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

Thesises

Adrienn Jókúti MD.

SEMMELWEIS UNIVERSITY
DOCTORAL SCHOOL OF MOLECULAR MEDICINE

BASIC AND CLINICAL IMMUNOLOGY

Tutor: Dr. Sára Tóth Ph.D.

Budapest, 2008.

INTRODUCTION

Nasal polyps are benign pedicled or sessile mucosal protrusions into the nasal cavity of multifactorial origin and are characterised by chronic mucosal inflammation. It 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 with eosinophil accumulation. The allergic fungal and eosinophilic inflammation has recently new insights on the pathogenesis of nasal polyposis. 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. Many cytokines (IL-4-6, 8, TGF-beta, GM-CSF etc.), chemokines (eotaxin, RANTES) and adhesion molecules (E- and P-Selectin, VCAM-1) have proven to be involved in the formation of polyps. It has already been clearly demonstrated, that IL-5

is upregulated in polyp tissue, and its production might be histamine-mediated. Accumulation and activation of eosinophils might be favoured by the overproduction of IL-5. Little is known about the presence and the role of histamine in human nasal mucosa and polyp. The contribution of tissue immunoglobulin E (IgE) in the pathogenesis of nasal polyps is not yet fully uncovered. Recent studies have 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. The relation of latent and active TGF-beta1 and its role in polyp growth and transdifferentiation might also be a particular interest.

AIMS

- The aim of our first studies was to study IgE, IL-5 and TGF-β1 in the pathogenesis of nasal polyposis and to localize these cytokines to relevant cells and structures in the polyp tissue. The special activation mechanisms of TGF-β1 have also been considered.
- One important mediator of IL-5 release may be histamine. For this reason we also wanted to estimate the role of histamine metabolism in the pathomechanism of nasal polyposis.
- 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.

MATERIALS AND METHODS

Materials

- 34 nasal polyp samples for the researches of IL-5, IgE, TGF-beta1, 15 for HDC, HNMT measurements and 11 for histamine receptors - removed during routine functional endoscopic sinus surgery (FESS) - were randomly selected from patients with chronic sinusitis.
- Normal healthy nasal mucosa (n=9 for IL-5, IgE, TGF-beta1, n=11 for HDC, HNMT measurements and n=9 for histamine receptors) was harvested from enlarged inferior turbinates in the process of routine septal operations. Allergy and other diseases, which might affect nasal mucosa, were excluded.
- Homogenisation was carried out, and then biopsy materials were stored at -20°C or -

80°C until used, depending on the following experiments.

- The studies were based in informed consent and ethical approval.

Methods

- IL-5 and TGF-beta1 ELISA
- Histology (Haematoxylin-eosine and Pappenheim's staining)
- IL-5, TGF-β1 immunohistochemistry
- HDC RT-PCR
- HDC Western blot
- Histamine RP-HPLC
- Indirect HDC immunohistochemistry
- HNMT activity
- H1, H2, H3, H4 Western blot analysis
- ECP ELISA

Statistical analysis

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

RESULTS

Local IgE

IgE was not present in controls compared to polyps and especially to atopic polyps. Interestingly in non-atopic patients with polyp we found a relatively great, though not significant amount of IgE compared to controls (p=0,08), but much less than allergic ones.

Serum IgE

The non-atopic group exhibits remarkable serum IgE level regarding the values in the normal population (0,1-1,0 μg/ml), the atopic group showed a significantly greater level though. (p=0,007). There is a good correlation between serum and tissue IgE in the polyp tissue (r=0,76; p<0,05) especially in the atopic group (r=0,8;

$p<0,05$) and a looser connection ($p=0,1$) in non-atopic patients.

IL-5

IL-5 is equally detectable in allergic and non-atopic polyps with no significant difference and its concentration doesn't exceed the detection limit (15 pg/ml) in controls. Non-atopic polyps and controls exhibit significant difference only with pooled T-test ($p=0,05$) but not with the Mann-Whitney test.

IL-5 and tissue IgE exhibited good correlation only in atopic polyps ($r=0,93$; $p<0,05$). There was no connection between IL-5 and serum IgE.

TGF-beta1

The quantity of tissue TGF-beta1 is relevantly higher in controls compared to polyps with no difference between atopic and non-atopic ones. After activation with heating 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.

Histology with Pappenheim's staining

Eosinophil cells were identified in a significantly greater amount in polyps compared to controls in the sections with Pappenheim's staining.

Localisation of IL-5 with immunohistochemistry

With immunohistochemical analysis IL-5 was detected in numerous - dominantly - eosinophil cells in polyp tissue.

Localization of TGF-beta1

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

HDC RT-PCR

HDC gene expression was found to be higher in polyp tissue than in normal nasal mucosa (p=0,07).

HDC Western blot

HDC protein content also seemed to be higher in polyp (p=0,07) than in the control.

HDC Immunohistochemistry

More HDC positive cells were in polyps than in normal mucosa sections by immunohistochemistry.

HNMT activity

HNMT activity was significantly (p=0,02) increased in polyp tissue compared to the control.

Histamine HPLC

There was no significant difference in the histamine content of polyp tissue and control mucosa.

Histamine receptors

The amount of H1 receptors and H4 receptors was elevated in the polyp tissue compared to the control nasal mucosa (H1 receptor p=0,045; H4 receptor p<0,001). The levels of H2 and H3 receptors were not increased significantly.

ECP

The concentration of eosinophil cationic protein (ECP) was significantly higher (p=0.002) in the polyp tissue (962.0 ng/ml) compared to controls (27.7 ng/ml)

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, while the concentration of ECP and the level of H4 receptor

demonstrated a considerable trend, although only at 10 % significancy level ($r=0.52$; $p<0,1$).

CONCLUSIONS

1. IL-5 plays a key role in the eosinophil recruitment and activation, but this process might be activated by both, allergic and non-allergic pathway.

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-β1 seem to be the eosinophils and macrophages.

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.

4. The altered histamine metabolism as a stimulator of IL-5 release can be an important factor in the pathomechanism of polyp formation.

5. 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.

PUBLICATIONS OF THE AUTHOR

1. Hirschberg A, Jókúti A, Darvas Z, Almay K, Répássy G, Falus A. The pathogenesis of nasal polyposis by immunoglobulin E and interleukin-5 is completed by transforming growth factor-beta1. Laryngoscope. 2003 Jan;113(1):120-4.

2. Jókúti A, Schwelberger H, Darvas Z, Páli Z, Bösze S, Falus A, Hirschberg A. Histamin metablism is altered in nasal polyposis. Inflamm Res. 2004 Mar;53 Suppl 1:S93-4.

3. Jókúti A, Hellinger E, Hellinger A, Darvas Z, Falus A, Thurmond RL, Hirschberg A. Histamine H4 receptor expression is elevated in human nasal polyp tissue. Cell Biol Int. 2007 Nov;31(11):1367-70.