget. A TGF-beta1 koncentrációt szignifikánsan magasabbnak találtuk kontroll nyálkahártyában. Immunhisztokémiai vizsgálataink során polipszövetben számos IL-5 pozitív eosinophil sejtet, illetve a lamina propriában TGF-beta1 pozitív sejtet találtunk. A HDC enzim génexpressziós és fehérje szinten is magasabbnak bizonyult polipszövetben. A HNMT aktivitást szintén polipszövetben találtuk emelkedettnek. A polip és kontrollnyálkahártyában mért hisztamintartalomban azonban nem volt különbség. Mind a H1, mind a H4 receptor szintjét polipszövetben magasabbnak találtuk, míg a H2 és H3 receptorokét nem. Az eosinophil cationic protein (ECP) szintén magasabb szinten volt jelen polipszövetben, mely kapcsolatban állhat a megemelkedett H4 receptor szinttel.

Az IL-5 fontos szerepet játszik az eosinophil felhalmozódásban és aktiválódásban. Feltehetőleg a szisztémás azonnali túlérzékenységi reakció nem játszik szerepet a kórkép kialakulásában, de a szöveti IgE termelődés lehet egy "lokális allergiás reakció" része. Az egészséges orrnyálkahártyában talált magas TGF-beta1 a sejtfelszíni aktív forma jelenléte nélkül, illetve a polipszövetben talált alacsony érték rámutathat a TGF-beta1 alapvető szabályozó szerepére a kórkép kialakulásában. Az IL-5 és TGF-beta1 forrásai az eosinophilek és a makrofág sejtek. A megváltozott hisztamin metabolizmus hatással lehet az IL-5 felszabadulására, és fontos szerepet játszhat az orrpolip kialakulásában is. A hisztamin hatásai elsősorban H1 és H4 receptorokon keresztül érvényesülnek polipszövetben. Feltételezhetjük, hogy a H4 receptoron keresztül kifejtett hisztaminhatás felelős az eosinophil sejtek felhalmozódásáért és aktiválódásáért az orrpolipózis kialakulásában.

# Summary

Nasal polyps are benign mucosal protrusions into the nasal cavity of multifactorial origin, characterised with chronic eosinophil inflammation. The suggested pathomechanisms comprise several important molecules and cytokines.

Our aim was to study IgE, IL-5, TGF-beta1, and histamine in the pathomechanism of nasal polyposis, as well as to find out which histamine-receptors play a role in the effect of histamine in nasal polyp tissue.

In our findings we found tissue IgE significantly higher in polyps compared to controls. Tissue IL-5 was significantly higher in polyp tissue compared to controls with no difference between allergic- and non-allergic polyps. TGF-beta1 proved to be significantly higher in controls than in polyps. Immunohistochemical analysis revealed numerous IL-5 positive eosinophil cells and TGF-beta1 positivity in the lamina propria of polyp samples, but none in controls. Both HDC gene expression and HDC protein were found to be higher in nasal polyps. HNMT activity was elevated also in nasal polyp tissue. There was no significant difference in the histamine content of NP and control mucosa. The expression of H1 and H4 receptors was elevated in polyp tissue, while the level of H2 and H3 receptors was not increased significantly. The concentration of eosinophil cationic protein (ECP) was significantly higher in polyp tissue and this elevation suggests an association with the increased H4 receptor expression. IL-5 plays a key role in the eosinophil recruitment and activation. Immediate hypersensitivity with systemic allergic reaction does not seem to be involved in the pathogenesis of this disease, but tissue IgE production might be due to local allergic mechanisms. High tissue TGF-beta1 quantity in healthy nasal mucosa without its active form on the cell surface and its low quantity in polyps may reflect its essential role in the regulatory mechanisms and pathogenesis of nasal polyposis. The main sources of IL-5 and TGF-beta1 are the eosinophils and macrophages. Histamine metabolism seems to be altered in polyp tissue, that can influence IL-5 release, and this may be an important factor in the pathomechanism of polyp formation. The histamine related mechanisms are preferentially mediated through H1 and H4 histamine receptors in the polyp tissue. Due to this point one may speculate, that the H4 receptor mediated histamine effects have a role in eosinophil accumulation and activation in inflammatory diseases of the nasal and paranasal sinus mucosa, like nasal polyposis.

# 1. INTRODUCTION

## 1.1. Definition

Nasal polyps are benign pedicled or sessile mucosal protrusions into the nasal cavity of multifactorial origin and are characterised by chronic mucosal inflammation.[1] (Fig. 1.)

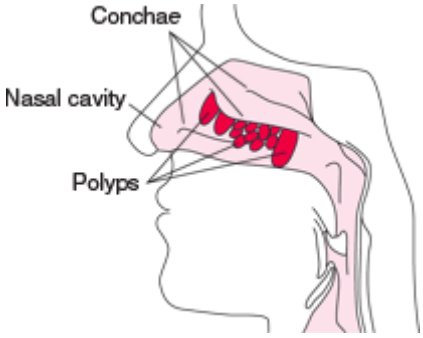


Fig. 1. Nasal polyp*

## 1.2. Epidemiology

Its incidence in normal population is around 2%[2], meanwhile in the study of Larsen, where transcranially removed nasoethmoidal blocks were screened in autopsy material, the incidence was found 25%[3]. The presence of nasal polyposis in certain systematic diseases – aspirin sensitivity, bronchial asthma, cystic fibrosis – is more often[4], and the tendency to recur is much higher (*Table 1.*).

* http://www.merck.com/media/mmhe2/figures/MMHE_19_221_01_eps.gif

*Table 1. The occurrence of nasal polyposis in systematic diseases*

| | |
|---|---|
| Aspirin/NSAID sensitivity | 36-72% |
| Adult asthma | 7% |
| atopic | 7% |
| non-atopic | 13% |
| Chronic sinusitis | 2% |
| atopic | 5% |
| non-atopic | 1.5% |
| Kartagener-syndrome | 5% |
| Primary ciliary dyskinesis | 5% |
| Young-syndrome | 5% |
| Cystic fibrosis | |
| children | 10% |
| adults | 10% |

## 1.3. Classification

According to the **clinical appearance**:

### 1.3.1. Extension:

- Unilateral or bilateral
    - isolated
        - choanal
            - antrochoanal
            - in the middle meatus
            - in the superior meatus
            - in the sinus sphenoidalis
    - multiple

- Extended form with rhinosinusitis
    - aspecific
    - allergic mycosis
        - isolated
        - diffuse
    - accompanied by systemic diseases

### 1.3.2. Predilection of origin:

- fissures of the middle nasal meatus (Fig. 2.)
- contact points of the middle concha and the nasal septum, and the lateral wall of the nasal cavity,
- frontal recess
- ethmoid cells
- superior meatus

### 1.3.3. Involvement of the nasal sinuses:

- ethmoid cells - about 90%
- maxillary sinus - about 65-70%
- frontal sinus - about 25-30%
- sphenoid sinus - about 8%

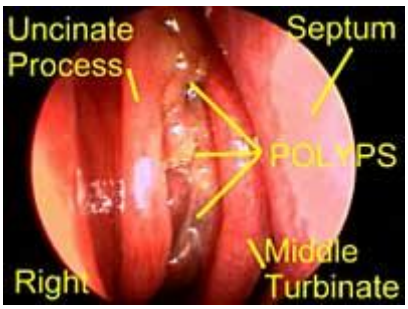


Fig. 2. Polyp in the middle meatus (endoscopic picture)*

* http://www.american-rhinologic.org/images/endoscopyarticle/12.jpg

## 1.4. Symptoms

The symptoms include mechanical obstruction to nasal breathing, mechanical anosmia, epiphora, colourless stringy or purulent secretion, postnasal catarrh, headache, snoring and rhinolalia clausa. Abnormal growth of the facial skeleton in children leads to a broad, bony nose. Chronic sinusitis may be caused by the obstruction of the sinus ostia. [1]

## 1.5. Diagnosis

The correct diagnosis helps to choose the appropriate therapy. The aim is to determine the extension of the polyp, to find other local or systematic diseases either are connected or not with the polyposis, and the localisation of anatomic variations, stenosis. The basic steps are the following:

1. Anamnesis
    - Systematic diseases
    - Fungal sinusitis
2. Physical examination, endoscopy
3. CT
4. Histological examination of removed specimens (exclusion of malignancy)
5. Other (allergy tests, functional examinations, biopsy, cytology)

The diagnostic management is based on endoscopy and CT scanning.[5]

## 1.6. Therapy

The aim of the therapy is to replace the normal nasal breathing and therefore the ventilation of the nasal cavity and the nasal sinuses, the improvement of smelling, the prevention and decrease of recurrence**.** The therapeutic management consists of the medical treatment options, which are given with evidence-based recommendations. Surgical treatment is indicated after failure of medical treatment and commonly

performed by endoscopy. Nevertheless medical therapy must be continued after surgery to prevent recurrences.[5]

### 1.6.1. Surgical therapy

Through surgical treatment the preservation of intact mucosa is very important, it should be carried out by endoscope. (FESS, functional endoscopic sinus surgery). Surgical treatment as monotherapy is satisfactory only is cases, where the polyp is isolated and not accompanied by rhinosinusitis.

### 1.6.2. Conservative therapy

The conservative therapy includes local, and in more severe cases oral steroid treatment. After 6 weeks of steroid therapy surgical treatment should be reconsidered. The steroids can be used also as pre- or postoperative therapy.

The follow up is necessary.

These treatments however, are not always sufficient and oral corticosteroids have several side effects. Surgery is often required, which in turn is not free of complications and recurrences. The recurrence of nasal polyposis in spite of any therapy is 40 %. Increasing insights in the pathophysiology of nasal polyp opens perspectives for new pharmacological treatment options.[6]

## 1.7. Histology of polyp tissue

Kakoi and Hiraide classified nasal polyps as soft tissue masses of oedematous (60%), glandular-cystic (27%), and fibrous (13%) types[7].

### 1.7.1. Epithelium

The stroma is always covered with epithelium. Between the cells of the epithelium desmosoma, and near to the surface „tight junction" type connecting structures can be observed. Intercellularly in some points there is oedema-formation. In the cytoplasm there are light-contented vacuoles, glycogen particles and fibrillar

bandages. The surface of the cells is covered with well-developed cilia and in some places instead of the cilia, microvilli.

Accumulation of neutrophils, mastocytes, eosinophils, lymphocytes, and mononuclear cells is characteristic in this layer. This region is significantly denervated.

### 1.7.2. Basal membrane

The stroma is separated by basal membrane from the epithelium. Dislike in normal mucosa, this basal membrane may contain cells, and is characterised by a loose net of collagen fibres. This oedematous layer differs from the cell-free, fine collagen-fibre net of the normal mucosa.

### 1.7.3. Stroma

Massive oedema fluid and pseudo-cyst formation is observed in the deeper layers[8] that can be explained by the open endothelial junctions of the venules. The polyp tissue is less vascular than normal mucosa, and the capillaries are often located near to the superficial epithelium, and next to the glands. Their diameter is smaller and there are less of them comparing to normal mucosa. The stroma is significantly denervated. The accumulation of inflammatory cells is very characteristic, eosinophils are dominant, but we can find also neutrophils, plasma cells, mastocytes, macrophages and lymphocytes. The number of mastocytes is double compared to the normal mucosa[9]. Most of the eosinophils are activated with prolonged survival[10,11] and the mast cells degranulated[12]. Plasma cells are present in groups, sometimes surrounded by basal membrane elements. In their nucleus vacuoles, in their cytoplasm crystalloids can be observed. Proliferation of connective tissue and epithelial cells[13], basal membrane thickening[14], fibrosis[15], deposition of fibronectin and albumin [7,16,17] are also described in the literature. (Fig. 3.)

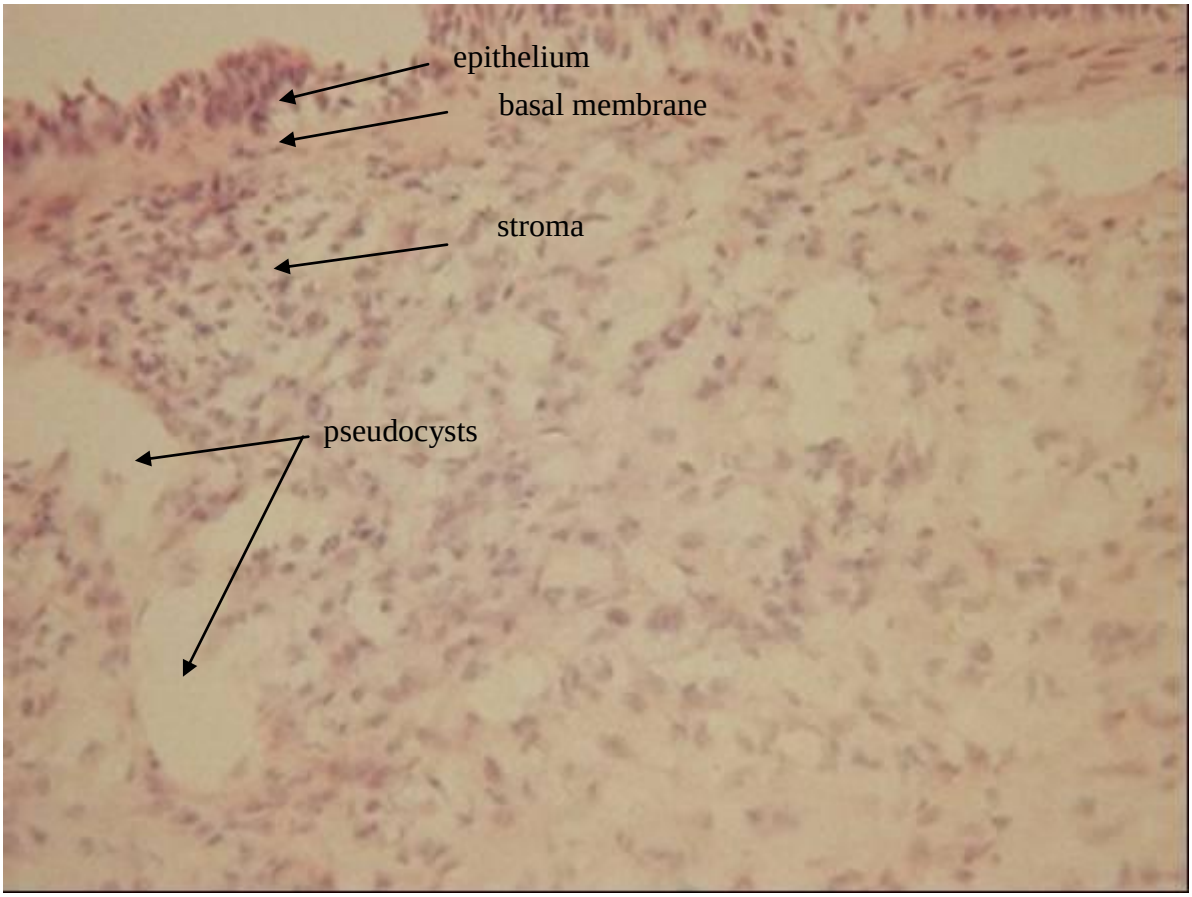


*Fig. 3. Nasal polyp tissue with haematoxylin-eosine staining – magnification x* 40

## 1.8. Mediators of interest in the pathogenesis of nasal polyposis

Nasal polyposis is a multi-factorial disease, of which the exact pathogenesis has not been discovered yet. It is the result of a chronic inflammation of the mucosa. 2/3 of the polyps are characterised by eosinophil accumulation. Many cytokines (IL-4, IL-6, IL-8, TGF-beta1, GM-CSF etc.), chemokines (eotaxin, RANTES) adhesion molecules (E- and P-Selectin, VCAM-1) and other mediators (IgE, histamine) have been described to be involved in the formation of polyps.

### 1.8.1. Immunoglobulin E and allergy

IgE is a 190 kDa antibody produced by plasma cells. It has the usual heterodimer structure of immunoglobulins, containing 2 heavy and 2 light chains. Its free level in healthy human blood in very low, because the majority of the formed molecules are attached to cell surfaces. Its function is the activation of mast cells, which causes the release of different mediators. It plays an important role in the defence against parasites, and has a key role in allergic reactions.[18]

The role of allergy as a pathogenetic factor in nasal polyposis was mentioned first in the beginning of the 1930s.[19] Later other authors has questioned this theory, saying, that they found more patients with nasal polyp from the group of non-atopics, than from the atopic group[2,] and showing, that there was less person, who has a multiple positive Prick-test from the patients with nasal polyp, than in normal healthy population.[4] Even though the 1/3 of patients with eosinophil polyp has allergic rhinitis, allergy does not seem to be a causative factor in the development of this disease. [20,21, 22]

However, the tissue IgE concentration, independent from the result of Prick-test, seems to be higher in patients with nasal polyposis, and this suggests the possibility of the local formation of IgE. [23]

Recent studies have also demonstrated a strong local upregulation of the immunoglobulin E (IgE) synthesis with the formation of specific IgE to Staphylococcus aureus enterotoxins, suggesting a possible role of superantigens in these pathologic processes.[24]

However, the role of IgE in the pathogenesis of nasal polyposis is not clear yet.

### 1.8.2. IL-5, Eosinophil Cationic Protein and eosinophilia

IL-5 is unusual among the T-cell-produced cytokines in being a disulphide-linked homodimeric glycoprotein. It is highly homologous between species, as indicated by the high sequence homology between mouse and human IL-5 and the cross-reactivity of the protein across a variety of mammalian species. Mature human IL-5 monomer comprises 115 amino acids (molecular weight [M,] of 12,000 and 24,000 for the dimer). The secreted material has an M, of 40,000 to 45,000, and thus nearly half the native material consists of carbohydrate, although this carbohydrate does not appear to be necessary for biologic activity in vitro.[25] Studies with mouse IL-5 indicate that the monomer has no biologic activity and has no inhibitory activity, suggesting that they do not form high-affinity interactions with the IL-5 receptor (IL-5R).[26] The dimer exists in an antiparallel (head to tail) configuration.[27]

IL-5 had been defined as a cytokine with specific action in the development, priming and survival of eosinophils.[28] IL-5 is the major, and possibly the only, cytokine involved in the production of specific eosinophilia. Experiments carried out in vivo clearly indicate the central role of IL-5 in eosinophilia. Classically eosinophilia is observed in a restricted number of diseases, most notably helminth infections and allergic diseases. However, although the increase in eosinophil numbers is less spectacular, there is increasing evidence for an involvement of eosinophils in a wider spectrum of diseases.

Since nasal polyposis is characterised by eosinophil accumulation, it seems to be reasonable to investigate the role of IL-5 in nasal polyposis. It has already been demonstrated, that IL-5 is upregulated in polyp tissue.[10,17,29,30]

Eosinophil cationic protein (ECP) is a low molecular-weight polypeptide (21000) rich in basic amino acids produced in eosinophils. For this reason it is a very suitable protein to detect the presence of eosinophils in different tissues. ECP is strongly implicated in the pathophysiology of upper airway inflammation observed in several conditions including asthma and allergy. It has cytolytic properties targeted against parasites and bacteria. It also activates haemostasis by neutralizing heparin and activating fibrinolysis. ECP is also neurotoxic.[31]

### 1.8.3. TGF-beta1

Transforming growth factor (TGF-beta), a generic name that refers to five structurally related growth factors, is unusual among the known cytokines as it is secreted as a latent complex and is found primarily in a latent form in vivo. [32]

TGF-beta1 is a 25-28 kDa homodimer connected by disulphide bindings.[18] TGF-beta1 is produced by lymphocytes, eosinophils[25,33], macrophages[34], fibroblasts and platelets[35]. The effects are pleiotrop, these factors regulate cell migration, adhesion, multiplication, differentiation and death in the life span of the organism. Many of these responses result from changes in the expression of key target genes. TGF-beta family members are multifunctional mediators, the nature of their effects depending on what has called the "cellular context".[36] It has a negative effect on the functions of the immune system, inhibits the lymphokine production and T-cell proliferation, the antibody production and B-cell proliferation, some functions of the monocytes, the endotoxic functions of natural killer (NK) cells, and the proliferation of early haemopoetic progenitor cells. It also has a basic role in tissue regeneration, and in fibrosis formation.[18]

Latency is one mechanism to control growth factor activity; it prevents the cytokine from eliciting a response until conversion to the active form and may also allow the cytokine to circulate and reach its target cell. Latency regulates TGF-beta bioavailability and may limit its diffusion from the secreting cell, thus modulating the autocrine and paracrine actions of the growth factor.[37] Latent TGF-beta1 is consisted of the matured dimer TGF-beta1 and LAP (Latency-associated Protein), a dimer proteolytic production fragment. This interaction is disrupted in vitro with heat and acidic pH in order to activate latent TGF-beta1. Latent TGF-beta1 binding protein (LTBP) is associated through covalent binding with LAP during secretion. (Fig. 4.)[32] The LTBP participates in the regulation of latent TGF-beta1 bioavailability by addressing it to the extracellular matrix (ECM).[38] Enzymatic processes by means of glycosidases, proteases (plasmin) and thrombospondin-1 (TSP-1) have also been reported to release active form from the latent complex. TGF-beta1 activity is then achieved in two consecutive proteolytic steps: first, releasing the latent complex from the ECM and second, cleaving LAP to allow the active form to attach to the cell surface. It is suggested, that plasmin and other proteases are involved in these

mechanisms, though other activation systems have also been described in less detail.[24] Latent TGF-beta activation can be induced independently of transcription.[39]

The relation of latent and active TGF-beta1 and its role in polyp growth and transdifferentiation might also be a particular interest.[40,41] Accumulation and activation of eosinophils is favoured by low TGF-beta1 concentrations and overproduction of IL-5 and eotaxin.[6]

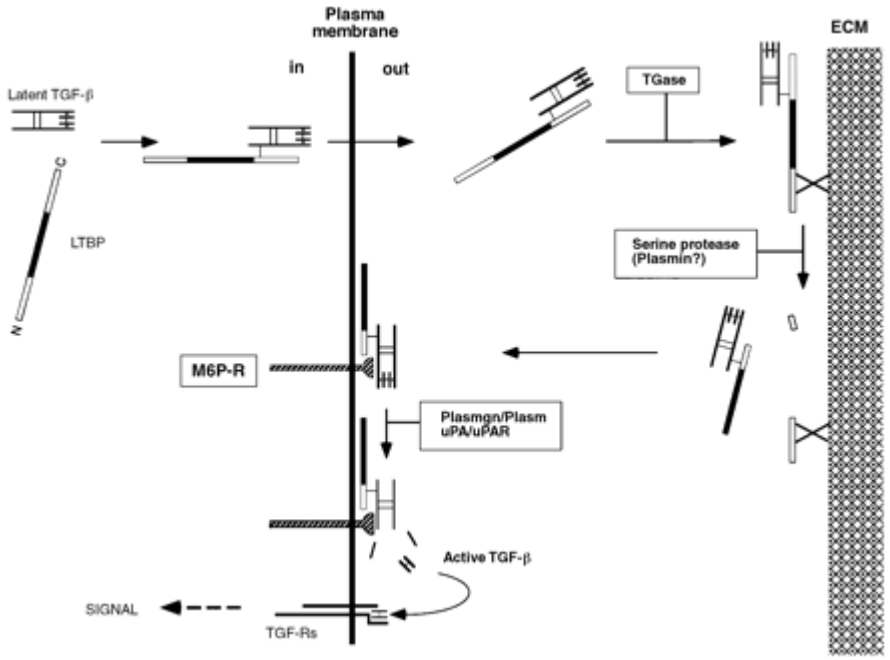


***Fig.4. A model for latent TGF-beta activation.****

*ECM: extracellular matrix protein; M6P-R: mannose 6-phosphate/IGF-II receptor; Plasm: plasmin; Plasmgn: plasminogen; uPA: urokinase type plasminogen activator; uPAR: urokinase type plasminogen activator receptor; TGase: transglutaminase; TGF-Rs: TGF-beta serine/threonine kinase receptors.*

### 1.8.4. Histamine metabolism

Histamine is considered as one of the most important immunomodulator molecules owing to being a mediator of allergy and inflammation processes. Moreover,

* Gleizes P-E, Munger JS, Nunes I, Harpel JG, Mazzieri R, Noguera I, Rifkin DB. TGF-ß Latency: Biological Significance and Mechanisms of Activation. Stem Cells 1997; 15: 190-197

histamine is a chemical messenger and aminergic neurotransmitter, playing an important role in a multitude of physiological processes in the central nervous system and peripheral tissues. Histamine is synthesized in a restricted population of neurons located in the tuberomammillary nucleus of the posterior hypothalamus implicated in many brain functions (e.g., sleep/wakefulness, hormonal secretion, cardiovascular control, thermoregulation, food intake, and memory formation). In peripheral tissues, histamine is stored in mast cells, basophils and enterochromaffin cells. Mast cell histamine plays an important role in the pathogenesis of various allergic conditions, e.g., histamine release leads to various well-known symptoms of allergic conditions in the skin and the airway system. Histamine can influence the tumor cell proliferation and promotion directly as a growth factor, e.g., in colon tumor, gastric tumor and melanoma, and indirectly via the modification of, e.g., cytokine production in the tumor and in the tumor-surrounding environment.[42]

Histamine is formed from L-histidine by histidine-decarboxylase enzyme (HDC) and it is degradated by two enzymes, the diamino-oxidase (DAO) and the histamine-N-metyl transferase (HNMT). In the nasal mucosa HNMT seems to be responsible for the degradation.[43] (Fig. 5.)

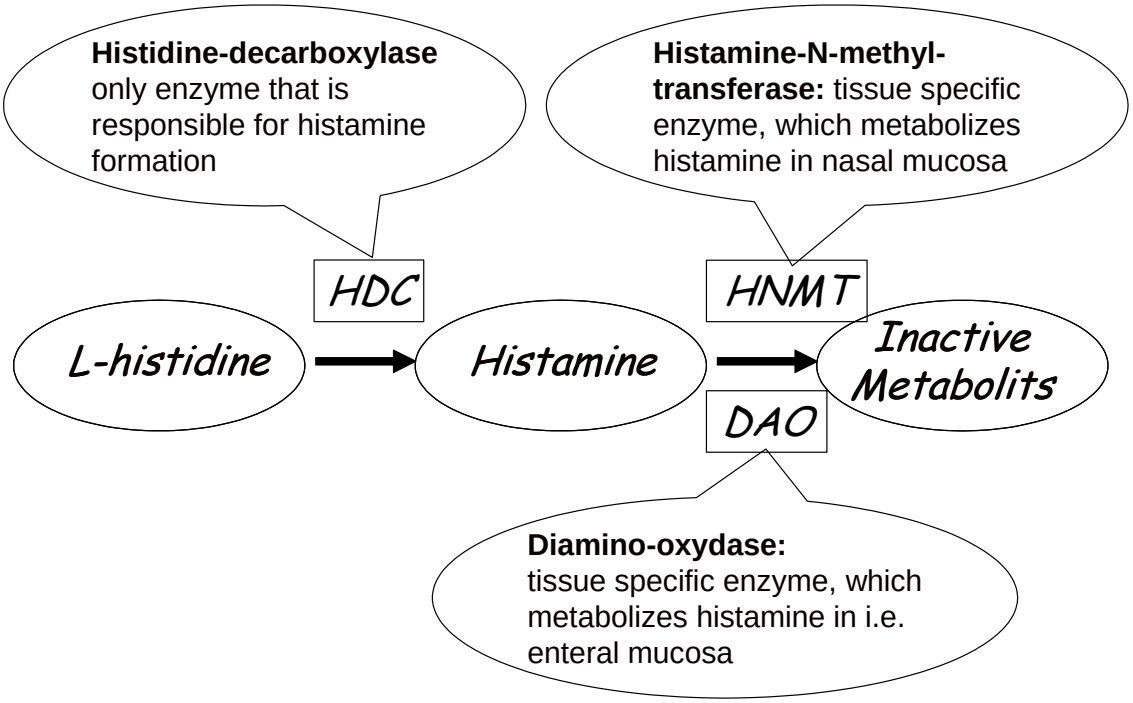


*Fig. 5. Histamine metabolism*

Histamine induces IL-5 production in Th2 cells through H2 receptors.[44,45,46,47] Little is known about the presence and the role of histamine in human nasal mucosa and polyp formation.[48,49]

### 1.8.5. Histamine receptors

The effects of histamine are mediated through four pharmacologically distinct subtypes of receptors, i.e., the H1, H2, H3, and H4 receptors, which are all members of the G-protein coupled receptor (GPCR) family. Histamine receptors display seven transmembrane (TM) domains, which are predicted to form helices that span the cell membrane, an extracellular N-terminus and a cytoplasmic C-terminus of variable length. The third and fifth TM domains of the receptors appear to be responsible for ligand binding, while the third intracellular loop is responsible for signaling pathway connection. Interestingly, the genes encoding the H1, H2, and H3 receptors share less

protein sequence identity with each other than with other biogenic amine receptor family members, e.g., with M2 muscarinergic receptor. Their overall homology is low (average 38%), suggesting that these histamine receptors evolved from different ancestor sequences.[50] Although lacking significant overall sequence homology, the histamine receptors apparently acquired crucial elements for the recognition of histamine during their evolution, e.g., in their ligand binding region.[42]

#### 1.8.5.1. H1 Receptor

Human H1 receptor (H1R) is composed of 487 amino acids and showed ~75–85% interspecies homology.

#### 1.8.5.2. The H1 Receptor Signaling Pathway

H1 receptor preferentially couples to the Gq/11 family of G-proteins and causes mobilization of intracellular Ca2+ in a pertussis toxin (PTX)-insensitive fashion. Histamine activates phospholipase C (PLC), which mediates the cleavage of the membrane phosphatidyl inositol diphosphate (PIP2), which results in formation of inositol triphosphate (IP3) and 1,2-diacyl glycerol (DAG). IP3 in turn mediates Ca2+ release from endoplasmic reticulum, and also increases Ca2+ influx from the extracellular space as a secondary but longer lasting event. This latter effect can be inhibited by nifedipine, suggesting the involvement of an L-type voltage-dependent Ca2+ ion channel. As a consequence of Ca2+ ion influx, a secondary breakdown of membrane phosphoinositides occurs. IP3 can be phosphorylated to produce IP4 which further increases the intracellular Ca2+ level. DAG activates a serine/threonine kinase, the protein kinase C (PKC) that can phosphorylate and activate other effector proteins in the cells. Additional secondary signaling pathways can be induced by the increased intracellular Ca2+ level and DAG. Via calcium/calmodulin (Ca2+/CAM)-dependent enzyme, nitric oxide (NO) activity is stimulated to produce an elevated NO level, which results in the activation. [42]

#### 1.8.5.3. Some Effects of Histamine Through H1 Receptor

As mentioned above, H1 receptor was the first membrane protein to be discovered as a mediator of histamine action. Its main activities include smooth muscle contraction and vascular permeability. It is the chief histamine receptor subtype involved in acute inflammatory and allergic disorders. H1 receptors have been found in

a wide variety of tissues including mammalian brain, airway smooth muscle, the gastrointestinal tract, genitourinary system, cardiovascular system, adrenal medulla, endothelial and various immune cells. Enhanced expression of H1R was observed in the nasal mucosa of patients with allergic rhinitis, in cultured aortic intimal smooth muscle cells of patients suffering from atherosclerosis, in the inflamed joints of rheumatoid arthritis patients, etc.

H1 receptor agonists do not appear to have any therapeutic potential according to our recent knowledge, but they are useful tools in research regarding the H1 receptor function in different experimental conditions. As for H1R antagonists, classical antihistamines included compounds such as mepyramine (also called pyrilamine) and triprolidine. They are highly potent H1 antagonists, and easily penetrate the brain, causing sedation. Many new so-called second-generation antihistamines are non-sedating H1 antagonists (e.g., cetirizine, astemizole and loratadine), and they are used to treat allergic conditions. [42]

#### 1.8.5.4. The H2 Receptor

The sole existence of the H1 receptor could not explain the effects of histamine on cardiac muscle and gastric acid secretion; therefore a second subtype was proposed, termed the H2 receptor. It is involved in a wide array of physiological histamine actions, including the relaxation of airway and vascular smooth muscle, regulation of right atrial and ventricular muscle of the heart, inhibition of basophil chemotactic responsiveness, various actions on immune cells and inhibition of prostaglandin E2-stimulated duodenal epithelial bicarbonate secretion. Its principal action from a clinical point of view is related to its role in the inhibition of gastric acid secretion.

Human H2 receptor protein contains 359 amino-acid residues, and its ligand binding site appears to be similar to the corresponding region of H1R. The most notable difference between the two receptors is the comparatively much shorter third intracellular loop and the longer C-terminus in the H2 receptor sequence.[51]

#### 1.8.5.5. The H2 Receptor Signaling Pathway

Histamine H2 receptors couple to adenyl cyclase via the Gs protein, and histamine stimulates cAMP production in many different cell types, e.g., the CNS and CNS-derived cells, gastric mucosa, cardiac myocytes, fat cells, vascular smooth cells, basophils and neutrophils.[51] Elevated cAMP concentration activates protein kinase A

(PKA), which is the downstream effector kinase of this pathway, phosphorylating a wide variety of proteins in the cells mentioned above. However, the H2R signaling pathway shows a dual face. In addition to the adenyl cyclase-mediated one, histamine through H2 receptor can increase the intracellular Ca2+ ion level in some cell types, e.g., gastric parietal cells and HL-60 leukaemic cell line. This action of histamine seems to be a direct effect mediated by another Gq member of the PTX-sensitive G-protein family. In contrast to other receptors that stimulate the dual signaling pathway, H2 receptor activates each pathway directly and the required histamine concentration for the stimulation of both systems is identical.

#### 1.8.5.6. Some Effects of Histamine Through H2 Receptor

Dual coupling of H2 receptors to Gs and Gq was demonstrated for the first time in cardiac myocytes. It represents a novel mechanism to augment positive inotropic effects by activating two different signaling pathways via one type of histamine receptor. Activation of the Gs-cAMP-PKA pathway promotes Ca2+ influx through phosphorylation of L-type Ca2+ channels. Simultaneous activation of Gq-signaling pathways might result in phosphoinositide turnover and Ca2+ release from intracellular stores, thereby augmenting H2-induced increases in the intracellular Ca2+ ion.[52]

H2 receptor agonists have been proven to be effective for acid peptic disorders of the gastrointestinal (GI) tract. Among H2 receptor antagonists, cimetidine was the first to be clinically used for the treatment of gastric and duodenal ulcers. Besides cimetidine, tiotidin and ranitidine are potent and more selective H2 receptor antagonists. Both compounds inhibit gastric acid secretion, but only ranitidine possesses therapeutic potential in gastric ulcer treatment because of the severe side effects of tiotidine.

Evidence has accumulated indicating that histamine exerts a variety of immunoregulatory actions. It inhibits CD4+ T cells and CD8+ T cells to produce histamine when activated with ConA.[53] Furthermore, histamine regulates cytokine production by T cells and macrophages.[54] Histamine affects the balance of cytokines from T helper type 1 (Th1) cells and T helper type 2 (Th2) cells by shifting cytokine production from a Th1 to a Th2 pattern.[55] It is known that in certain circumstances, stress mediators, as well as histamine and adenosine, can be increased, and due to histamine effects the upregulated Th2 cytokines may also play a role in induction and progression of certain processes, e.g., in allergic/atopic reactions and in tumor growth.

Modulation of IL-12 and IL-10 secretion by histamine involved the H2 and H3 receptors of antigen presenting cells. IL-10 production was enhanced by histamine through either an H1 receptor- or an H2 receptor-dependent fashion.[56] In addition, histamine stimulates IL-10 production in Th2 cells which was reversed by either H1 receptor or H2 receptor antagonists, and the release of IFN-$\gamma$ and IL-13 was enhanced by histamine in both H1 receptor- and H2receptor-deficient spleen cells.[54] Inhibition of IFN-$\gamma$ synthesis by histamine was reversed by either cimetidine, an H2R antagonist, or pyrilamine, an H1R antagonist.[57]

#### 1.8.5.7. H3 receptor

As for H1 and H2 receptors, H3 receptor was initially identified on a pharmacological basis. The first report of the H3 receptor came from Arrang et al. who found a new histamine receptor that acted as an autoreceptor which mediated histamine release from neurons.[58] They identified a sequence that encoded a 445-amino acid coding region with low homology to other biogenic amine receptors, which may have allowed it to elude discovery for so long. However, it did have an aspartic acid residue in transmembrane domain 3 which is a conserved residue for receptors that bind to primary amines. The H3 receptor binds to histamine with a high affinity (Kd ~5 nM), which is consistent with its role as an autoreceptor. Most agonists of the receptor are imidazole derivatives related to histamine. Much more work has been done in developing H3 receptor antagonists, since they appear to have more therapeutic utility. The early antagonists were also imidazoles like the agonists. Recently, there has been much work to develop non-imidazole antagonists. The piperazine amides have been shown to be efficacious in mouse obesity and dipsogenia models.[59,60] The expression of the H3 receptor based on mRNA appears to be mainly restricted to the central nervous system. The human receptor mRNA has been found in human brain samples, with the highest expression being in the caudate, cortex and thalamus and little expression seen in peripheral tissues.[61]

#### 1.8.5.8. The H3 receptor Signaling Pathway

There had been some studies indicating that the receptor is coupled to Gi/o G-proteins.[61,62,63,64] The receptor can also signal via increases in intracellular calcium.[65]. Furthermore, it has been shown that activation of rat H3 receptor can increase p44/p42 MAP kinase phosphorylation and arachidonic acid release in transfected cells.[63,64] As

for the cAMP response, both of these responses are PTX-sensitive. Finally, there is some evidence in transfected systems that the H3 receptor can inhibit the activity of Na+/H+ exchangers.[66] One interesting aspect of H3 receptor signaling is that it appears to possess a high degree of constitutive activity; that is, activity in the absence of agonists. This opens up the possibility that H3 receptor ligands that were initially characterized as antagonists may in fact be inverse agonists, i.e., they inhibit the constitutive activity of the receptor.

#### 1.8.5.9. Some Effects of Histamine Through H3 Receptors

The H3 receptor was initially identified as an autoreceptor that mediated histamine release from neurons.[58] In histaminergic neurons, the H3 receptor controls both the release and the synthesis of histamine.[67] The H3 receptor has also been shown to act as a heteroreceptor where it affects the release of other neurotransmitters. Activation of the H3 receptor has been shown to inhibit the release of acetylcholine, glutamate, noradrenaline, orepinephrine, dopamine, serotonin, substance P, and GABA. This effect on neurotransmitter release has several physiological consequences. The H3 receptor has been linked to changes in sleep/wake cycle, memory and learning. H3R has also been implicated in food intake.

#### 1.8.5.10. H4 Receptor

Cloning of the H3 receptor led directly to identification of the histamine H4 receptor.[68,69] The primary amino acid sequence of the H4 receptor clearly identifies it as a member of the GPCR family. The 390 amino acid protein is predicted to have seven transmembrane regions, DRY sequence at the end of transmembrane helix 3 and an aspartic acid residue in the second transmembrane domain, which indicate that it is a member of the biogenic amine GPCR family. The aspartic acid residue at position 94 in transmembrane helix 3, which is conserved among the other histamine receptors, is crucial for the binding of histamine.[70] The H4 receptor has highest sequence homology to the H3 receptor, but its homology to H1 and H2 receptors is actually lower than that to other GPCRs.[71] Like the H3 receptor, the H4 receptor has a high affinity for histamine, with Kd values of approximately 8 nM, which is not surprising given that the sequence homology in the transmembrane region is 58% between the two receptors. Like the H3 receptor, the H4 receptor has a restricted expression pattern and seems to be limited to cells of the hematopoietic lineage.[72,73,69,50] There are hints of

expression on many hematopoietic cell types, but it is clearly expressed on eosinophils, mast cells, basophils, dendritic cells, and T cells.[69,74,75] While this expression pattern differs from that of the H3 receptor, there is significant overlap with H1 and H2 receptor, whose expression has also been reported on these cell types. However, expression of H4 appears to be restricted to these hematopoietic cells, whereas the H1 and H2 receptors are more widely expressed. Eosinophils, mast cells, basophils, dendritic cells and T cells and all major players in the inflammatory response and presence of the H4 receptor on these cell types suggest that it plays a role in inflammation.

#### 1.8.5.11. The H4 receptor Signaling Pathway

In primary cells, H4 receptor signaling is mediated by increases in intracellular calcium levels. Raible et al. showed that histamine can induce calcium mobilization in human eosinophils and that this effect is not mediated by H1,H2, or H3 receptors, implying a role for the H4 receptor.[76,77] In mouse primary mast cells, histamine induces calcium mobilization from intracellular stores which can be blocked by H4 receptor antagonists but not by antagonists for other histamine receptors.[78]

Thus, in primary cells, activation of H4R leads to increases in intracellular calcium, which in mast cells is mediated by G.i/o proteins and phospholipase C.

#### 1.8.5.12. Some Effects of Histamine Through H4 Receptors

The biological consequences of H4R activation are also still being elucidated. Histamine can induce eosinophil chemotaxis, which can be blocked by thioperamide and the H4 receptor-specific antagonist JNJ 7777120.[79,80,81,82,83] In addition, the chemotactic responses of eosinophils to other chemokines such as eotaxin can be enhanced by activation of the H4 receptor.[84,80] Chemotaxis requires the polymerization of G-actin to F-actin and histamine has been shown to induce actin polymerization in eosinophils. This effect can be blocked by thioperamide, suggesting that it is mediated by the H4 receptor.[84] Adhesion molecules on the surface of eosinophils such as CD11b/CD18 (Mac-1) and CD54 (ICAM-1) can be upregulated by histamine.[80,84] This effect can be blocked by H4 receptor antagonists, implying that the H4 receptor is involved.[80,84] Thus, in eosinophils, the H4 receptor mediates many of the events important for localization of eosinophils to sites of inflammation, like chemotaxis and adhesion molecule upregulation. As for eosinophils, the H4 receptor mediates

histamine-induced chemotaxis in mast cells. Histamine-induced migration of mast cells can also be observed in vivo.[85] (Fig. 6.) As seen with histamine inhalation, it is known that there is a redistribution of mast cells to the epithelial lining of the nasal mucosa in response to antigens.[86,87] In addition, mast cell numbers increase at sites of inflammation in a number of different pathophysiological conditions such as asthma, rhinitis, rheumatoid arthritis, psoriasis, and cardiovascular disease.[88,89,90,91,92,93,94,95,96] Histamine-induced chemotaxis via the H4 receptor may play a role in the local migration of mast cells to sites of inflammation. The H4 receptor appears to be expressed on dendritic cells, as well as both CD4+ and CD8+ T cells, but little is known about its function in these cells. In T cells, the H4 receptor may control cytokine release.

#### 1.8.5.13. Uses of H4 Receptor Antagonists

While no H4 antagonists are currently used in the clinic, the current literature suggests several possible indications. Histamine is a major mediator in allergic rhinitis where H1 receptor antagonists are useful but not completely effective. One indication that H4 antagonists may have a therapeutic use in this condition comes from the fact that neither H1 nor H2 antagonists can inhibit histamine-induced congestion.[97] Furthermore, the major role that mast cells play in allergic rhinitis, coupled with the expression of H4 on these cells, may also suggest that H4 receptor antagonist will be useful for treating this condition.

Another aspect where the H4 receptor may be of benefit is in the allergic hyper-responsiveness developed upon repeated exposure to antigens which is characterized by increased sensitivity to allergens. An increase in the number of mast cells and other inflammatory cells in the nasal mucosa is thought to be responsible for this response. Therefore, if H4 receptor antagonists can inhibit mast cell and eosinophil accumulation at sites of inflammation, they may be effective in reducing allergic hyper-responsiveness.

The expression and function of the H4 receptor in mast cells, eosinophils, dendritic cells and T cells open up the possibility of use in a number of therapeutic areas. These cell types play a major role in the etiology of asthma. Histamine is known to be released in asthma; however, H1 receptor antagonists have only modest effects[98,99,100] leaving open the possibility that H4 receptor antagonists may be useful.

Autoimmune diseases, such as rheumatoid arthritis, multiple sclerosis, type I diabetes and systemic lupus erythematosus, are other are as that deserve further study in order to elucidate the role of the H4 receptor, since these conditions are driven by dendritic cells and T cells.

Finally, itching associated with conditions like atopic dermatitis and urticaria is also thought to be partially mediated by histamine[101], and although H1 antagonists are useful in the treatment of urticaria, they are ineffective in other conditions like atopic dermatitis.[102]

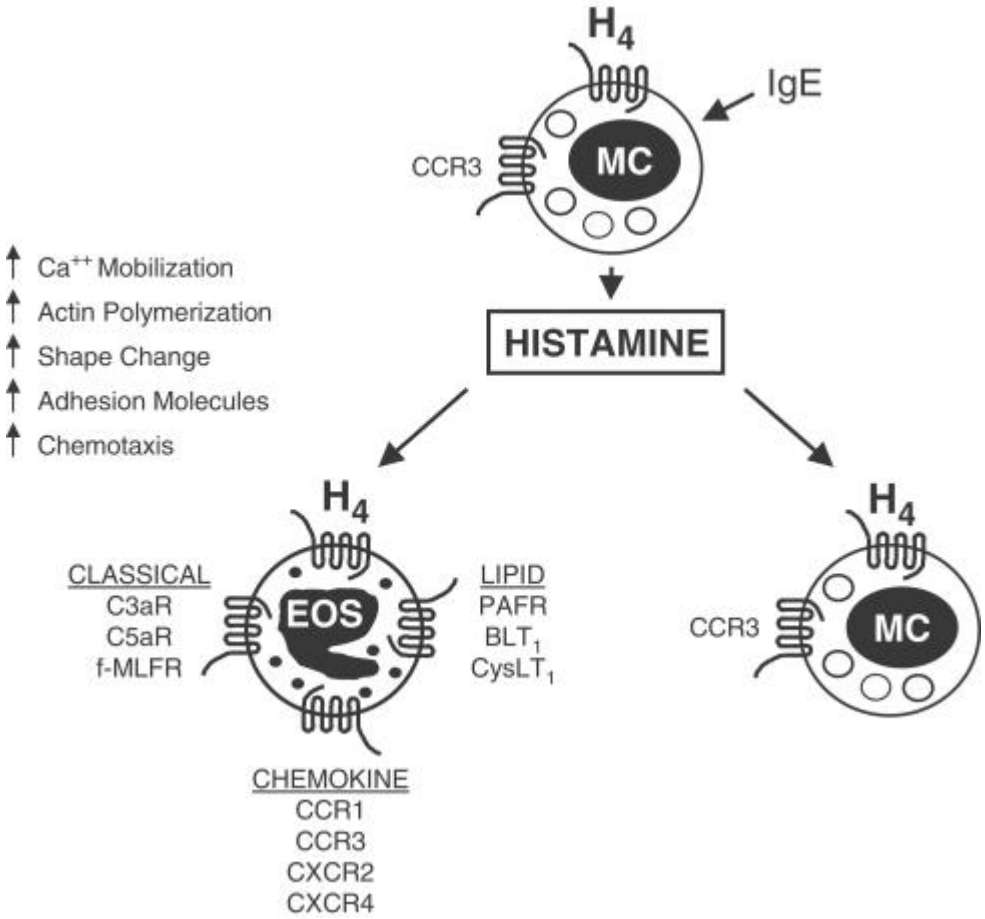


*Fig. 6. Mechanism of histamine-induced recruitment of eosinophils and mast cells*[*]
*Abbreviations: EOS, eosinophil; MC, mast cell; IgE, immunoglobulin E; $H_4$, histamine receptor type 4; PAFR, platelet activating factor receptor; $BLT_1$, leukotriene $B_4$ receptor type 1; $CysLT_1$, leukotriene $D_4$ receptor type 1; CCR1, C-C chemokine*

[*] Bruce L. Daugherty. Histamine H4 antagonism: a therapy for chronic allergy? Br J Pharmacol. 2004; 142: 5-7

*receptor type 1; CCR3, C-C chemokine receptor type 3; CXCR2, C-X-C chemokine receptor type 2; CXCR4, C-X-C chemokine receptor type 4; C3aR, complement 3a receptor; C5aR, complement 5a receptor; f-MLFR, N-formyl-methionyl-leucyl-phenylalanine receptor.*

### 1.8.6. Other theories for polyp formation

Enterotoxins derived from Staphylococcus aureus have been implicated recently by Bachert et al. in the pathophysiology of nasal polyps as disease-modifying factors; recent findings using therapeutic proof-of-concept approaches support this hypothesis. The cytokine environment is decisive regarding the impact of Staphylococcus aureus derived enterotoxins, which function as superantigens. Staphylococcus aureus enterotoxin B further shifts the cytokine pattern in nasal polyps toward T-helper-2 cytokines (increases greater than twofold for interleukin-2, interleukin-4 and interleukin-5), but it disfavours the T-regulatory cytokines interleukin-10 and transforming growth factor-beta1. Furthermore, Staphylococcus aureus derived enterotoxins influence local immunoglobulin synthesis and induce polyclonal immunoglobulin E production, which may contribute to severe inflammation via activation of mast cells.[103,7]

The role of viral[104] or bacterial infection[105] and/or allergy is still debated, but seems to be secondary, though it might activate mucosal defence mechanisms.

The allergic fungal inflammation has recently new insights on the pathogenesis of nasal polyposis.[106]

# 2. AIMS

Considering the fact, that nasal polyposis is characterised by chronic eosinophil inflammation it seemed to be reasonable to investigate IL-5 in the pathogenesis of nasal polyposis.

The histological picture of nasal polyps shows fibrosis, that assumes the presence of TGF-beta1 in the pathogenesis.

The theories analysing the role of allergy in the pathomechanism are not unique, for this reason we wanted to take a closer look at the serum and tissue IgE.

Therefore we decided to investigate the presence of the above mentioned cytokines and to localize them to relevant cells and structures in the polyp tissue. The special activation mechanisms of TGF-beta1 have also been considered.

One important mediator of IL-5 release may be histamine, as it is well described in other tissues. For this reason we also wanted to investigate the histamine metabolism in the pathomechanism of nasal polyposis. We measured HDC content, HNMT activity and histamine content in normal and polyp tissues.

We also wanted to determine, which histamine receptors play the most important role in mediating the function of histamine in nasal polyp tissue and to obtain additional data on the connection between that and eosinophil accumulation. To detect the presence of eosinophils we used the measurement of ECP.

Evaluation of these results might lead to a better understanding of the formation of nasal polyposis.

# 3. MATERIALS AND METHODS

## 3.1. Materials

### 3.1.1. IL-5, IgE, TGF-beta1

34 nasal polyp samples - removed during routine functional endoscopic sinus surgery (FESS) - were randomly selected from patients with chronic sinusitis. (Fig. 7.)

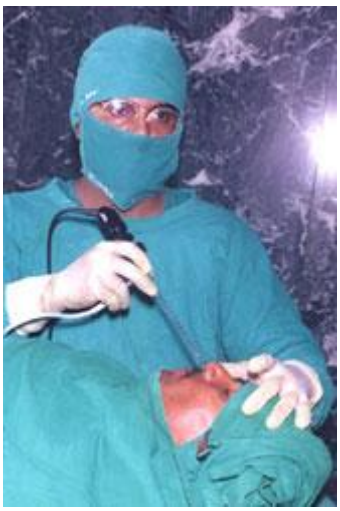

*Fig. 7. FESS (functional endoscopic sinus surgery)*[*]

Normal healthy nasal mucosa (n=9) was harvested from enlarged inferior turbinates in the process of routine septal operations, where mucosal reduction was planned preoperatively to improve nasal breathing (allergy and other diseases, which might affect nasal mucosa, were excluded). Atopic patients (n=16/34) were identified by means of skin prick test, history and serum IgE detection. (Fig. 8.) Biopsy specimens were stored at -20°C until used.

[*] http://www.kkrenthospital.org/images/img_endosinus.jpg

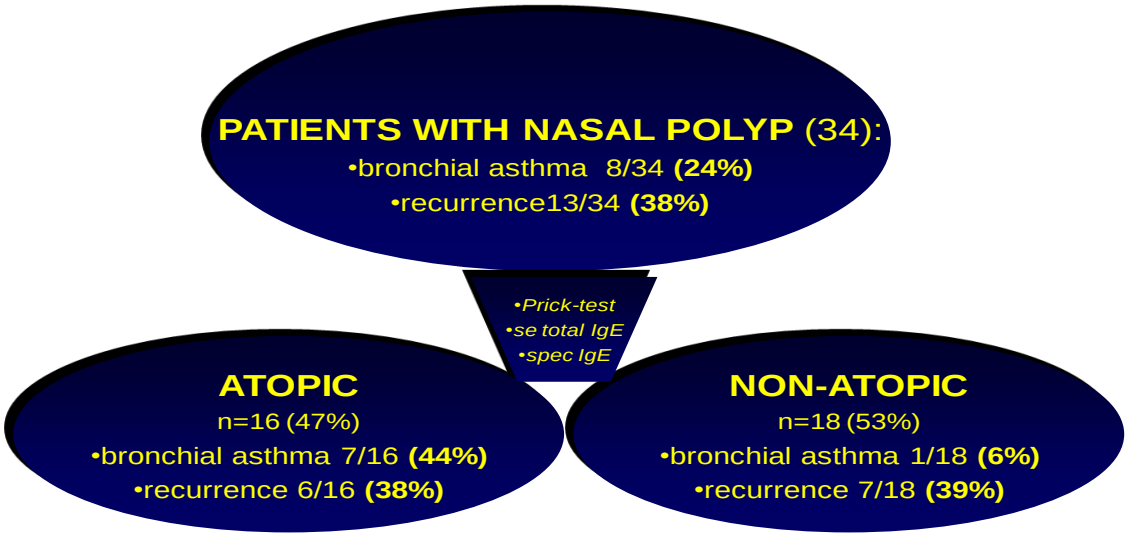


*Fig. 8. Clinical data*

### 3.1.2. HDC, HNMT

15 human nasal polyp samples (NP) - removed during routine functional endoscopic sinus surgery - and 11 normal healthy nasal mucosa harvested from enlarged inferior turbinates in the process of routine septal operations were used. Biopsy materials were stored at -80°C until used.

### 3.1.3. Histamine receptors

Eleven nasal polyp samples - removed during routine endoscopic sinus surgery - were taken from patients with chronic rhinosinusitis. Normal healthy mucosa (n=9) was harvested from enlarged inferior turbinates in the process of routine septal operations, in which mucosal reduction was planned to improve nasal breathing. The patients had neither allergy, nor any other rhinological disease. Biopsy materials were stored at -20ºC until used.

The studies were based in informed consent and ethical approval.

## 3.2. Methods

### 3.2.1. IgE, IL-5 and TGF-beta1 ELISA measurements

Biopsy materials were weighed, chopped into pieces of 1 $mm^3$, and homogenized in 0.9% sodium chloride solution on ice, 1 ml solution was added to 100 mg tissue. Suspensions were centrifuged at 4°C at 3000 rpm for 10 minutes, and supernatants were stored in aliquots at -20°C until used. Local IL-5 (n=22), TGF-beta1 (n=27) and IgE (n=13) concentrations were measured with ELISA technique in homogenised polyp tissue and in control mucosa (n=8, 7, 5) samples. The same technique was used to measure serum IgE level (n=13).

IL-5 and TGF-beta1 cytokine concentrations were measured by using commercially available solid phase sandwich ELISA kit (OptEIA$^{TM}$ human IL-5 kit; BD PharMingen, USA), and set (OptEIA$^{TM}$ human TGF-beta1 set; BD PharMingen, USA) TGF-beta1 activation was achieved by means of acidic incubation (1:25x 1N HCL, 60 min; 4°C) and with heating (10 min; 80 °C) before and after homogenisation (polyp n=4; control n=4).[32] Immunogolbulin E was measured by means of Sevatest ELISA IgE Micro II (Sevapharma, Czech Republic).

In every case two parallels of each sample were examined.

### 3.2.2. Histology and IL-5, TGF-beta1 immunohistochemistry

Five frozen tissue specimens (3 polyps and 2 controls) were processed into cryomatrix (Shandon, UK), serially sectioned by cryomicrotome (Jung CM 1500; Leica, Germany) at 4 μm thickness and stored at -20°C until used. One section of each sample was stained with haematoxylin and eosin for histomorphological analysis, and another with Pappenheim's staining (using May-Grünwald-, and Giemsa-solution at 35°C) to detect eosinophils. For immunohistochemical investigation one section of each sample was incubated with anti-human IL-5 monoclonal antibody (rat) in 1:50 working dilution, - 10 μg/ml of final concentration in PBS 1% for BSA (BD PharMingen, USA; clone: TRFK5, concentration: 0,5 mg/ml) -, and another was incubated with anti-human TGF-beta1 polyclonal antibody (rabbit) in 1:50 working dilution, - 20 μg/ml of final concentration in PBS 1% for BSA (Promega, USA;

concentration: 1 mg/ml) - as primary antibodies. It was stained with biotin-streptavidin-peroxidase system (Universal Immunostaining Kit; Immunotech, France), in which biotinylated polyvalent antibody was used as secondary antibody. As positive control one section was incubated with kappa/lambda antibody (Universal Immunostaining Kit, Immunotech, France) staining any plasma cells. Non-immune rat $IgG_1$, and omission of the primary antibody served as negative controls. The sections were first fixed in acetone at 4°C for 5 minutes, and then treated with 3% hydrogen peroxide in 70% methanol (ratio of hydrogen peroxide and methanol was 1:36) for 30 minutes to quench endogenous peroxidase activity. After washing in deionised water and rehydration in PBS for 10 minutes, the sections were preincubated with protein blocking agent (Universal Immunostaining Kit; Immunotech, France) for 30 minutes and then incubated with the primary antibodies in humidity chambers at room temperature for 60 minutes. After washing in PBS 0.05% for Tween-20, 3 times for 5 minutes, they were incubated with the secondary antibody for 30 minutes at room temperature in humidity chambers. They were washed again in the same buffer, and subsequently conjugated by streptavidin-horseradish-peroxidase for 45 minutes at room temperature. After washing again, the IL-5-labeled sections were stained for peroxidase reactivity with VIP kit (Vector VIP SK-4600; Vector, USA), counterstained with methylene-green, the TGF-beta1-labeled sections with amino-ethylcarbazole (AEC; Universal Immunostaining Kit; Immunotech, France) and counterstained with haematoxylin. The specimens were examined with a light microscope (Olympus BX50) equipped with digital camera (Olympus Camedia C-2020ZOOM).

### 3.2.3. HDC RT-PCR

Biopsy materials were weighed, chopped into pieces of 1 $mm^3$, and homogenized in 0.9% sodium chloride solution on ice, 1 ml solution was added to 100 mg tissue. Suspensions were centrifuged at 4°C at 3000 rpm for 10 minutes, and supernatants were stored in aliquots at -80°C until used. Total RNA isolation from the samples was carried out with TRI REAGENTT$^M$ (Sigma) and HDC RT-PCR with Access RT-PCR System (Promega). We used 2 microgram of total RNS from each sample. The following PCR program was used: 48°C for 45 minutes, 94°C for 2 minutes, 40 times (94°C for 30 seconds, 45°C for 1 minute, 68°C for 2 minutes) 68°C for 7 minutes. Two specific primers were applied (Pharmacia Biosystems): 5'-primer,

5’-AATCTTCAAGCACATGTC and 3’- primer, 5’-CTGGATAGTGGCCGGGATGA. For positive control we used mRNA isolated from WM35 primer melanoma cell line, for negative control deionised water. The results were evaluated with Chemiluminager 2000 gel documentation system. Densitometry was carried out with the computer program ChemiImager™ 5500 (Alpha Innotech).

### 3.2.4. HDC Western blot

Biopsy materials were weighed, chopped into pieces of 1 $mm^3$, and homogenized in 0.9% sodium chloride solution on ice, 1 ml solution was added to 100 mg tissue. Suspensions were centrifuged at 4°C at 3000 rpm for 10 minutes, and supernatants were stored in aliquots at -20°C until used. Protein concentration was measured with Bradford method. 10 μg of protein was run on a 10% SDS-PAGE (sodium-dodecyl-sulphate-polyacrylamide gel) and transferred for one hour onto a nitrocellulose membrane. For blocking the membrane was dipped into 2.5 % slim milk solution for one hour. First antibody (polyclonal rabbit, anti human HDC, ICN) was used in 1:10000, and second antibody (goat, anti rabbit-HRP, Sigma) in 1:1000 dilution. Following ECL plus (Amersham) detection, using Chemiluminager 2000 gel documentation system, densitometry was carried out with ChemiImager™ 5500 (Alpha Innotech).

### 3.2.5. Histamine RP-HPLC

Tissues were homogenized (Bis–Tris-HCl, pH=7,0), then centrifuged ($4^oC$, 10 min, 10000 rpm). Following addition of 60% perchloric-acid, then KOH and OPA the samples were injected onto the chromatograph (Hypersil SCX Duet, 5μm). The eluents were A: 50 mM Na- acetate buffer pH=7,0, B: methanol. The gradients were: 15% B at 0 min., 65% B at 20 min, 40% B at 30 min, 70% B at 50-55 min. Speed was 0,8 ml/min. Fluorescent detector (Shimadzu) was used on 330/450 nm wavelength.

### 3.2.6. Indirect HDC Immunohistochemistry

4 μm cryostate sections were treated anti- HDC polyclonal antibody (rabbit, ICN, 1:1500, 30 min). Biotin labelled secondary antibody (goat, DAKO, 1:100, 30 min), then streptavidin-peroxidase conjugate (DAKO, 1:400, 30 min) was used. For

visualization Vector VIP substrate -kit (Vector labs) was used. Background staining was 0.5% methylgreen.

### 3.2.7. HNMT activity

Biopsy materials were weighed, chopped into pieces of 1 $mm^3$, and homogenized in 0.9% sodium chloride solution on ice, 1 ml solution was added to 100 mg tissue. Suspensions were centrifuged at 4°C at 3000 rpm for 10 minutes, and supernatants were stored in aliquots at -20°C until used. HNMT activity was determined by radiometric HNMT assay. The homogenates (20mM Bis-Tris-HCl buffer pH=7,0) were centrifuged (2 min, 4000g, 4°C). Supernatants were centrifuged again (10 min, 4°C, 23000g) and these supernatants were used. Reaction mixtures contained: 50 µl of supernatant + 20µl water, 1M Na –phosphate (pH=7,5) buffer + 10µl of histamine (histamine concentration = 37.5µmol/µl) or + 10µl water instead of the histamine. The reaction was started with the addition of 14C-SAM (1nCi/µl). The samples were incubated on 37°C for 30 min. The isotope labelled product was extracted with Toluene: isoamylalcohol / 0,17%PPO. Dpm was measured for 4 min. Specific activities were calculated in µU/ mg protein where 1 µU converts 1 pmol of histamine per minute at 37 °C.

### 3.2.8. H1R, H2R, H3R, H4R Western blot analysis

The removed tissues were chopped in pieces of 1 $mm^3$ and homogenized in lysis buffer solution on ice. The lysis buffer solution contained 20-30 mmol TRIS, 1% Triton X-100, 1 mmol Na-ortho-vanadate, 1 mmol PMFS, 5 mmol NaF, 1 mmol EGTA and 10 µg/ml leupeptin, and 10 µg/ml aprotinin. One ml solution was added to 100 mg tissue. Suspensions were centrifugalized at 4ºC at 3000 rpm (2770 g) for 10 minutes, and supernatants were stored in aliquots at -20ºC until used. Protein content of the samples was measured with Bradford method. 10-15µg of protein was run on a 10% sodium-dodecyl-sulphate-polyacrylamide gel and transferred for one hour onto a nitrocellulose membrane. For blocking the membrane was dipped into 2.5 % slim milk solution for one hour. The membrane was incubated with the first antibody (polyclonal rabbit anti human H1R, H2R, H3R and H4R antibodies, Alfa Diagnostic Intl. Inc; San Antonio, TX, USA) in 1:1000 dilution according to the manufacturer's instructions for one hour or rat anti-yeast α-tubulin (1:2500, Serotec, Kidlington, United Kingdom).

After rinsing, the membrane was incubated with the secondary antibody (goat antirabbit-HRP, Promega; Madison, WI, USA) in 1:2500 dilution, or rabbit antirat IgG$\alpha$ and $\lambda$ chain – HRP, Promega; Madison, WI, USA 1:10,000 dilution). After another rinsing electrochemoluminescence detection system (ECL plus, Amersham; Fairfield, CT, USA) was used. Results were evaluated by densitometry carried out with Chemiluminager 2000 gel documentation system (ChemiImager™ 5500, Alpha Innotech, San Leandro, CA, USA) and relative protein expression levels were calculated after normalization with the respective housekeeping $\alpha$-tubulin signals.

#### 3.2.9. ECP ELISA measurement

Biopsy materials homogenized and centrifuged as mentioned above. Eosinophil cationic protein (ECP) concentrations were measured by using commercially available solid phase sandwich ELISA kit (Mesacup ECP Test, Medical and Biological Laboratories Co. LtD; Nagoya, Japan), according to the manufacturer's instructions. In every case two parallels of each sample were examined.

## 3.3. Statistical analysis

Mann-Whitney U test and two-sampled t-test were used.

# 4. RESULTS

## 4.1. Local IgE ELISA measurement

IgE was not present in controls compared to polyps and especially to APs. Interestingly NAPs contain a relatively great, though not significant amount of IgE compared to controls (p=0.08), but much less than allergic ones. For statistical analysis Mann-Whitney U test was used. (Table 2., Fig. 9.)

*Table 2. Results of local IgE ELISA (NP=nasal polyp, AP=atopic polyp, NAP= non-atopic polyp, CNM=control nasal mucosa, SD=standard deviation)*

| **LOCAL IgE (µg/ml)** | NP | AP | NAP | CNM |
|---|---|---|---|---|
| AVERAGE | 84.77 | 139.14 | 21.33 | 1.00 |
| SD | 156.33 | 201.95 | 27.25 | 0.00 |

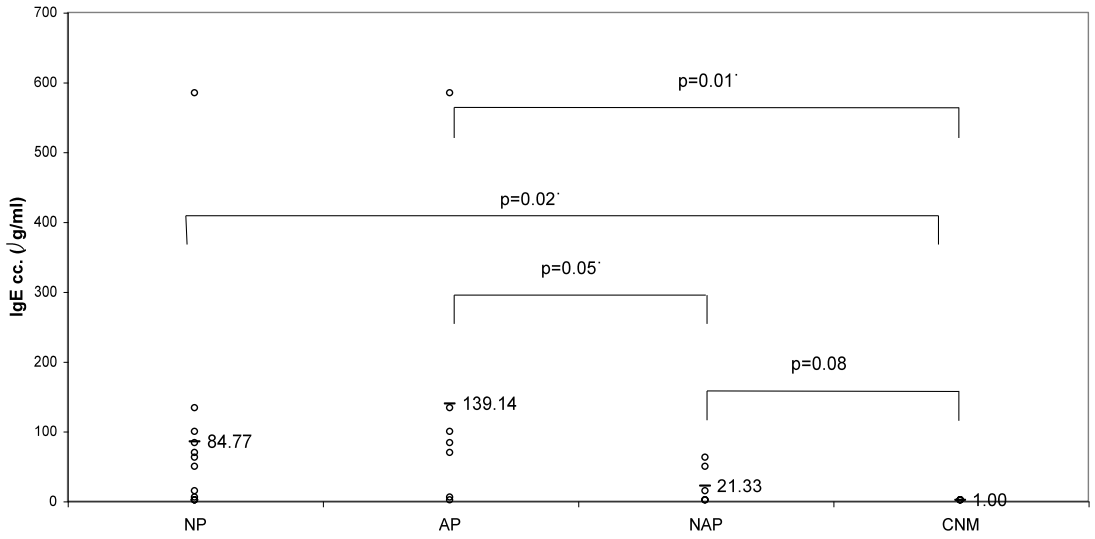


*Fig.9. Local IgE concentrations (NP=nasal polyp, AP=atopic polyp, NAP= non-atopic polyp, CNM=control nasal mucosa)*

### 4.2. Serum IgE ELISA measurement

Even the NAP group exhibits remarkable serum IgE level regarding the values in the normal population (0.1-1.0 μg/ml).[18] The values of the AP group are significantly higher compared to NAP. (Table 3., Fig. 10.) For statistical analysis Mann-Whitney U test was used.

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*Table 3. Results of serum IgE ELISA (NP=nasal polyp, AP=atopic polyp, NAP= non-atopic polyp, SD=standard deviation)*

| **SERUM IgE (μg/ml)** | NP | AP | NAP |
|---|---|---|---|
| AVERAGE | 283.00 | 489.71 | 41.83 |
| SD | 431.32 | 512.95 | 33.74 |

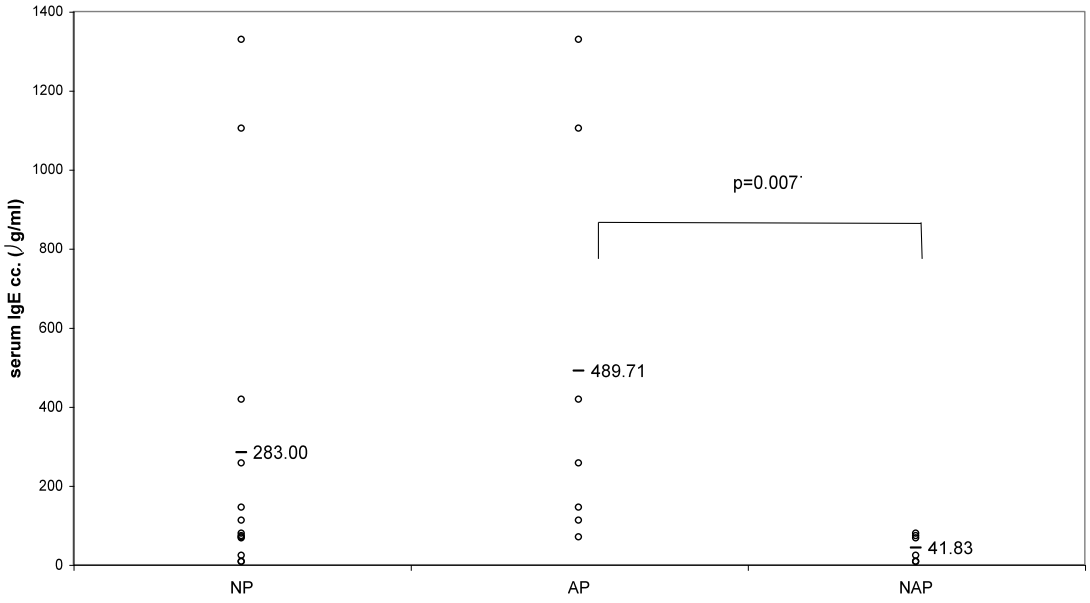



*Fig. 10. Serum IgE concentrations(NP=nasal polyp, AP=atopic polyp, NAP= non-atopic polyp)*

There is a good correlation between serum and tissue IgE in the NP (Fig. 11.) and AP (Fig. 12.) groups and a looser connection (p=0.1) in NAPs (not shown).

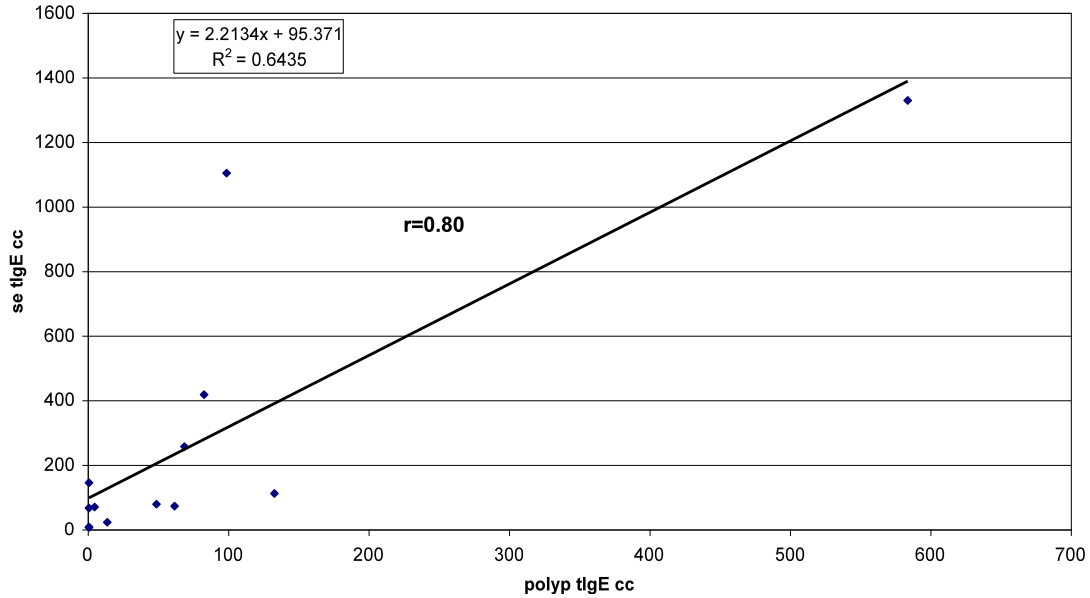



*Fig. 11. Correlation between serum and local IgE in patients with nasal polyp (se tIgE c c= serum total IgE concentration, polyp tIgEcc = polyp tissue IgE concentration)*

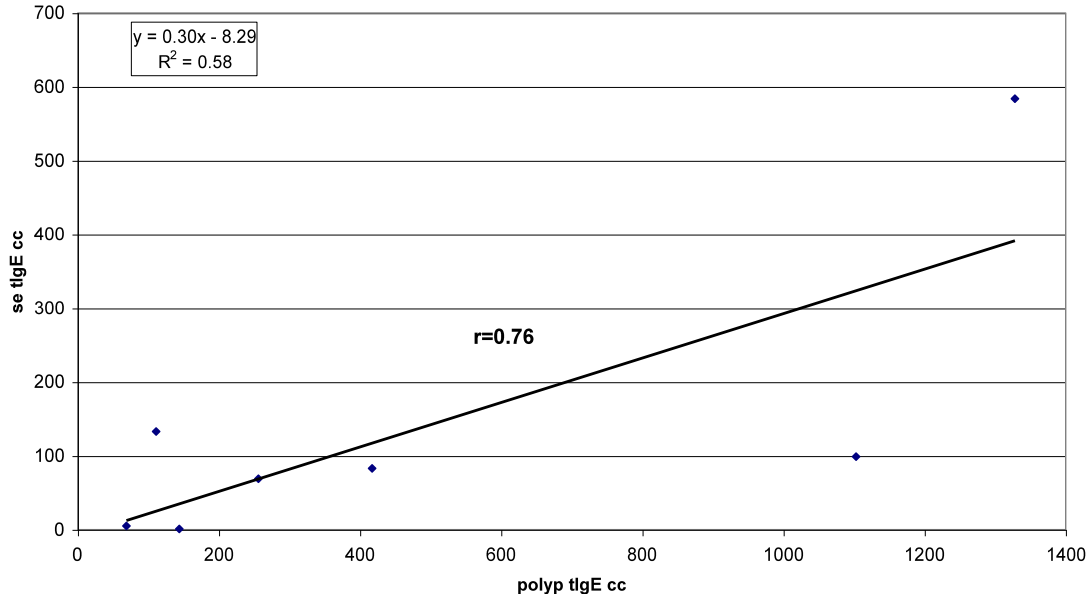



*Fig. 12. Correlation between serum and local IgE in patients with atopic polyps (se tIgE cc= serum total IgE concentration μg/ml, polyp tIgEcc = polyp tissue IgE concentration μg/ml)*

### 4.3. IL-5 ELISA measurement

IL-5 is equally detectable in allergic and non-allergic polyps with no significant difference and its concentration doesn't exceed the detection limit (15 pg/ml) in controls. (Table 4., Fig. 13.) In NPs (p=0.04) and separately the AP group (p=0.01) exhibit significant difference compared to the controls with the Mann-Whitney U test.

*Table 4. Results of IL-5 ELISA (NP=nasal polyp, AP=atopic polyp, NAP= non-atopic polyp, CNM=control nasal mucosa, SD=standard deviation)*

| **IL-5 (pg/ml)** | NP | AP | NAP | CNM |
|---|---|---|---|---|
| AVERAGE | 46.56 | 50.01 | 42.44 | 15.06 |
| SD | 42.64 | 43.34 | 43.72 | 16.71 |

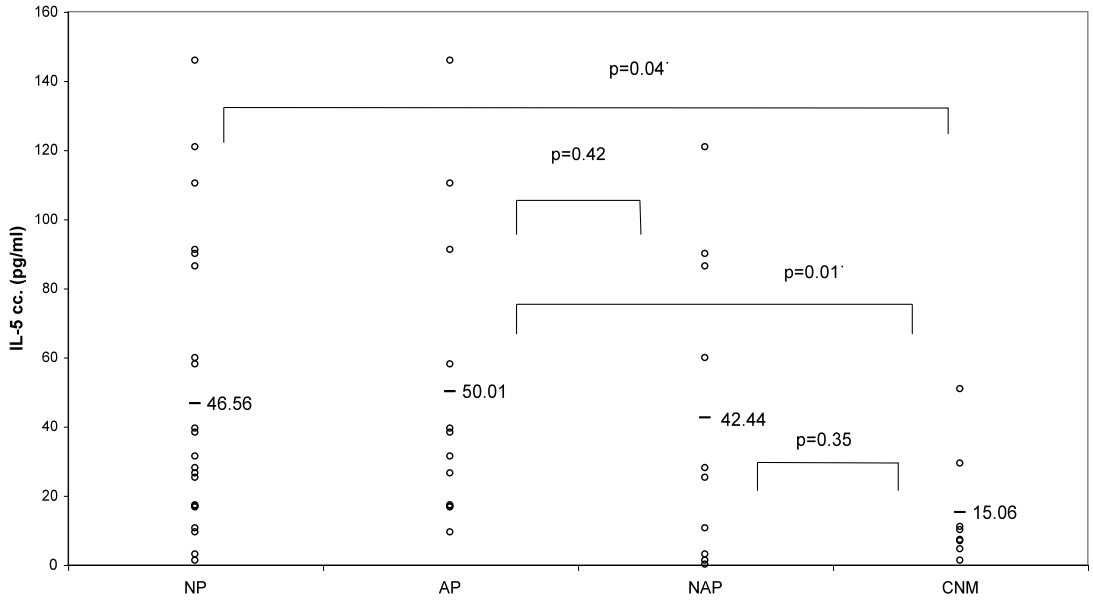



*Fig. 13. IL-5 concentrations (NP=nasal polyp, AP=atopic polyp, NAP= non-atopic polyp, CNM=control nasal mucosa)*

IL-5 and tissue IgE exhibited good correlation only in APs (Fig. 14.). There was no connection between IL-5 and serum IgE.

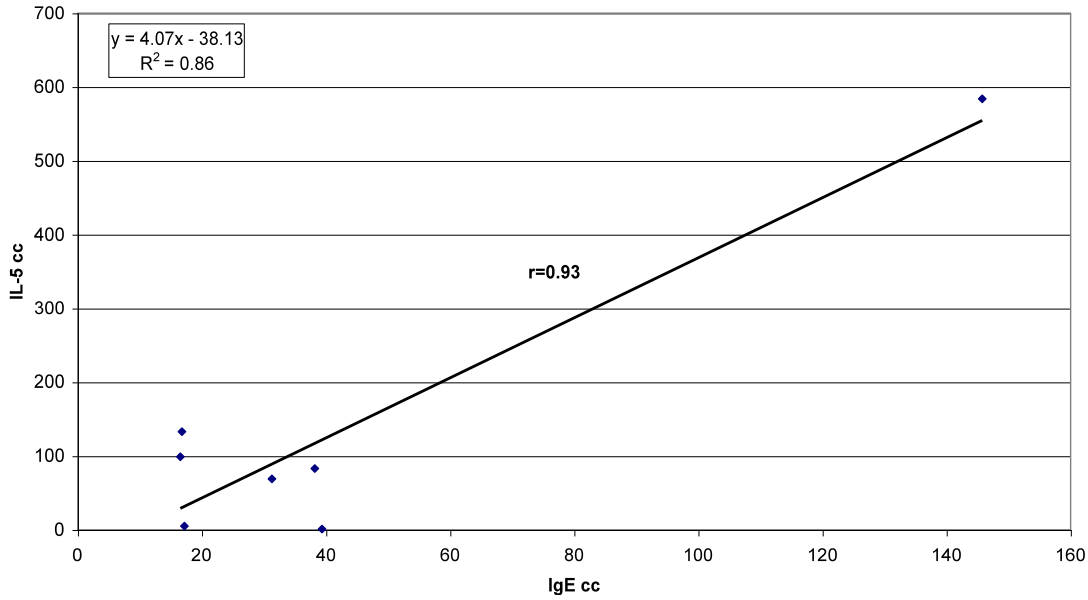



*Fig. 14. Correlation between local IgE and IL-5 concentration in allergic polyp tissue (IL-5 cc= IL-5 concentration, pg/ml, IgE cc=IgE concentration, μg/ml)*

### 4.4. TGF-beta1 ELISA measurement

The quantity of tissue TGF-beta1 is relevantly higher in controls compared to NPs with no difference between APs and NAPs (Table 5., Fig.15.). For statistical analysis Mann-Whitney U test was used. After activation with heating[32] before homogenisation there was no detectable quantity of TGF-beta1 neither in polyps (n=4) nor in controls (n=4), while in the non-activated reference samples (n=4) it was 78.79 and 122.00 pg/ml in polyps and controls. There was no difference in these results, if activation procedures were done after homogenisation.

*Table 5. Results of TGF-beta1 ELISA (NP=nasal polyp, AP=atopic polyp, NAP= non-atopic polyp, CNM=control nasal mucosa, SD=standard deviation)*

| **Active TGF-beta1 (pg/ml)** | NP | AP | NAP | CNM |
|---|---|---|---|---|
| AVERAGE | 430.17 | 336.08 | 481.40 | 783.90 |
| SD | 355.76 | 298.71 | 407.09 | 445.27 |

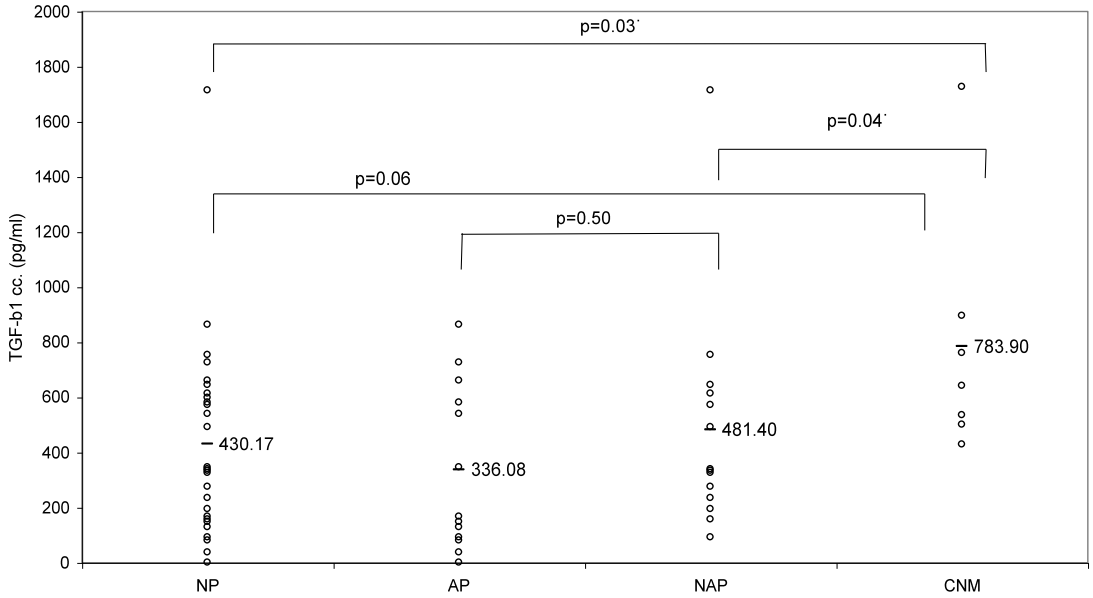



*Fig. 15. TGF-beta1 concentrations (NP=nasal polyp, AP=atopic polyp, NAP= non-atopic polyp, CNM=control nasal mucosa, SD=standard deviation)*

### 4.5. Histology of nasal polyp tissue with haematoxylin and eosin

To demonstrate the histomorphological structure of our polyp samples we show our haematoxylin and eosin stainings. (Fig. 16.)

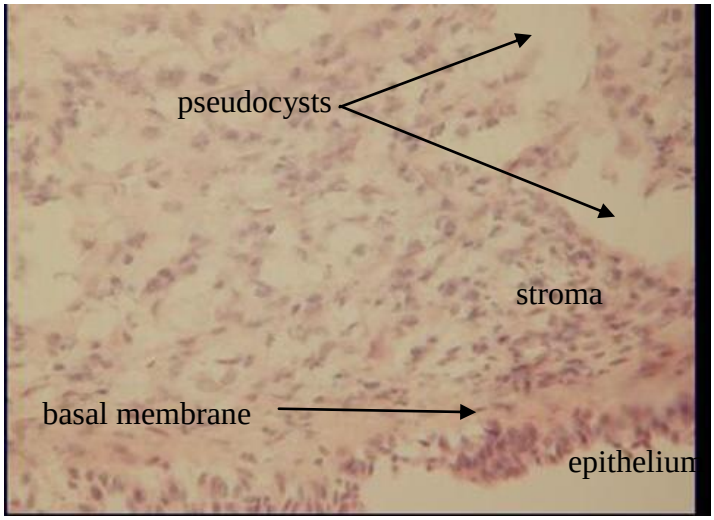



*Fig. 16. Haematoxylin and eosin staining in nasal polyp tissue. (Original magnification x 500 )*

### 4.6. Histology of nasal polyp tissue with Pappenheim's staining

Eosinophil cells were identified in a greater amount in polyps compared to controls (not shown) in the sections with Pappenheim's staining. (Fig. 17., Fig 18.)

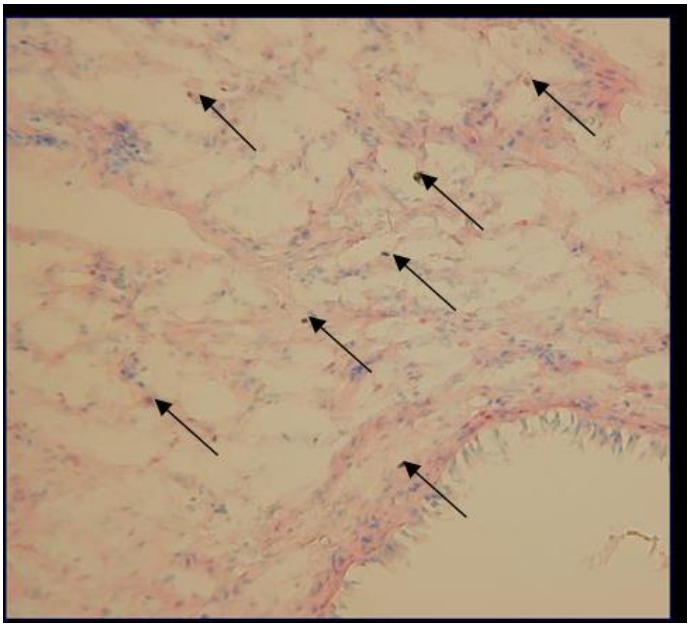


*Fig. 17. Pappenheim's staining, nasal polyp tissue (eosinophil cells - arrows – original magnification x 500)*

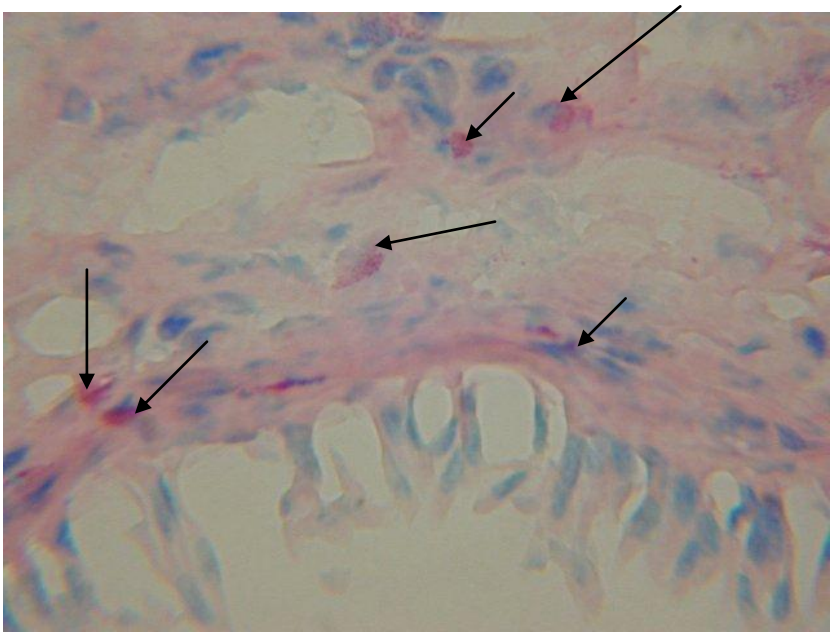


*Fig. 18. Pappenheim's staining, nasal polyp tissue, eosinophil cells with the characteristic nuclei and garunules (arrows) – original magnification x 1000)*

### 4.7. Localisation of IL-5 with immunohistochemistry

With immunohistochemical analysis IL-5 was detected in numerous - dominantly - eosinophil cells in polyp tissue. (Fig. 19., Fig. 20.)

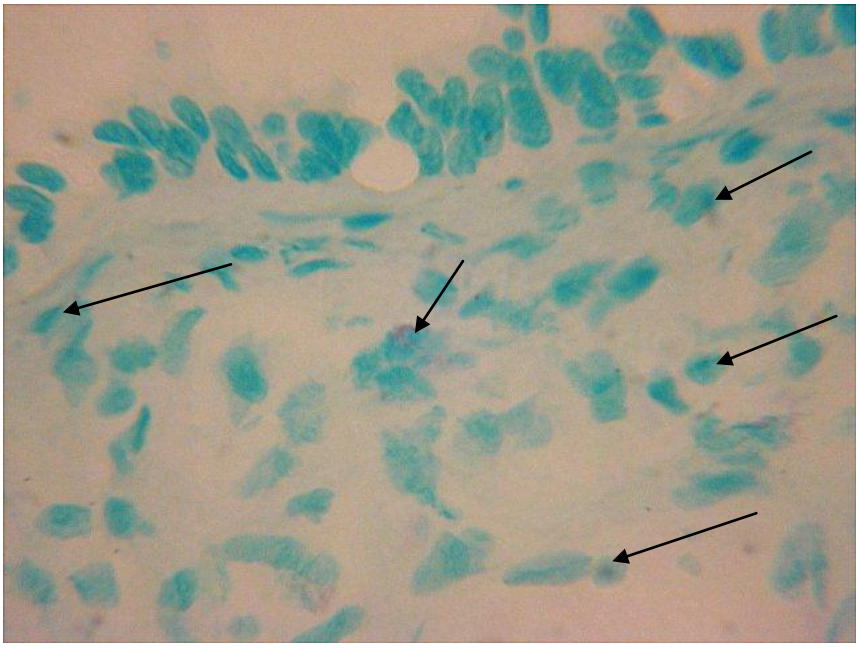


*Fig. 19. Immunhistochemical analysis for IL-5 in polyp tissue. IL-5 positive cells (arrow) counterstained with methylene-green. – (original magnification x1000)*

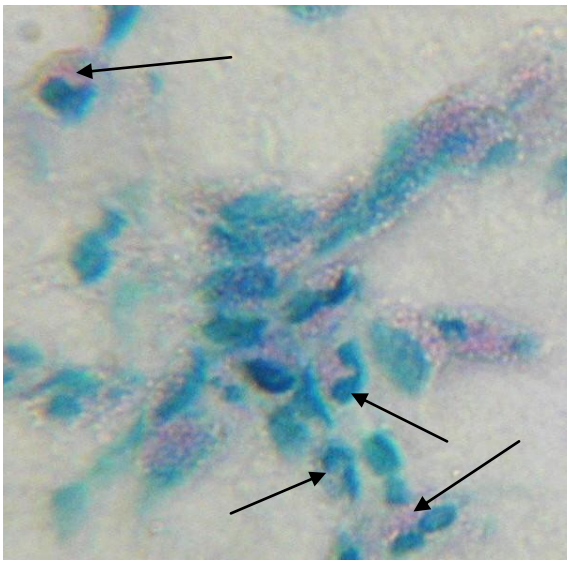


*Fig. 20. Immunohistochemical analysis for IL-5 in polyp tissue. IL-5 positive cells (arrow) counterstained with methylene-green. – (original magnification x1000)*

In control mucosa no acitivity was found. (Fig. 21.)

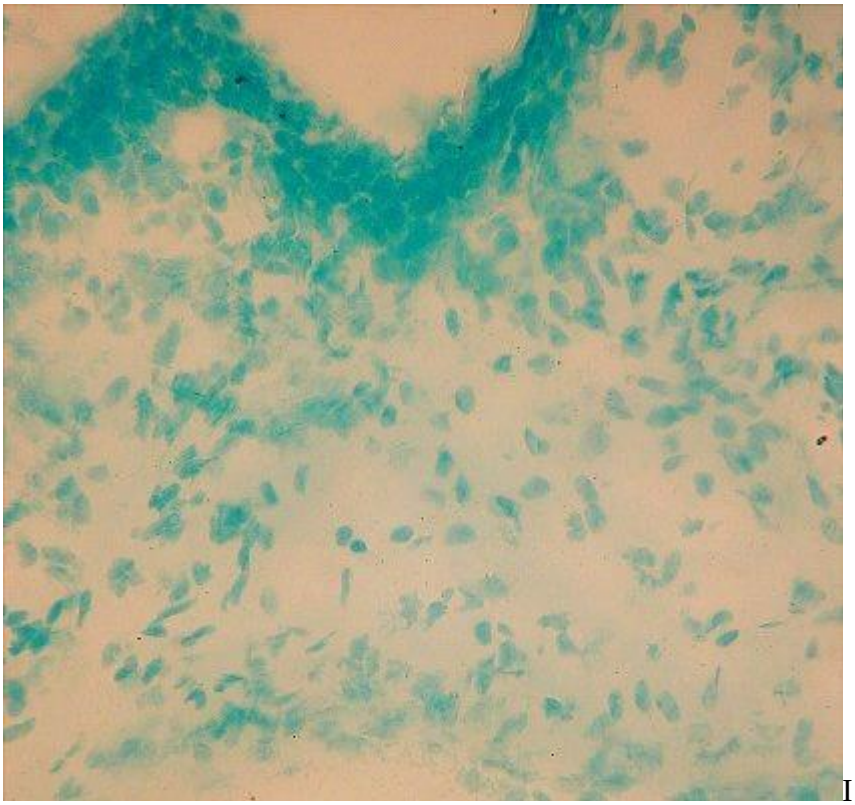


*Fig. 21. Immunohistochemical analysis for IL-5 in control mucosa. There are no IL-5 positive cells. Counterstained with methylene-green. – (original magnification x400)*

### 4.8. Localization of TGF-beta1

TGF-beta1 in nasal polyp tissue was detected in eosinophils and macrophages respectively, dominantly in the lamina propria, (Fig. 22.) while no activity was found in controls (Fig. 23.).

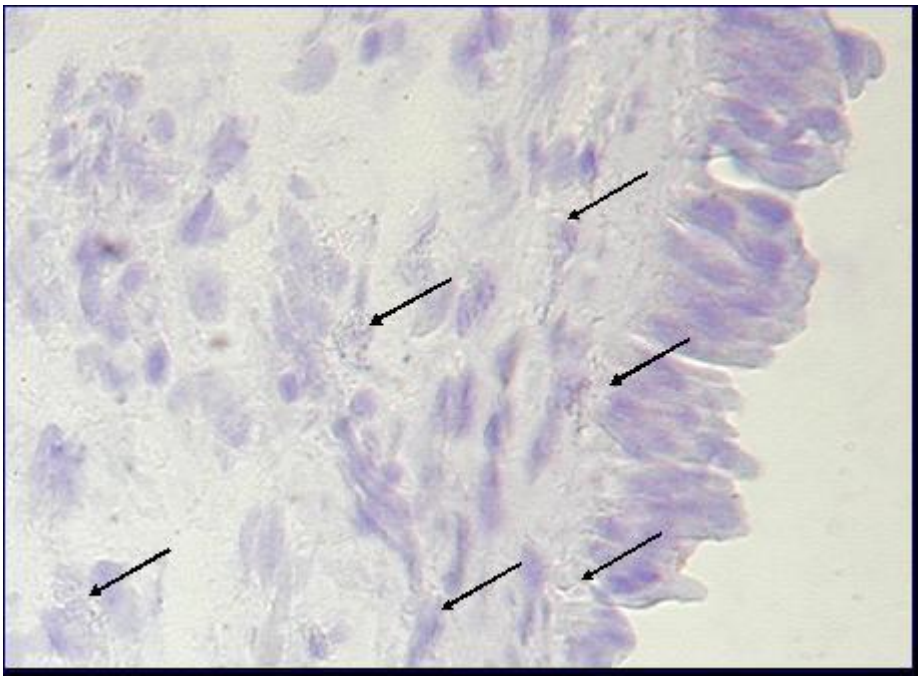


*Fig. 22. Immunohistochemical analysis for TGF-beta1 in nasal polyp tissue. TGF-beta1 (arrows) positive cells counterstained with haematoxylin. (original magnification x1000)*

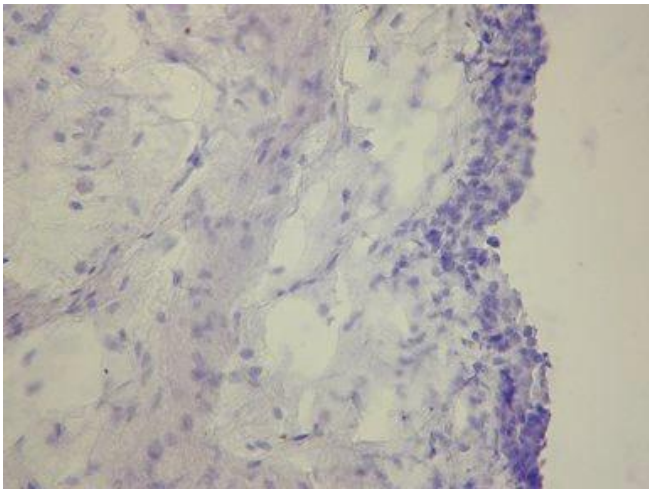
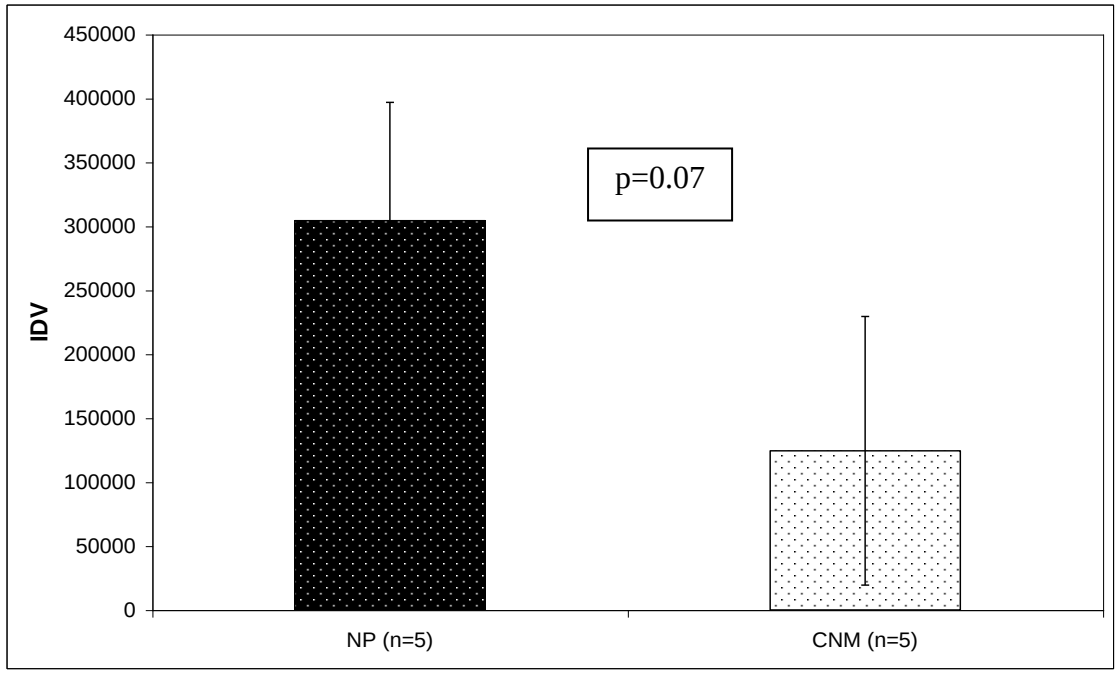


*Fig. 23. Immunohistochemical analysis for TGF-beta1 in control mucosa. No TGF-beta1 positive cells were found. Counterstained with haematoxylin. (original magnification x400)*

### 4.9. HDC RT-PCR

HDC gene expression was found to be higher in NP tissue than in normal nasal mucosa in 10% significancy level with Mann-Whitney U test. (p=0.07). (Fig. 24.)



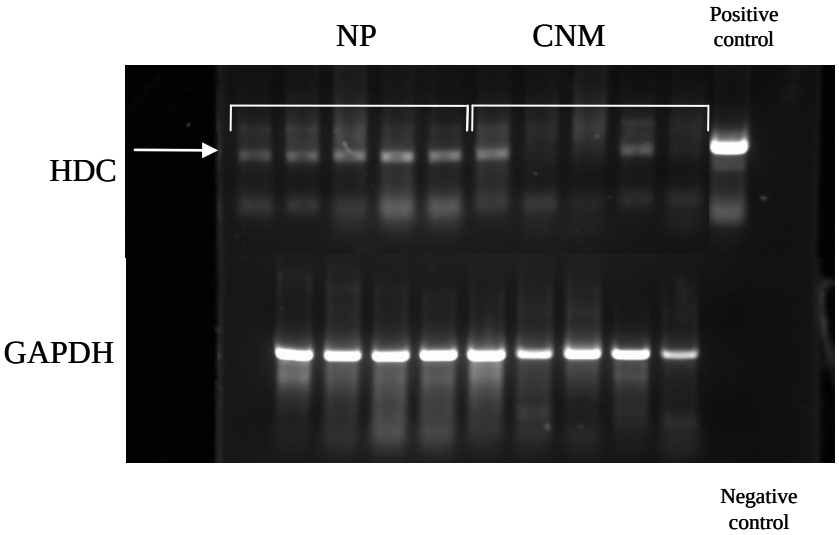




*Fig. 24. HDC (208 base pair) (RT-PCR, Positive control - WM35 primer melanoma cell line, Negative control - deionised water, IDV= integrated density value)*

### 4.10. HDC Western blot

HDC protein content also seemed to be higher in NP at 10% significancy level (p=0.07) than in the control with Mann-Whitney U test. (Fig. 25.)

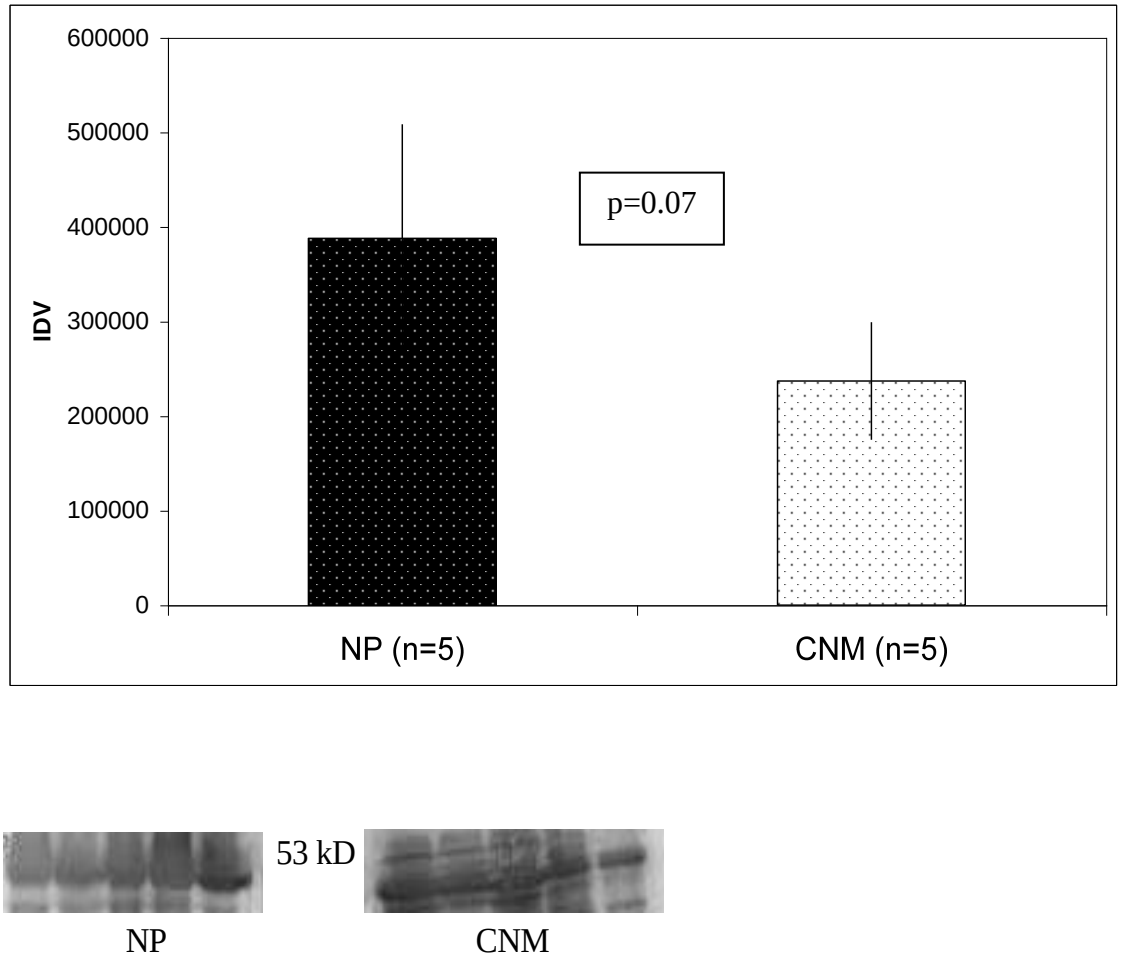



*Fig. 25. HDC protein (Western blot, IDV= integrated density value)*

### 4.11. HDC Immunohistochemistry

We found numerous HDC positive cells were in NP (Fig. 26.), while in normal mucosa sections we could not detect HDC positive cells (Fig. 27.).

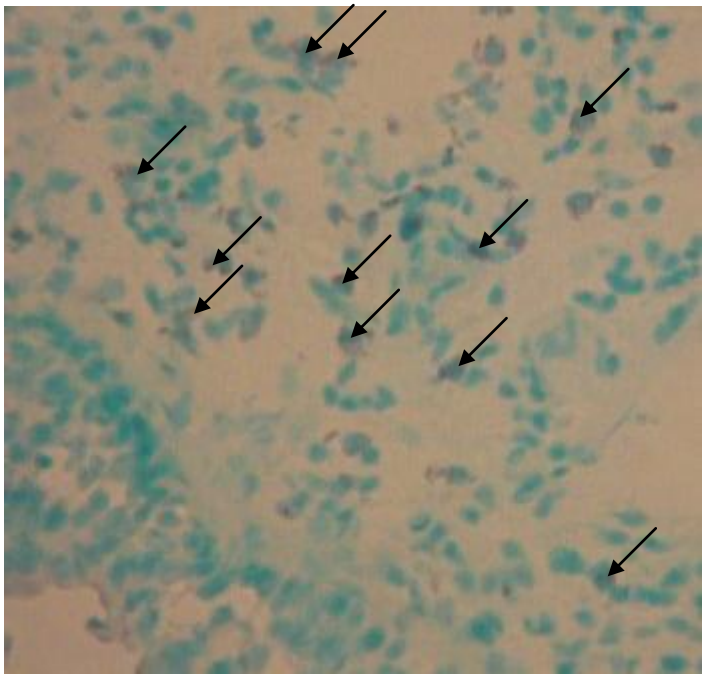


*Fig. 26. HDC immunopositive cells in nasal polyps (arrows). (Original magnification x400)*

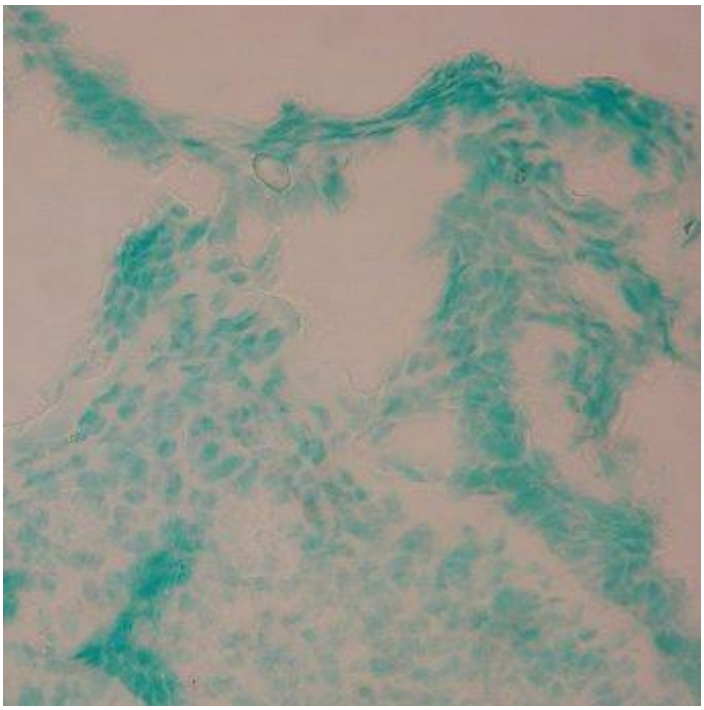


*Fig. 27. No HDC immunopositive cells detected in control nasal mucosa. (Original magnification x400)*

### 4.12. HNMT activity

HNMT activity was significantly (p=0.02) increased in NP compared to the control. (Fig. 28.). For statistical analysis Mann-Whitney U test was used.

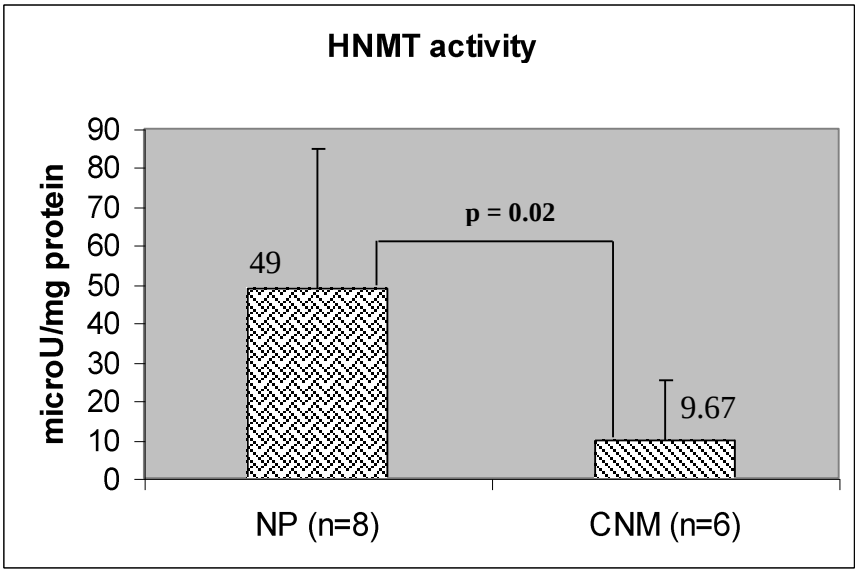



*Fig. 28. HNMT activity (average+SD)*

### 4.13. Histamine HPLC

There was no significant difference in the histamine content of NP and control mucosa. (Fig. 29). For statistical analysis Mann-Whitney U test was used.

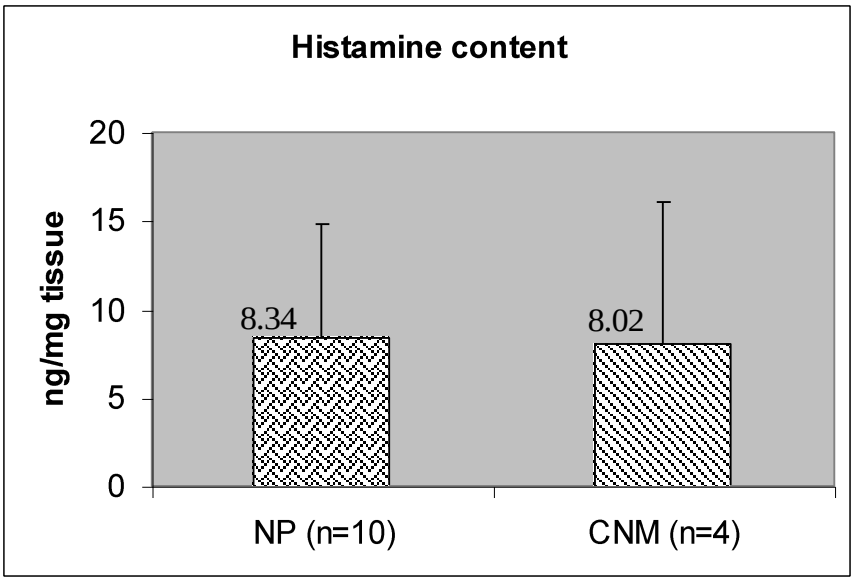



*Fig. 29. Histamine content (averages+SD, HPLC).*

### 4.14. Histamine receptors - Western blot

The amount of H1 receptors (Fig. 30A, Fig. 31A) and H4 receptors (Fig. 30D, Fig.31D) was elevated in polyp tissue compared to the control nasal mucosa (H1 receptor $p=0.045$; H4 receptor $p<0.001$). The expression of H2 (Fig. 30B, Fig. 31B) and H3 receptors was not increased (Fig. 30C, Fig. 31C). The differences of the mean levels were analyzed non-parametrically with the Mann-Whitney U test.

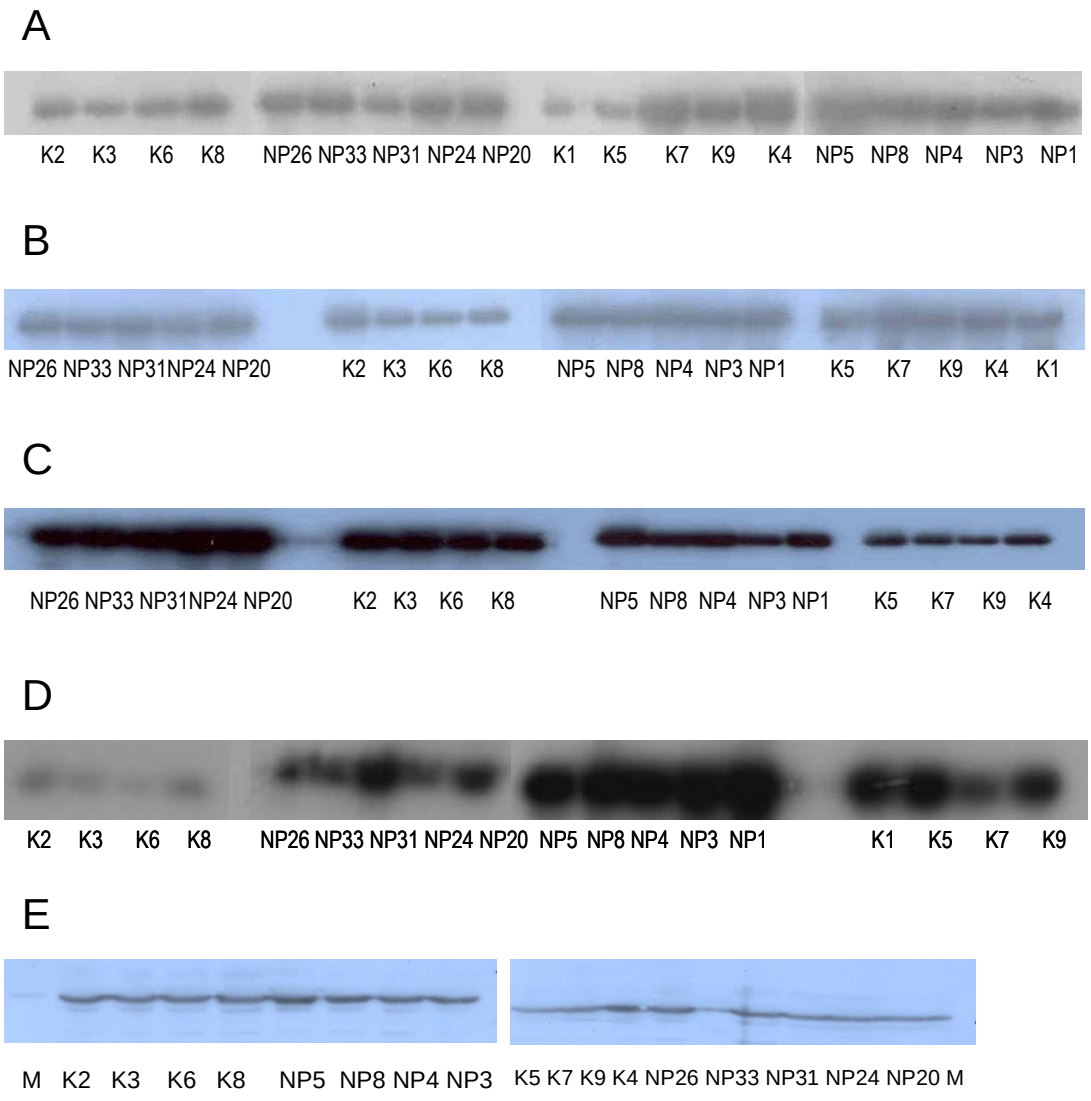



*Fig. 30. Western blot analysis for histamine receptor expression. Immunoreactive bands of histamine H1 (A), H2 (B), H3 (C) and H4 (D) receptors from individual samples (K1-9: controls, NP 1,3,4,5,8,20,24,26,31,33: nasal polyp speciments) – and immunoreacitive bands from alpha-tubulin housekeeping protein (E)*

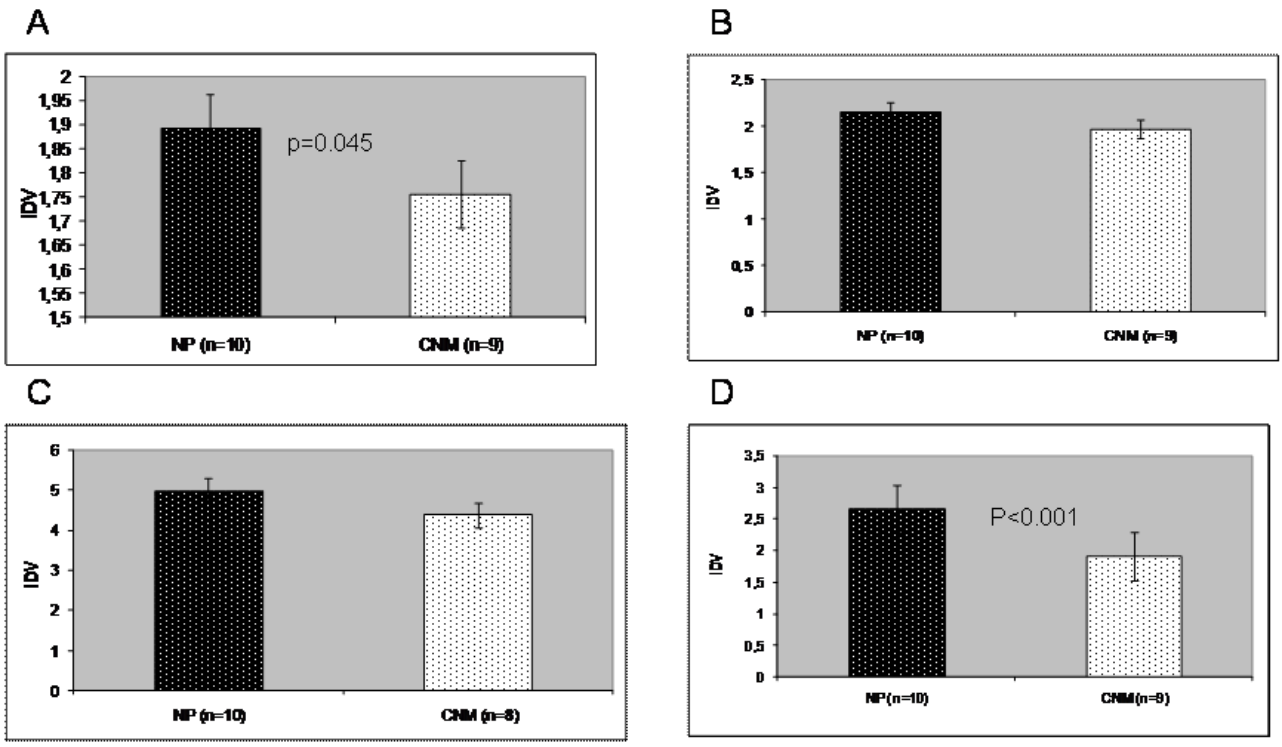



*Fig. 31. Densitometric analysis of histamine receptor protein expression. IDV normalized to alpha-tubulin housekeeping protein. (IDV= integrated density value) H1R (A), H2R (B), H3R (C), H4R (D)*

### 4.15. ECP ELISA measurement

The concentration of eosinophil cationic protein (ECP) was significantly higher (p=0.001) in polyp tissue (962.0 ng/ml) compared to controls (27.7 ng/ml) (Fig. 31.). For statistical analysis pooled T-test was used.

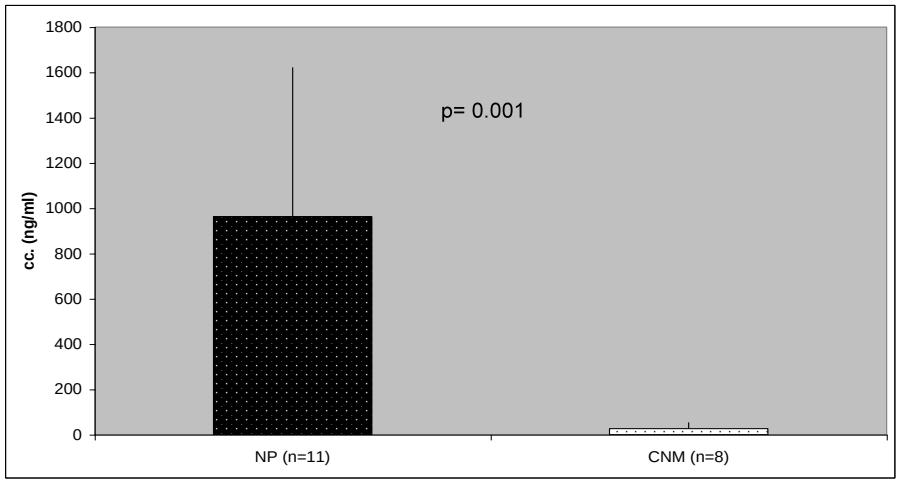



*Fig. 32. ECP concentrations in polyp tissue and control mucosa (ELISA, averages + standard deviation)*

Neither the correlation between the concentration of ECP and the level of H1 receptor, nor the correlation between H1 and H4 receptor expressions were not significant (not shown), while the concentration of ECP and the level of H4 receptor demonstrated a considerable correlation at 10 % significancy level ($r=0.52$; $p<0.1$; Fig. 33.).

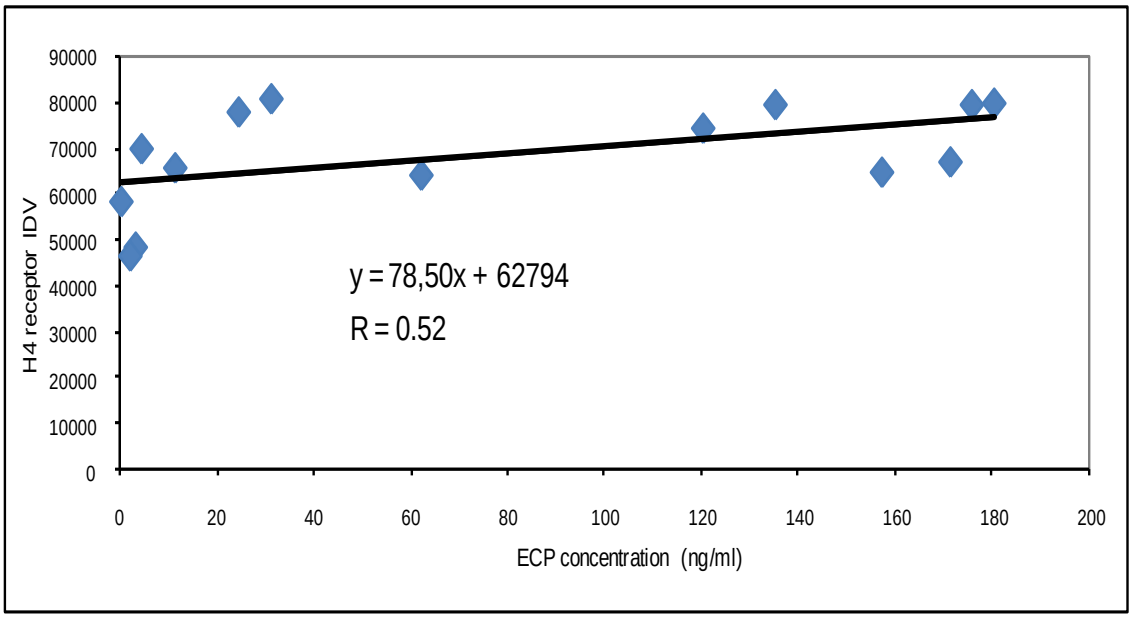



*Fig. 33. Correlation between ECP level and H4 receptor content.*

# 5. DISCUSSION

## 5.1. IgE

In our findings tissue IgE was significantly elevated in nasal polyps compared with nonpolyp tissue, and it was correlated with IL-5, as also described by Bachert et al.[30] Those authors demonstrated that atopy had a marked impact on tissue IgE levels in nonpolypous tissue, but not present in nasal polyps, suggesting that total and specific IgE are unrelated to skin Prick test positivitiy in nasal polyps. However our data, consistent with others[107,108], suggest that atopy might have an impact on tissue IgE levels even in nasal polyps. This is supported by the observation that total IgE was below the detection limit in nonatopic control mucosa samples, similar to the majority of nonatopic polyps. According to recent findings, Staphylococcus aureus superantigens might induce local IgE production, associated with provoking or modifying nasal polyp disease.[6,24,30] In our case tissue IgE levels did not correlate with total IgE. Asthma and polyp recurrence was absent when total IgE was below the detection limit.

## 5.2. IL-5

IL-5 is the most well-known growth factor of eosinophil granulocytes that is produced in $T_H2$-cells, mast cells and eosinophils. (Fig. 34.)

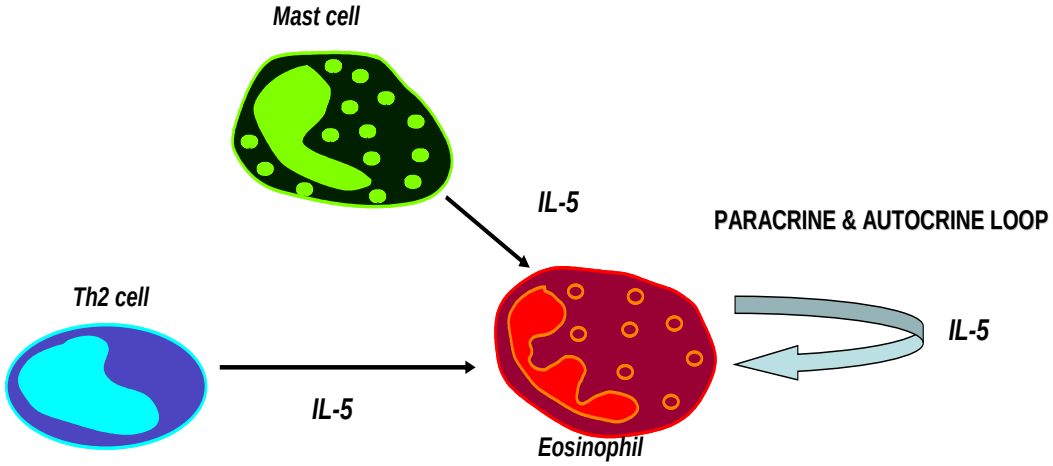



*Fig. 34. The role of IL-5*

The role of eosinophils in the pathogenesis of nasal polyposis has become obvious a long time before,[109,110] for this reason it seemed to be reasonable to suggest the pathogenetic role of IL-5, since it is an important factor in the formation and maintenance of eosinophil imflammation. The key role of IL-5 was emphasised by other authors too.[17,29]

In healthy control nasal mucosa the quantity of IL-5 - a $T_H2$-type cytokine - is not exceeding the detection limit,[8,21,22] while in NPs - independently from immediate hypersensitivity - it is detectable in relevant concentrations, as it was also described recently.[17,29] The high concentrations and good correlation of IL-5 and IgE in APs might be due to allergic mechanisms, but interestingly, these molecules are found in higher concentrations in both the tissue and serum in NAPs compared to controls. Tissue eosinophilia seems to be related to IL-5, which might be induced by IgE and IL-4 mediated allergic-atopic diseases or by other "local allergic" mechanisms triggered via unknown antigens with the formation of nasal polyps at the end.[21,22]

Antagonizing the effect of IL-5 is a potential new treatment strategy in patients with nasal polyps. Researches, that has been done to try this showed, that a single injection of anti-IL-5 reduces the size of nasal polyps for 4 weeks in half of the patients, and nasal IL-5 levels predict the response to anti-IL-5 treatment.[111]

### 5.3. Transforming growth factor-beta1

Transforming growth factor-ß1 turned out to be an essential regulator of the immune system. Downregulation of the inflammatory, upregulation of the tissue regeneration mechanisms and tumour formation seem to be its primary roles. It also has a basic role in the production and secretion of the ECM molecules (e.g. fibronectin) and fibrosis, especially by enhancing fibroblast and myofibroblast[35] activity. According to Nakagawa[16] there is a correlation between eosinophil infiltration, the oedematous feature, size and CT scores of NPs and the expression of fibronectin. Epithelial fluid absorption could be due to enhanced sodium transport as indicated by Bernstein et al.[112] in a study, in which transepithelial potential differences were measured in cultured cells. Little at al. described, that TGF-beta1 expression in polyp tissue can have dual effects. One role is to act as an anti-inflammatory agent shown by the ability to inhibit pro-inflammatory mRNA and protein production. At the same time, TGF-beta1 expression leads to increases in factors involved in fibrosis and angiogenesis, promoting remodelling and cell growth.[113] Coste et al.[38,114] demonstrated

increased cell proliferation via epithelial repair processes and secreting growth factors such as platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) in human nasal polyp epithelium. TGF-beta1 could limit the proliferative effects of other growth factors to control the epithelial cell proliferation in polyps. Loss of TGF-beta1 expression or the inactivation of TGF-beta1 receptors may give an explanation of the hyperproliferative disorders.

The ELISA and immunostaining techniques detect only the active form. The higher quantity in normal mucosa proved to be reproducible (three independent experiments), similarly to literature data.[35,106,115] There may be differences in the localization depending on the observation, that anti-TGF-beta1-3 monoclonal antibodies detect secreted form of TGF-beta1, while TGF-beta1 isoform-specific antibodies detect both secreted and matrix-associated TGF-beta1 forms. It is supposed, that the homogenisation procedure activated the LTBP either by yielding proteolytic enzymes to cleave the complex or by direct effect. Since there is no TGF-beta1 activity in controls in frozen sections but there is a great amount of that in the homogenised tissue, it seems to be evident, that normal nasal mucosa has significant latent TGF-beta1 concentration. The radically decreased quantity of the latent form in the polyp tissue might be due to an enhanced TGF-beta1 activation mechanism resulting in the shortage of the latent TGF-beta1. Neither of the in vitro, non-enzymatic activation procedures[32] before and after homogenisation resulted in an increased TGF-beta1 activation, moreover it decreased its concentration under detection level in both, the polyp and the normal samples. Heating and acidic incubation might have damaged LTBP and TGF-beta1 respectively in these settings.

### 5.4. Histamine metabolism

Numerous – sometimes contradictory – observations have already been made about the role of histamine in the nasal mucosa. Takeuchi and co-workers found, that histamine upregulates IL-8 mRNA expression and downregulates zonula occludens-1 (ZO-1) mRNA expression in cultured human nasal epithelial cells.[116] Di Lorenzo found that histamine in nasal fluids from patients with nasal polyposis was higher than that observed in patients without polyposis.[117] In contrast, Abe observed that neither histamine content nor HDC activity did increase in nasal polyp tissue.[43] Bachert could not demonstrate significant difference in the histamine concentration of polyp and non-polyp nasal tissue.[30]

As we saw from our studies and from other researches, nasal polyps are characterized by eosinophilic infiltration and by the presence of inflammatory mediators, such as tissue IgE, ECP and cytokines, especially IL-5.[118]

Some authors have found, that histamine induces IL-5 release from Th2 cells.[30,45,46,48]

In our findings there was no significant difference between the histamine content of nasal polyp and normal mucosa. Histamine metabolism although seems to be changed in nasal polyposis. First of all this is supported by the elevated levels of the only histamine forming enzyme, histidine-decarboxylase (HDC) in nasal polyp, which was detected in both gene expression and protein level. Beside mast cells other HDC positive cell types were detected in polyp tissue, that are very likely to be also histamine-source in polyp tissue. The reason that histamine level was not found to be elevated, in spite of the increased formation, seems to be, that histamine degradation is also more active. The tissue specific histamine degradating enzyme, histamine-N-methyl-transferase (HNMT) activity was detected to be elevated in nasal polyp, and this can be responsible for the faster histamine turnover.

The pathogenetic significance of this altered metabolism could be the increased IL-5 release from Th2 cells, as described by some authors previously in other tissues.[30,45,46,48] It also can play a role in the accumulation and activation of eosinophils, therefore might be one of the links between the "local allergic reaction" and the eosinophil inflammation.

### 5.5. Histamine receptors

Nakaya (and co-workers) first described the presence of all four histamine subtypes in the nasal mucosa. Interestingly, H3 and H4 receptors were found to be localized in the neurons of the turbinate tissue, though it is believed that H4 receptors are responsible for eosinophil and leukocyte trafficking.[119,120]

We were also able to demonstrate the presence of all four histamine receptors both in normal nasal turbinate mucosa and in nasal polyp tissue. The increased level of H1 and H4 receptors in polyp tissue might indicate, that the histamine related mechanisms are mediated preferentially through these receptors, and not by H2 and H3 receptors in the polyp tissue.

Eosinophil cationic protein (ECP) is a characteristic protein, that can only be detected in eosinophil cells. We found a tendency for correlation between the

expressions of ECP and H4 receptor, that might indicate the hypothesis that H4 receptor mediated histamine effects - beside other effects - may also have a role in eosinophil accumulation and activation in inflammatory diseases of the nasal and paranasal sinus mucosa, like nasal polyposis.

H4 receptors seemingly have a more significant role in mediating histamine effects in nasal diseases compared to H2 and H3 receptors. Histamine - the well-known mediator in allergic diseases – could be involved in the eosinophil accumulation of nasal polyp tissue through H4 receptors. (Fig. 35.) Elucidation of the role of receptors in the local cell biological regulation of tissues of nasal polyposis might give rise to possible clinical applications of future H4 antagonists in the medical treatment of nasal polyps. Morover, polypous tissues, as non-malignant models for highly activated local cellular activities, may serve for better understanding of histamine related cell functions.

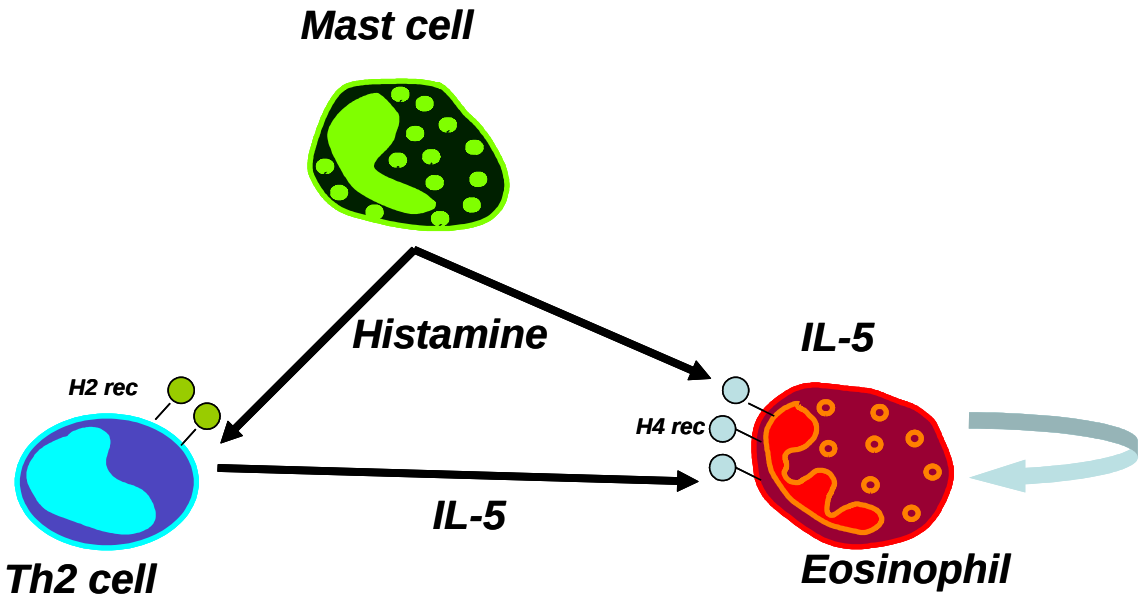



*Fig. 35. The role of histamine in nasal polyp tissue*

# 6. CONCLUSIONS

**6.1.** IL-5 plays a key role in the eosinophil recruitment and activation – described by other authors too - in nasal polyposis, and this process might be activated by both, allergic and non-allergic pathways.

**6.2.** Immediate hypersensitivity with systemic allergic reaction does not seem to be involved in the pathogenesis of this disease, but tissue IgE production might be due to local allergic mechanisms. The main sources of IL-5 and TGF-beta1 seem to be the eosinophils and macrophages.

**6.3.** TGF-beta1 seems to be an essential factor in the regulatory mechanisms and pathogenesis of nasal polyposis. Integrity of the healthy nasal mucosa is maintained - besides other factors - by TGF-beta1, while the balance of this microenvironment could be changed radically by local immunologic and allergic mechanisms triggered by unknown factors, which might end up in polyp formation.

**6.4.** The altered histamine metabolism – elevated formation by HDC and increased degradation by HNMT - as a stimulator of IL-5 release can be an important factor in the pathomechanism of polyp formation.

**6.5.** In nasal polyp tissue only the level of H1 and H4 receptors are increased, the H2 and H3 receptor levels are not. Therefore H4 receptors might have a more significant role in mediating histamine effects in nasal diseases than H2 and H3 receptors. In nasal polyposis the relevant and well-known eosinophil accumulation might be navigated by histamine, which seems to be an important link between the “local allergic reaction” and the eosinophil inflammation. Elucidation of the role of H4 receptors in nasal polyposis might give rise to the possible clinical applications of future H4 antagonists in the medical treatment of nasal polyps.

# 7. PATHOMECHANISM

As a response to an unknown trigger – that may be fungi, different kinds of inhalative allergens, or Staphylococcus aureus superantigen – a "local allergic reaction" starts, that is mainly characterised by IgE production originating from plasma cells. Eventhough it is possible that mechanisms other than an IgE-mediated response could cause degranulation of mast cells in nasal polyps,[121] it has been shown, that IgE is able to induce histamine synthesis in bone marrow mast cells even in the absence of antigen by the upregulation of HDC.[122] Therefore the role of the "local allergic mechanism" seems to be not in the release of histamine, but in the enhanced metabolism. As our studies have showed we did not find elevated histamine levels, but we found as well as the formation as well as the degradation of histamine to be increased. The elevated level of H4 receptors suggests the role of histamine in the activation[84] and recruitment[85] of eosinophils in nasal polyposis. We were also able to demonstrate the presence H2 receptors although its level did not increase significantly in nasal polyp tissue. It has been shown, that histamine induces IL-5 release from Th2 cells through H2 receptors.[30,45,46,48] In summary, the altered histamine metabolism mainly through H4 receptors - affecting directly the eosinophils-, but also through H2 receptors - inducing IL-5 release of Th2 cells - results in the autocrine loop of eosinophils is maintained by IL-5. The role of the other two histamine receptors in nasal polyposis is not clear yet. Again, transforming growth factor-beta can modulate fibroblast function and thus contribute to eosinophil infiltration and stromal fibrosis. [123] Active TGFbeta1 helps the epithel proliferation and hyperplasia, the deposition of fibronectin, thickening of the basal membrane, accumulation of ECM, vasodilatation, increased vascular permeability, oedema formation, and deposition of plasma proteins. These phenomena lead to the formation of nasal polyp. (Fig. 36.)

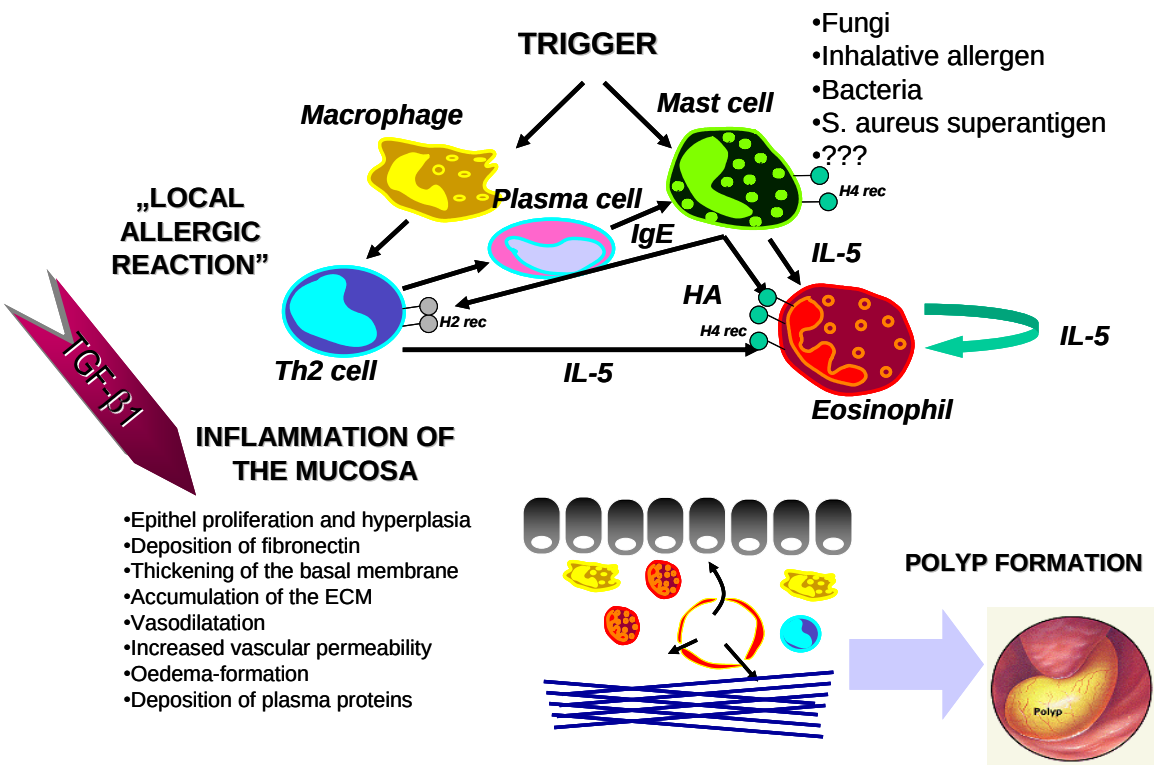



*Fig. 36. Formation of nasal polyp*

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# 9. PUBLICATIONS

**Publications related to the PhD dissertation**

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